WTEC Panel Report on

INTERNATIONAL ASSESSMENT OF RESEARCH AND DEVELOPMENT IN RAPID VACCINE MANUFACTURING

Joseph Bielitzki (Chair)
Stephen W. Drew
Cyril Gerard Gay
Terrance Leighton
Sheldon H. Jacobson
Mary Ritchey
WTEC PANEL ON RAPID VACCINE MANUFACTURING

Sponsored by the National Science Foundation (NSF), the National Institute of Biomedical Imaging and Bioengineering (NIBIB) of the National Institutes of Health (NIH), the National Institute of Standards and Technology (NIST), and the U.S. Department of Agriculture (USDA).

Dr. Joseph Bielitzki (Chair)
Chief Operations Officer
LensAR, Inc.
250 Park Ave S. Suite 310
Winter Park, FL 32789

Dr. Stephen W. Drew
Science Partners LLC
126 Mountain Avenue
Summit, NJ 07901

Cyril Gerard Gay, DVM, PhD
National Program Leader, Animal Health
National Program Staff, Animal Production and Protection
USDA – Agricultural Research Service
5601 Sunnyside Avenue
Beltville, MD 20705-5148

Dr. Terrance Leighton
Senior Staff Scientist
Children's Hospital Oakland Research Institute
5700 Martin Luther King Jr. Way
Oakland, CA 94609

Dr. Sheldon H. Jacobson
Professor, Willett Faculty Scholar
Director, Simulation & Optimization Lab
Department of Computer Science
University of Illinois
201 N. Goodwin Avenue (MC258)
Urbana, IL 61801-2302

Dr. Mary Ritchey
Ritchey Associates, Inc
206 Somerset Road
Norwood, NJ 07648

WTEC Mission

WTEC provides assessments of international research and development in selected technologies under awards from the National Science Foundation (NSF), the Office of Naval Research (ONR), and other agencies. Formerly part of Loyola College, WTEC is now a separate nonprofit research institute. Michael Reischman, Deputy Assistant Director for Engineering, is NSF Program Director for WTEC. Sponsors interested in international technology assessments and related studies can provide support for the program through NSF or directly through separate grants or GSA task orders to WTEC.

WTEC’s mission is to inform U.S. scientists, engineers, and policymakers of global trends in science and technology. WTEC assessments cover basic research, advanced development, and applications. Panels of typically six technical experts conduct WTEC assessments. Panelists are leading authorities in their field, technically active, and knowledgeable about U.S. and foreign research programs. As part of the assessment process, panels visit and carry out extensive discussions with foreign scientists and engineers in their labs.

The WTEC staff helps select topics, recruits expert panelists, arranges study visits to foreign laboratories, organizes workshop presentations, and finally, edits and publishes the final reports. Dr. R. D. Shelton, President, is the WTEC point of contact: telephone 410-467-9832 or email Shelton@ScienceUS.org.
**ABSTRACT**

Vaccine protection from infectious diseases represents decades of investment in basic research, manufacturing processes, and regulatory oversight. Vaccines are the most cost-effective and enduring medical countermeasures that science has developed; their efficacy has dramatically reduced illness, death, and epidemics worldwide. Today, emerging and accelerating public health threats require new vaccines to address a greater number of challenging diseases. To meet society’s expanding needs and mounting global disease pressure, vaccine production—currently slow and meticulous—needs to embrace innovation to become significantly more agile, modular, and rapid. For this to occur, manufacturers urgently need new engineering approaches that can rapidly and safely incorporate scientific discovery into scalable, adaptive vaccine manufacturing processes.

The WTEC study on research and development in rapid vaccine manufacturing evaluated the current status and trends in this field in the United States and Europe through workshops and site visits with the goal of identifying new areas of scientific and engineering opportunity and reward. It explored the specific context of vaccine manufacturing for mitigation of a major pandemic threat as the vaccine industry’s—and the world’s—most significant challenge in this field. The study included a focus on technologies for the manufacture of animal vaccines in terms of how those technologies might augment human vaccine production. It also examined economic incentive structures that currently favor legacy vaccine production processes over innovative ones.

This report discusses basic research for vaccine manufacturing, the regulatory process, and the economics of vaccine production and distribution as they currently impact the vaccine industry. It describes a number of promising models for more rapid vaccine manufacture already under investigation in a number of laboratories in the United States and Europe. Among the most important avenues to improve the rapidity of vaccine manufacturing and delivery, especially for a pandemic, are modular and disposable fermentation systems; validation tests that are part of an inline processing system; non-animal-based *in vitro* test systems; new adjuvants and immunogen delivery systems; application of microreactor technologies already used in the pharmaceutical industry; new vaccine-centered education, training, and compensation programs; improved synergy between animal and human vaccinology research and manufacturing operations; improved funding and incentive mechanisms; and increased focus on global harmonization of the regulatory process. The report concludes that there are salient new opportunities for overcoming bottlenecks and constraints within the existing vaccine production system through imaginative cross-disciplinary solutions, and that a more focused, coordinated domestic and international effort can accelerate progress toward more rapid production of safe, effective, and affordable vaccines.
ACKNOWLEDGMENTS

We at WTEC wish to acknowledge and thank all the panelists for their valuable insights and their dedicated work in conducting this international benchmarking study of rapid vaccine manufacturing R&D, and also to thank the presenters at the North American baseline workshop and all the site visit hosts for so generously sharing their time, expertise, and facilities with us. For their sponsorship of this important study, our sincere thanks go to National Science Foundation, the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health, the National Institute of Standards and Technology, and the Department of Agriculture. The subject of this study is of intimate concern to people everywhere; we believe this report provides a valuable overview of the field that can help citizens and policymakers around the world better understand and more effectively address requirements for new vaccine manufacturing technologies and systems so as to more rapidly produce effective vaccines that are reliable, affordable, and available as needed.

R. D. Shelton
President, WTEC
FOREWORD

We have come to know that our ability to survive and grow as a nation to a very large degree depends upon our scientific progress. Moreover, it is not enough simply to keep abreast of the rest of the world in scientific matters. We must maintain our leadership.\(^1\)

President Harry Truman spoke those words in 1950, in the aftermath of World War II and in the midst of the Cold War. Indeed, the scientific and engineering leadership of the United States and its allies in the twentieth century played key roles in the successful outcomes of both World War II and the Cold War, sparing the world the twin horrors of fascism and totalitarian communism, and fueling the economic prosperity that followed. Today, as the United States and its allies once again find themselves at war, President Truman’s words ring as true as they did a half-century ago. The goal set out in the Truman Administration of maintaining leadership in science has remained the policy of the U.S. Government to this day: Dr. John Marburger, the Director of the Office of Science and Technology (OSTP) in the Executive Office of the President made remarks to that effect during his confirmation hearings in October 2001.\(^2\)

The United States needs metrics for measuring its success in meeting this goal of maintaining leadership in science and technology. That is one of the reasons that the National Science Foundation (NSF) and many other agencies of the U.S. Government have supported the World Technology Evaluation Center (WTEC) and its predecessor programs for the past 20 years. While other programs have attempted to measure the international competitiveness of U.S. research by comparing funding amounts, publication statistics, or patent activity, WTEC has been the most significant public domain effort in the U.S. Government to use peer review to evaluate the status of U.S. efforts in comparison to those abroad. Since 1983, WTEC has conducted over 60 such assessments in a wide variety of fields, from advanced computing, to nanoscience and technology, to biotechnology.

The results have been extremely useful to NSF and other agencies in evaluating ongoing research programs, and in setting objectives for the future. WTEC studies also have been important in establishing new lines of communication and identifying opportunities for cooperation between U.S. researchers and their colleagues abroad, thus helping to accelerate the progress of science and technology generally within the international community. WTEC is an excellent example of cooperation and coordination among the many agencies of the U.S. Government that are involved in funding research and development: almost every WTEC study has been supported by a coalition of agencies with interests related to the particular subject at hand.

As President Truman said over 50 years ago, our very survival depends upon continued leadership in science and technology. WTEC plays a key role in determining whether the United States is meeting that challenge, and in promoting that leadership.

Michael Reischman
Deputy Assistant Director for Engineering
National Science Foundation

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\(^1\) Remarks by the President on May 10, 1950, on the occasion of the signing of the law that created the National Science Foundation. *Public Papers of the Presidents* 120: 338.

\(^2\) [http://www.ostp.gov/html/01_1012.html](http://www.ostp.gov/html/01_1012.html).
# TABLE OF CONTENTS

Foreword.................................................................................................................................................. i
List of Figures ......................................................................................................................................... v
List of Tables .......................................................................................................................................... vi
Executive Summary .............................................................................................................................. vii

1. **Introduction**  
   *Joseph Bielitzki and Terrance Leighton*
   
   Overview ........................................................................................................................................... 1  
   Vaccinology: Definition and Scope ................................................................................................. 2  
   Vaccine Development Issues ........................................................................................................... 3  
   Vaccine Manufacturing Issues ........................................................................................................ 4  
   WTEC Report: International Assessment of Rapid Vaccine Manufacturing ............................. 5  
   Acknowledgments ............................................................................................................................ 8

2. **Regulation and Control**  
   *Mary B. Ritchey*
   
   Background on Vaccine Regulation and Control .......................................................................... 9  
   The General Regulatory Process ................................................................................................. 10  
   United States Human Vaccine Regulations ................................................................................ 10  
   United States Animal Vaccine Regulations ................................................................................ 12  
   European Union Human and Animal Vaccine Regulations ....................................................... 12  
   Harmonization of Regulations ...................................................................................................... 14  
   Concepts for Optimizing the Regulatory Process for Speed and Efficiency .............................. 15  
   Special Considerations in a Pandemic Situation ........................................................................... 17  
   How Fast Is Fast? ......................................................................................................................... 18  
   Analytical and Control Testing ..................................................................................................... 21  
   Strategies to Increase the Speed and Efficiency of Analytical and Control Testing .................. 22  
   Summary and Conclusions ............................................................................................................ 23  
   References ...................................................................................................................................... 25

3. **Discovery and Development of Effective and Safe Vaccines**  
   *Terrance Leighton and Joseph Bielitzki*
   
   Discovery-Based Vaccine Development ....................................................................................... 27  
   Adaptive and Modular Production Processes ................................................................................ 31  
   The End-To-End Vaccine Discovery-Development-Market Approval Process ............................ 33  
   References ....................................................................................................................................... 35

4. **Platforms for Vaccine Manufacturing**  
   *Stephen W. Drew*
   
   Introduction .................................................................................................................................... 37  
   Vaccine Design .............................................................................................................................. 38  
   Strategies and Decisions for Vaccine Manufacturing Platforms .................................................. 38  
   Manufacturing Systems .................................................................................................................. 42  
   Modulating the Onset and Development of an Influenza Pandemic ............................................ 45  
   Manufacture of Animal Vaccines .................................................................................................. 50  
   New Technologies for Rapid Development and Delivery of Vaccines ........................................ 51  
   Conclusions .................................................................................................................................... 53  
   References ....................................................................................................................................... 53
# The Economics of Vaccine Delivery

*Sheldon H. Jacobson*

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background: Immunization and Vaccines</td>
<td>55</td>
</tr>
<tr>
<td>Routine Pediatric Immunization</td>
<td>55</td>
</tr>
<tr>
<td>Pediatric Combination Vaccines and Pricing</td>
<td>57</td>
</tr>
<tr>
<td>Pediatric Vaccine Shortages and Stockpiling</td>
<td>58</td>
</tr>
<tr>
<td>Influenza Vaccine Production and Demand</td>
<td>59</td>
</tr>
<tr>
<td>Vaccine Distribution and Pandemic Influenza Response</td>
<td>60</td>
</tr>
<tr>
<td>Pandemic Influenza Vaccine Production Issues</td>
<td>61</td>
</tr>
<tr>
<td>Questions and Challenges: The Future</td>
<td>62</td>
</tr>
<tr>
<td>References</td>
<td>64</td>
</tr>
</tbody>
</table>

## APPENDIXES

**A. Panelists' Biographies** ........................................................................................................ 67

<table>
<thead>
<tr>
<th>Site Reports—Europe</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Baxter Vaccines AG (Austria)</td>
<td>70</td>
</tr>
<tr>
<td>DECEHEMA (Society for Chemical Engineering and Biotechnology, with Mikroglas, Merck, and MicroChemTec; Germany)</td>
<td>73</td>
</tr>
<tr>
<td>European Centre for Disease Prevention and Control (Sweden)</td>
<td>76</td>
</tr>
<tr>
<td>European Collection of Cell Cultures (UK)</td>
<td>78</td>
</tr>
<tr>
<td>GlaxoSmithKline Biologicals (Belgium)</td>
<td>80</td>
</tr>
<tr>
<td>Health Protection Agency, Centre for Infections (UK)</td>
<td>84</td>
</tr>
<tr>
<td>Institut für Mikrotechnik Mainz GmbH (IMM; Germany)</td>
<td>87</td>
</tr>
<tr>
<td>Institute for Animal Health (UK)</td>
<td>91</td>
</tr>
<tr>
<td>Intervet and Nobilon International (The Netherlands)</td>
<td>93</td>
</tr>
<tr>
<td>Karolinska Institute and the Swedish Institute of Infectious Disease Control</td>
<td>97</td>
</tr>
<tr>
<td>National Institute for Medical Research (UK)</td>
<td>103</td>
</tr>
<tr>
<td>Novartis Vaccines (Italy)</td>
<td>105</td>
</tr>
<tr>
<td>Pfizer Animal Health s.a. (Belgium)</td>
<td>110</td>
</tr>
<tr>
<td>PowderMed, Ltd. (UK)</td>
<td>116</td>
</tr>
<tr>
<td>Scientific Institute of Public Health (Belgium)</td>
<td>120</td>
</tr>
<tr>
<td>University of Oxford (UK)</td>
<td>123</td>
</tr>
<tr>
<td>University of Siena (Italy)</td>
<td>127</td>
</tr>
<tr>
<td>University of Vienna and Bird-C GmbH &amp; CoKEG (Austria)</td>
<td>131</td>
</tr>
<tr>
<td>World Health Organization (Switzerland)</td>
<td>134</td>
</tr>
</tbody>
</table>

**B. Definitions of Terms** ......................................................................................................... 139
# List of Figures

2.1. Stages of U.S. vaccine review and regulation by CBER (FDA) ..........................................................11
2.2. Stages of EU/EMEA centralized procedure for review of vaccine license applications, both human and animal. ..................................................................................14
2.3. One possible approach for making vaccine available in an emergency ...........................................20
2.4. Example of lead times for manufacturing, testing, and review .........................................................22
2.5. The “lab-on-a-chip” concept. ................................................................................................................24
2.6. Application of “lab-on-a-chip” to ELISA assays. ..................................................................................24
2.7. Artificial immune system concept ........................................................................................................25

3.1. Scanning electron micrograph of an *E. coli* bacterial ghost ..........................................................28
3.2. Diagram of Dr. Rappuoli’s tripartite R&D approach to bridging the science and engineering gaps in the end-to-end process of vaccine production.........................................................35

4.1. Structural diagram of the influenza virus ..........................................................................................47
4.2. Three pandemic waves: Weekly combined influenza and pneumonia mortality, United Kingdom, 1918–1919.............................................................................................................47
4.3. Siena/Rosia Egg-based manufacturing ..............................................................................................49
4.4. Components of human skin .............................................................................................................52
4.5. Particle Mediated Epidermal Delivery (PMED™) device by PowderMed, Ltd. .................................52

B-1. Merck’s micromixing system relies on laminar flow .........................................................................74
B.2. Representative microprocessing plant with components available from IMM ..................................88
B.3. IMM’s lab-on-a-chip provides a suite of integrated processes ................................................................89
B.4. Novartis Vaccines research campus in Siena, Italy .........................................................................105
B.5. Novartis Vaccines and Diagnostics: global presence .................................................................106
B.6. Novartis Italian operations, Siena site .............................................................................................106
B.7. Novartis Italian operations, Rosia site ..............................................................................................107
B.8. Example of closed, automated systems, Pfizer ..............................................................................111
B.9. Formulation tank, Pfizer .....................................................................................................................111
B.10. Formulation, Pfizer .............................................................................................................................112
B.11. Filling, Pfizer ........................................................................................................................................112
B.12. Freeze-drying, Pfizer ...........................................................................................................................113
B.13. Packaging robot, Pfizer .......................................................................................................................113
B.14. Sterility Testing, Pfizer ......................................................................................................................114
B.15. PMED™ DNA vaccination .................................................................................................................117
B.16. Town center, Siena, Italy ....................................................................................................................127
LIST OF TABLES

1.1. Topics and Presentations at the WTEC North American Vaccine Manufacturing Workshop........7
1.2. European Sites Visited by WTEC Panelists.................................................................8

2.1. Highlights of Regulatory Review Time Frames, United States and European Union ..........19

4.1. Examples and Characteristics of Major Concepts for Design and Scale-Up of New, Improved Vaccines..................................................................................................................40
4.2. Molecular Innovations in North America and Europe that Improve Clinical Safety &/or Efficacy..................................................................................................................................43
EXECUTIVE SUMMARY

BACKGROUND
New infectious disease emergence, the threat of bioterrorism, and the increasing risk of global pandemics have focused public attention and expectations on innovations in vaccinology. Despite the best efforts of dedicated laboratories, industries, and governmental organizations, the often cumbersome systems for manufacturing and delivering new vaccines do not enable rapid response to public health emergencies. We have no assurance that vaccines can be developed, manufactured, approved, and distributed in time to prevent catastrophic morbidity and mortality.

The impetus for development of new vaccine manufacturing processes has eroded due largely to caution and costs. Still-dominant legacy models include industry’s continued use of alum as an adjuvant (first described and used in the mid-1920s) and of eggs for the production of vaccine against influenza virus (first used in 1933). Many current vaccine technologies predate the discovery of DNA. Total development time for a new vaccine can extend to well over a decade. While billions of dollars are being invested in the search for new protective technologies for infectious disease, there is no clear consensus or industry concurrence regarding a path forward to new, more rapid vaccine development and manufacturing models.

Roadblocks to preparedness for a pandemic disease are complex, and solutions are multidimensional and multidisciplinary. In the case of a bona fide pandemic, the failure to deliver vaccine in a timely manner will result not only in significant morbidity and mortality but also in loss of public confidence in science, industry, and government. Key impediments to achieving effective, rapid vaccine manufacturing include:

- Public perceptions that include near-zero tolerance of contraindications in vaccines as prophylactic health products and lack of understanding of the considerable costs involved in vaccine development
- Complex and costly regulatory requirements for new and modified vaccines and their associated manufacturing processes
- Inadequate government funding and/or incentives for innovation in vaccine manufacturing
- Economic and political structures that inhibit cooperation and collaboration, including inadequate protections for manufacturers (e.g., with regard to intellectual property, liability, and cost recovery)
- Lack of standardized, validated, and predictive immune assays, which limits clinical trial data comparisons
- Inadequate animal models of infectious disease and human immunity
- High production costs of clinical trial materials that limits translation of vaccine research to clinical development
- The global nature of disease and the need for integration of multinational vaccine development, manufacturing, and distribution systems
- Intellectual property claims and positions that constrain the use of new vaccine technologies
- Lack of formal education and training programs in vaccinology-related disciplines, combined with inadequate recruitment and career development options
- Insufficient focus on the zoonotic origins of human disease, on the potential for disruption of egg-based vaccine production, and on information- and resource-sharing among veterinary and human health experts
- Insufficient built-in surge capacity, production agility, adaptability, and modularity to enable sufficiently responsive vaccine manufacturing in case of emergency or unanticipated needs

There are ongoing, concerted international efforts to address these and related issues. Developments in advanced bioengineering, protective antigen identification, novel adjuvants, and novel delivery systems offer tools and technologies to transform how vaccines are developed and produced. Infusion of these new technologies into mainstream vaccine development will require substantial efforts to systemically incorporate leading-edge practices into discovery, development, commercialization, and regulatory compliance. This WTEC report addresses these issues and discusses solutions being proposed in North America and Europe to significantly accelerate and improve vaccine manufacturing capabilities.
THE WTEC STUDY: PURPOSE AND SCOPE

This international study of research and development in rapid vaccine manufacturing was sponsored by the National Science Foundation (NSF), the National Institute of Biomedical Imaging and BioEngineering (NIBIB) of the National Institutes of Health (NIH), the National Institute of Standards and Technology (NIST), and the U.S. Department of Agriculture (USDA). It was administered by the World Technology Evaluation Center, Inc. (WTEC).

The purpose of the study was to assess engineering methods, advanced technologies, and regulatory procedures for rapid vaccine manufacturing in Europe and to compare them to U.S. R&D programs, with the expectation that this review will inform policies and planning for response to a public health emergency. Although not intended to be exhaustive, the study reviewed a broad cross-section of innovations, bottlenecks, and hurdles that must be considered to achieve fast, agile, and responsive vaccine manufacturing. Veterinary vaccine issues were considered, due to the vital connections between animal and human vaccine protections. This report distills the findings of the WTEC panel. Its principal findings and recommendations are as follows.

STUDY FINDINGS

Regulation and Control

While vital to ensuring safety and efficacy, the regulatory process for vaccines is complex and challenging, frequently lasting longer than vaccine development times and contributing to the reluctance of manufacturers to incorporate new technologies. Vaccines are more highly regulated than other biologic products, with validation mandated for the product, the facilities and equipment, and the process of manufacture, and inspections conducted by experts in both the “product” and in good manufacturing practices. Clinical trials may extend over many years to establish both safety and efficacy, with subject numbers in the tens of thousands. An improved understanding of the key product attributes that control vaccine safety and efficacy, combined with faster, standardized and definitive assays for these attributes, would minimize transit time through the manufacturing-distribution chain.

The multinational use of vaccines requires manufacturers to submit to each national regulatory agency for approval and product release. The European Medicines Agency (EMEA) has been working for some time, with considerable success, to harmonize national regulations in Europe. It is now possible to request marketing authorization for a vaccine in all of the EU countries using a single application. The International Committee on Harmonization (ICH) is an EU-U.S.-Japanese effort to make the required information similar or identical in those nations. ICH activities have led to some progress, but much work remains to be done. In both the United States and Europe, efforts are being made to better define and accelerate regulatory procedures, with special considerations for pandemic or other emergency situations.

A major difference in regulation and control between the United States and Europe is in the organization of responsibilities with respect to human and animal vaccine products and processes. U.S. veterinary vaccines are regulated by the United States Department of Agriculture, whereas human vaccines are regulated by the Food and Drug Administration; each has different regulations for product approval and release. In the European Union, EMEA oversees production of both veterinary and human vaccines. Under this system, cross-training for production specialists and regulators is facilitated by the common regulatory framework, and cross-use of manufacturing facilities is possible—an important source of surge capacity in an emergency.

The panel identified the following avenues to reducing the time and cost burdens of regulatory review while maintaining the safety and efficacy of vaccine products:

- Increase fundamental scientific understanding of immunity elicitation, and implement \textit{in vitro} engineered human immune systems that provide standardized correlates of protection, to mitigate the inadequacies of current animal models.
- Extend understanding of key product attributes that control vaccine safety and efficacy, and develop fast, standardized, and definitive assays for critical product characteristics (minimize the number of tests).
- Incorporate micromanufacturing technology and small-scale, high-throughput bioreactors to provide well controlled and scalable systems for high-density, continuous production processes that overcome many of the current batch production process limitations.
Incorporate validated and mass-produced inline diagnostic/validation systems with disposable reactors and process components; disposable fermentation systems provide an opportunity to improve agility, modularity, and flexibility of immunogen production while maintaining standards at the GMP level.

Harmonize or mutually recognize U.S. and European regulatory processes to reduce the burden of preparing multiple documents and additional testing as required by separate regulatory agencies.

Harmonize U.S. animal and human vaccine regulations to promote innovation, adaptability, and increased surge capacity

Expand education, training, and career incentive programs in regulatory agencies and in the production and optimization phases of vaccine development and manufacturing to better support faster problem-solving, regulations that are more scientifically based, and resiliency in the vaccine enterprise.

**Discovery and Development of Effective and Safe Vaccines**

Vaccine discovery research ranges from foundational studies on epitopes necessary for immunogen selection, to how the immune synapse transfers information during antigen processing and amplification. Considerable investments are being made in vaccine discovery research, although few clinical candidates have emerged.

Effective response to an emerging disease requires the rapid identification of the pathogen, including its genotype, serotype, and pathotype. New technologies are available that use high-throughput PCR or DNA sequencing systems to rapidly classify both known and unknown threats. These technologies have also enabled reverse vaccinology (pioneered by Novartis Vaccines, Italy), where the pathogen genome is used to predict highly immunogenic epitopes. Such innovations have the potential to dramatically shorten vaccine discovery and development.

One new approach is determining how at a molecular level microbes are able to alter key signal transduction pathways to rapidly produce protective immunity; Toll-like receptors (TLR) are often the target of these new classes of immune stimulants or modulators. This has led to development of new adjuvants increasingly capable of reducing the antigen dose needed to induce immune response and the number of vaccinations required for protective immunity, as well as increasing the percentage of recipients who respond to a vaccine.

Concepts of antigen presentation are changing as carriers are developed that provide improved antigen processing. New systems for introducing immunogen to antigen presenting cells have seen sizeable investment and innovation. Current research focuses on the use of natural or synthetic particulates. Advanced delivery systems include “bacterial ghosts” (BIRD-C GmbH & CoKEG and University of Vienna), virus-like particles (Karolinska Institute), genetically modified viruses (University of Oxford), and synthetic cellular organelles and polymer particles (PowderMed)—with or without adjuvants.

Another area of research is how to rapidly produce large quantities of defined immunogens in standardized systems that can be readily approved by regulatory agencies. New methods include large, modular, disposable fermenters that adapt to a variety of cleanroom settings (Wave, Inc.). New microreactor technologies such as those under investigation at Mikroglas, IMM, and DEHEMA may be adaptable to high-throughput vaccine production needs, providing new paradigms for designing and evaluating pilot plants. Immunogen production research is leading in many new directions from use of transgenic chickens to cell-free synthetic systems (Stanford University: J. Swartz, 2007, http://wtec.org/vaccmfg/workshop-na/Proceedings/11-Swartz.pdf). Lastly, a new method being developed for evaluating vaccines is an in vitro artificial human immune system (VaxDesign, Inc.) capable of mimicking both cellular and humoral responses.

Vaccine discovery (exciting work, often with public accolades), development, and production (clinical trials, product launch, mass production, public use) have been redefined by Dr. Rino Rappuoli of Novartis Vaccines as (1) conceptualization, (2) gestation, and (3) growth and maturity. The development or gestation phase—the slow process of optimization and validation essential to product success and deeply linked to quality assurance and control (QA/QC)—is generally undervalued and underfunded; it is this phase that most hinders rapid development of new vaccines. Solutions include vastly improved training of professionals across the field and development of new academic disciplines (Rappuoli suggests “Structural Immunology”) focused on this phase. Specific, ongoing work towards accelerated discovery and development of effective and safe vaccines includes the following:

- Rapid identification of the causative agent following the index case
- Rapid identification of immunogenic epitopes for optimization
Executive Summary

- Identification of genetically stable epitopes for long-term vaccine production
- New systems that produce immunogens in transgenic plants and animals, optimized cell-based systems, microbial systems, or immunogens produced in food items
- New delivery systems that utilize natural or synthetic particles that carry both adjuvant and immunogen
- New methods for introducing the immunogen to the body that exploit natural epithelial barriers, such as transdermal patches, ingestion, or inhalation
- Basic research that identifies both innate and adaptive pathways beyond Toll-like receptors for activating an appropriate immune response in 100% of vaccinates
- New classes of adjuvants
- Defined and standardized correlates of protection that are recognized at a multinational level
- Alternatives to animals to produce equivalent or improved prediction of vaccine efficacy for humans
- Evaluation of microreactor technology for accelerated production of immunogens and for inline validation
- Greater commitment by academia to provide training in the area of vaccinology to increase the numbers and quality of workers in the field

Vaccine Manufacturing Platforms

Decisions on antigen selection and vaccine type (e.g., live whole-unit, recombinant, sub-unit, conjugated), on development specifics, and on means of delivery to the immune system must be translated into corresponding scalable, repeatable manufacturing procedures—manufacturing “platforms”—that meet rigorous regulatory requirements. Innovative manufacturing platforms are being pursued both in Europe (e.g., at Karolinska Institute, Oxford University, and the University of Vienna) and in the United States (e.g., Michigan State University and VaxInnate). At the same time, evolutionary improvements are being made to existing manufacturing platforms in which manufacturers are already heavily invested (e.g., at GlaxoSmithKline Biologicals [GSK] and Novartis Vaccines, both historically invested in egg-based manufacture). Process innovations include modular microprocessing systems for line design, configuration and modeling, mixing, filtration, separation, and formulation; and inline, real-time optical and chemical sensor systems that monitor information on microbial or cellular growth and multiple product characteristics.

An important consideration affecting manufacture is the vaccine delivery system; the traditional needle and syringe is slow, may not stimulate an ideal immune response, and can be problematic in terms of wastes. New targets for innovative vaccine delivery systems being studied in both the United States and Europe include the respiratory system, digestive system, urogenital system, and the skin. For example, the Particle Mediated Epidermal Delivery (PMED™) system of PowderMed, Ltd. (UK) uses high-pressure helium to deliver gold particles coated with DNA vaccine to dendritic cells in the skin.

Pandemic threats pose extraordinary challenges to systems for vaccine formulation and manufacture. Several approaches are promising to help speed response time and increase the number of available vaccine doses in a pandemic. EMEA has defined a regulatory procedure for “core dossier” approval of pre-pandemic vaccine manufacture based on substrains of avian influenza already known to have potential to infect humans. If only slight modifications to the vaccines and manufacturing procedures are needed, associated approval and scale-up processes can be considerably shortened. Novartis and Sanofi Pasteur have received EMEA approval for pre-pandemic vaccines and GSK for a “mock-up” whole virus alum-adjuvanted vaccine while a pre-pandemic vaccine is under review. Baxter has a cell-culture-based pre-pandemic vaccine in clinical trials. GSK is designing adjuvants to reduce the antigen dose needed to induce an immune response and thus increase proportionately the number of available doses of vaccine. The state-of-the-art facility of Intervet-Nobilon International (now part of Schering-Plough) in The Netherlands meets EMEA standards for both human and veterinary vaccine production; it can rapidly cycle between product types to provide flexible surge capacity.

Critical to improved and faster vaccine production will be cost-effective engineering improvements:

- Miniaturization, automation, and process integration
- Improvements in bioreactor technologies, including more rapid culture expansion methods that provide consistent growth environments
- Inactivation of cultures without degrading the important immunologic determinants
Executive Summary

- Transfer of materials in a sterile contained and controlled system (particularly lyophilization) with assured security and validation
- Improved waste collection, handling, and disposal
- Stabilization of the final product, including validation of stability and shelf life
- Improvements in delivery of the vaccine to the patient
- Improvements in product packaging and shipping
- Single-use and disposable equipment for batch production
- Further application of microprocessing technologies and use of integrated microfluidic quality control processes as validated inline test and validation systems
- Storage facilities to bank both master cultures and seed cultures with appropriate safety systems to prevent contamination; more rapid methods for culture expansion
- Utilization of new permissive cell lines and the continued use of established cell lines
- New culture media, free of human- or animal-derived components

Co-location of R&D, pilot, and production facilities, as in several plants in Europe, will facilitate rapid incorporation of engineering advances and better understanding in the plant of the nature of process changes.

The Economics of Vaccine Distribution

Vaccination unequivocally saves lives and allays the financial and other burdens of disease on individuals, families, and society. Yet the vaccine industry is small compared to other healthcare industries, vaccine supplies are fragile, and vaccine distribution continues to fail significant in-need populations worldwide.

In recent years, the number of vaccine manufacturers has fallen (there are now only four pediatric vaccine manufacturers that provide vaccine in the United States); many vaccines are produced by only one manufacturer; and there have been periodic global shortages of routine vaccines. The reasons are complex for the declining numbers of vaccine manufacturers and the rising potential for vaccine shortages. They include high facilities and validation costs, limited profit margins, unstable pricing constructs for multivalent vaccine, unpredictability in the annual pediatric immunization schedule; the complexity and long time frames for regulatory approval/reapproval of facilities and processes; and shortages of well-trained personnel. The U.S. General Accounting Office and Centers for Disease Control support creating six-month rotating vaccine stockpiles as a partial solution to these problems. (A helpful 2005 change in accounting rules allows firms to list vaccine put into stockpiles as revenue from sales.) Stockpiling is also part of EU strategy.

Under normal circumstances, both the U.S. and EU public health systems coordinate the public and private scheduled vaccination of millions of children born each year. Even these routine immunization systems have economic and other complications brought about by packaging of childhood vaccine in different multivalent combinations by different manufacturers (combination pediatric vaccines are more widely approved and used in the EU than in the United States); by new vaccine requirements for emerging problem diseases such as hepatitis A and B; and by philosophical, ethical, cost, and convenience questions on the part of parents. In general, production needs are determined by annual trends and limited to meet market demands.

Compared to pediatric vaccine, production and delivery of annual influenza vaccine is a risky, unstable process, because the vaccine must be reestablished each year due to virus mutation and because there are only three manufacturers that produce influenza vaccine for the U.S. market (Sanofi Pasteur, Chiron, and GSK). Production and compliance complications caused massive vaccine shortages in the 2004–5 flu season.

In the case of a pandemic, vaccine distribution systems would be tasked to deal with greatly expanded demand bases. U.S. healthcare infrastructures might have to handle an unprecedented need to vaccinate 80 million people in a 60-day period. Global planning for such an eventuality is taking place at the national and international levels, for local execution and implementation. Planning must account not only for creation, approval, manufacture, delivery, and stockpiling of appropriate vaccine, but also for defining priority immunization policies; distribution of supplies and handling of hazardous wastes; training and protection of healthcare workers; recording of statistical data; management of quarantine scenarios; management of information; and maintenance of public order and confidence in government. Some nations’ laws will forbid export of vaccine in a pandemic, suggesting schemes to broaden global distribution of vaccine manufacturing capabilities. Rapid vaccine administration technologies with less biowaste will be important tools in a global pandemic, as will be availability of adjuvants that reduce dosage requirements.
Several measures could improve the overall health and reliability of vaccine distribution businesses/systems:

- Expand development and use of computer-based tools to optimize, track, record, and predict
- Incorporate better predictability into the routine pediatric vaccination schedules and better cross-policy solutions between nations
- Implement pricing procedures that allow manufacturers to appropriately recapture investment costs
- Address requirements noted in earlier sections to speed vaccine discovery, approval, and manufacture, which can curb both costs and time to market and support more entrants into the market
- Optimize the distribution supply chain to promote flexibility/adaptability to meet surge demand

Several measures could improve preparation for a pandemic:

- Provide incentives and support to build surge capacity into manufacturing operations
- Amplify pre-pandemic planning activities both within nations and between nations
- Include broad representation in pre-pandemic planning to better generate consensus and anticipate and emplace measures to effectively, safely, and fairly meet the wide range of needs in an emergency
- Develop systems that integrate vaccine availability with outbreak biosurveillance, provide citizen protection by social distancing, and stratify at-risk populations
- Explore extraordinary measures to overcome commercial proprietary or intellectual property concerns that could delay production of the best vaccine for a pandemic disease
- Focus research on establishing the degree (if any) of cross-protection for pandemic virus afforded by childhood immunization and/or annual influenza vaccine

Public Health and Veterinary Vaccines

It is a rare infectious agent that is not shared between humans and animals. The veterinary vaccine industry produces a greater variety of vaccines and greater quantities of vaccine than does the human vaccine industry; it represents significant experience and expertise in vaccines and their manufacture. This expertise is infrequently accessed by the public health community in the United States. As mentioned earlier, it would be a great benefit to harmonize U.S. veterinary and human vaccine development and manufacturing standards.

Chickens and other wild and domestic fowl have a long history of infection with influenza virus. In the case of pandemic influenza, birds will likely be the initial hosts. This is an ongoing threat to poultry production but also a threat to human health should a virus mutate to allow transmission to and between humans, as in the three major influenza outbreaks of the 20th century. In addition, current influenza vaccine production is heavily dependent on chicken eggs for growing the virus necessary for vaccine production. In order to protect humans, the chickens must also be immunized, and quality assurance must determine that they are specific-pathogen-free for vaccine use. Overall, the interplay between human and veterinary health is substantial.

As an example of veterinary vaccine manufacturing practices that might be profitably translated to manufacturing of human vaccine, a number of veterinary vaccines are produced with specialized functions being distributed, for example immunogen production being separated from the fill and finish process. Human vaccines are typically produced as an end-to-end process. Also, veterinary vaccine manufacturing facilities typically safely manufacture a variety of products, while most human vaccine facilities are committed to a single product for the life of the facility.

Changes in U.S. human vaccine procedures and protections related to those for animal vaccines are overdue:

- Facilitation of greater communication between the public health community and the veterinary health community should be a priority.
- Modularity and flexibility are attributes of veterinary vaccine manufacture that should be carefully considered for human vaccine facilities.
- Engineering standards for all vaccine production facilities should be identical in order to enable utilization of veterinary vaccine production facilities for surge capacity in a human health crisis.
Final Observations

The WTEC panel found a consistent dedication throughout the vaccine manufacturing community to the goal of producing vaccines that are safe, effective, and absolutely constant in formulation from dose to dose. However, insufficient incentives for innovation or opportunities to recapture investment have severely constrained the number of vaccine manufacturers worldwide and global capacity to produce even traditional vaccine in sufficient quantity, let alone rapidly produce and deliver adequate amounts of vaccine in a pandemic situation. The need to support a more robust industry is increasingly apparent. There must be incentives for academic institutions, industry, regulatory agencies, and delivery agencies to embrace innovation through the full extent of vaccine discovery, manufacture, and distribution. It must be recognized that these activities are multidisciplinary and that cross-fertilization will be critical to success; incentives for collaboration are vital. Engineering expertise at every level from chemical to systems engineering can make significant contributions to improving this process. Workforce development is sorely needed at every level.

Fundamental to any systemic change to attain more rapid vaccine manufacturing will be broader appreciation on the part of policymakers and the general public of the value of immunization to national security and individual and global health, and the complexity of the routes to those goals—before a biological crisis or a pandemic should occur. Rapid, safe vaccine discovery, manufacture, and distribution will be the nation’s—and the world’s—best hope for rapid containment and for minimization of morbidity and mortality. We hope that this study contributes to advancing these important processes.

The WTEC Panel on Rapid Vaccine Manufacturing
December 2007
CHAPTER 1

INTRODUCTION

Joseph Bielitzki and Terrance Leighton

OVERVIEW

Despite vaccinological advances in the 20th Century that significantly enhanced disease prevention and health maintenance capabilities, converging demographic and medical trends are already bringing new potential for catastrophic spread of diseases worldwide in the 21st Century. During the period between 1950 and 2025, the human population of the earth will have tripled from 2.4 to 7.5 billion persons. Previously undisturbed and uninhabited geographic areas are now subject to intensive agriculture, forestry, mineral extraction, and other forms of environmental disruption. New transportation systems allow people to move anywhere on the planet within a day. The human population has significantly wider contact with animal populations and the environment. People are also in more frequent contact with each other, as urban lifestyles have replaced agrarian ones. People are living longer, but although many previously fatal diseases (e.g., autoimmune disease, HIV/AIDS) now have treatments that effectively extend life (e.g., cancer therapies, organ transplants), these tend to compromise immune function. These and other factors serve both to increase human exposure to disease and to reduce the effectiveness of animal and human immune response systems.

Pandemic health threats became increasingly evident in the last century and may pose even greater danger now. Several major infectious diseases already have global distributions and affect millions of individuals annually. World Health Organization (WHO) data indicate that stable pandemics such as malaria result in the annual deaths of more than 3,000,000 people, a large percentage of whom are children. Human Immunodeficiency Virus infected 2,900,000 people in 2006, and approximately 39,500,000 people are living with the infection. New and expanding pandemics are appearing on the horizon. Avian influenza continues to infect both birds and humans around the world. Ebola virus and Severe Acute Respiratory Syndrome (SARS)—both believed to be of animal (zoonotic) origin—are of significant concern, even though their distribution so far remains within confined geographic areas. The relationship between infectious animal diseases and human diseases is ever more evident; most new infectious agents considered biological threat agents are zoonotic diseases. Besides these naturally occurring biological threats, there are potential threats from accidentally or deliberately released biological agents. These trends are well known; disease emergence is a common topic of discussion for the press and the public as well as for the public health community.

While the medical community recognizes the increasing human vulnerability to biological agents, its ability to rapidly produce a vaccine against an existing or emerging pathogen is limited. Vaccine development times typically range between 11 and 23 years. This time frame is unacceptable in the face of emerging pandemics and weaponized bioagents. If biotechnology is to meet the public’s needs and expectations for protection against infectious diseases, our approach to vaccine development and production must change.

This report addresses the issues of vaccine manufacturing and innovations that may provide improvements to this process. It looks at regulation issues and at the full process of vaccine discovery through vaccine development, including optimization, evaluation, and scalable production of a product for release to the public. Finally, it considers the issues of distribution and economics that drive the system.

1. Introduction

VACCINOLOGY: DEFINITION AND SCOPE

Vaccinology is a complex science combining knowledge of immunology, molecular biology, biochemistry, and infectious diseases. Vaccines hold great promise for managing future biothreats; molecular biology and genomics have opened new methods of inquiry into how we might provide better protection against the microbial world in which we live.

The manufacturing of vaccines is equally complex, combining engineering and biology in a highly regulated and absolutely defined process. The process used to manufacture a vaccine is determined by the vaccine’s formulation and not by the type of available hardware or design of an existing plant. For vaccine manufacturing to be cost-effective, the plant and processes are optimized to achieve the formulation of the final product.

The vaccines currently available in the United States cover diseases with long histories of significant human morbidity and mortality. These infectious diseases remain a continuous threat to human health and have a high prevalence in susceptible populations. Vaccination has resulted in a dramatic reduction in the frequency of these infectious diseases, and in both morbidity and mortality. Smallpox has been eradicated; polio and pertussis are uncommon in the United States. Just under 60 specific vaccine products are available in the United States, providing protection to 25 known pathogens. Vaccines continue to improve the quality of life for Americans and for the rest of the world. Generally, vaccine supply has met vaccine demand.

Vaccines and improved sanitation have saved more lives than any other technology or medical breakthrough in the history of humankind. Yet vaccines have advanced slowly, with new products coming to market only after extended periods of development.

A Brief History

As early as the twelfth century, the Chinese used variolation to provide active immunity against smallpox. In the West, Edward Jenner, in 1791, inoculated the eight-year-old child James Phipps with exudates from the hand of a milkmaid who had cowpox. Six weeks later, Jenner challenged the child with smallpox; the child was effectively protected. This began the age of vaccinology.

Following Jenner, vaccine development increased, albeit haltingly. Louis Pasteur developed the vaccines against chicken cholera in 1870 and rabies in 1884. New vaccines were available before 1930 for diphtheria, tetanus, and tuberculosis. In 1932, yellow fever vaccine became available. The following year, chicken embryos were used to produce large quantities of virus, leading to the first two influenza vaccines, made available in 1936. In 1944, diphtheria, pertussis, and tetanus vaccines appeared as a combined product.

The inactivated polio vaccine Jonas Salk released in 1955 and the live, attenuated oral polio vaccine Albert Sabin released in 1962 began the widespread popularization of vaccines, because the immediate decline in the number of polio cases was so dramatic. The concept of active immunity through vaccination has dominated immunology over the last six decades. There has been an expectation that vaccines could be developed for all infectious agents, known and unknown, and that these products would be effective in all people, could be produced economically, and would be provided to the global public at minimal cost.

Most of today’s vaccines are combination products of an immunogen and an adjuvant; the adjuvant is added to enhance protective immunity. In the 1920s, Gaston Ramon combined tapioca, the first adjuvant, with tetanus toxin and reported improved protection; several years later, A.T. Glenny, following Ramon’s lead, mixed aluminum salts with tetanus toxin. Alum was again used with Salk’s polio vaccine as an adjuvant, effectively improving protective immunity.

The Changing Vaccine Environment

Today’s vaccines have been developed over time—the standard methods are at least 50 years old. These vaccines are focused on common infectious diseases with well-defined histories: polio, measles, mumps, diphtheria, pertussis, and so on. The most effective vaccines are those directed at pathogens with stable immunogens that produce a consistent immune response and a predictable level of protective immunity. For the most part, these vaccines have met the needs of the general public, who put their trust in the products. In addition, up to now, vaccines have generally remained a “black box” to a majority of the public (that is, vaccine input and immunity output are more important than the intervening processes). Vaccines are regarded as prophylactic, given to healthy individuals with the expectation that the product will provide long-
lasting protection with no adverse reactions. The reality is that there can be significant variation in an individual’s immunological response to vaccines, and adverse events can and do occur.

As standard vaccines were developed and released for the common infectious agents, demand has grown for development of new vaccines for less common or newly discovered agents. The presently rising era of disease emergence, the release of anthrax as a biological weapon, and the threat of avian influenza or other infectious agents leading to a true pandemic have increased the urgency for new methods to be developed in vaccine development, manufacturing, and distribution. The existing development timetable, often in excess of ten years to go from an identified pathogen to an approved vaccine, is unacceptable. In the case of a new threat agent, like SARS, that has pandemic potential, long development times translate to significant human morbidity and mortality. Necessity dictates that for new or emerging diseases, our ability to identify the infectious agent and produce and deliver an effective vaccine must outpace morbidity and mortality as the disease spreads through susceptible populations. The best estimates for a pandemic influenza are that even with an identified and preapproved production system for a vaccine, that vaccine would not be available for a minimum of four months and that at current production maximums, shortages would exist for five years.

VACCINE DEVELOPMENT ISSUES

While the need for significant improvement in the vaccine development process is reaching critical levels, numerous factors work against change. Vaccine producers are, for the most part, multinational corporations with significant investment in product development. Long development times and a rigid path through the regulatory approval process, often in more than one country, increase costs and reduce the willingness of these producers to take risks with any part of the manufacturing process. These factors play heavily on our continued use of gallinaceous eggs for viral growth, a method developed in 1933, and on our continued use of alum as an adjuvant, first identified in the mid-1920s. Technology over the last eighty years has made significant advances throughout every scientific discipline, yet vaccinology remains wedded to the proven methods of tradition (low risk) and regulatory approval (low cost).

Change comes with return on investment rather than merely with new discoveries and innovation. The vaccine producer must be able to recover the costs associated with introducing a new method into an existing and validated production system and remain confident that the resulting product is as safe and effective as products produced with earlier processes. Likewise, cost recovery and/or cost savings drive technology R&D. Most vaccine manufacturers do some internal discovery-based research and development, but more often, this type of research is funded by the Federal government. Academia and small biotechnology firms participate to a significant degree in advancing new concepts for vaccines and adjuvants. However, there is often a disconnect between a discovery and the ability to incorporate it into an existing manufacturing process. With a stronger linkage between discovery, development, and scalable manufacturing should come a systematic approach to incorporating innovation into manufacturing processes.

Most vaccine manufacturers locate their production facilities with both labor costs and ease of distribution to target markets as significant considerations. The vaccine industry is seeing significant expansion of facilities in India, China, Brazil, and a number of Eastern European nations. Outsourcing for vaccine production is becoming commonplace, especially for vaccine with production needs in the hundreds of millions of doses. There are significant risks with outsourcing in terms of meeting national needs for vaccine during a crisis or a period of reduced vaccine availability. These should be thoughtfully addressed as a matter of national policy.

Finding qualified personnel to work in the vaccine manufacturing industry is difficult. Vaccine manufacturing at the development level is series of optimization and standardization activities ranging from epitope identification and antigen production and formulation, through the final fill and finish process and into distribution. Many of these process steps require personnel with advanced degrees and both scientific knowledge and technological know-how. There is a limited number of qualified vaccinologists and technologists to meet this need. These positions are often seen by new PhDs as being unexciting or detrimental to career development. This severely limits the number of qualified professionals entering and remaining in vaccine production. Likewise, few formal academic programs exist in vaccinology or vaccine manufacturing in either the United States or in the European Union. As a result, workforce development is an area requiring focused and continued support as vaccine needs increase.

Since vaccines are generally seen as prophylactic rather than therapeutic, there is a greater burden on the manufacturer to minimize adverse events and to optimize efficacy. The fact that vaccine recipients are
healthy when the product is administered means that any associated illness or complication is seen as a more significant risk than for a product where an improvement in patient health is anticipated with a therapy.

Vaccines are regulated in the United States by the Food and Drug Administration (FDA), using Chapter 21 Code of Federal Regulations (CFR) and Chapter 9 Code of Federal Regulations, which defines the good laboratory practices (GLP) for both preclinical and clinical trials and the good manufacturing practices (GMP) necessary for manufacturing. The regulatory process is difficult but necessary to ensure public health and safety and to maintain public confidence in a program of vaccination. In addition to the requirements of the CFR, there are a minimum of 14 specific guidance documents for producing viral vaccines.

Each vaccine goes through the regulatory process for release and licensure. This process may take as long as 10 to 15 years and involve more than 70,000 subjects. By the end of this process, a producer has made significant investments in the process. Since safety and efficacy are linked to both the GLP trials and to GMP manufacturing, any changes to the process would require revalidation and testing of the product. This is an expense that a profit-motivated business is often unwilling to incur without promises of significant financial benefit. This alone limits and restricts changes to our available vaccines, regardless of the potential benefit.

From a regulatory perspective, the United States and the European Union (EU) differ on a number of points. Most notably, the same EU regulatory agency regulates and sets standards for both veterinary and human vaccines, which theoretically might allow veterinary facilities to be converted to human vaccine production during periods when existing human manufacturing facilities could not meet the public health needs. In the United States, veterinary products are regulated by the United Stated Department of Agriculture, whereas human vaccines are regulated by the FDA. It seems that the European system might be more appropriate for meeting surge capacity during a crisis, but many important questions would need to be addressed. Currently, four manufacturers in the world produce influenza vaccine for the poultry industry. Could these manufacturers rapidly switch to production for pandemic influenza? If this is possible, how vulnerable to avian influenza infection would their specific-pathogen-free flocks be that are needed for egg production for virus growth? What would be the consequences of loss of these flocks should they succumb to avian influenza? Would we need vaccines for both the poultry industry and the human population?

VACCINE MANUFACTURING ISSUES

U.S. Regulatory Requirements

The scalable production of vaccines employs manufacturing methods that have proven effective and consistent over time. These methods are approved by the FDA and must meet specific standards directed both at product safety and efficacy and at assuring product quality through every step of the manufacturing process. The vaccine itself, as released, must meet standards for identity, purity, potency, safety, efficacy, stability, and consistency. The vaccine manufacturing process, as regulated, must meet standards for

- source and quality of the starting materials, especially when of human or animal origin
- characterization of the cellular substrate for growth, including considerations of identity, origin, passage history, adventitious agents, endogenous agents, and tumorigenicity
- characterization of seed stocks, again considering identity, origin, and adventitious agents
- validation for inactivation or removal of adventitious agents
- in-process testing with defined specifications and standard operating procedures
- release testing of both bulk and finished product for defined specifications
- stability studies to define the shelf life of the product.

Changes to the manufacturing process must be reported to the FDA, and significant changes in the process require the submission of a license amendment application. In addition, testing is required to validate the amended process, including the evaluation and comparison of batches made before and after the modification; this includes the potential for additional animal testing. The approval process is of course necessary and valuable, but it is also costly from a financial perspective and a significant contributor to the time it takes to make a new or improved product available to the public.
Vaccine Formulation

The manufacturing system is dependent on the type of vaccine needed and the complexity of its formulation. As examples, vaccines may be produced from:

- killed inactivated virus (the Salk polio vaccine) or bacteria
- modified live attenuated virus (measles-mumps-rubella vaccine)
- subunits of organisms that represent specific immunogenic epitopes (pertussis)
- conjugation with specific polysaccharides (meningococcus)
- peptide vaccines for certain parasites; recombinant vaccines (hepatitis B)
- DNA

Vaccines are becoming more complex as the number of approved adjuvants increases and as vaccines are beginning to combine more than one immunogen from an organism to provide better protection. New methods of immunogen presentation are being developed as synthetic particles, virus-like particles, bacterial ghosts, and cellular components are produced by new methods. Research continues to define the process by which protective immunity is acquired and how infectious agents interact with the body during this process. In an ideal situation, our understanding would move from protective immunity to the best form of protective immunity. This requires answers to questions concerning antigen processing and responses at the level of innate immunity and what balance of cellular and humoral responses produce an ideal level of protection in a given individual against a given pathogen.

Each new discovery will require modification and innovation in the existing manufacturing methods so that they are equivalent to or better than the standards for the released product. Each new discovery will consider economic impact, time to delivery, rate of delivery, testing, validation, and final cost of the product. At present, innovations are difficult to incorporate because of the fixed costs associated with existing production plants, their anticipated functional life, and the cost of modifications. The regulations and revalidation costs inevitably reduce the frequency of incorporation of new technologies into the system. Methods can be introduced into production systems that are cost-effective, provide better products, and increase the public’s confidence in vaccines, but such change will require impetus and support by governments and the public if we are to move away from the current market model.

Vaccine Facilities

Issues related to existing production facilities impact improvement in vaccine production in several ways. The first is cost: it may cost $500 million for the production of a single vaccine product. A second is facility design. Most facilities are unable to produce multiple vaccines or move with any agility to a second vaccine, due to plant design. A third is capacity. Most plants already produce vaccine at or near full capacity and are unable to increase production during periods of vaccine shortage. A fourth is that there is a significant concern with the potential cross-contamination of a product when multiple agents are used in a facility. (Interestingly, multiple veterinary vaccines are often produced in the same facility, including the fill and finish process, without this concern.)

The difficult question remains as to how the vaccine manufacturing industry can rapidly and effectively respond to the public’s requirement for protection against a biological threat, starting from the identification of the index case to the release of a safe and effective vaccine product in the face of an emerging pandemic. The answer requires cross-cutting research in manufacturing, biomedical engineering, systems modeling and other disciplines, quality standards, clinical testing, and education of responders and the general public, as well as training of and incentives for high-quality researchers and technicians for the industry of the future.

WTEC REPORT: INTERNATIONAL ASSESSMENT OF RAPID VACCINE MANUFACTURING

Scope of the Study

This study considered current issues and trends worldwide in vaccine manufacturing science and technology and related systems that may be of benefit to government and industry policymakers if agile, modular, rapid, and large-scale vaccine manufacturing is to be reliably available for protection of life and health in response to disease emergence, pandemic threats, and the specter of weaponized bioagents. The study sponsors are the National Science Foundation (NSF), the National Institute of Biomedical Imaging and BioEngineering
(NIBIB) of the National Institutes of Health (NIH), the National Institute for Standards and Technology (NIST) and the U.S. Department of Agriculture (USDA). The study was administered by the World Technology Evaluation Center, Inc. (WTEC). This report is the final product of the study.

It is hoped that the study results presented in this report will provide guidance to R&D program managers worldwide, including those in NSF; USDA; NIST; NIH and other agencies of the Department of Health and Human Services (HHS), including the Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC); the Department of Homeland Security (DHS); the Department of Defense (DOD); and various international organizations concerned with public health.

The study and the report address the following key questions:

1. What is the status of international R&D on rapid vaccine manufacturing and the development-deployment systems chain?
2. How do U.S. activities in this area compare to those of other countries?
3. What ideas from other countries are worth exploring in U.S. R&D programs, and vice versa?
4. What technologies and systems are most likely to promote progress in vaccine manufacturing?
5. What are the needs for government promotion of general progress in vaccine manufacturing?
6. What opportunities exist for international collaboration?

The study analyzed and compared the research in the United States with that being pursued in Canada and selected countries in Europe. (A study of vaccinology R&D in Asia may follow.) It is not the intention of this panel or its report to describe the “sum of all efforts” in rapid vaccine manufacturing but rather to present an overview of some of the most innovative, rapid, or advanced efforts.

The Study Team

WTEC assembled a team of six experts in the field of vaccinology to conduct the study, drawing from academia, private business, and government:

- **Joseph Bielitzki** (Panel Chair), Chief Operations Officer, LensAR, Inc.
- **Stephen W. Drew**, Science Partners LLC (New Jersey)
- **Terrance Leighton**, Senior Staff Scientist, Children's Hospital Oakland Research Institute
- **Sheldon H. Jacobson**, Professor, Department of Computer Science, University of Illinois at Urbana-Champaign (UIUC), and Director, UIUC Simulation and Optimization Laboratory
- **Mary Ritchey**, Ritchey Associates, Inc.

The panelists’ biographies are included in Appendix A. WTEC participants in the panel’s site visits were Hassan Ali, Director of WTEC International Study Operations, and Grant Lewison, Advance Contractor.

Study Elements and Chronology

**Kickoff (20 October 2006)**

After WTEC’s selection of the panel, a meeting was held at NSF on 20 October 2006 between sponsors, panelists, and WTEC representatives in order to charge the panelists. The panelists then began a literature search and prepared for a baseline workshop of the status of North American vaccine industries. WTEC managed planning for the initial workshop, for the panelists’ visits to sites in Europe, and for the follow-up workshop and editing of this report.

**North American Vaccine Manufacturing Workshop (23 January 2007)**

The initial study workshop at NSF in Arlington, VA, on 23 January 2007 was intended to provide a baseline for the international study of rapid vaccine manufacturing by inviting U.S. and Canadian speakers, researchers, program managers, and industry representatives to discuss present status, issues, and concerns relative to the vaccine manufacturing industry on the North American continent. Table 1.1 lists the topics and presentations. The presentations are available online at http://www.wtec.org/vaccmfg/workshop-na/.
Table 1.1. Topics and Presentations at the WTEC North American Vaccine Manufacturing Workshop

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<thead>
<tr>
<th>Topic/Presentation</th>
<th>Moderator/Presenter</th>
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<tr>
<td>Regulation of Vaccines: Challenges and Opportunities</td>
<td>Erik Henchal (USDA)</td>
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<td>Rotavirus Vaccines: A Case Study</td>
<td>Dr. John Boslego (PATH)</td>
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<td>Topic II. Can Vaccine Manufacture be Agile, Modular, &amp; Responsive?</td>
<td>Dr. Mary Ritchey (commentator/moderator)</td>
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<td>Large-Scale Vaccine Manufacture</td>
<td>Dr. Shou-Bai Chao (Wyeth Biotech)</td>
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<td>Single-Use Bioreactors; Rapid Process Development and Scale-Up</td>
<td>Dr. Vijay Singh (WAVE Biotech LLC)</td>
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<td>What Limits the Rate of Vaccine Development and Scale-Up?</td>
<td>Dr. Alan Shaw (VaxInnate)</td>
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<td>Topic III. Incorporating Innovation in Bioprocessing</td>
<td>Joe Bielitzki (commentator/moderator)</td>
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<td>The Front-End of Vaccine Manufacturing: Getting Good Candidates</td>
<td>Dr. Bill Warren (Vax Design)</td>
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<td>Measuring and Optimizing Process Performance</td>
<td>Dr. Govind Rao (U. Maryland Baltimore Cty.)</td>
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<tr>
<td>Miniaturizing Development and Manufacture of Biologics</td>
<td>Dr. Klavs Jensen (MIT)</td>
</tr>
<tr>
<td>Topic IV. The Changing Face of Vaccine Development: The Systems Approach to Vaccine Manufacture</td>
<td>Steve Drew (commentator/moderator)</td>
</tr>
<tr>
<td>Concepts Regarding Adenovirus-Based Vaccine Systems</td>
<td>Dr. Andrea Amalfitano (Michigan State U.)</td>
</tr>
<tr>
<td>Total Synthesis and Assembly of a Virus-Like Particle</td>
<td>Prof. Jim Swartz (Stanford University)</td>
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</tbody>
</table>

Panel Visits to Sites in Europe (26 February–2 March 2007)
To obtain a first-hand appreciation of the current status and trends and concerns of vaccine manufacturers, industry, and government managers in other countries, panelists visited a number of premier laboratories and offices in Europe in late February and early March. Table 1.2 lists the sites that the WTEC panel visited.

Workshop Presentation and Discussion on European Findings (30 Mar 2007)
The WTEC panel presented its findings of its international assessment of the work at vaccine labs in Europe and its assessment of issues and possible solutions at a public workshop held at the National Science Foundation in Arlington, Virginia, on 30 March 2007. The international assessments and follow-up workshop discussions provided the foundation for this report. Workshop presentations are available online at http://www.wtec.org/vaccmfg/workshop/.

Overview of the Report
This report broadly follows the outline of the November 2006 workshop:

- Chapter 2 addresses issues of regulation and control (Mary Ritchey)
- Chapter 3 addresses pathways and paradigms for discovery and development of safe and effective vaccines (Joe Bielitzki and Terry Leighton)
- Chapter 4 discusses platforms for the manufacture of vaccines (Stephen Drew)
- Chapter 5 discusses the economics of vaccine delivery (Sheldon Jacobson)
- Appendixes:
  - A: Biographies of the panelists
  - B: Site reports on the institutions the panelists visited in Europe
  - C: Glossary of acronyms used in this report
  - D: Glossary of terms used in this report

Additional documentation on all phases of the WTEC International Assessment of Rapid Vaccine Manufacturing is available on the WTEC website at http://www.wtec.org/vaccmfg/.
Table 1.2.
European Sites Visited by WTEC Panelists*

<table>
<thead>
<tr>
<th>Site</th>
<th>Country</th>
<th>Date (2007)</th>
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<tbody>
<tr>
<td>European Centre for Disease Prevention and Control</td>
<td>Sweden</td>
<td>Feb. 26</td>
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<tr>
<td>Institut für Mikrotechnik Mainz GmbH (IMM)</td>
<td>Germany</td>
<td>Feb. 26</td>
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<tr>
<td>Karolinska Institute and the Swedish Institute of Infectious Disease Control</td>
<td>Sweden</td>
<td>Feb. 26</td>
</tr>
<tr>
<td>European Collection of Cell Cultures (ECCC)</td>
<td>U.K.</td>
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<tr>
<td>Health Protection Agency (HPA)</td>
<td>U.K.</td>
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<tr>
<td>National Institute for Medical Research</td>
<td>U.K.</td>
<td>Feb. 27</td>
</tr>
<tr>
<td>Novartis Vaccines</td>
<td>Italy</td>
<td>Feb. 27</td>
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<tr>
<td>University of Siena</td>
<td>Italy</td>
<td>Feb. 27</td>
</tr>
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<td>DECHHEMA (Society for Chemical Engineering and Biotechnology)</td>
<td>Germany</td>
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<tr>
<td>Mikroglas Chemtech GmbH (part of DECHEMA visit)</td>
<td></td>
<td></td>
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<tr>
<td>Merck KGaA-Germany (part of DECHEMA visit)</td>
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<tr>
<td>Institute for Animal Health</td>
<td>U.K.</td>
<td>Feb. 28</td>
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<tr>
<td>PowderMed, Ltd.</td>
<td>U.K.</td>
<td>Feb. 28</td>
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<tr>
<td>University of Oxford</td>
<td>U.K.</td>
<td>Feb. 28</td>
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<tr>
<td>GlaxoSmithKline (GSK) Biologicals</td>
<td>Belgium</td>
<td>Mar. 1</td>
</tr>
<tr>
<td>Pfizer Animal Health</td>
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<td>Mar. 1</td>
</tr>
<tr>
<td>Scientific Institute of Public Health</td>
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<td>Mar. 1</td>
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<tr>
<td>Baxter Vaccines AG (Austria)</td>
<td>Austria</td>
<td>Mar. 2</td>
</tr>
<tr>
<td>Intervet and Nobilom International (part of Schering-Plough as of Nov. 2007)</td>
<td>Netherlands</td>
<td>Mar. 2</td>
</tr>
<tr>
<td>University of Vienna and BIRD-C GmbH &amp; CoKEG</td>
<td>Austria</td>
<td>Mar. 2</td>
</tr>
<tr>
<td>World Health Organization (Teleconference)</td>
<td>Switzerland</td>
<td>Mar. 6</td>
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</tbody>
</table>

*See Appendix B for site reports on the WTEC panel’s visits to these organizations.

ACKNOWLEDGMENTS

The panelists wish to extend our gratitude and appreciation to all presenters and hosts for their generous sharing of their time, expertise, insights, and facilities with us, and for the stimulating discussions that have informed the preparation of this report. We also wish to thank Mr. Hassan Ali and Dr. Grant Lewison for their outstanding support during the panel discussions and interactions, as well as the sponsors who made this study possible: the National Science Foundation, the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health, the National Institute of Standards and Technology, and the United States Department of Agriculture. We hope that the process of conducting this study and the resulting report will serve to extend mutual cooperation between practitioners in the field and contribute to advancing the science, technology, and associated systems of rapid vaccine manufacturing for the benefit of world populations in years to come.
CHAPTER 2

REGULATION AND CONTROL

Mary B. Ritchey

Vaccine development and commercialization are complex processes. Regulation and control are significant contributors to the safety and effectiveness of vaccination programs but also contribute to the length of time it takes to get vaccines to target populations. This chapter gives background on the complexity and controls that are a part of regulating vaccines, reviewing key components of the existing regulations for the United States and Europe for both human and animal vaccines and key analytical and control testing requirements for obtaining an initial license and for marketed products. It discusses aspects of the regulatory process that are designed to enhance efficiency and speed, along with potential challenges for both the regulatory process and testing. It also describes the potential for future improvement of testing technologies to improve the speed of the overall process. Finally, it reviews special considerations that exist for a pandemic situation.

BACKGROUND ON VACCINE REGULATION AND CONTROL

Vaccines occupy a special place in the healthcare system. In contrast to most prescription pharmaceuticals, vaccines are used to prevent disease rather than to treat existing conditions. As such, they are given to healthy individuals or to individuals with preexisting chronic health problems who are especially vulnerable to the disease the vaccine is targeted to prevent. Target populations for vaccines span all age ranges from newborn infants to the elderly. Rather than being optional, administration of vaccines to individuals is often dictated by national, state, or local laws, or is a requirement for admission to school or for employment in specialized workplace environments. Because of the broad age ranges at which vaccines are given, a large number of recipients do not give their own consent, even if a particular vaccine is optional.

The purpose of vaccination also adds complexity to the selection and use of vaccines. In addition to preventing a particular disease in a vaccinated individual, vaccination is also intended to protect the population at large. This is accomplished by what is known as “herd immunity.” Diseases that most vaccines are designed to prevent are caused by infectious agents that spread from individual to individual in various ways. If a population has a high immunization rate against a specific infectious disease, it is more difficult for an infectious agent to spread in that population. A few unvaccinated individuals in such a population are protected by virtue of a significantly less likelihood of exposure to the infectious agent.

Depending upon the design of a vaccine, the herd immunity it provides can be more or less powerful. Agents used in vaccination programs that are live and attenuated tend to have more effective herd immunity than agents that are inactivated, because they are more likely to mimic the natural disease agent without causing disease, and they can both immunize against infectious agent replication in the vaccinated individual and provide immunity against the disease it produces. Live attenuated poliovirus vaccine is a good example. Public health policymakers often choose a live, attenuated vaccine over an inactivated vaccine because of the extra benefit it provides, especially when vaccination rates in a given population are not optimal. In such a policy there is a potential downside to individual recipients. An individual who has an unrecognized immunodeficiency or another unknown condition that is incompatible with use of live attenuated vaccine may experience an adverse event that is less likely to occur with the use of an inactivated vaccine.
Culture contributes to attitudes and policies toward vaccination. Adverse events that result from an active attempt to prevent disease are considered unacceptable, especially in the United States; these subject the vaccine manufacturer, healthcare providers, and sometimes even governments to excessive product liability, even if the events are exceedingly rare. Because vaccines are given to very young individuals, potential liability can exist decades beyond the immunization event. Administration of vaccines to the very young, whose full medical and developmental status is not or cannot be known, can also lead to the attribution of a developmental disorder to an immunization event. A current example is the proposed link between autism and measles vaccine and/or thimerosal, a common preservative used in vaccines. Although the science does not support this theory, the perception can still be explored via litigation, at least in the United States.

Given the place that vaccines occupy in the healthcare system and the lack of tolerance for adverse events, especially when young vaccine recipients are involved, it is no surprise that vaccine products are highly regulated and have a number of requirements over and above what is typical for other pharmaceutical products. One example is the requirement for the administrative release of each lot of vaccine by both the manufacturer and a government control authority. Additional examples will be highlighted below in describing the current regulatory and control processes in the United States and the European Union.

A final concern that contributes to a high level of vaccine testing and controls that has become part of the vaccine development and manufacturing landscape is the nature of the vaccine products themselves. Traditional vaccines and some of the more recent conjugates are all considered “not well characterized” by most regulatory authorities. Manufacturing begins, in the majority of cases, with growth of the target organisms or derivatives and ends with a biological product that is as well characterized as possible, but not to the extent that pharmaceuticals based on small molecules can be described. Thus, controls and additional testing are put in place to ensure that the marketed product remains consistent with the product testing in human clinical studies.

The purpose of animal vaccines is similar to that of human vaccines with respect to protection against disease of both individual animals and large populations. In terms of regulation and control of these veterinary products, there are both similarities and differences when compared to human vaccines.

THE GENERAL REGULATORY PROCESS

The basic regulatory process begins with a request for permission to conduct clinical studies and continues during development, licensure, and marketing of the final product. Well before clinical studies can be conducted, the sponsor needs to have an understanding of the requirements that need to be met to secure permission to study the candidate vaccine. These requirements include the appropriate level of characterization and testing of the candidate vaccine, along with an appropriate level of control and cleanliness when the clinical supplies are manufactured (good manufacturing practices). Clinical plans for initial studies are required along with appropriate permissions for sites that will conduct the studies.

Good clinical practices must be adhered to during all of the clinical study procedures. Regulatory interaction occurs as clinical results are obtained and next steps in clinical studies are designed (Phases 1-3, see Figure 2.1). The level of good manufacturing practices that are implemented for the product itself will increase as the development program progresses so that full compliance and validation are achieved and documented and made part of the license application for marketing the final product.

Post licensure, a manufacturer will continue surveillance for adverse events (often with prospective studies), continue to monitor product stability, and submit each lot intended for sale to a government control authority for release. As regulations change or products are improved, the manufacturer must make changes and modify the license documents. These supplements must be submitted for approval to the control authority.

UNITED STATES HUMAN VACCINE REGULATIONS

The basis for regulating human vaccines is found in the following key U.S. regulations: the Public Health Service Act (42 USC 262-3); The Food Drug and Cosmetic Act (21 USC 301-392); the FDA Modernization Act (1997); and Title 21 of the Code of Federal Regulations. In Title 21, there are sections that provide the requirements for product standards (section 600); human clinical studies and the application for permission to study a candidate vaccine in human subjects, called an Investigational New Drug Application (NDA) (section 300); good manufacturing practices (section 200); Institutional Review Boards (required for
institutions that conduct clinical studies) and protection of human subjects (section 50); and environmental impact and assessment (section 25). There are also a variety of Guidelines and Guidance to Industry and Points to Consider documents that provide guidance on subjects such as cell line characterization, DNA vaccines, vaccines produced from recombinant DNA technology, combination vaccines and clinical testing, and preparation of the chemistry and manufacturing and the establishment sections of a Biological License Application. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) also publishes documents that provide guidance, for example, stability studies and viral validation and safety.

Vaccines are regulated by the FDA’s Center for Biologics Evaluation and Research (CBER) Office of Vaccines Research and Review. The CBER regulatory stance is to take a case-by-case approach and use sound scientific principles and appropriate risk management in regulating the initial application for licensure and post-marketing control. The goals are to ensure the safety and rights of clinical trial subjects, protect the public health, and facilitate innovation in a balanced, flexible, and responsive way. CBER reviews and approves applications for human clinical studies, issues licenses to market products, conducts tests related to release of lots, and issues lot releases. Facility inspections are carried out prior to issuing a license, as part of a review of a supplement to an existing license, and on a routine basis to ensure compliance with the regulations. Inspections are carried out by Team Biologicals, which draws inspectors from the product specialists at CBER and FDA’s Office of Regulatory Affairs. The CBER stages of vaccine review and regulation are captured in the Figure 2.1.

![Figure 2.1. Stages of U.S. vaccine review and regulation by CBER (FDA).](image)

Highlights of requirements that must be met to obtain permission to study the candidate vaccine in human subjects include characterization of the candidate vaccine and source materials, animal toxicology studies of the antigen and antigen-adjuvant combinations conducted under good laboratory practices (GLP) conditions, pharmacokinetics for control-based delivery systems such as microspheres and animal, and/or other studies to support safety and type of immune response that might be expected in a human study. This process may take months or years, depending upon the initial state of knowledge about the disease and the proposed antigen. Once permission to perform human clinical studies is granted, the development of a full picture of the product characterization, formulation, assays, validation, specifications, manufacturing process, and controls is completed during the first three “Investigational New Drug” (IND) phases of clinical study.

Following the IND phases, all the clinical data, nonclinical data, and supporting information on the large-scale process and manufacturing facility are submitted in support of the “Biologics License Application” (BLA). Before the BLA is approved, an inspection of the facility and data is conducted, sometimes including inspection of sites that conducted human clinical studies. CBER may assemble an advisory committee of experts who review various parts of the submission and assist CBER in making its decision.
Post-licensure, regulations require routine plant inspections to ensure ongoing compliance with current good manufacturing practices (GMPs) and often require Phase 4 human studies to continue to validate the safety and efficacy of the product. Adverse event data collection goes on for as long as the product is marketed. BLA “supplements” are required to document any changes made in processes, tests, or facilities relative to the original BLA. These supplements are submitted to the FDA; depending on the magnitude or the type of change, many require FDA’s approval before they can be implemented. Some manufacturing changes may require additional clinical studies for approval.

Collection and documentation of the required information at every stage of review and regulation of a vaccine product is a complex process that requires many years, even decades, of effort. The total duration of regulating product and process development depends in large part on the scientific knowledge available at the starting point. In addition, a commitment to continuously document changes to procedures and product performance in the field continues as long as the product is on the market.

There are some significant differences in requirements for vaccines compared to those for other pharmaceutical products. Overall, more extensive control exists for vaccine products. FDA’s initial license approval requires studies in many more subjects and requires more time than for a typical small-molecule pharmaceutical product. Extensive field studies involving thousands of subjects are often necessary to look for safety (adverse events) and efficacy (proof that the vaccine provides protection against disease). Unless a true correlate of immunity to the targeted disease is known, a study in a population experiencing disease is necessary to demonstrate efficacy. At least one lot of vaccine made at the final manufacturing scale must be represented in human clinical studies. Validation of the large-scale process (generally requiring the manufacture of three lots) must be completed and submitted as part of the BLA rather than be submitted post-approval. Post-licensure requirements include the release by both the manufacturer and the CBER of each lot intended for sale. This process involves preparing a protocol summarizing the manufacture and testing of each lot and submitting it along with samples of the lot to CBER. CBER has the option to test the samples, but it must issue a certificate of release for the lot before it can be marketed. For some products, there is a CBER release requirement for intermediate stages of production. For most products, there is at least one animal test required.

UNITED STATES ANIMAL VACCINE REGULATIONS

U.S. regulation of animal vaccines is based on the Virus-Serum-Toxin Act and Title 9 of the Code of Federal Regulations (mainly sections 101–118). The Virus-Serum-Toxin Act of 1913 was designed to prevent the importation and shipment of contaminated or harmful veterinary biologicals. It was amended in 1985 to include all biological products for animal use that are shipped into, within, and from the United States.

The regulatory framework for animal vaccines is under the control of the U.S. Department of Agriculture. The Center for Veterinary Biologics (CVB) within the USDA’s Animal and Plant Health Inspection Service (APHIS) is the main group that deals with veterinary biologics. The CVB mission includes ensuring that biologics are free of disease-producing agents and that products are pure, safe, potent, and effective. CVB’s role includes developing standards and procedures for product release, issuing licenses and permits, monitoring and inspecting products and facilities, and controlling field tests and release of veterinary biologicals (APHIS n.d.; Robinson 2004).

The International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Products also publishes guidelines that are applicable to vaccines.

EUROPEAN UNION HUMAN AND ANIMAL VACCINE REGULATIONS

The European Medicines Agency (EMEA) has oversight for both human and animal vaccines that are marketed in the European Union. It is a decentralized body with headquarters in London; its main responsibility is the promotion of public and animal health through the evaluation and supervision of medicines for human and veterinary use. A balancing of risks versus benefits is a key EMEA principle in reviewing applications and ongoing issues. The agency works through a network of over 4000 experts who
come from EU member countries and those of the European Economic Area (EEA) and the European Free Trade Association (EFTA).²

The EMEA’s Committee on Medicinal Products for Human Use (CHMP) through its Vaccine Working Party has oversight for human vaccines; the Committee for Medicinal Products for Veterinary Use (CVMP) has oversight for animal vaccines. There is a common set of good manufacturing practice standards (GMP Directive 200/94/Ec and Annex 16) plus some additional standards for veterinary products (Directive 91/412/EEC). There are centralized procedures for scientific review and approval of license applications and lot release post-licensure. Each country within the EU may have some additional considerations that are addressed at the end of the centralized procedure for licensure.

As in the United States, there are a variety of guidance documents that need to be considered during the process of developing a vaccine that is to be marketed in the European Union. Besides the EMEA standards, other guidelines that need to be considered include those published by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH), and the World Health Organization (WHO). The EU regulations also specify that each vaccine manufacturing entity must have a “Qualified Person” within its quality assurance unit who has a legal responsibility to ensure that products released meet all requirements. This individual must take specialized training and be certified to serve in this role.

Although there is a centralized procedure to apply for EMEA vaccine licensing, vaccine manufacturers must apply to individual countries for permission to conduct clinical studies, and these studies are conducted as required for each particular country. The EMEA publishes guidelines on the conduct of clinical studies and summarizes some of the individual country requirements. The applicant performs preclinical and characterization studies in a manner similar to that described above for U.S. regulation and continues to implement additional GMP standards to the product and assays as the clinical studies continue.

Figure 2.2 depicts the basic elements of the EMEA’s centralized procedure for the Marketing Authorization (license) Application (MAA) itself. Highlights of this process are as follows. Presubmission activities start well before the dossier is submitted. As early as 36 months before, “scientific advice,” a formal process for obtaining advice on the science of the overall program, is engaged. Interactions continue to set the stage for formal submission and review of the final application.

To officially start the EU licensing process, the applicant must submit a dossier of scientific information on quality, efficacy, and safety to support the application. The CHMP appoints two of its members to act as rapporteurs, who along with each of their teams of experts will evaluate the dossier and provide a report to the CHMP and the applicant (Day 80). By day 120, the primary evaluation is completed, including the formal CHMP review, and a consolidated list of questions is sent to the sponsor. At this point, the clock stops and the applicant answers questions. The secondary evaluation is based on answers to the questions and can include a hearing if needed. The final opinion is given by day 210. The remaining days in the process, if the opinion is positive, are used to notify and work with individual countries for the specific country marketing authorizations.

The EMEA also uses its member experts to conduct inspections of an applicant’s facilities. The initial inspection for a product license takes place during the clock stop period, and then routine inspections and inspections related to supplements or for cause occur during the post-marketing period. During the process, control labs are also selected that will be responsible for testing and lot release of each manufactured lot that will be sold in the European Union. These control authorities will release the lot for all countries within the EU. Changes that are made post licensure may require preapproval, and some may require additional clinical study, even though indications are not changed.

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² EEA includes EU members and Iceland, Liechtenstein, and Norway; EFTA includes those countries and Switzerland.
HARMONIZATION OF REGULATIONS

Efforts to harmonize vaccine regulations are taking place between countries of the EU, Japan, the United States, Latin America, and elsewhere, to varying degrees. Harmonization of regulations and controls provides opportunities for the harmonizers to learn from each other to develop efficient procedures that promote both public safety and rapid delivery of needed vaccines to the marketplace. Harmonized licensing procedures also facilitate the ability for new vaccines to be available to multiple countries in the same time frame using a single license application. A cautionary note, however, is that the potential exists for the harmonized procedures to be more complex than procedures for an individual country, with potential for delayed entry or nonentry into the marketplace of a good candidate vaccine.

Europe has had a long-standing initiative to harmonize procedures across EU member states, including mutual recognition of licenses and inspections between countries and some centralized procedures that exist today. Both veterinary and human vaccines can be approved for use in the whole of the European Union by submitting a single dossier. In addition, release of lots for sale after marketing authorization has been obtained can be accomplished by submitting a single document and lot samples to a single control authority.

Vaccine regulatory procedures across the United States and Europe are not harmonized, nor is there mutual recognition of licensing or lot release, despite ongoing efforts by the regulatory authorities in the United States and the European Union to look at harmonization. Regulatory authorities in both regions request license applicants to look at ICH guidelines, thus forming a partial basis for harmonization.

A notable contrast to the European practice is that in the United States human and animal vaccines are regulated by different government agencies (FDA and USDA, respectively) and thus do not have harmonized procedures for both types of vaccines, as does the EU. Thus the benefits of cross-training and sharing of ideas or sharing of facilities for product manufacture are more difficult to achieve in the United States than in the European Union.

A role for facilitating harmonization is played by several international associations. The ICH, as noted above, provides guidelines for human pharmaceutical, including biological, products. It was launched in 1990 to bring together the regulatory authorities of Europe, Japan, and the United States and experts in the industry to discuss scientific and technical aspects of product registration. The objectives of harmonization are more
economical use of human, animal, and material resources, and avoidance of delays in licensing new medicines while maintaining safeguards for the public. The VICH, the counterpart of the ICH for veterinary products, was launched in 1996 and based its mission and role on the ICH. In addition, the International Association for Biological Standardization (IABS) also promotes harmonization (see IABS n.d.; IABS 2006). IABS is an independent, nonprofit scientific organization founded in France in 1955 that now has members in over 50 countries. It provides an international forum for bringing together control authorities, manufacturers, academic researchers, and public health officials to develop consensus on issues of standardization and control of biological medicines for both human and animal use. The organization’s publications and international conferences are employed to disseminate information and to foster collaboration.

CONCEPTS FOR OPTIMIZING THE REGULATORY PROCESS FOR SPEED AND EFFICIENCY

As can be surmised from the forgoing descriptions, it is a daunting task to assess and synthesize all of the regulations and guidance documents that need to be considered when developing a vaccine. Few organizations have the expertise and resources to develop a vaccine from start to finish and market it globally. Most small companies, academic institutions, or other small organizations are unlikely to have the resources to complete the initial steps to prepare and test material suitable for study in humans. This can be accomplished only through partnerships with other larger organizations or government institutions. The WTEC panel noted one example of partnering in its visit to the University of Vienna. Dr. Werner Lubitz and his colleagues established a small company, BIRD-C GmbH & CoKEG, based on the discovery of bacterial ghosts as a potential vaccine carrier platform and are in the process of partnering with the U.S. Walter Reed Army Institute of Research to prepare human-clinical-grade materials for study.

Other possibilities to optimize the regulatory process include developing government tax incentives or grants that are (or can be) based in part on partnerships between small companies, larger companies, and universities. This could be one step toward facilitating the overall economic health of the vaccine industry.

A step toward minimizing the regulatory review time can be made by building on elements the regulators have already reviewed and found acceptable, such as a prior, proven technology. Several strategies follow.

Share Platforms

Common technologies are often referred to as “platforms” (see Chapter 4), and companies already take advantage of them by establishing platforms that can be used for more than one product. If a vaccine has been licensed or has an approved IND using a particular technology such as Vero cells, a second application may be filed without repeating the studies performed for the first product, without jeopardizing the safeguards of the regulatory process. The same cell line strain and conditions would need to be used for the second product, and if the second application provided reference to the first product, or a master file was in place on the Vero cells, the information should not need to be submitted again. Examples from Europe include the Vero cell substrate for influenza vaccine that Baxter Vaccines has developed. Because Vero cells support the growth of many different viruses and Baxter has built its Czech Republic facility to Biosafety Level 3 standards, the company has a great deal of flexibility with respect to the types of viral vaccines that it can produce in this facility with this technology. It also has a chicken embryo cell aggregate platform that is currently used to produce a vaccine against tick-borne encephalitis that can be used for some of its pipeline products.

Other examples of platforms that are in development that could potentially be used to support more than one vaccine candidate include adenovirus vectors being developed at the University of Michigan, as described by Dr. Andrea Amalfitano (2007) at the WTEC North American Workshop, and bacterial ghosts developed at the University of Vienna (see Chapters 3 and 4 and the University of Vienna site report in Appendix B).

Use Immune Correlates

The first vaccine against a disease often takes the longest to be licensed, as the technology may be new and correlates of protection are not available. In such a case, a field study needs to be performed in an area of the world where disease is occurring at a rate that makes a clinical study feasible. If a correlate of immunity is developed, then other companies may be able to build on the published literature to avoid a field study and rely on laboratory documentation of achieving the correct immune response in vaccine subjects. This can
save time and expense and, indeed, may be the only way to introduce additional entries into the marketplace, since the first entry may have reduced disease to an extent that a field study to demonstrate efficacy is no longer practicable.

This concept was used to license a third vaccine developed to protect against *Haemophilus influenzae* disease. It is also used to license viral influenza vaccines and associated annual product updates for vaccines that stimulate antibody production against the hemagglutinin and/or neuraminidase proteins that reside on the surface of this virus.

The U.S. “Animal Rule” also allows a license applicant to use animal data to demonstrate efficacy under conditions where a human clinical study is not feasible and a suitable animal model exists. Vaccines against Anthrax and SARS are examples of where the Animal Rule could be applied. Human safety would still have to be demonstrated for such vaccines.

**Use Currently Licensed Facilities**

Another method to achieve faster results is to use facilities or contractors that already are licensed to perform some aspect of either the manufacturing or the testing that the product requires. These organizations need to have already filed documentation with the regulatory authorities that can support a vaccine license, and new data that needs to be submitted is limited to the application of the methods to the specific vaccine. Europe has the added advantage of a common set of GMP regulations for human and animal vaccines, thus making it theoretically possible to produce human and animal vaccines in the same facility. Although this is not commonly practiced, at least one organization, Nobilon, is designing facilities to serve both purposes (see site report in Appendix B on Intervet and Nobilon International).

**Fill in the Science Gaps**

A major issue with expediting the process of review and licensure of a vaccine is the diversity in nature of pathogens and the diseases that they cause. It is often very difficult to find common means of dealing with this diversity, considering both the manufacturing and testing issues and the clinical response that is required to generate immunity to disease. This is mainly due to the lack of scientific knowledge of the immune system. Dr. Rino Rappuoli (Novartis Vaccines, Italy) provides an excellent overview of the history of vaccine development and a future for vaccinology that seeks to improve the process by filling in the scientific gaps (see Chapter 3). Understanding the immune system in a way that provides for a more generic approach for how to induce immunity using vaccine products is the real key to reducing the amount of case-by-case regulatory intervention that is practiced today.

**Enhance the Personnel Selection and Cooperation Parts of the Process**

Efficient and effective regulation depends upon the knowledge, skills, and motivation of people who choose regulatory careers and the cooperation between the regulators, developers, and producers of vaccines. Key to this process is ensuring that the regulatory staff has sufficient status and training and the disposition to work effectively with discovery scientists, vaccine developers, manufacturers, and clinicians. The current rewards and incentives for pursuing careers in public service related to vaccines are not optimized to achieve this goal. More attention needs to be given to this area so that innovation can be appropriately but more quickly evaluated for translation into useful vaccine products.

Companies and scientists need to take advantage of the opportunities to interact with regulators during the process of developing and licensing a vaccine. The United States provides for a standard set of meetings with industry, and the EU has a scientific advice process. Although interactions with regulators are frequent during the final stages of the licensing process, taking advantage during early stages of both formal and informal (e.g., scientific conferences) interaction opportunities can help a company meet regulatory expectations more efficiently. Such meetings can also help facilitate interaction between regulators and companies on proposed changes in regulation that affect the vaccine development and licensure process.

In addition, education on the regulatory process and requirements needs to be available to small companies and academic institutions that are interested in applying their discoveries to vaccines. Although workshops are available, extended coursework and internships would provide much better preparation for careers in the vaccine industry. Incentives for universities or others to offer suitable education or to establish training centers would be beneficial.
Press Ahead on Harmonization of Regulations

There is potential for additional harmonization of regulations between Europe and the United States (as well as other countries) to facilitate more rapid development of new vaccines. A system of mutual recognition for applications for human clinical studies would enable companies to select the best location for studies via one application. Similarly, a mutual recognition system for product licenses would make products more generally available, sooner. Ideally, in the long run, a common application for both processes would be beneficial. It would also be beneficial if countries would adopt similar immunization schedules, especially for infants and children.

Harmonization should also include having common points to consider and guideline documents. Implementing regulatory and manufacturing reciprocity between the United States and the EU has been estimated to cut development costs by 20 percent or more and to decrease time to market by 6 months (Rappuoli et al. 2002). Additionally, facilitating cross-talk between the animal and human vaccines communities can provide opportunities for each discipline to learn from the other.

Develop and Use Electronic Tools

Electronic submission of data is now possible and is practiced in some instances for regulatory submissions. Continued development of these tools to allow online review for multiple users should become a standard practice. Additionally, development of easily searchable databases to locate and view relevant regulatory documents would be beneficial. Continued development of databases and electronic capture of data within research and manufacturing organizations can also speed the process of collecting and analyzing data.

SPECIAL CONSIDERATIONS IN A PANDEMIC SITUATION

Both the United States and Europe have put in place measures that are designed to speed the approval process for vaccines in the event of a pandemic. The potential for an influenza pandemic caused by an avian influenza strain, events related to appearance of SARS disease, and the potential for bioterrorism have resulted in laws and regulations that can be used in special circumstances.

European Union

Within Europe, the EMEA has established procedures that cover the formation and responsibilities of various crisis teams that would act to provide central guidance to the members of the EU in the event of a pandemic. These include a Gold Crisis Team that is responsible for confirming the onset of a pandemic and working within the framework of “Business Continuity Planning”; a Pandemic Silver Crisis Team that mobilizes resources based on information received from the Gold Team; and the Pandemic Bronze Team that runs the program, including approval of pandemic influenza vaccines.

In addition, a procedure was established that allows an influenza vaccine manufacturer to submit a “core dossier” in advance of a pandemic that describes the processes it would use for manufacturing and testing an influenza virus pandemic vaccine strain. Influenza vaccine manufacturers have submitted three such dossiers that could provide vaccine for countries in Europe. The data for the dossier is developed on available pre-pandemic strains. The dossier provides a set of procedures and test results, including human clinical evaluation, of a “mock-up” vaccine that can form the basis for marketing a vaccine for a true pandemic strain, upon submission and approval of a variation to the core dossier to incorporate the actual strain causing the pandemic. Such a dossier will help both the manufacturer and the regulatory authority provide vaccine more rapidly using the pandemic strain, because of the experience gained during the process of developing the mock-up vaccine. (The major limitation of this process is that there will be less overall knowledge of the pandemic vaccine, when compared to interpandemic year vaccines, concerning the characterization and human response to the actual pandemic vaccine strain.) In addition, the vaccine developed against the pre-pandemic strain can also be stockpiled and possibly used in the event of an outbreak, if it is close enough in its characteristics to the strain the ultimately causes a pandemic.

Under European law related to a pandemic situation, each country’s public health ministry is able to make an independent decision on how much information is required to allow the pandemic vaccine to be used in its various populations. This law can also be used for other emergency situations than an influenza pandemic, such as that of a SARS epidemic, if necessary.
Europe can also take advantage of the fact that is has a pool of regulatory authorities who have common procedures for reviewing vaccine license application dossiers and for testing and release of vaccine lots post marketing authorization. This gives Europe the ability to focus a large number of resources in one area or on one product if needed. Part of the European readiness plan is cross-training to ensure an adequate number of individuals are prepared to assist in reviewing vaccines related to pandemics.

The EMEA planning also extends to animal influenza vaccines, especially for chickens and ducks. Based on the planning for human vaccines, it has established a process to authorize emergency use of veterinary vaccines against highly pathogenic avian influenza viruses in birds and is adopting a core dossier approach called a “multistrain dossier” that is better suited to veterinary medicines. The multistrain dossier approach includes specifications for excipients, adjuvants, maximum number of antigens, and maximum amount of antigen per dose. It assumes the ability to extrapolate data between strains.

United States

In the United States, the Homeland Security Council has published a National Strategy for Pandemic Influenza (2005) and a critical infrastructure guide (HHS 2006b) that describes contingency planning for a pandemic. The Department of Health and Human Services (HHS) has published a Pandemic Influenza Implementation Plan with a chapter devoted to vaccines (HHS 2006a, 186-187; see also HHS 2007, various sections). Stockpiling of vaccines prepared using pre-pandemic strains is part of the strategy. Several manufacturers have been contracted to provide supplies. The chapter provides a discussion of regulatory considerations.

Under Homeland Security law (Project BioShield Act of 2004, Public Law 108–276), there is also the potential for emergency use authorization (EUA) of a vaccine if national security could be affected. The Secretary of HHS can authorize emergency use after the Secretary of Defense, Homeland Security, or HHS determines that an emergency (or potential for one) exists. Anthrax vaccine was authorized for emergency use in December of 2005 for high-risk individuals. In addition, once an IND has been filed and approved for initiating clinical studies in humans, the regulations allow use in humans under special circumstances such as a pandemic.

CBER has also published a guideline on requirements for licensing a pandemic flu vaccine and a guideline on annual renewal of licenses that already exist. As with the EU core dossier plan, these documents and the manufacturing experienced gained during the pre-pandemic years will allow for more rapid vaccine manufacturing and approval should a true influenza pandemic arise.

Not in place yet but under consideration is another aspect of speeding up the regulatory process for licensing of vaccines against pandemic strains, which would be to provide a means of sharing or cross-referencing information between vaccine manufacturing companies. Intellectual property and trade secret considerations would make this difficult, but finding a way to facilitate this would be helpful. Several manufacturers in Europe noted to WTEC panelists that more efficient and rapid sharing of information and strain candidates from the U.S. and European government agencies that initially develop them would speed up the vaccine development and approval process, especially in the event of an emergency.

Partnerships between animal and human vaccine development companies can also facilitate the rapid availability of pandemic influenza vaccines. Facilities can be qualified in advance by regulatory authorities for manufacture of both types of vaccines. Although this is more easily accomplished in the EU because both classes of vaccines have common regulatory guidelines for facilities, it can be accomplished in the United States, if initiated well before a pandemic occurs.

HOW FAST IS FAST?

Speeding up the time for a new vaccine to get to market is important in both normal and emergency situations, but the goal of “fast” vaccine availability is relative to existing time frames and what is practicable in terms of both science and safeguards. A review of currently used vaccine products indicates that overall time frames for development, clinical study, and licensure take at least ten years, longer in most cases. Vaccines described at the WTEC North American Workshop (2007) spanned 13 to 28 years for development and licensure.
Vaccine development is discussed in Chapter 3; however, it should be noted here that the regulatory review period can itself constitute a significant portion of the time it takes for a vaccine to reach the market. After submission of an application for licensure, U.S. regulatory review can often take up to two years, including resolution of issues with the license applicant; this is in addition to the review time required during the progression in development from initial clinical studies through Phase 3 of the IND application.

In order to speed up regulatory review times for vaccines, FDA and EU regulatory authorities are currently committed to firm timetables for review of submissions at certain stages. These timetables include shorter times for priority and fast track review if justified by the proposed use of the vaccine. Table 2.1 gives highlights of both standard and expedited timetables. These time frames do not include the time that it takes for applicants to answer questions or resolve issues raised in the regulatory review.

### Table 2.1

<table>
<thead>
<tr>
<th>Application for Human Clinical Studies</th>
<th>Standard License Application</th>
<th>Changes to Original License App.</th>
<th>Priority Review</th>
<th>Fast Track</th>
<th>Accelerated Approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human vaccines only</td>
<td>30 days (IND)</td>
<td>10 months (BLA)</td>
<td>CMC*: 4 mos. Clinical: 10 mos.</td>
<td>6 months</td>
<td>6 months; rolling review, more frequent communication</td>
</tr>
<tr>
<td>European Union</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centralized procedure, both human and animal vaccines</td>
<td>Apply to member states; centralized guideline exists, but timing varies by country</td>
<td>210 days for opinion (180 if no significant issues), plus 67 days for final authorization (MAA)</td>
<td>30–90 days; depends on the type of change to the original application</td>
<td>Opinion process is shortened to 150 or 120 days if no outstanding issues</td>
<td></td>
</tr>
</tbody>
</table>

* CMC = The quality data, i.e., chemistry, manufacturing, and controls sections, of biologics license applications

Time frames in Table 2.1 are given for submissions that are determined to be complete. In the EU process, the clock is stopped at day 120 to allow for applicants to answer questions. In the United States, applicants formally respond to questions at the end of the review.

Some recent examples provide evidence that the expedited processes that exist for review and resolution of issues do work, although many months are still required. Prevnar®, a vaccine for pneumococcal pneumonia in infants, in an example of a vaccine that was given a fast track status by FDA, which resulted in a rolling review as data were submitted; the review time once all data were submitted was six months.

In addition to the expedited processes for normal regulatory review, FDA has also put in place an “Animal Rule” that allows for efficacy to be demonstrated in animals rather than humans under an appropriate set of circumstances. This will not necessarily speed up an approval, but it can provide a means for developing a vaccine that would otherwise never be developed if there were no appropriate studies that could be carried out in humans to demonstrate efficacy. Anthrax vaccine is an example of an agent where the animal rule can be applied, because it meets certain criteria set forth in the Animal Rule, such as the need for disease prevention in certain settings, lack of a suitable setting for human efficacy studies, and ability to use animal models and other laboratory data in addition to human safety data to assess its suitability for human use.

The EU centralized, accelerated procedure for animal vaccines resulted in a positive opinion for an animal, avian influenza vaccine, Nobilis Influenza H7N1, in 120 days. Statistics on review completion time frames on various types of applications can be found on the CBER and EMEA websites.

In considering accelerated overall time frames for vaccines against pandemic flu or other agents, regulatory agencies will need to balance risks versus benefits when employing the various accelerated procedures that have been put in place as described above. This is more easily done when the vaccine properties bear close resemblance to those in other licensed vaccines. Pandemic influenza vaccine strains could potentially be available for human use as early as four months after the decision on which strain should be included in a pandemic vaccine. This time frame is based on the current guidelines in place and the work that has already
been done by manufacturers and the regulatory authorities using the pre-pandemic strains, combined with the ability to invoke laws that allow early vaccine use in a pandemic situation. This time frame is supported by comments that GSK hosts made in presentations at the WTEC panel’s European site visit.

In a situation where an agent is not well characterized and there is no current vaccine in place, the process would require more time. A moderate example is the SARS situation. If it was determined that a SARS vaccine program should be put in place, there is potential in the United States to begin emergency IND use as early as 8 months post identification of the strain, according to one scenario suggested by Dr. Jesse Goodman, CBER Director, at a CDC conference in March 2006 (Goodman 2006). Use of the vaccine under the EUA rule could potentially begin as early as 10–11 months post identification of the strain. Key to the entire process is a continuous assessment of benefit versus risk and ongoing communication with stakeholders, especially regarding the public’s expectations regarding safety, efficacy, and availability.

It is recognized that in a true emergency, even accelerated approaches may fall short. This is especially true when the disease-causing agent is novel and thus even less well studied than in the moderate example of SARS described above. One solution suggested by Goodman (2006) is illustrated in Figure 2.3. The figure describes a hypothetical emergency roll-out program for a novel vaccine. The key elements are integrating manufacturing and broadening clinical studies, coordinated simultaneously with initial use. This approach involves more risk and increased cost to obtain data quickly, but it would deliver vaccine more quickly than what is typical, even for an accelerated program on an unknown agent. As shown in the figure, vaccine would begin clinical testing in humans five months after identification of a suitable seed strain, followed by emergency use (at 7 months) under an IND in a large number of people, along with extensive clinical evaluation of the vaccine. Again, continuous assessment of benefit versus risk and communication with stakeholders would be needed to ensure success.

The above discussion indicates that much work is going on to improve the time frames for new pandemic vaccine release; however, even the best scenarios require months. Continued focus is necessary on understanding the science and developing technologies that allow for rapid identification of pathogens, rapid manufacturing, and rapid analysis.
ANALYTICAL AND CONTROL TESTING

A key component of the regulatory submission and control of vaccines during their lifecycles is analytical and control testing. Assays are needed to evaluate the potency, purity, and stability of the vaccine. A great deal of analysis is required to understand the candidate vaccine and ensure that it has a very high probability of being safe and effective. It is also necessary to analyze the manufacturing process in order to understand the key process parameters that must be controlled in order to consistently produce a safe and effective product. The final process and associated equipment must be validated at the lot size that the manufacturer intends to use to provide large quantities of vaccine for sale. Validation ensures that the processes and equipment that are used to manufacture the vaccine consistently yield a product with the desired quality attributes.

To accomplish these goals, it is necessary to adapt existing assays and analytical techniques to the candidate product, in addition to developing new ones. Each assay that is performed must be itself validated, along with the associated equipment, just as the production process is validated. Validation for an assay includes such items as understanding the limits of the assay, determining the precision that can be obtained, and ensuring that the assay appropriately detects and/or determines the concentration of the components in a mixture. For many assays, it is necessary to ensure that they will be sufficiently sensitive to detect stability issues. For any given antigen or vaccine component, it is necessary to develop a series of assays to be able to detect the specific antigen at various stages in manufacturing, as a finished purified entity, and then in the final formulation that will be given to the vaccinee. Each assay will also require its own set of reagents, reference standards, and controls; these must be produced, standardized, maintained, and controlled.

As the development program progresses, assays evolve, and there needs to be a way to link a new assay to the old assay in order to compare results. In addition, if multiple labs and/or more than one individual will be performing the assay, validation is required to ensure that all of the participating labs and individuals obtain comparable results. When assays are transferred to the control authority that will be testing product on a routine basis for release, another series of exercises must be done to ensure that all parties obtain comparable results on a given assay for a given sample. This assay work is also required for studying the immune response to the candidate vaccine in the clinic. Indeed, given the amount of assay work that is required both during development and for monitoring the marketed product, it easy to see why analytical control and testing is probably the most time-consuming element in the overall process of developing and commercializing a vaccine.

To place the testing time in perspective, Figure 2.4 (from the Pfizer vaccine plant the WTEC team visited in Belgium) can be used to compare the actual manufacturing time with the time it takes to test and review the lot for release. It can be seen that more time is required for evaluation than actual production. This is a timeline that represents the process after all of the development work has been done. Products in the development stage can take much longer to test and evaluate, because the testing regime is not yet fixed and assays are not yet fully developed.

Facility Controls and Testing

Testing is also required to ensure that facilities used to manufacture clinical supplies and marketed products are validated and meet good manufacturing practices standards throughout their use. Validation is a complex series of tests that ensures that each piece of equipment and each area used in the process does what it is intended to do in the way of controlling the process or cleanliness. Because most vaccines are injectibles and their components are not naturally inhibitory to potential microbial contaminants, they must be handled in an aseptic or extremely clean environment to meet today’s regulatory standards.

It can take many months or years to complete validation requirements consistent with current regulatory expectations. Once validation is complete, ongoing monitoring is required. The air quality, water, and gasses used in the facility must be monitored. Testing covers such items as air pressure differentials between various areas of the facility, particulates and microbials within the air system, and any gasses used in the processing. Water must meet standards for chemical content, pyrogens, and microbial content. All instruments and equipment need an appropriate calibration and preventive maintenance testing program.
All of these items are subject to review as part of the control authority facility inspection process, and in many cases, the data collected is submitted to the control authority. In instances where facility data are not as expected, an investigation must be conducted to determine the impact on the product to determine its final disposition. Allowing time to review facility data before product release is part of the process for getting product to market or released for clinical study. The time that it takes to collect and analyze samples becomes part of the overall timeline.

STRATEGIES TO INCREASE THE SPEED AND EFFICIENCY OF ANALYTICAL AND CONTROL TESTING

Conduct Testing on the Production Line

The ability to test during the manufacturing process and get immediate results without having to collect a sample, send it to a laboratory, and wait for results before proceeding to the next step can save a lot of time. It can also avoid a complicated investigation process if the manufacturing process cannot be stopped to wait for test results. Highly automated plants such as those seen at Pfizer in Belgium and Intervet in the Netherlands have many automated data collection features for their facilities and much of their equipment. Fermentation/bioreactor operations also have the capability to monitor inline parameters such as pH, dissolved oxygen, and glucose consumption. Disposable sensing patches are also in use in research settings (e.g., at the University of Maryland, Baltimore County; Rao 2007) However, most operations do not have the ability to determine potency of the key components online and in real time; thus, hold steps are introduced into processes to wait for these results. The main reason for this is that the antigen concentration at the earlier stages of production is often too low to quantitate with the developed assays. Effort needs to be devoted to designing assays that detect microscale quantities or to designing production systems that are not largely water (see Chapters 3 and 4).
Test in Microquantities (Inline/Onsite)
The ability to test microquantities inline can shorten testing time frames by eliminating the time it takes to collect a sample and send it to a lab for concentration and manipulation to obtain results. IMM in Germany (see site report, Appendix B) has developed a “lab-on-a-chip” concept that allows assays to be done inline or at the site where the sample is collected. The system is based on the processing of the smallest fluid volumes in microchannels to include metering, mixing, pumping, filtering, and concentration. IMM has applied the concept to biological assays such as PCR testing for DNA and ELISA (Enzyme-Linked ImmunoSorbent Assay) assays. Figures 2.5 and 2.6 depict the ELISA application of the “lab on a chip." Research to adapt these types of systems for potency assays used in vaccine development and manufacturing areas would be of great benefit in saving time and costs. U.S. research is also going on in this area, for example, the work of Dr. Klavs Jensen (2007) at MIT on miniaturizing development and manufacturing of biologics.

Select Tests with Shorter Time Frames
Tests that can be run within minutes or hours are preferable to more typical ones with time frames of days or weeks. Often the type of test selection is based on the technology selected for manufacturing, as was pointed out by Dr. Shaw of VaxInnate at the WTEC Workshop on Science and Technology in North American Rapid Vaccine Manufacturing (Shaw 2007). Avoiding the use of animals can save the time that it takes to procure a reliable supply of animals and the weeks that it often takes to immunize an animal and wait for the immune response to develop. A better understanding of the immune system and an investigation of animal systems to find correlates to use rather than the animal test itself are necessary to limit the amount of animal testing required for a final product.

Limit the Number of Required Tests
Understanding the critical process parameters that influence a product’s quality attributes is key in minimizing the number of tests that must be done at each stage to ensure quality. Process Analytical Technology (PAT) is supported by regulatory agencies as a means of analyzing a process so that sufficient knowledge is gained to minimize routine testing. This technology is currently difficult to apply to complex processes, but miniaturization and better detection tools (described above) can facilitate collection of the data needed to fully understand a manufacturing process. Systems such as the SimCell Platform described by Dr. Klavs Jensen (2007) at the WTEC Workshop on Science and Technology in North American Rapid Vaccine Manufacturing can also facilitate the rapid collection of data. Key to developing these systems is the interaction of appropriate engineering and life sciences disciplines.

Develop Artificial Immune Systems
At the WTEC North American Workshop, Dr. Bill Warren of VaxDesign (Orlando, FL) described the development of artificial immune systems that can be constructed to look at the immune response to candidate antigens (Warren 2007). These systems have the advantage of eliminating the use of animals for the test and minimizing the time to obtain answers. One can envision that in the future such systems can provide a means to shorten or substitute for some of the human clinical phases of vaccine development and also provide shorter testing time frames overall. Figure 2.7 depicts VaxDesign’s high-throughput in vitro clinical trial model that can test the efficacy of the artificial immune system immunocytes and biomolecules against the disease (functional assays/disease model). (See also Chapter 3.)

SUMMARY AND CONCLUSIONS
Vaccine development and manufacturing are highly regulated processes that require extensive testing and control efforts to arrive at a final vaccine and continue to meet new standards throughout their life cycles. Public concerns for safety have a major influence on the control process. Current regulatory strategies seek to balance risk and benefit, a task that is difficult, as the welfare of both individuals and the collective public health must be considered. Vaccines currently in use took many years to develop and have production and testing cycles that are long and regulatory requirements that make it difficult to expand capacity or introduce new technologies quickly. Regulatory authorities, manufacturers, and many research organizations recognize the limitations and are working toward solutions that will make vaccine development and commercialization occur more quickly. There are laws in both the United States and the European Union that allow for emergency use of vaccines under special circumstances.
Chip-based-Lab

Characteristics:
- Modules for individual task
  - Fluidic control
    (pumps, valves, ...)
  - Metering and mixing
- Amplification
  (PCR, NASBA, ...)
- Detection
  (Electrochemical, Fluorescence, ...)
- Compatibility to lab equipment
- Computer control for automatization

Figure 2.5. The “lab-on-a-chip” concept (courtesy IMM).

Design of the ELISA Chip

Outlet → Section of a 5x Elisa Chip

Cuvette for Detection

Cover Foil Sample

Mirror LED Fluorescence Detector

Detector (Transmission)

Scheme of the light path in the optical detection chip

Sample in

Figure 2.6. Application of “lab-on-a-chip” to ELISA assays (courtesy IMM).
The major key to limiting the amount of regulatory intervention required is filling in the science gaps that exist. If science could uncover a unique means of conferring immunity to a broad range of pathogens or even of ensuring that immunity is generated to variants of the same pathogen, vaccines could be delivered to the public much more rapidly.

Ensuring that the right people and training exist for regulations will contribute enormously to speed and efficiency. Additional harmonization across the EU and the United States and more exchanges between the animal and vaccine community could facilitate ideas for improvement. Meanwhile, efforts to build on what is already available in the way of manufacturing platforms, vaccine facilities, and electronic tools can aid in rapid regulatory review of submissions.

Analysis and control testing occupies the greatest amount of time in the development and commercialization processes. As with regulatory intervention, understanding the science of the processes is key to ultimately limiting the amount of testing effort required. The vaccine industry could make enormous advances if some of the miniaturization techniques and “lab-on-a-chip” concepts could be adapted to vaccine processes. This requires a multidisciplinary approach involving scientists trained in a variety of engineering and biological fields. Meanwhile, making improvements to current online testing capabilities, careful selection of test methods, and thorough process evaluation can make testing efforts more efficient.

REFERENCES


CHAPTER 3

DISCOVERY AND DEVELOPMENT OF EFFECTIVE AND SAFE VACCINES

Terrance Leighton and Joseph Bielitzki

DISCOVERY-BASED VACCINE DEVELOPMENT

The Basic Principles of Immune Response

Discovery-based research in both infectious diseases and basic immunology has contributed significantly to the field of vaccinology. Major contributions have been made in our understanding of antigen presentation and the signaling pathways that are essential for inducing both an innate and an adaptive immune response.

Innate Immune Response

Innate immunity is found in such organisms as eukaryotes, prokaryotes, invertebrates, and plants. The innate immune response is activated by the chemical properties of the antigen. This innate response nonspecifically provides an immediate defense against a wide variety of pathogens but does not provide long-lasting protection. The primary defense in innate immunity is a function of the skin and mucous linings resisting pathogen entry or trapping invading pathogens. The secondary defense in innate immunity includes phagocytes such as macrophages that engulf pathogens. Other secondary defenses include antimicrobial proteins and the complement system that coats pathogens to enhance phagocytic engulfment.

In the early 1990s, a Toll gene was identified as a key component of the innate immune system of Drosophila (“fruit fly” species). In the mid-1990s, the mammalian equivalents, Toll-like receptors (TLR), were identified. TLRs recognize molecules known as pathogen-associated microbial patterns (PAMP) that are broadly conserved in pathogens and released as cellular degradation products following microbial death, but that are distinguishable from host molecules. When PAMP binds to TLR, the intracellular portion of TLR interacts with MyD88 (a primary response gene), resulting in NFkB-activated transcription of cytokines and interferons. This signal transduction pathway is necessary for antigen processing with the presentation of antigen to T lymphocytes as adaptive immunity is initiated.

Adaptive Immune Response

Adaptive immunity is found in higher vertebrates. The adaptive immune response is specific to the pathogen and provides long-lasting protection. Once an antigen has been processed and recognized, the adaptive immune response selectively expands a large number of cells specifically designed to attack the antigen. The main players in adaptive immunity include leukocytes (white blood cells). One arm of the adaptive immune system is the humoral response, where B cells produce specific antibodies in response to bacteria and viruses. The other arm is cell-mediated immunity, where T cells produce antibodies in response to virus-infected cells.

Immunity develops as a series of events that starts with the introduction of an antigen—either endogenous or exogenous—into the body and its recognition by T lymphocytes. Endogenous antigens are generated within the cells, including proteins expressed by viruses that have invaded the cells or mutant proteins expressed by mutant genes. Exogenous antigens enter the body from the environment via inhalation, ingestion, or skin wounds, etc.

Endogenous antigens are degraded and processed into short peptides within the cell, loaded onto Class I histocompatibility molecules (MHCs), and then displayed on the cell surface where they can be recognized by CD8+ T cells. CD8+ T cells are cytotoxic and can destroy the infected cell.
Exogenous antigens are engulfed by antigen-presenting cells (APCs), including B lymphocytes and phagocytes such as dendritic cells (DCs) and macrophages. The antigens are degraded and processed into short peptides within the APC. These peptides are loaded onto Class-II major histocompatibility complex (MHC) proteins and then displayed on the surface of the APC, where they can be recognized by CD4+ helper T cells. Those CD4+ cells that recognize peptides displayed on macrophages and dendritic cells release lymphokines to attract other cells to the area in what is known as cell-mediated immunity. Those CD4+ cells that recognize peptides displayed on B cells stimulate the development of the B cells into plasma cell clones that secrete antibodies against the specific antigenic peptide. This is known as antibody-mediated immunity.

Approaches to Development of New Vaccines

New vaccines attempt to mimic the process of antigen presentation. Newer adjuvants—substances that elicit a stronger immune response when mixed with the immunogen—target the TLRs of the dendritic cells. Antigen presentation seems most efficient when this natural sequence is activated. How antigens in a vaccine are presented to the DCs affects the quality of the immune response, the formulation of the vaccine, the manufacturing process, and even the regulatory path to product approval. Several areas of research are not covered in this report, including the use of genetically engineered foods as a method for vaccination. Plant-based vaccines, including bananas and potatoes, have been developed, but their ability to induce a protective immune response requires further study. Recent studies with tobacco leaf tissue suggest that this plant system has considerable potential for recombinant antigen production. New eukaryotic systems, including chickens, quail, and shrimp, are being developed for rapid production of high-quality immunogens.

Bacterial Ghosts

WTEC panelists visited Dr. Werner Lubitz at Vienna University (see site report, Appendix B, and Figure 3.1) to learn first-hand about a new technology he and his group uses, so-called “bacterial ghosts,” to present antigen to the DC. This product uses genetically engineered Gram-negative bacteria that incorporate specific antigens from the target pathogen into the cell wall or membranes of the bacterial ghost. The bacteria are then killed using the controlled expression of the cloned gene E of the lytic phage PhiX174. The action of the gene E product of the phage results in rupture of the bacterial cell wall and the loss of the internal cellular contents. Bacterial ghosts are a killed preparation and pose no risk to the recipient. They can carry foreign guest proteins or immunogenic epitopes and, since they begin as microbes, they can carry a variety of adjuvants in the form of naturally occurring PAMPs. Bacterial ghosts offer an opportunity to naturally stimulate both innate and adaptive immunity. Small particle size, being a killed product, the ability to incorporate adjuvants, and the possibility for polyvalent antigens contribute to the strengths of the bacterial ghost as a potential method of antigen presentation.
Virus-Like Particles

In a similar vein, “virus-like particles” (VLP) is a concept for an engineered delivery system that carries specific immunogenic epitopes needed to produce effective immunity (see site report for Karolinska Institute and the Swedish Institute of Infectious Disease Control in Appendix B). Saccharomyces cerevisiae and Baculovirus can be engineered to produce specific antigens that resemble the intact microbe immunologically and produce a robust immune response at both the cellular and humoral level. These virus-like structures lack nucleic acids and the ability to replicate. As is the case with the bacterial ghost, antigen presentation using VLPs also uses naturally occurring mechanisms within the DC, primarily MHC Class I presentation, to initiate both an innate and an adaptive response. Vaccine constructs for hepatitis B and human papilloma virus use VLPs and are approved by the U.S. Food and Drug Administration (FDA). A research construct of an Ebola virus-like particle is showing promise at the experimental level. VLPs are being considered for other infectious diseases such as Hepatitis C and HIV. Vaccine constructs have been produced for a number of infectious diseases of interest to the veterinary community with good response.

Cell-Free Production

At the WTEC Workshop on Science and Technology in North American Rapid Vaccine Manufacturing, Dr. James Swartz (2007) of Stanford University presented his work to produce protein using a cell-free environment. The cell-free production system provides an opportunity to rapidly and inexpensively produce a high quantity of antigens with high purity. Much of this work has explored the production of tumor antigens, but the cell-free method applies equally well to immunogens of infectious agents. The goal is to couple the cell-free production of antigens—produced and carried in a biologically relevant three-dimensional structure—with new delivery systems to initiate an immune response.

Synthetic Particle Systems

Synthetic particles have been developed by a number of facilities that combine the strengths of polymer chemistry at the microscale with the incorporation of both adjuvants and immunogen onto the particles at the nanoscale. These systems provide the opportunity to better control the amount of antigen provided and the rate at which the adjuvant and antigen are released. The particle size and configuration may also be modified to alter the response to the vaccine. Particulate delivery systems provide an opportunity to move beyond the response caused by a native pathogen and to initiate responses that are optimally protective by better controlling the response to the immunogen. Free protein and nucleic acids often lack the desired immunogenicity necessary to produce a robust immunologic response. The synthetic particle system appears to provide the size and configuration necessary to improve vaccine responses and the combinatorial flexibility to optimize protection.

Current limitations of the concept include (1) the relatively few polymers approved as carriers, (2) the difficulty associated with producing monodispersed particles of consistent particulate size, and (3) the regulatory complexity associated with the combinatorial product itself. PowderMed, Inc. (see site report, Appendix B) has developed a needle-free delivery system, referred to as Particle-Mediated Epidermal Delivery (PMED™), for particulate systems. When coupled with the use of synthetic particles as carriers of both the immunogen and the adjuvant, this system has significant promise in the production of safe, specific, and robust immunity.

Adjuvants

Adjuvants, the small molecules that prime innate and adaptive immune responses are an active area of research. The Toll-like receptors (TLRs) respond to degradation products of bacterial cells, that is, lipopolysaccharides (LPS), nucleic acids, flagellin, cell walls, and others to trigger specific signal transduction pathways that improve vaccine efficacy. This understanding has led to the development of new adjuvants capable of reducing the antigen dose needed to induce an immune response, reducing the total number of vaccinations required for protective immunity, and reducing the number of individuals who do not respond to a vaccine.

Requirements for Progress in Vaccine Development

Repeatedly, members of the vaccine manufacturing community worldwide expressed to WTEC panelists the same basic requirements for progress in vaccine development:
1. Better correlates of protection
2. Systems for product testing and development that are not heavily dependent on rodent models
3. Improved selection criteria for immunogens
4. New methods to evaluate single or multiple antigens with adjuvants prior to clinical trials

**Better Correlates of Protection**

Correlates of protection are often defined in terms of neutralizing antibody, T cell responses, and the relative cytokine profiles seen following immunization. These correlates may indicate an active response to an antigen, but that response may not confer an ideal immune response to an individual. “Protective immunity” occurs if after a subject’s exposure to a pathogen, the agent is unable to cause a defined disease state in the subject. Protective immunity is a balance between T and B cell responses, and for each pathogen there should be an ideal ratio of these responses. At present, “protection” is defined loosely as exposed subjects not becoming infected with any appreciable degree of morbidity or mortality; it lacks an idealized response.

A goal of vaccine formulation is to produce a robust and protective response in 100% of the vaccine recipients with 0% adverse events. The genetic variation of the human population in the configuration of Human Leukocyte Antigen (HLA) allows each person to differentiate self from non-self. However, this benefit also results in variation at the binding site of antigens with MHC Class II proteins. The binding of an antigen with the MHC Class II molecule affects the efficiency and quality of the presentation of the antigen to the lymphocyte population and the resulting immune response.

**Alternatives to Product Testing on Animals**

Vaccine development is ethically constrained under the principle of beneficence. This principle prevents researchers from causing harm to the human research subject. Consequently, infectious disease and vaccine research is heavily dependent on animal models throughout both the discovery and development stages. This is problematic. Animals demonstrate varying susceptibility to human pathogens. The course of an infectious disease in animals may resemble the human disease but is infrequently identical to the disease in humans. The immunologic response of the mouse, while well defined, is not identical to a human response. Animal models represent the best option in vaccinology for establishing the potential protective benefit of an antigen. The shortcomings of the model result in many good immunogens for developing protective immunity in mice, but these same immunogens provide significantly less immunogenicity when tested in humans. Vaccines and immunogens are best evaluated in the species for which the final product is intended.

Few alternatives to animal use exist. VaxDesign, Inc., a biotechnology company in Orlando, FL, is developing a human artificial immune system (AIS) that provides an opportunity to evaluate many of the early phases of vaccine testing and development in the target species. It may provide new concepts for evaluating actual human responses to an immune challenge and the kinetics and process of developing protective immunity for both naïve and recall responses. The AIS provides a system that is the target of both the infectious agent and the vaccine. The system uses donor peripheral blood mononuclear cells in a 96-well format to recapitulate exposure to antigen, antigen processing, immune synapses, transfer of information to lymphocytes, clonal selection and the functional outputs of immunity, antibody production, and cellular responses.

**Improved Selection Criteria for Immunogenicity / New Methods for Looking at Multifactor Vaccines**

The AIS system combines immunology and engineering and reduces the immune system to the set of lowest common denominators necessary for functional and appropriate immune responses. The AIS starts with peripheral blood mononuclear cells collected from a large and diverse donor pool. These cells are matured into type 1 DCs exposed to antigen and then exposed to a population of T and B lymphocytes where lymphocytes produce both humoral and cellular responses. During the WTEC North American Workshop, Drs. William Warren and Eric Eisenstadt of VaxDesign suggested that coupling these essential immune functions to a genomics-based identification system for antigenic epitopes through the concepts of reverse vaccinology might provide an end-to-end system for rapidly testing and identifying immunogens (Warren 2007). Reverse vaccinology is leading to vaccine formulations with immunogens representing several epitopes and an adjuvant. The ability to determine new epitopes based on the genome of the pathogen will continue to improve vaccine efficacy. This is especially true for pathogens with rapid antigenic drift, where conserved immunogens are identified that in the past were unrecognized as contributing to immune response.
The VaxDesign AIS provides a mechanism for evaluating antigens, adjuvants, naïve and recall responses, human variation, and the protective response. Such a system provides a method for pursuing high-throughput screening of vaccines for immune responses, with the capability of doing combinatorial vaccine design with side-by-side evaluation of the hosts’ responses.

The immune system is highly complex and interacts with every tissue within the body. Fundamental science and new technology can improve the sophistication of vaccine formulation by providing new components or merely by providing additional beneficial immune modulators. This will require collaboration and cooperation among the cadre of investigators committed to improving human health and eliminating infectious diseases by vaccination. Each new discovery that is incorporated will run the gamut of the regulatory process to ensure consumer safety. These discoveries will require a systems approach as to how and where in the process they might be incorporated, and each will have a cost that will determine its relative merits compared to the tried and proven systems currently in place.

**ADAPTIVE AND MODULAR PRODUCTION PROCESSES**

The global spread of H5N1 highly pathogenic avian influenza in wild birds and domestic poultry has raised concerns that if the virus crosses species barriers and evolves efficient human-to-human transmission capabilities, it could cause the fourth major pandemic in recent history (the three preceding occurrences being the 1918, 1957, and 1968 pandemics). These global public health apprehensions have accelerated “pre-pandemic” vaccine research and development. The United States intends to produce 20 million doses of pre-pandemic vaccine (HSC 2006) to protect a U.S. population of 300 million. Several European companies (Novartis, GlaxoSmithKline) have created pre-pandemic vaccine and "mockup" vaccine processes for EU countries (EVM 2006), and the World Health Organization (WHO) is also creating stockpiles for pandemic interdiction at nodes of disease emergence (WHO 2006b).

Current annual global interpandemic vaccine production capacity is approximately 350 million doses of trivalent influenza vaccine (WHO 2006a). Even if all of this capacity could be redirected to pandemic vaccine manufacture, it would be massively insufficient to protect a global population of 6.6 billion. The long time lags of 5–14 years in construction, development, and licensing of new vaccine production facilities (Chao 2007) would further exacerbate these supply shortcomings.

Modern vaccine manufacturing relies on a complex cross-disciplinary fusion of basic, applied, and empirical sciences. The existing manufacturing infrastructure was challenged by the demand for large supplies of influenza vaccine in 2003, and the withdrawal of one supplier of diphtheria vaccine seriously constrained supply of that vaccine. These recent supply shortfalls illustrate the fragile and inelastic nature of the current vaccine production enterprise. There is a compelling case for developing more adaptive, agile, and modular vaccine manufacturing technologies and platforms. Improvements in vaccine delivery, dosing, filling, packaging, and distribution systems are all required. The first part of this chapter discussed rapid antigen production systems. This section will focus on new developments in microstructured reactors, integrated in-line QA/QC control/monitoring and associated technologies that could overcome these deficiencies in vaccine production processes: poor volumetric productivity; nonstandard custom process solutions; lack of real-time process monitoring tools; lack of scalability; inflexibility; high costs of production; and separate batch processes versus continuous manufacturing.

**Microstructured Reactors and Integrated Bioproduction Processes**

WTEC panel visits to the Institut für Mikrotechnik (IMM) in Mainz, Germany, and DECHEMA in Frankfurt, Germany, revealed vibrant and innovative approaches to the use of microreactor and microanalytic technologies for large-scale manufacturing (see site visit reports in Appendix B). At IMM, microfluidics and joining disciplines are integrated to produce full-scale manufacturing systems that integrate microreactors, fluid handling, mixing, filtration, separation, optical and electrochemical sensing, simulation, process modeling, and control systems. This suite of technologies allows rapid commissioning of small-dimension, modular, adaptable plants that lower investment risks and allow shorter payback periods. IMM has found that many conventional processes can benefit from these innovations through faster development and optimization, continuous and simplified processing, higher product purity, scalable and portable production options, and fast adaptation of process parameters. These multiscale technologies allow smart dimensioning of plant investments and products. Applications of these technologies in the fine and bulk chemicals sectors
have resulted in orders-of-magnitude impacts on process scale, production efficiencies, and product throughput (Hessel et al. 2006). All of the system modules are standardized and integrated inline with embedded and disposable analytics (Drese, von Germar, and Ritzi 2007).

The potential application of these revolutionary IMM technologies and those developed by Jensen and coworkers at MIT (Jensen 2007) to vaccine manufacturing could enable

- Modular, agile and adaptive vaccine plants
  - Microreactor cell concentrations and productivity could be increased by $10^2$ per unit volume
  - Cell architecture and environmental control could increase production 10 times
- Fast process ID, modeling, and control
  - Inline cell separation, product isolation, and formulation could streamline the antigen-to-vaccine pathway to market release

IMM participates in the MicroChemTec Initiative, which is led by Drs. Thomas Dietrich of Mikroglas and Alex Bazzanella of DECHHEMA (see DECHHEMA site report, Appendix B). This is a consortium that includes 40 companies and research institutes working cooperatively to address critical process engineering gaps:

- Poor volumetric productivity
- Nonstandard custom process solutions
- Lack of real-time process monitoring tools
- Lack of scalability
- High costs of production
- Separate batch processes vs. continuous manufacturing

MicroChemTec is addressing these gaps by developing an architecture of compatible microcomponents and standardized interfaces that can be assembled with backbone frames and interfaces into integrated macrosystems. Merck has successfully applied these design principles to real-world, full-scale chemical processes. Available MicroChemTec modules of interest include bioreactors/fermentation; mixing/emulsifying/suspending; extractors; chromatographic and electrokinetic separators; pressure, flow, temperature, O$_2$, conductivity, and optical sensors; and high-performance liquid chromatography (HPLC), gas chromatography (GC), and capillary electrophoresis (CE) analyzers. The MicroChemTec consortium is pioneering innovative solutions to real-world process engineering problems. There are very attractive opportunities for extending these concepts and technologies to rapid vaccine manufacturing applications.

**Rapid Identification of Threat Agent Variation and Genotype/Serotype Prediction**

The accelerating scope, scale, and consequences potentially associated with future infectious disease outbreaks are unprecedented. For example, a recent quantitative study of an influenza pandemic similar to the 1918 outbreak predicted that 62 million people would be killed (Murray et al. 2006). The lack of a coherent global system for facile detection, characterization, diagnosis, management, and prevention of known and emerging diseases increases the probability of adverse events (King et al. 2006; Lancet 2006). There is a severe mismatch between the genetic and ecological complexity of disease emergence and our ability to identify root causes of epidemics. Faster and finer-grained systems are required to identify agents, genotypes, sources, and spread of priority pathogens.

At present, infectious disease outbreaks are only recognized by epidemiological investigation after their occurrence. There is an urgent need to drive the epidemic continuum from the reactive era into a proactive era that recognizes disease emergence at the earliest possible stage and extinguishes disease pressure prior to outbreak occurrence or spread. There is an unmet need for broadband, high-throughput pathogen surveillance systems that can track, in near real time, genetic variation that can lead to antigen shift and drift. Once pre-pandemic vaccine stockpiles are filled, they will need to be replenished due to H5N1 strain drift. Similar problems confront interpandemic vaccines designed to protect against seasonal influenza. Several new technologies could address the current public health and veterinary surveillance vacuum that constrains the detection of and response to disease outbreaks.

TIGER (Triangulation Identification for Genetic Evaluation of Risk) is a rapid, high-throughput molecular method for broadband pathogen surveillance that utilizes an analytical methodology based on analysis of
multiple polymerase chain reaction (PCR) amplicons using PCR/ESI-MS to determine base compositions of complex mixtures of amplicons. High-resolution genotyping of specific bacterial and viral species is accomplished by utilizing species-specific primers that interrogate regions of high intraspecies variability to distinguish closely related strains, pathotypes, and biotypes.

For example, to assess the landscape of bacteria present in diagnostic or clinical samples, a set of 16 broad-range surveillance primers are used that allow PCR amplification and quantitative identification of many different bacterial pathogens in a single assay. These broadband primers were chosen by computational analysis of sequence alignments of all available ribosomal DNA operons and 160 broadly conserved protein encoding housekeeping genes. The ribosomal DNA-targeted primers have the broadest range of bacterial coverage. For example, four designed primer pairs targeted to 16S ribosomal DNA match 98% of the bacterial sequences in the Ribosomal Database Project when allowing for two to three mispairings under permissive PCR cycling conditions. The primers targeted to protein-encoding housekeeping genes have breadth of coverage at the level of major bacterial subdivisions (e.g., beta proteobacteria, bacilli, streptococci, staphylococci, etc.). Although any single primer target region might have an overlap of base compositions with other species, combined information from multiple primer pairs provides unambiguous organism-specific signatures for all major bacterial pathogens.

Similar strategies have been developed for broadband characterization of viral samples, including adenoviruses, filoviruses, SARS, and influenza virus strains (Sampath et al. 2007). Panmicrobial microarrays offer an alternative approach to PCR-based unbiased pathogen discovery and surveillance (Palacios et al. 2007). Variants recognized by these advanced detection systems subsequently can be fully characterized by large-scale genome sequencing (Obenauer et al. 2006).

While these advanced pathogen detection technologies can identify the emergence of new genomic variants, they cannot directly determine the effects of these sequence alterations on viral serology. The central problem is that flu genetic change is gradual, while antigenic change is epochal. For example, human influenza A viruses have limited hemagglutinin and antigenic diversity determined by clusters that emerge and replace each other and establish strain cross-immunity. Koelle and coworkers (2007) have developed a novel neutral network model that allows flu genotype-to-phenotype mapping (at least for H3N2). The model captures well-known as well as previously unrecognized features of interpandemic influenza dynamics and evolution, including the epochal pattern of viral evolution, periods of antigenic stasis during which genetic diversity grows, and punctuated episodic diversity contraction during antigenic cluster transitions. These approaches may provide a path forward to deconvolve genotype/serotype relationships for H5N1 and enable knowledge-based selection of strains for vaccine research and development.

**THE END-TO-END VACCINE DISCOVERY-DEVELOPMENT-MARKET APPROVAL PROCESS**

WTEC panelists were extremely fortunate to spend several hours with Dr. Rino Rappuoli, Global Head of Vaccines Research for Novartis in Siena, Italy. Dr. Rappuoli and his colleagues have been seminal thought and technology leaders in vaccinology for the past several decades (Ulmer, Valley, and Rappuoli 2006; Serruto and Rappuoli 2006). For example, his group pioneered the application of genomics and reverse vaccinology to antigen discovery (Mora et al. 2006; Giuliani et al. 2006). He has also been engaged in a very deep and rich analysis of the current state of vaccinology science and technology. Dr. Rappuoli was preparing a major review of this topic at the time of the WTEC team’s visit, but he generously shared many of his pivotal conclusions and insights with the WTEC team prior to publication of his writings.

**History**

From a historical perspective, vaccinology has changed little for over the past one hundred years (circa Pasteur). Most vaccines are produced by the principles that Pasteur articulated: *Isolate – Inactivate – Inject the Causative Agent*. These approaches have been very successful for pathogens that present stable antigenic structures to the immune system: diphtheria, pertussis, tetanus, polio, H. influenza type b (Hflu), hepatitis B virus (HBV), measles, mumps, and rubella. Vaccine development for pneumococcal and meningococcal diseases has been more challenging due to the much greater diversity of antigenic structures presented by these pathogens. These pathogens typically change major circulating antigen types once a decade. This rate of change is approximately the time currently required to develop a new vaccine, so vaccinology has been able to maintain protective immunity to emerging variants. Influenza changes antigenic variants on yearly
cycles and is a stressing case for vaccine protection. Human immunodeficiency virus (HIV) changes antigenic variants once a day and has confounded all attempts to develop vaccine protection. Tuberculosis (TB) and malaria are similar cases.

**Major Science, Engineering, and Education Gaps**

Dr. Rappuoli ascribes the failure to develop successful HIV, TB, and malaria vaccines to a lack of understanding of how the immune system works. In particular there are major scientific gaps in knowledge concerning

- Mechanisms of mucosal immunity
- Mechanisms of non-antibody cellular protection
- The interplay between cell- and antibody-based protection
- Mechanisms of adjuvant immune system programming
- Strategies to reveal and defeat antigen cloaking

Dr. Rappuoli suggests that a new discipline of vaccinology, “Structural Immunology,” is required to elucidate the molecular and atomic interactions between target antigens and the immune system. He mentioned the recent success in determining the three-dimensional structure of the conserved neutralizing epitope of HIV gp120 (Zhou et al. 2007) as providing a path forward to the genesis of a Structural Immunology discipline. These studies revealed that the gp120 surface is protected against antibody recognition by a dense array of carbohydrate camouflage and a shape-shifting mechanism that only exposes the conserved neutralizing epitope once it reaches the CD4 T cell receptor (antigen cloaking).

When Novartis acquired Chiron Vaccines in April 2006, its scientists carefully studied the company’s state of vaccinology science and technology (S&T) relative to Novartis’ plans to create a new Vaccines and Diagnostics division under Dr. Rappuoli’s leadership. They advised Dr. Rappuoli that they considered Chiron vaccinology S&T similar to Pharma S&T of the 1950s. Dr. Rappuoli took it as his challenge to address the vaccinology deficiencies that were noted: lack of knowledge of target-based mechanisms for protection and screening, the inability to elicit balanced T and B cell responses, nonavailability of *in vitro* surrogate markers for protection, nonavailability of single-dose oral or mucosal delivery systems, the inability to produce life-long protection, and the inability to protect against all antigenic variants.

Another important factor Novartis recognized is that the current economics of the vaccine market severely undervalues the long-term contributions of vaccines to reductions in morbidity and mortality and provides a disincentive for innovation. (The advent of emerging and pandemic infectious disease threats may substantially alter these market forces.) Dr. Rappuoli is a strong proponent of innovation in vaccinology; he also emphasizes that vaccines offer a very attractive alternative strategy for enduring control of multi-drug-resistant bacterial pathogens.

Beyond addressing these science gaps, Dr. Rappuoli has turned his attention to addressing translational science and engineering gaps that affect the end-to-end process of vaccine production, which he has divided into three conceptual elements (Figure 3.2 and following list):

1. **Conception:** The “hot” basic discovery science front-end of vaccine development is well funded, has an attractive academic career path, but rarely produces products of human utility.
2. **Gestation:** The “boring and tedious” development engine of systematic discovery and optimization of vaccine candidates, production scale-up, establishment of GMP production and formulation conditions, safety and toxicology testing, and proof-of-concept in man is underappreciated in the academic community. This results in all of the education, training, and career development occurring in only five major industrial settings. At the time of the WTEC study, there was only one, fifteen-day vaccinology course offered in the world, and it was continuously oversubscribed. This is a major gap in vaccine manufacturing education and training, particularly since there is no existing surge capacity to meet the challenges of a global pandemic. *Gestation is the choke point for rapid vaccine manufacturing.*
3. **Growth & Maturity:** These involve Phase II & III clinical studies, large-scale GMP manufacturing, licensing, lot release, and commercialization, and they follow well-established career paths and competencies in the pharmaceutical industry.
Dr. Rappuoli’s concept of a path forward to “structural vaccinology” emphasizes that the insights that vaccinology has progressed little beyond Pasteur; that we do not understand the rules of immune system response, regulation, and adjuvant programming; and that there is an unmet need for rational selection and design of antibody responses (3D epitopes) are in fact all high-reward opportunities for innovation.

Dr. Rappuoli’s abstraction of the vaccine development pathway from conception to gestation to maturity is very powerful in illuminating the translational disconnections between these three essential elements of vaccine development. In the event of a pandemic, it will be extremely difficult to meet surge demand in the absence of an educational and training infrastructure that provides a pool of gestation practitioners beyond those already employed in industry. This is a critical area for scientific and policy focus.

Figure 3.2. Diagram of Dr. Rappuoli’s tripartite R&D approach to bridging the science and engineering gaps in the end-to-end process of vaccine production (courtesy of Dr. Rino Rappuoli).

REFERENCES


CHAPTER 4
PLATFORMS FOR VACCINE MANUFACTURING

Stephen W. Drew

INTRODUCTION

Vaccines are substances that stimulate the body’s immune response with the goal of preventing or controlling an infection. They present an antigenic component that defines the specificity of the induced immune response. Other attributes of vaccine-induced immunity, such as the kinetics of immune response, the longevity of the response (in terms of evidence of continued immunogenicity and protection), and even the specific type of immune response, may depend on formulation of the vaccine and its delivery to antigen processing cells in the immune system cascade. Maintenance of the safety, efficacy, and quality of the vaccine requires a manufacturing process that begins at the molecular level (e.g., the nature of the antigen and its interaction with the immune system) and continues to the point of use. The development of a successful manufacturing process requires insight, innovation, concerted effort by dozens of disciplines, construction and use of complex facilities, good judgment, long-horizon planning, and extraordinary patience.

Figure 3.2 in Chapter 3 shows a conceptual path from discovery to marketed vaccine, identifying several of the steps along the way. At first glance, the path seems linear, with each step following the other sequentially. The reality is that each step presents myriad alternative choices. Each decision narrows, defines, and sharpens the range of options for scale-up and manufacture of vaccine materials. Early decisions on which antigens to pursue, how to manipulate the antigens and present them to the test immune systems of animals and humans, and the nature of immune responses sought can severely restrict the range of options for manufacturability.

Yet modern approaches to the design of successful vaccines encourage an iterative process in which stakeholders all along the path from concept to clinical deployment participate to achieve optimum results in minimum time and at minimum cost. The efficiencies that are captured by integration along this path can often enable overlapping strategies to combat disease through multiple approaches. The WTEC panelists saw much evidence of this in our study of North American and European vaccine manufacturing. In each and every case studied, the overarching goal has been to create safe and effective vaccines for the treatment of serious disease. Safety and efficacy are the universal arbiters of decisions that aim to establish vaccine quality (e.g., identity, potency, purity, immunologic response, protection and longevity, and vaccine stability) and manufacturability (e.g., scale-up, process robustness and reliability, worker and environmental safety, cost, the rates at which a manufacturing process can be developed, and the rates at which it can provide vaccine).

This chapter builds on the fundamental sciences of vaccinology described in Chapter 3 to assess some of the current manufacturing options, directions, and capabilities in Europe. This WTEC study aimed to identify the best strategies and practices in vaccine design and scale-up with special emphasis on (1) the types of vaccines under development, (2) vaccine presentation to the immune system (how they are formulated and delivered), (3) the implications for manufacturing facilities and unit operations, and (4) the factors that control the rate of progress and manufacturing capability. Throughout the WTEC panel’s studies in Europe, we used the framework example of vaccine manufacturing response to the threat of an influenza pandemic to test the most demanding edge of the manufacturing envelope.
VACCINE DESIGN

New and improved vaccines against diseases of public health importance are being conceptualized and designed along several basic lines. In general, vaccine design is based either on disease-related whole organisms that have been rendered clinically nonpathogenic or on components (subunits) of disease-related organisms that, by themselves, will not cause the disease. Single, simple components seldom elicit an adequate immune response by themselves. Successful subunit vaccines are often designed to present the antigen in particulate form (e.g., live or inactivated recombinant microbial cells or virus vectors, self-assembled mimics of cell or virion structure) or as subunits conjugated with other macromolecules that enhance immunogenicity (e.g., polysaccharide-protein conjugates). Table 4.1 lists several examples of vaccine platforms designed along these strategies.

Again, the two irrefutable criteria for success are safety and efficacy in the target population(s). An “ideal vaccine” might have some of the following characteristics, or others that address the two dominant standards of safety and efficacy:

- Broad safety profile across all ages, both sexes, and those with suppressed immune systems
- Stimulates protection against the target disease within 2 weeks of administration
- Will not cause disease in others
- Elicits effective herd immunity
- Elicits a high level of long-lived efficacy, including in young infants and the elderly
- Clears itself from the recipient’s system so that it does not retain residual pathogenicity
- Can be manufactured reliably and reproducibly at large scale by uncomplicated, economical processes
- Is effective against all strains or serotypes associated with the disease
- Requires one or two doses, administered over a short time, to confer protection
- Can be combined with other vaccines in a common formulation or coadministered with other vaccines
- Can be administered without a hypodermic needle and syringe
- Can be produced in formulations that are stable for long periods of time at high and low temperatures
- Can be designed, scaled-up, and manufactured in a time frame that will effectively intercept the progression of the disease

In the panel’s observation, no vaccine meets all of these criteria, but some closely approach individual ideals.

STRATEGIES AND DECISIONS FOR VACCINE MANUFACTURING PLATFORMS

A manufacturing platform is a repeatable component of vaccine manufacturing that aims to reduce the time and cost of development of a new vaccine while providing confidence that the product will be safe, efficacious, reliably available, and within the required unit-dose cost parameters.

Triggering Immune Response

The first manufacturing platforms arose with work of Edward Jenner (1749–1823) and Louis Pasteur (1822–1845) who established the immunologic cross-protection against disease (e.g., smallpox, anthrax, rabies) by live agents similar to the disease agent and attenuated strains of the disease agent (Brown 1996). Their processes for manufacture were relatively simple cultivation of the strains and/or infectious material and their use without further purification. The principles of live bacterial and viral vaccine design have changed little over the intervening century and a half, but manufacturing methodology has greatly advanced. Aseptic techniques, factory design, fermentation and cell culture scale-up, formulation, and storage/delivery have advanced to produce whole-organism vaccines that are safe, effective, and available throughout the world. Live vaccines tend to be more immunogenic than nonliving antigens because they can proliferate and elicit strong innate and adaptive immune responses. Directed attenuation (based on an understanding of the virulence genetics and proteomics of a wildtype infectious agent) is beginning to work its way to the clinic for viral vaccines (Vialat et al. 1997).

Some of the concerns regarding the use of live vaccine agents have been discussed earlier. Sidebar 4.1 provides a brief summary of some of the issues related to the safety of all manufactured vaccines. Caution with regard to the long-term safety and efficacy of live vaccines has lead to process changes during clinical
development and very long approval times for initial use of many of these vaccines. However, once approved, the vaccines generally are considered to be safe and effective platforms for further development of combination vaccines. For example, the development of a varicella vaccine for chickenpox by Merck & Co., Inc., required more than 23 years to evolve the process, test the product, and gain FDA approval. Addition of the vaccine to measles, mumps, and rubella required only 12.2 months of FDA review time to gain approval (FDA 2005). The advantages of live vaccines, an increasingly molecular level of understanding about viral growth and persistence in vivo, and the ability to tightly control the manufacturing processes, guarantee that safe and effective live vectors for recombinant vaccine delivery will be part of the human vaccine platform arsenal.

**SIDEBAR 4.1. Safety Issues Regarding Vaccine Components Derived from Cell Substrates, Viral or Bacterial Components, and Manufacturing Media**

Vaccine safety and efficacy are the interdependent goals of immune intervention. Each vaccine platform offers advantages in specificity and breadth of immune response, ease of manufacturing, and longevity of immune response. Each also presents the potential for safety risk. Some of the areas of concern include

- Potential for oncogenicity of cellular components (e.g., cellular DNA, latent viruses, residual viral DNA/RNA)
- Viral adventitious agents
  - Are test methods adequate and validatable?
  - Is sampling of the bulk pool adequate when individual components (e.g., individual eggs in inactivated viral vaccines) cannot be tested for adventitious agents?
- If complex animal materials are used in any phase of the manufacturing process, are viral clearance and inactivation procedures adequate and validated? Are the testing methodologies sensitive enough to detect viruses in the bulk materials?
- What methodologies could be used to assess the presence of spongiform encephalopathy agents?

The complexities of these issues have lead toward the elimination of animal-derived components (the largest risk factor) wherever possible. Totally defined chemical media are possible even for complex cell culture systems and offer the next level of relief from other adventitious agents, as well as improved manufacturing process control, and in many cases, enhanced productivity.

*Recombinant vector vaccines* are based on recombinant microorganisms such as viruses or bacteria that do not cause disease in humans or on natural disease-related organisms that have been attenuated to the point that they no longer cause disease. Recombinant viruses or bacteria carry one or more recombinant genes from the target infectious agent, but not enough of its components to represent a threat of disease. The recombinant organisms are used as vectors, or carriers, to deliver the recombinant genes to the immune response processing cells of the body. The body (or the recombinant microbe) produces proteins from these genes, and these proteins stimulate an anti-infectious agent immune response. Some of the recombinant vectors are of particular interest since they may specifically recruit adaptive immunity involving T-cell and B-cell response. Others may have advantage by not being usual infective agents for humans and therefore will not face a resident immunity to the vector in the clinical cohort.

*Subunit vaccines* are those created from the components of disease-causing organisms. Their goal is to maintain immunologic response and establish protection with reduced side effects and an increased safety margin. An example of a complex, successful subunit vaccine is the hepatitis B surface antigen (HBsAg) produced during hepatitis B infection in humans. Chronic hepatitis B infection and resultant liver disease may lead to formation of incomplete viral particles comprised of the primary surface antigen of the virus self-assembled into 22 nm particles containing lipids and carbohydrate from the host. These particles, termed Australia Antigen (Mathews and Mackay 1970; Hilleman 2002), circulate in the blood of infected patients but are not infectious in their own right. During the late 1970s, Merck & Co., Inc., developed a manufacturing process for a subunit vaccine based on isolation of Australia Antigen from infectious human blood plasma. The manufacturing process was complex, requiring full containment of materials, aseptic processing, and multistep inactivation of blood-borne microbial and viral pathogens. The vaccine, adsorbed to alum, was licensed in the United States in 1981.
### Table 4.1.
Examples and Characteristics of Major Concepts for Design and Scale-Up of New, Improved Vaccines

<table>
<thead>
<tr>
<th>Platforms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live Attenuated Bacterial and Viral Vaccines (e.g., S. typhi shigella varicella measles)</td>
</tr>
<tr>
<td>Inactivated Wildtype Bacterial Vaccines (e.g., DPT, animal vaccines)</td>
</tr>
<tr>
<td>Inactivated Wildtype Viral Vaccines (e.g., animal vaccines, annual influenza) (GSK, Novartis, MedImmune)</td>
</tr>
<tr>
<td>Replicating Nonpathogenic Bacterial Vectors (e.g., recombinant commensal bacteria, oral typhoid, animal vaccines, experimental cancer vaccines)</td>
</tr>
<tr>
<td>Replicating Nonpathogenic Viral Vectors (e.g., cancer vaccines, HIV vaccines, animal vaccines)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Whole Organisms</th>
<th>Sub-Units</th>
<th>Bulk Process Complexity</th>
<th>Formulation Complexity</th>
<th>Requires BSL-3 Process Containment</th>
<th>Transmissible to Non-Vaccinees</th>
<th>Immunologic Advantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attenuated wildtype organisms</td>
<td>“Split” vaccines are processed to remove core components, leaving surface antigens</td>
<td>Minimum complexity; fermentation/cell culture, often without purification</td>
<td>May require lyophilization with stabilizing agents</td>
<td>(Minimum BSL-2+)</td>
<td>Yes</td>
<td>Very low dose; innate and adaptive immunity; long duration of immunity</td>
</tr>
<tr>
<td>Egg-adapted current strains; cell culture may use wildtype</td>
<td>Egg-based process – complex; cell culture process – less complex</td>
<td>Yes (BSL-2/3)</td>
<td>No</td>
<td>Not infectious; innate and adaptive immunity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg-based process</td>
<td>Recombinant heterologous antigens</td>
<td>Minimum complexity; fermentation</td>
<td>May require lyophilization with stabilizing agents</td>
<td>No</td>
<td>Yes</td>
<td>Recombinant DNA vaccine platforms that stimulate both innate and adaptive immunity; may be very-low-dose systems</td>
</tr>
</tbody>
</table>
Table 4.1 (con’t.).
Examples and Characteristics of Major Concepts for Design and Scale-Up of New, Improved Vaccines

<table>
<thead>
<tr>
<th>Platforms</th>
<th>Whole Organisms</th>
<th>Sub-Units</th>
<th>Bulk Process Complexity</th>
<th>Formulation Complexity</th>
<th>Requires BSL-3 Process Containment</th>
<th>Transmissible to Non-Vaccinees</th>
<th>Immunologic Advantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Replicating Viral Vectors (e.g., alphavirus replicons, RNA and DNA vaccines)</td>
<td>Recombinant heterologous antigens</td>
<td>Moderate to complex</td>
<td>May require stabilants or cross-linking; used in conjunction with adjuvants</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Recombinant DNA vaccine platforms that stimulate both innate and adaptive immunity</td>
</tr>
<tr>
<td>Particulate Vaccines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Infected wildtype viruses may require BSL-3</td>
<td>No</td>
<td>Mimic whole virus vaccines in recruiting innate and adaptive immunity</td>
</tr>
<tr>
<td>(viruses-like particles, bacterial ghosts, self-assembled sub-unit antigens)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>Generally poor immunogens</td>
</tr>
<tr>
<td>Purified Proteins, Peptides and Polysaccharides</td>
<td></td>
<td>Moderate to complex</td>
<td></td>
<td>Minimal; many adsorbed to alum</td>
<td>No</td>
<td>No</td>
<td>Improved immune response to the purified antigens</td>
</tr>
<tr>
<td>Conjugated Proteins, Peptides and Polysaccharides (e.g., pneumococcal, meningococcal, and H. influenzae conjugates)</td>
<td></td>
<td>Complex; two fermentations/isolations/purifications; separate conjugation chemistry and purification steps</td>
<td>Minimal; many adsorbed to alum</td>
<td>No</td>
<td>No</td>
<td>Can be designed and scaled-up in very little time; recruit innate and adaptive immune response; effective as “primer” in “prime-boost” strategies</td>
<td></td>
</tr>
<tr>
<td>DNA Vaccines</td>
<td></td>
<td>Typically recombinant gene(s) amplified in bacterial plasmids</td>
<td>Minimum complexity</td>
<td>Minimum to moderate complexity</td>
<td>No</td>
<td>No (?)</td>
<td></td>
</tr>
</tbody>
</table>
The world’s first recombinant subunit vaccine was created by Merck to replace the Australia Antigen product and was licensed in the United States in 1986. The recombinant vaccine was produced by inserting a plasmid containing the gene for HBsAg into Baker’s yeast (Saccharomyces cerevisiae). Yeast cells produce HBsAg, which is harvested and purified to more than 95% HBsAg protein (5 to 40 μg/mL). The product may contain yeast-derived proteins of up to 5% of the final product, yeast-derived lipid, but less than 10 μg yeast DNA in the vaccine. This vaccine represented a significant improvement in the margin of safety, since it could not result in hepatitis B viral infection or other human viral disease because no potentially infectious viral DNA or complete viral particles are produced in the recombinant system. As with the surface antigen from infectious blood plasma, the recombinant HBsAg is adsorbed to aluminum hydroxide (aluminum hydroxyphosphate sulfate), which acts as an adjuvant. Recombinant hepatitis B vaccine is produced by Merck (Recombivax HB) and GlaxoSmithKline (GSK) Pharmaceuticals (Engerix-B) in the United States.

Native and recombinant subunit vaccines are now commonplace, but not all subunits are as immunogenic as proteins. Polysaccharide-based vaccines often require conjugation with another moiety such as a protein or protein complex or a toxoid to stimulate sufficient immune response to generate protection. Such conjugate vaccines have two components, a poorly immunogenic T-cell-independent antigen (polysaccharides and some peptides) covalently linked to a highly immunogenic protein (e.g., the outer membrane protein complex of N. meningitidis, tetanus, or diphtheria toxoids). The conjugate becomes T-cell-dependent, leading to immunologic memory. Another type of subunit vaccine is called a virus-like particle vaccine (VLP or pseudovirion vaccine). Virus-like particles are noninfectious particles that self-assemble to form structures that contain one or more, but not all, virus proteins and resemble the virion itself.

DNA vaccines introduce recombinant DNA to the human immune system. Unlike recombinant vector vaccines, naked DNA containing some, but not all, of the recombinant genes of the target infectious agent is injected directly into the body. Cells take up this DNA and use it to produce proteins from the infectious organism. The proteins trigger an anti-infectious-organism immune response.

Although different approaches to vaccine design offer different immune response characteristics (see Table 4.1), not all diseases respond to a single approach. Combination strategies may be required to protect against particularly rapid disease progression (e.g., HIV/AIDS, viral influenza) or as treatments for rapidly progressing metabolic disease (e.g., cancer). Heterologous “prime-boost” vaccination is a strategy that introduces the same antigen at different times through fundamentally different vaccines or through different routes of administration. The total immune response to a prime-boost regimen often significantly exceeds response to the individual component vaccines. The goal of this approach is to stimulate different kinds of immune responses that enhance the body's overall immune response to the disease.

MANUFACTURING SYSTEMS

Many factors influence the direction that manufacturing design may take. The first of these are the “investments” of individuals and companies. One example of an “investment” is the promise of innovation that will achieve safe and effective clinical impact for the control of disease. WTEC panelists interviewed several of the world’s leaders in vaccine design. Table 4.2 lists some of the innovative vaccine design systems and scientific insights that will influence vaccine evolution over the next decade. In each case, the basic approach to eliciting immune response points the way to the range of manufacturing systems that will be needed to support their production, formulation, and delivery.

For example, both adenoviruses and alphaviruses specifically recruit B-cell and T-cell involvement in immune response. They will ultimately require master and working cell and virus seed banks that are prepared with minimum dependency on animal-derived products (serum or co-factors). The master and working banks will need to be held in multiple geographically distinct cold storage systems to minimize the manufacturing risk of loss of the banks. Both recombinant vectors will require contained cell culture bioreactors that can be scaled up to meet the material requirements of clinical trial and qualification and beyond to full-scale manufacture of a licensed vaccine. Conditions for containment of live materials will be required until the stage at which the vaccines are inactivated. Live recombinant viral vectors will require both containment (closed system vessels, closed system transfer from vessel to vessel, contained air handling systems, dedicated and isolated waste collection and treatment systems) and aseptic transfer methodology throughout the manufacturing processes to the final closed unit dose container. Lyophilization and/or other
stabilizing formulation processes may be required to provide adequate shelf life for vector viability and/or antigen presentation.

Decisions of how the recombinant viral vectors will be developed (live viral vectors, inactivated viral vectors) and deployed (purity of the live or inactivated vectors, formulation of the vectors with stabilizing agents, frozen/refrigerated/dried-and-reconstituted formats) will define the unit manufacturing operations; their configuration and layout; the majority of analytic requirements for characterization, in-process control, and product release; and ancillary systems (encapsulation and buffers for oral delivery, aerosol delivery and buffers and for nasal delivery; and adjuvants to stimulate broad innate and adaptive immunity). Clearly, the range of manufacturing technologies is immense, even for this one example.

**Table 4.2.**

Molecular Innovations in North America and Europe that Improve Clinical Safety &/or Efficacy

<table>
<thead>
<tr>
<th>Innovation / Insight</th>
<th>Innovator &amp; Institution</th>
<th>Bioreactor Design</th>
<th>Facility Design</th>
<th>Formulation &amp; Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engineered Non-Replicating Adenovirus</td>
<td>Dr. A. Amalfitano, Michigan State University</td>
<td>Cell Culture</td>
<td>BSL-2 (+) containment on scale-up; Clean air and steam; Water for injection; Moderate isolation and purification</td>
<td>Stabilizers to avoid aggregation and maintain immunologic properties</td>
</tr>
<tr>
<td>Alphavirus Replicons</td>
<td>Prof. P. Liljestrom, Karolinska Institute/SMI</td>
<td>Cell Culture</td>
<td>BSL-2 (+) containment on scale-up; Clean air and steam; Water for injection; Moderate isolation and purification</td>
<td>Stabilizers to avoid aggregation and maintain immunologic properties</td>
</tr>
<tr>
<td>Chimpanzee Adenovirus</td>
<td>Prof. A. Hill, Oxford University</td>
<td>Cell Culture</td>
<td>BSL-2 (+) containment on scale-up; Clean air and steam; Water for injection; Moderate isolation and purification</td>
<td>Stabilants to preserve viability</td>
</tr>
<tr>
<td>Bacterial Ghost Adjuvant/Carriers</td>
<td>Prof. W. Lubitz, University of Vienna; BIRD-C GmbH</td>
<td>Fermentation</td>
<td>BSL-2 (+) containment on scale-up; Clean air and steam; Water for injection; Moderate isolation and purification</td>
<td>Complex adsorption with exogenous antigens; recombinant expression of antigens</td>
</tr>
<tr>
<td>Rotavirus Reassortants</td>
<td>Dr. J. Boslego, PATH</td>
<td>Cell Culture</td>
<td>BSL-2 (+) containment on scale-up; Clean air and steam; Water for injection; Moderate isolation and purification</td>
<td>Buffers for oral delivery, other stabilants to preserve viability</td>
</tr>
<tr>
<td>Broadened Cross-Protection Correlates with the use of Adjuvants</td>
<td>Prof. A. Hill Oxford; Dr. E. Hanon, GSK; Dr. R. Rappuoli, Novartis</td>
<td>Chemical Processing Train</td>
<td>Closed system processing; Water for injection; Solvent handling; May separate fermentations to generate adjuvant sub-unit</td>
<td>Sterile filling, spray drying, or lyophilization</td>
</tr>
<tr>
<td>Soluble Protein (may confer immunity to viral influenza)</td>
<td>Dr. A. Shaw, VaxInnate Corp.</td>
<td>Cell Culture &amp;/or Fermentation</td>
<td>BSL-2 (+) containment on scale-up; Clean air and steam; Water for injection; Moderate to complex isolation and purification</td>
<td>May require combination with adjuvants; stabilants or lyophilization</td>
</tr>
</tbody>
</table>
In some cases, the degree to which a company has an established manufacturing platform may influence the technology with which the company moves forward to meet a vaccine development challenge. GlaxoSmithKline Biologicals, Novartis Vaccines, and Intervet International all have historic investments in egg-based vaccine manufacture that influences the timing of their strategies and decisions about vaccines and vaccine manufacture.

Rapid manufacturing response may be the difference between a vaccine that modifies the progression of a disease and one that arrives in clinical use too late to alter disease progression or save lives. The decision to pursue a particular process or a particular manufacturing technology is often driven by the potential to respond quickly to an urgent need and the opportunity for new revenue to appear on a faster time-track. One example is the longer-term trend toward cell-culture-based viral vaccines as a platform distinct from egg-adapted whole virus vaccines. All of the companies that the WTEC panel interviewed are moving toward cell culture systems to achieve lower cost, improved process control, and shorter manufacturing cycles. Some of the companies have taken the leap to install Biosafety Level 3 containment in their large-scale cell culture facilities. This capability allows a company to move forward to a new viral vaccine using wildtype infectious agents rather than delaying until attenuated strains can be created.

Rapid development of new vaccines can also be served by strategies that do not need to wait for the construction of sophisticated, expensive manufacturing facilities. Where dedicated pilot plant facilities do not exist, many researchers and start-up companies rely on disposable bioreactors and processing systems to jump-start scale-up. This approach bypasses the build-out of expensive, slow-to-construct facilities to make enough material to test in animals or even in Phase I or Phase II human clinical trials. A potential downside to this approach is that it may delay the development of robust manufacturing processes that will be needed if scale-up ultimately requires large fixed facilities.

**Predicting the “Right” Vaccine Manufacturing Approach**

With so many competing promises, attributes, time advantages, and available infrastructures, what path will vaccine development take? The forecasting of technologies and implementation strategies is a complex science that seeks to define the paths forward from a point in time. Professor H.C. Co at California State Polytechnic University, Pomona, presents a useful framework for evaluating how individual factors and progressive decisions define the spectrum of technical possibilities and the path(s) that may be taken to realize long-term goals (Co 2006). The figure used by Co and reproduced in Sidebar 4.2 (Porter 1991) suggests the myriad of possible technical paths from innovative concept to commercial entity. Any particular path forward is defined both by opportunities and by the topology of resources, constraints, and urgencies.

This concept projects a single path forward as an example, but the reality that WTEC panelists observed in our study is that the innovators in vaccine design, development, scale-up, full-scale manufacturing, and delivery to the patient bring forward parallel, often overlapping, technology pathways to address prevention of disease. That is, no single path is likely to dominate for all disease applications. Neither will a single vaccine or vaccine manufacturing strategy be universal for the more complex disease targets (e.g., those requiring recruitment of both innate and adaptive immune responses and the ability to cope with genetic shift by the disease agent) that face us today. This observation carries over to manufacturing operations, where both Novartis and GSK rely on egg-based vaccine manufacture for annual influenza vaccine while simultaneously developing sophisticated cell culture capability for the next generation of viral vaccines.
For a forecaster, the future fans out as a wedge-shaped terrain of peaks and valleys of threats and opportunities. The probability of following any one given pathway into the future is small, but the sum of the probabilities of all the different discrete pathways through the terrain is fairly unified. The forecaster’s job is to map out the contours (threats and opportunities) of the future’s terrain and show the potential routes through it so the decisionmaker can judge the best path. The forecaster’s dilemma is that the finer the detail used to describe the pathway through the terrain, the lower the probability of that exact pathway being followed and of that particular terrain being traversed as the future unfolds.

The successful forecast accomplishes the following:

- Identifies limits beyond which it is not possible to go.
- Establishes feasible rates of progress, so that the plan can be made to take full advantage of such rates; the plan does not demand an impossible rate of progress.
- Describes the alternatives that can be chosen.
- Indicates possibilities that might be achieved if desired.
- Provides a reference standard for the plan. The plan can thus be compared with the forecast at any later time to determine whether it can still be fulfilled or whether, because of changes in the forecast, the plan must be revised.
- Furnishes warning signals that can alert the decision-maker that it will not be possible to continue the present activities.

*Image courtesy of Alan Porter, Georgia Tech., from Porter et al. 1991, figure 4.1, © John Wiley and Sons, Inc., used with permission; analysis based on a 2006 presentation to the NRC TIGER Standing Committee by Prof. Henry Co of California State Polytechnic University.

MODULATING THE ONSET AND DEVELOPMENT OF AN INFLUENZA PANDEMIC

The presenters at the North American Workshop on Vaccine Manufacture, held January 2007 in Arlington, VA, and leading academic, government, and industrial experts whom the WTEC team met in Europe identified many powerful new and evolving strategies to maximize the impact of vaccines in fighting important infectious disease:

- miniaturization and automation of vaccine discovery and evaluation tools
- single-use process equipment that can accelerate the production of vaccines for clinical testing
- novel vaccine vectors and adjuvants that tailor immune response
- delivery systems that place vaccines in cells responsible for antigen presentation
- facilities and staff configured to learn most rapidly from experts in both human and animal vaccine development.
Across this broad landscape, the WTEC panel used a particularly demanding scenario for vaccine development and manufacture as a framework for discovering best practices. That framework scenario is the development of a vaccine to protect against an anticipated influenza pandemic. We used manufacturing response to pandemic influenza as an edge-of-the-envelope framework to test rate-limiting steps, technology gaps, and systems integration that control the speed of vaccine design, development, and deployment.

Seasonal influenza epidemics are an annual occurrence and can spread around the world in weeks to months. Symptoms are generally mild with rapid onset of fever as high as 104ºF, cough, and headache. The fever dissipates by the third day after infection, and the disease has run its course within a week. Influenza Type A undergoes genetic variation such that a new vaccine is required on average every year. Effective vaccines are available and are manufactured mostly in processes that use chicken eggs as a medium for growth.

Influenza Type A viruses infect humans and can also cause disease in aquatic birds (avian influenza) and other species. The virus is normally avirulent in the natural reservoir of wild aquatic birds but can be transmitted to chickens and turkeys, where further genetic drift may occur. Influenza A virus subtypes are defined by two surface glycoproteins, the hemagglutinin (H) and neuraminidase (N). At least 16 hemagglutinin and 9 neuraminidase influenza A subtypes circulate in birds (Olsen et al. 2006).

Influenza A viruses may be either spherical or filamentous. The surface glycoproteins (four hemagglutinins to one neuraminidase) are embedded in a lipid bilayer membrane. Eight segments of the viral genome enable reassortment between co-infected subtypes and contribute to the rapid rate of genetic shift (Figure 4.1). If Type A influenza undergoes reassortment in an animal population such that it (a) emerges in a human population with no preexisting immunity, (b) replicates enough to cause disease, and (c) is easily transmitted and maintained in the population pool, we will face the potential of a true pandemic infection. The World Health Organization Global Influenza Preparedness Plan notes that, “On average, three pandemics per century have been documented since the 16th century, occurring at intervals of 10–50 years. In the 20th century, pandemics occurred in 1918, 1957 and 1968” (WHO 2005). The three pandemics in the last century were caused by Type A subtypes H1N1, H2N2, and H3N2 respectively.

Figure 4.2 indicates the rate at which pandemic influenza can advance through a population. The graph presents the weekly deaths from influenza and pneumonia in the United Kingdom during the three waves of pandemic influenza 1918–1919 (Jordan 1927; Taubenberger and Morens 2006). Sidebar 4.3 provides a concept of what might be at stake in a true pandemic. The pandemic of 1918 and 1919 swept across a world of modest-sized cities and limited trans-global transportation compared to today’s world of large cities and commonplace international travel. The WTEC panel chose the concept of response to pandemic influenza for analysis of current vaccine manufacturing capability because the kinetics of progression of an influenza pandemic are today so rapid that they exceed the normal pace of new vaccine introduction (other than incremental updates of annual influenza) by more than an order of magnitude. If manufacturing is to be effective in stemming an emerging pandemic, new strategies, new processes, new logistics, and new economics must prevail.

**Vaccine Manufacturing in the Context of an Influenza Pandemic**

The figure in Sidebar 4.2 above suggests that technologies evolve along a path defined by opportunity and constraints. Vaccines to combat annual influenza exist today but will almost certainly not confer cross-protection to strains that could arise as pandemic agents. However, the know-how to produce large amounts of vaccine in chicken eggs is broadly established. Both GlaxoSmithKline (GSK) and Novartis have established facilities and infrastructures for manufacture of annual influenza vaccines produced in chicken eggs and are major global suppliers of these vaccines. Although WTEC panelists were not able to visit Sanofi Pasteur, it also has an established capability to produce the annual influenza variant vaccines. This strong base of installed conventional capacity that could be called into play to address a pandemic is an important cornerstone for a pandemic vaccine strategy but is clearly not sufficient, since the combined capacity of GSK and Novartis is a small fraction of that needed to address a truly global pandemic.
Figure 4.1. Structural diagram of the influenza virus (illustration by Chris Bickel in *Science* 2006).

Figure 4.2. Three pandemic waves: Weekly combined influenza and pneumonia mortality, United Kingdom, 1918–1919 (Taubenberger and Morens 2006).
There is widespread concern among policymakers and public health experts about the possibility of a worldwide epidemic of avian influenza. Such pandemics are not new: there were three in the 20th century, of which one, the 1918–1919 Spanish flu outbreak, is estimated to have killed over 500,000 people in the United States and up to 50 million worldwide. Public health concerns arise because of the challenge of creating the public health infrastructure in the United States and other countries that would be adequate to meet the challenges of a severe pandemic.

Although a pandemic could be caused by any of several influenza strains, scientists are particularly worried about H5N1, a strain that has caused repeated epidemics with high mortality among poultry in Asia, has spread from Southeast Asia to flocks in Central Asia and Europe, and has made the jump from birds to humans, causing the deaths of over 60* people. Moreover, viruses of the H5 subtype are not known to have ever circulated among the human population, which means that there would be little immunity to it. To date, close contact with infected poultry is thought to be required for human infection, but the danger exists that the virus will evolve in a way that allows for efficient human-to-human transmission. If the virus does acquire that capability, a worldwide epidemic, or pandemic, could occur. Depending on the virulence of the particular strain of flu, such an outbreak could have substantial consequences for people and economic activity around the world.

Infectious diseases are, however, unpredictable. It is impossible to say for sure whether another pandemic will arise, whether it will involve H5N1, and, if it does, when it will happen or whether it will be mild or severe. The H5N1 virus could mutate in a way that caused a severe pandemic next year or a mild epidemic in a decade or two. Or it could evolve in a way that rendered it harmless, and a pandemic could arise from an entirely different virus subtype.”

— U.S. Congressional Budget Office 2005, 1

*Note: H5N1-related deaths now exceed 100 people.

Established Manufacturers of Influenza Vaccines

Novartis and others have conceptualized—with the European Center for Disease Control (ECDC), the national authorities such as the Health Protection Agency (HPA, U.K.), and the European Agency for the Evaluation of Medical Products (European Medicines Agency, EMEA)—a pre-pandemic vaccine based on Type A substrains of avian influenza known to infect man in some portion of the world. The EMEA established guidelines for the manufacture of a pre-pandemic vaccine and its qualification up to commercial marketing (see Chapter 3). WTEC panelists’ hosts referred to these guidelines as “pre-pandemic core vaccine dossiers” or “pre-pandemic vaccine mock-up filings” (See Chapter 2). The concept allows the development and testing of a potential pandemic vaccine, but, more importantly, allows the prelicensing of the strains, the processes to make them and formulate them, and the manufacturing facilities to manufacture them.

Novartis began development of a pre-pandemic vaccine based on its expertise and infrastructure for egg-based annual influenza vaccines in the late 1990s (Figure 4.3), commencing with early clinical studies of H5N3 and H9N2 vaccines and continuing to production and stockpiling of two types of H5N1 vaccines. The vaccines have been tested in humans, with and without the proprietary MF59 oil in water emulsion as adjuvant, and were found to be safe and to elicit an immune response that is protective against both the vaccine strain and also against other H5N1 clades, which are generated by the antigenic drift of the virus. The confidence on the safety of the adjuvant is high, since this has been already used for ten years for seasonal influenza and more than 30 million doses have been administered. Novartis Vaccines was the first company to receive EMEA core dossier approval for a pre-pandemic influenza vaccine.

GlaxoSmithKline Biologicals followed a similar path to prequalify an egg-based process for a pre-pandemic vaccine. Its researchers chose the H9N2, H5N1, and H3N2 strains, adapted the strains to grow in eggs, prepared the virus by conventional egg-based processes, inactivated them with formalin, and prepared them as vaccines using the current process for annual influenza vaccine. The vaccines were tested in humans (with and without adjuvant) and found to be safe and effective in raising immune response in humans to a level that correlates with protection against annual influenza. The EMEA granted a Core Dossier License to GSK for a whole virus alum-adjuvanted mock-up vaccine in December 2006. GSK submitted a file to EMEA for a pre-pandemic split adjuvanted vaccine in late 2006 and expects it might obtain the license in 2008.
Figure 4.3. SIENA/ROSIA FLU egg-based manufacturing; at Step 3, a vaccine can be fairly quickly made ready for use in case of a pandemic (courtesy, Novartis).

The pre-pandemic core dossier licenses allow the manufacturers to have a preapproved manufacturing process in place in the event of a pandemic influenza outbreak. It also allows individual nations in the EU to elect to stockpile vaccine under the key assumption that a pre-pandemic vaccine made using one to three strains would confer cross protection against other strains. (Discussions with the ECDC, the HPA, and others suggest that the pandemic could arise from H5N1 or an H7 variant). Both Novartis and GSK Biologicals have concluded that coadministration of an adjuvant will be required to enhance cross-protection and to reduce the effective dose of the inactivated vaccines so as to increase the number of doses available.

Recent GSK clinical trials with an adjuvanted (proprietary oil-in-water adjuvant) “split” Vietnam strain H5N1 vaccine demonstrated a strong cross-immune neutralizing antibody response in humans against the Indonesian strain of the virus. The adjuvant also reduced the dose (administered twice, three weeks apart) to 3.8 μg HA antigen per dose. If confirmed, this could increase vaccine capacity by more than a factor of ten.

In a separate preclinical study in animals, GSK demonstrated that the adjuvanted vaccine containing the Vietnam H5N1 strain provided protection against a Vietnam H5N1 challenge and also provided 96% cross-protection against a lethal challenge with the genetically drifted Indonesia strain of H5N1 (ISRVI 2007).

Sanofi Pasteur reported in January 2007, that a two-dose schedule of 90 μg of its subvirion H5N1 Influenza Virus Vaccine could be effective in preventing H5 influenza in healthy adult recipients (Sanofi Pasteur 2007). Based on the data to date, it seems likely that adjuvants will play an increasing role in broadening and enhancing immune response to life-saving vaccines.

Cell Culture versus Egg-Based Manufacture of Influenza Vaccines

The well-understood processes for annual influenza vaccine manufacture and the installed capacity for large-scale manufacture have enabled Novartis, GSK Biologicals, and Sanofi Pasteur to move forward very quickly to develop candidate egg-based pre-pandemic vaccines and qualify for preapproval of their vaccine platforms. However, this rapid activity exposes the considerable inertia in the vaccine industry. All of the large vaccine manufacturers recognize the intrinsic limitations of egg-based vaccine systems, whether for whole inactivated influenza vaccines or for subunit (“split”) influenza vaccines:

- Egg-based manufacturing is intrinsically variable due to egg-to-egg and flock-to-flock variations
- The key raw material, embrionated eggs from healthy flocks, cannot be stockpiled for long time periods
- Although master virus seed banks and working virus seed banks are prepared in specific pathogen-free eggs, the demand for large numbers of eggs for manufacturing to meet a pandemic challenge means that standard eggs from established and monitored farms will be used

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3 Although aluminum hydroxide adjuvants have been used successfully and safely in many vaccines for decades, oil-in-water adjuvants have been slow to gain acceptance, partly due to pain and inflammation at the injection site.
Platforms for Vaccine Manufacturing

- Egg-based manufacturing generates large amounts of hazardous waste
- Since Type A influenza is a pathogen to chickens as well as to humans, an adaptation or attenuation process is required to prepare new strains for productive culture in eggs

Although the WTEC panelists did not discuss in detail cell culture of influenza viruses with either Novartis or GSK Biologicals, both companies are rapidly pursuing second-generation pre-pandemic vaccines utilizing this technology advance.

Baxter Vaccines AG has developed its vaccine business through acquisition of two companies, Immuno (Austria) and North American Vaccines (U.S.) and does not have the facilities or logistic networks for egg-based vaccine manufacturing. This has allowed it to enter vaccine manufacture with cell culture as its primary technology for virus cultivation. It is the only company in Europe to have a cell-culture-based pre-pandemic vaccine in clinical trials. Baxter’s commercial-scale cGMP manufacturing facility is built to Biosafety Level 3 (BSL-3) containment standards that will allow it to manufacture vaccine from wildtype virus without developing attenuated or egg-adapted strains. This advantage means that a new or emerging strain could begin scale-up as soon as it is clearly identified. Baxter’s process utilizes Vero cells cultured on microcarriers and is scaled-up from partially automated roller bottles to 6000 L stirred bioreactors. Closed systems with automated cleaning and sterilization dominate unit operation process equipment configurations. Virus propagation at full scale is complete in two days, and downstream processing includes ultracentrifugation, filtration, and concentration. Two inactivation steps using formaldehyde and ultraviolet light complete the process to produce a whole virus, inactivated vaccine bulk. Phase I and II clinical studies with the H5N1 vaccine indicate that the safety is equivalent to that of annual influenza vaccines. Baxter managers believe that the unmodified antigenic structure of Baxter’s wildtype H5N1 whole virus vaccine will confer broader, more complete immunity than the attenuated viral subunit vaccines. The company is participating in the European plan to develop a pre-pandemic core license dossier based on currently available H5N1 strains as a means of making the approval process more rapid in a pandemic situation.

MANUFACTURE OF ANIMAL VACCINES

Veterinary vaccines in Europe fall under the same directive for GMPs as human vaccines (Directive 2003/94/EC of 8 October, 2003). Thus, the possibility exists of using veterinary facilities for human vaccine production for EU markets. This directive also allows products to be released for multiple countries using a common release protocol and test regime, although countries may have some individual requirements. U.S. regulations governing animal vaccines, however, are notably dissimilar to those in Europe.

Established Animal Vaccine Manufacturers

Pfizer is the world’s largest manufacturer of animal vaccines. Most of the antigens are manufactured in bulk at its Lincoln, Nebraska, facility through processes that include advanced cell culture for recombinant vector vaccines and self-adjuvanting vectored recombinant vaccines. The Pfizer facility at Louvain-la-Neuve in Belgium makes 8 of the 37 antigens that the company formulates, fills, packages, and distributes for animal use. Its facility utilizes closed system unit operation when handling and inactivating live agents. Process areas are largely fixed installations, but disposable bags are in use for media and viral harvest.

Pfizer’s aseptic formulation capabilities are extensive. It has the ability to prepare simple mixtures just prior to filling or to formulate complex mixtures of many components, including adjuvants. Emulsions are also prepared for some of the formulations. Filling capabilities include single and multidose lines with the ability to freeze-dry if needed. The lines are highly efficient, automated, and integrated, with speeds up to 40,000 vials per hour. Vials to be filled are passed through a washer, depyrogenation tunnel, and a contained area for filling and automatic stoppering. For freeze drying operations, the vials are diverted to a freeze dryer with fully automatic loading and unloading under aseptic conditions prior to capping. Automatic monitoring of the freeze-drying cycle parameters is built into the system. Most of the products are presented in glass vials. There is one filling line capable of processing plastic vials.

The Pfizer animal vaccine manufacturing capability is as sophisticated as most human vaccine facilities and approaches many of the cGMP requirements for human vaccine manufacture in the United States. Pfizer managers and researchers are experts at global logistics and have an extraordinary knowledge base in vaccine design and manufacturing that spans virtually all of the current vaccine platforms under consideration for human vaccines.
Intervet International was in 2006 the third largest veterinary pharmaceutical company in the world behind Merial (#2), and Pfizer (#1). As of November 2007, Intervet is part of Schering-Plough; based on 2006 sales figures, it is one of the leading veterinary pharmaceutical companies in the world. Like Pfizer, Intervet has fully modern automated facilities for animal vaccine manufacture and a very strong knowledge base in vaccine development. Intervet representatives indicated that the company produces 300 animal vaccines and 600 antigens using manufacturing platform technologies that range from egg-based antigen production to Baculovirus recombinant vector and subunit vaccines.

Intervet’s sister company in human pharmaceuticals is Organon. In 2002 Intervet and Organon created a new company, Nobilon International BV, which combines the vaccine know-how of Intervet with Organon’s expertise in the human pharmaceutical market. For example, Nobilon uses the technology of a licensed Intervet live vaccine for influenza in horses, in part, as the basis for development of a human live influenza vaccine in its Boxmeer development laboratories in The Netherlands. In both cases, the processes are commercial egg-based; however, Nobilon is also developing a cell culture process for the human vaccine. (As of November 2007 Intervet, Organon, and Nobilon are all part of Schering-Plough.)

Again, the juxtaposition of research and manufacturing, and in this case, different and complimentary disciplines has lead to creative thinking and unusual accomplishment. An example of the synergy that can arise from innovative thinking is the manufacturing facility that Nobilon has constructed for both animal and human vaccines. The design work for this facility began in January 2002 and construction commenced on a greenfield site in May 2002. The facility was completed and commissioned (EMEA-approved for GMP operation) in April 2004 at a cost of €30 million. The facility is designed for unidirectional flow of material and people with separate removal of waste materials such that new and spent materials do not cross. There is a single train starting with a cell culture bioreactor and progressing to two suites of isolated and independent rooms for downstream processing; each room has completely separate waste-collection and waste-handling capability. (In principle, a single cell culture cycle might source two tandem or staggered isolations, but the WTEC visiting team was told that this is not yet done.) The facility utilizes clean-in-place (CIP) and sterilize-in-place (SIP) strategies and is designed and licensed by the EMEA for both bacterial and viral vaccines and for both human and animal vaccines based on validated shut-down/clean-out/restart procedures.

To date Nobilon has focused only on viral vaccine campaigns in this facility, but at the time of the WTEC visit, it had undergone 3 cycles of manufacturing animal virus vaccines followed by facility shut-down and cleaning and then by restart for human viral vaccine manufacture. The EMEA and Ministry of Health inspected this facility three times during this period and found the facility and its performance satisfactory.

This facility was conceptualized and designed in a cooperative effort between the process engineering and facility engineering staffs in animal vaccines (Intervet) and human vaccines (Nobilon). WTEC panelists asked how much adjustment in design criteria for animal vaccines or human vaccines was required to achieve the final cGMP design approved by the Ministry of Health. Our hosts indicated that less than 5% redesign was required between the first and final design criteria. This facility is MI III (BSL-3) validated for both influenza and rabies.

The Pfizer and Intervet–Nobilon facilities are world-class, modern, and automated. Their global manufacturing organizations combine rich resources of animal vaccine knowledge. Nobilon has fully integrated this expertise with strategies for human vaccines that support an aggressive vision for vaccine development and record-setting manufacturing.

**NEW TECHNOLOGIES FOR RAPID DEVELOPMENT AND DELIVERY OF VACCINES**

The skin is a rich source of antigen processing cells called Langerhans cells. These dendritic cells are concentrated in the epidermis of skin (Figure 4.4) and migrate to the draining lymph node where they present polypeptide fragments of antigens for further processing by the immune system. Antigens carried by Langerhans cells can trigger both innate and adaptive immunity. DNA vaccines take advantage of processing by these dendritic cells by providing recombinant antigen genes that express the target antigen within the maturing Langerhans cells. A simple yet elegant way to engage Langerhans cells is to deliver naked recombinant DNA in the form of supercoiled plasmids directly to the cells by ballistic penetration of the outer skin layer, the stratum corneum.
PowderMed, Ltd., has achieved this through its Particle Mediated Epidermal Delivery (PMED™) technology. This device, designed and manufactured for the delivery of DNA vaccines, is a single-use, disposable system that uses high-pressure helium gas to deliver gold particles (coated with the DNA vaccine) to the dendritic cells in the skin (Figure 4.5). PMED is conceptualized as a safe and efficient platform for rapid delivery of any DNA vaccine. The amount of gold used with each dose administered is small (one milligram of gold per dose) and carries 1 to 4 micrograms of DNA. A single dose of the DNA-gold particles is placed in a plastic cylindrical cassette whose ends are capped with a flexible membrane. The particles are discharged to the epidermis by explosive decompression of helium that ruptures the upper membrane, entrains the micron-sized gold particles, ruptures the lower membrane, and develops particle velocities approaching supersonic speeds. The excess helium vents to the atmosphere before reaching the skin, and the gold particles impact the skin, penetrating through the stratum corneum to the epidermis.

PowderMed estimates that the cost of DNA delivery by this approach will be comparable with other DNA vaccine delivery methods. Preparation of the recombinant DNA plasmid in E. coli is efficient and well established. Production of recombinant DNA supercoiled plasmids from E. coli fermentations can reach 40 grams of recovered plasmid per 300 L fermentation batch. An H5N1 vaccine challenge trial in mice, with and without an encoded heat-labile E. coli enterotoxin as an auto-adjuvant, showed 100% survival after two doses using this system for ballistic delivery (Sharpe et al. 2007). The PMED™ system has been used to
administer 1500 deliveries of DNA to human patients across 10 clinical trials. Three influenza studies in humans were ongoing, as of February 2007, with administration of 1–4 μg of DNA per exposure.

Other studies with DNA as a vaccine have demonstrated its safety at doses that are at least 1000 times higher than those used by PowderMed for its influenza trials (Wang et al. 1998). Immunogenic response has been clearly demonstrated. Demonstration of protective immunity will place this approach to vaccine development among the most rapid that WTEC panelists have seen. The time needed to respond to a pandemic threat could be shortened for a DNA-based vaccine developed from a typed and characterized influenza strain, because of the ease of preparing, formulating, and delivering the appropriate DNA. PowderMed researchers believe that PMED technology could mount an effective response, carrying out the molecular biology, initial development, and scale-up of a commercial-scale process based on the company’s existing delivery system, in as little as 3 months, followed by production of the vaccine. (DNA vaccines are being developed by other companies as well.) The stratum corneum can be made permeable to DNA by hydration, abrasion, or electroporation in addition to ballistic delivery. Antigens and adjuvants applied directly to skin treated by these methods can result in the induction of strong immune responses (Levine and Sztein 2004).

CONCLUSIONS

WTEC panelists were impressed by the vision, strategy, and best-in-practice tactics evident at all of the major contributors to vaccine manufacturing technology whose operations are outlined above. Clearly, the individuals that we met at each location are thought-leaders in their companies and in the international vaccine community at large. Still the energy, drive, and resources applied to vaccine development along simultaneous parallel paths impressed us as unusual in the global biologics industry. We saw evidence of early involvement of manufacturing expertise in the choice and design of both near-term and long-term vaccine platforms. Programs developing novel adjuvants that extend immunological response and stretch vaccine supply suggest research-to-manufacturing discussions as well.

A characteristic shared by most if not all of these companies is the co-location, at least at one of their sites, of research and development, pilot plants, testing laboratories, and manufacturing operations. This fortuitous juxtaposition may uniquely support rapid innovation and development.

Because of the complexity of manufacturing and control of vaccines, a highly educated staff is required to execute development and operate facilities. Thus, in order to increase manufacturing capacity and speed up the development of the next generation of vaccines, it is necessary to consider the ability to recruit qualified staff. Novartis and Pfizer were particularly sensitive to this issue and highlighted education and training as rate-limiting steps in reaching faster, more effective vaccine manufacturing.

A step change in the rate of current vaccine development will require

- Development and acceptance of new rules (e.g., EMEA core “mock-up” dossier)
- New processes (e.g., cell culture of infectious viral strains for preparation of inactivated vaccines)
- New approaches (e.g., recombinant vectored systems or precise delivery of antigens and/or the DNA encoding antigens)
- New economics that help stakeholders recover up-front costs (early capital investment and at-risk operating capital) and minimize liability risk (see Chapter 5 for a more in-depth analysis of the economic barriers to more rapid vaccine development)
- Safe and effective adjuvants that stimulate broad cross-protection while balancing adverse reaction at the injection site may be required to reach the next level of vaccine efficiency
- Synergy between individuals, organizations, industries, and agencies to accelerate the necessary improvements.

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CHAPTER 5
THE ECONOMICS OF VACCINE DELIVERY

Sheldon H. Jacobson

BACKGROUND: IMMUNIZATION AND VACCINES
The World Health Organization (WHO) states that immunization against infectious diseases has had an enormous impact on world health (Plotkin and Orenstein 2004). Immunization spares millions of children each year from contracting potentially debilitating and sometimes fatal infectious diseases. One estimate is that pediatric immunization prevents three million deaths in children each year worldwide (Diekema 2005). In 1966, there were approximately twenty million cases of smallpox worldwide, while by 1980, vaccination prompted the WHO to declare that smallpox was no longer an infectious disease threat (Mackay and Rosen 2001). Today many healthcare professionals still regard the eradication of smallpox as one of the greatest accomplishments of public health (Cohen 2000; Mackay and Rosen 2001). In the United States, since vaccines became available, there has been no case of indigenous poliomyelitis and a 99% decrease in the number of cases of diphtheria, measles, mumps, and rubella.

Besides its health impact, pediatric immunization prevents an enormous cost burden—both tangible and intangible—for individual children, families, and society-at-large (Cohen 2000). For example, the 2005 National Immunization Survey, administered by the United States Centers for Disease Control and Prevention (CDC), estimates a savings of $27 in direct and indirect costs for every dollar spent on vaccinating against diphtheria, tetanus, and pertussis (Cochi 2005). Similar favorable cost-to-benefit ratios make pediatric immunization an excellent investment in support of a nation’s public health infrastructure.

There remains much work in the area of vaccine development and distribution, despite the enormous progress of the past fifty years. In 1998, over 20% of the deaths worldwide (over 13 million of 54 million deaths) were due to infectious disease (Cohen 2000), with an estimated 1 million of these deaths attributable to measles—a disease with an available vaccine at a relatively modest cost; (Cohen 2000; Plotkin and Orenstein 2004). Moreover, the emergence of new infectious diseases such as human immunodeficiency virus (HIV) and Lyme disease, the resurgence of diseases such as tuberculosis, and the recent threats of bioterrorism (e.g., weaponized anthrax and smallpox) and pandemic influenza highlight the need for continued vigilance in the effort to combat infectious diseases and move forward with the creation of new vaccines to protect the lives of people worldwide (Binder et al. 1999; Plotkin and Orenstein 2004).

ROUTINE PEDIATRIC IMMUNIZATION
Each year, based on recommendations from the United States Advisory Committee on Immunization Practices (ACIP) and the American Academy of Family Physicians (AAFP), the National Immunization Program (NIP) publishes a Recommended Childhood Immunization Schedule that outlines vaccination requirements for children through adolescence (CDC 2006a). Similar schedules exist for other countries around the world; see http://www.euvac.net/graphics/euvac/vaccination/vaccination.html for a list and description of childhood immunization schedules for over thirty European Union (EU) countries. Although all these schedules have several common elements, there are a variety of political, economic, and social issues that have resulted in slight differences in how immunization is implemented.
The first U.S. immunization schedule was presented in 1983, while the first harmonized schedule (i.e., all the stakeholder organizations [ACIP/CDC, American Academy of Pediatrics, and the AAFP] agreed to the same immunization schedule recommendations) was made available in 1995. The United States Recommended Childhood Immunization Schedule outlines the vaccines required to protect a child against several infectious diseases that pose a risk to children living in the United States. (Note that the ACIP has also put forward an adult immunization schedule; see CDC 2006c.)

The pediatric vaccines routinely recommended by the NIP are

- inactivated polio (IPV)
- measles-mumps-rubella (MMR)
- *Haemophilus influenzae* type b (Hib)
- hepatitis B (HBV)
- hepatitis A (HAV)
- varicella (VAR)
- diphtheria-tetanus-acellular pertussis (DTaP)
- pneumococcal conjugate (PCV)
- a pentavalent combination vaccine (DTaP-HBV-IPV)

Pediatric vaccines are typically delivered by injection during scheduled wellness check-ups at healthcare clinics. For example, infants are scheduled to receive vaccine doses for hepatitis B, diphtheria, tetanus, pertussis, *Haemophilus influenzae* type b, polio, and pneumococcus at their two-month wellness check-up. Using currently licensed vaccines, children may receive as many as five injections during this particular check-up. Under extreme conditions, a fifteen-month-old child may receive as many as eight injections in a single clinic visit. The resulting crowding and complexity of the Recommended Childhood Immunization Schedule is only likely to grow worse as new infectious diseases emerge and/or new vaccines are developed.

These complexities increase the likelihood that a parent/guardian will reject or delay some vaccinations, resulting in noncompliance with the Recommended Childhood Immunization Schedule and associated risks. The cost of vaccinating a child also contributes to the underimmunization of children—the opportunity cost of time for a parent/guardian to make clinic visits, as well as the monetary cost of vaccination (Plotkin and Orenstein 2004). These costs often contribute to either missed clinic visits or missed vaccine doses. The 1990-1992 measles epidemic in the United States involved 28,000 cases of measles, most of which were due to inadequate vaccination of these patients when they were under two years of age (Mackay and Rosen 2001). Clearly, noncompliance with the Recommended Childhood Immunization Schedule puts children at risk of contracting numerous infectious diseases.  

The extensive healthcare delivery system in the United States, through private and public clinics and facilities, has facilitated the delivery of pediatric vaccines to the over four million children born in this country each year. Immunization registries have also made it easier for healthcare providers and parents to track a child’s up-to-date immunization record, and hence, determine when vaccines have been missed or delayed. A stated goal of the NIP is to achieve and sustain a 95% immunization coverage rate for all children by the time they enter school, for the first six diseases listed in the Recommended Childhood Immunization Schedule (CDC 2006a; 2006b). Details of pediatric immunization guidelines are regularly disseminated throughout the medical community (e.g., see Kroger et al. 2006 or http://www.cdc.gov/nip), and hence, pediatric immunization has become a mainstay and foundation for the nation’s public health infrastructure.

The strong and well-developed public health infrastructure in EU countries likewise has been the mainstay of their pediatric immunization programs. For example, in the United Kingdom, the Health Protection Agency provides guidance on vaccines and immunization policies and procedure, although the United Kingdom Department of Health implements such policies. This creates a natural “checks and balance” framework that provides multiple levels of control and guidance for the system.

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4 For further discussion on vaccine injection overcrowding, schedule complexity, and the cost of vaccinating a child, see Weniger (1996).
PEDiATRIC COMBINATION VACCINES AND PRiCiNG

Given that combination (multivalent) vaccines reduce the number of required injections and may be more economical, pediatricians, public health policymakers and administrators, and parents/guardians will likely choose combination vaccines over multiple single antigen (monovalent) vaccines. However, using combination vaccines may inject a child with antigens they have already received in the recommended quantity and timing sequence. For example, injecting a child with a DTaP-HBV-IPV (diphtheria, tetanus, pertussis, hepatitis B, and polio) combination vaccine at age 4 months would provide extraimmunization for hepatitis B, because (according to the Recommended Childhood Immunization Schedule, assuming a dose of hepatitis B was administered) no dose of vaccine is required at that age. Such extraimmunization poses biological risks and amplifies philosophical concerns with immunization in general. Biologically, extraimmunization of some antigens increases the risk of adverse side effects, as is the case with diphtheria and tetanus vaccines (CDC 1999).

Philosophically, many people challenge the safety and effectiveness of vaccinating children and particularly object to the use of combination vaccines, since they believe injecting a child with multiple antigens simultaneously overwhelms the infant immune system; extraimmunization due to combination vaccines only increases these fears (Edwards and Decker 2001; Chen et al. 2001). This philosophical barrier to vaccination is an increasing concern for pediatricians and public health administrators. For example, a recent national survey reported that 54% of pediatricians had encountered parents over a 12-month period that refused to vaccinate their child, citing safety concerns as the top reason for this refusal (Flanagan-Klygis et al. 2005). In another survey, 70% of pediatricians had encountered a parent in the 12-month period preceding the survey that refused at least one immunization for their child (Diekema 2005).

In addition to these biological and philosophical concerns, the economic toll of extraimmunization is significant. For example, the annual societal cost burden of providing one additional dose of a vaccine for each child born in the United States is over $28 million, assuming a birth rate of 11,100 births per day (Jacobson et al. 2006b) and a vaccine cost of $7, both of which are conservative estimates.

Given such obstacles and challenges, the practical advantage of having combination vaccines available for immunization (and hence, the opportunity to administer fewer injections) has resulted in vaccine manufacturers becoming more adept at creating new combination vaccines, which can make for fierce competition between such products, particularly when a single vaccine formulary (i.e., the set of vaccines that are stocked and available for immunization) must be stocked with only one of several such products. For example, Pediarix®, a combination vaccine manufactured by GlaxoSmithKline that immunizes against diphtheria, tetanus, pertussis, polio, and hepatitis B, gained FDA approval in December 2002. In addition, Pentacel®, a combination vaccine manufactured by Sanofi Pasteur that immunizes against diphtheria, tetanus, pertussis, polio, and Haemophilus influenzae type b, is positioned to gain FDA approval in late 2007 or early 2008.

On the surface, having several combination vaccines to choose from would appear to be a good thing for pediatricians, healthcare administrators, and the public health community. However, the fact that these two vaccines are partially overlapping makes them ill-suited to be stocked within a single pediatric vaccine formulary. Therefore, pediatricians, healthcare administrators, and the public health community, in general, must choose between such combination vaccine products.

The economic issue faced by vaccine manufacturers is how to price their vaccines (particularly their combination vaccine, since it will form the backbone of any pediatric vaccine formulary) so as to maximize their overall revenue. Given that the pediatric vaccine market in the United States is a $2+ billion industry, each 1% shift in market share translates into $20+ million of revenue. Therefore, it is in the best interest of each company to appropriately price their combination vaccines (or appropriately set the price premium inherent in a combination vaccine, where this price premium is the difference between the price of a combination vaccine and the sum of the prices of the individual vaccines that comprise the combination vaccine). However, since any change in price of one combination vaccine can be responded to by a change in price in a partially overlapping (and hence, competing) combination vaccine, a natural question to ask is whether there exist equilibrium prices for combination vaccines. Once two or more of such products become available, market forces will operate to move towards and reach an equilibrium market share for all these products, as well as price premiums that support such equilibriums.
Combination vaccines have been more aggressively incorporated into immunization schedules within the EU than in the United States. For example, in the United Kingdom, the diphtheria, tetanus, pertussis, polio, and *Haemophilus influenzae* type b vaccine is the vaccine of choice for the months 2, 3, and 4 immunizations. As noted above, as of this writing, this vaccine has yet to gain FDA approval within the United States, hence is unavailable here. In Austria, the diphtheria, tetanus, pertussis, polio, hepatitis B, and *Haemophilus influenzae* type b vaccine is the vaccine of choice for the months 3, 4, and 5 immunizations. Also as of this writing, this vaccine has yet to gain FDA approval within the United States. Clearly, longer U.S. regulatory approval times make it more difficult for vaccine manufacturers to launch products in the United States that have been deemed safe for use in many EU countries. This creates a more fragile vaccine manufacturer and supplier infrastructure and an associated suboptimal immunization environment.

**PEDIATRIC VACCINE SHORTAGES AND STOCKPILING**

**United States**

Over the past decade, vaccine production interruptions in the United States have lead to widespread vaccine supply shortages, resulting in children not being fully immunized according to the Recommended Childhood Immunization Schedule. Numerous factors have contributed to this vaccine supply shortage crisis (Sloan et al. 2004). First, over the past decade, there has been a downward trend in the number of pediatric vaccine manufacturers (NVAC 2003), which means that any single vaccine production interruption is more likely to lead to widespread vaccine supply shortages. In 2007, only four pediatric vaccine manufacturers (GlaxoSmithKline, Merck, Sanofi Pasteur, and Wyeth/Lederle) provided all the vaccines needed to meet the immunization requirements for the (over) four million children born in the United States each year.

Second, since the United States Federal Government purchases over one-half of all pediatric vaccines that are delivered, they have negotiated contract prices that are significantly lower than those paid in the private sector. The resulting limited profit margins make it economically unattractive for new vaccine manufacturers to enter the pediatric vaccine market or for existing manufacturers to increase production capacity through either new investments or reallocation of production capacity. This has also made it difficult to appropriately price pediatric combination vaccines, which often require high research investments, yet whose prices are closely tied to their monovalent vaccine components that are already priced and available in the market (Jacobson et al. 2003a; 2003b; 2005).

Third, the United States Food and Drug Administration (FDA) has become increasingly more stringent when certifying vaccine manufacturing facilities (FDA 2004). Therefore, if a production facility is cited for production violations and is temporarily shut down by the FDA, it must go through a rigorous recertification process that can delay and limit production even further.

Fourth, when new vaccines gain FDA approval, vaccine manufacturers may not be able to accurately predict the rate at which pediatricians and other healthcare providers will welcome the use of such products. The very nature of the resulting uncertain (stochastic) demand function makes it highly challenging to determine reasonable vaccine production runs without creating either shortages or inventory surpluses in the process.

Lastly, if the ACIP makes either modifications to the Recommended Childhood Immunization Schedule that result in additional dose requirements for a particular vaccine, or a change in the timing for administering a particular vaccine, this may create unpredictable demand surges to which vaccine manufacturers may be unable to respond in a timely manner. All these issues must be considered when designing and controlling the vaccine manufacturing production process.

The National Vaccine Advisory Committee (NVAC) formed the working group, "Strengthening the Vaccine Supply of Routinely Recommended Vaccines" (NVAC 2003), which held a workshop in 2002 with industry representatives, regulatory authorities, public health officials, purchasers, providers, consumers, legislators, and academic investigators to discuss and identify solutions to the pediatric vaccine supply shortage problem. The outcome of this deliberation led to several recommendations, including providing financial incentives for vaccine manufacturers, instituting policy and regulation changes, and growing the rotating pediatric vaccine stockpiles (USGAO 2002). Sloan et al. (2004) present the structure for a pediatric vaccine subsidy system that could serve as a catalyst to attract more pharmaceutical companies into the vaccine production industry, and hence, create a more stable vaccine supply environment.
Officials of the United States General Accounting Office (GAO) and CDC believe that a national pediatric vaccine stockpile is the best avenue to protect against vaccine production interruptions and the ensuing supply shortages (USGAO 2002). It is worth noting that the first national pediatric vaccine stockpiles were created in 1983 to address short-term vaccine supply interruptions. The CDC has gone on record that these stockpiles have eliminated or reduced the impact of vaccine supply shortages on at least eight different occasions since 1983.

Given the challenges that exist in creating and maintaining pediatric vaccine stockpiles, it is imperative that most, if not all, economic and production barriers be minimized, and hopefully, completely eliminated. As recently as 2005, an economic disincentive existed for vaccine manufacturers to create and maintain pediatric vaccine stockpiles, since accounting rules and procedures did not permit vaccine manufacturers to list such vaccine stockpiles as revenue from sales. After much deliberation and negotiation, on December 5, 2005, the Securities and Exchange Commission (SEC) stated that vaccine manufacturers could recognize revenue on vaccines placed into government stockpiles instead of waiting to count such payments until when the vaccines are physically taken out of the stockpile (SEC 2005). National policy changes as such provide a more business-friendly and welcoming environment for vaccine manufacturers to work with government agencies to provide the necessary public health protection afforded by pediatric vaccine stockpiles.

The goal of the national vaccine stockpile program is to maintain a six-month rotating inventory for all routinely recommended pediatric vaccines (see the list in the section above, Routine Pediatric Immunization). To increase the existing vaccine stockpile levels to the recommended levels (Manning 2004), the United States Federal budget allocated over $170 million for fiscal year 2003 to provide partial funding to support the necessary expansion of the national vaccine stockpile program. Jacobson et al. (2006a; 2006b) analyze the proposed vaccine stockpile levels using a stochastic inventory model. They use this model to examine the adequacy of the proposed six-month rotating pediatric vaccine stockpile levels, as well as to provide insights into what the appropriate pediatric vaccine stockpile levels should be to achieve prespecified vaccination coverage rates. The level of funding needed to create such pediatric vaccine stockpile levels is also reported and discussed.

European Union

Each EU country maintains full autonomy in managing its immunization programs, including setting its routine pediatric immunization schedules, ordering vaccines, negotiating prices and costs for such products, setting its own pediatric vaccine stockpile policy and maintaining vaccine stockpiles. Given the strictly advisory role of the European Centres for Disease Prevention and Control (ECDC), they can facilitate communication between the various countries and help to coordinate efforts in addressing immunization and public health issues and crises; however, each country acts independently and autonomously. This parallels each state in the United States receiving guidance from the CDC, but ultimately setting its own immunization agenda within the accepted national immunization schedule. On the other hand, the United States Government maintains a single stockpile for the entire country, which it releases as needed and appropriate. Therefore, the EU is ultimately more decentralized than the United States in how it is prepared to deal with vaccine shortages.

INFLUENZA VACCINE PRODUCTION AND DEMAND

Routine pediatric vaccines account for a large proportion of vaccine manufacturing activities. However, such vaccines are considered mature or well developed, with no (or little) changes needed to the vaccine production process. Therefore, vaccine production interruptions can be mitigated through vaccine stockpiling and inventory management. On the other hand, the seasonal (annual) influenza vaccine uses a conventional manufacturing process that requires the identification of three virus strains that are likely to be the influenza virus in the ensuing influenza period (which changes each year, due to antigen shift/drift). Vaccine manufacturers then use eggs to grow the vaccine components (Gerdil 2002). Given that there are only three vaccine manufacturers that provide the influenza vaccine in the United States (Sanofi Pasteur, Chiron, and most recently, GlaxoSmithKline), the stability of the influenza vaccine supply chain rests at the production source.

This heavy reliance on producers for availability of influenza vaccine became most apparent during the 2004–2005 influenza season, when production problems and compliance issues created a massive vaccine
shortage. The resulting supply shortage prompted academic researchers to investigate what brought on this crisis and how it may be avoided in the future. Corbett and Deo (2007) show how the interaction between yield uncertainty in the influenza vaccine production process and corporate strategy contribute to the small number of vaccine manufacturers and the resulting reduction in production output. They consider the tradeoff between risk pooling (by diversifying the number of suppliers) and the economies of scale (by avoiding any duplication of fixed investment costs). Computational results are reported with real-world parameters to assess the impact of yield uncertainty on the United States influenza vaccine market, and conclude that increases in fixed costs have most likely been the most significant factor that has led to fewer suppliers, rather than yield uncertainty. Chick et al. (2007) show that influenza production risks lead to inadequate vaccine supply. Their analysis focuses on the middle of the vaccine supply chain, namely, the design of business contracts that allow vaccine manufacturers to remain profitable and government entities to balance cost with public health needs.

Recent studies suggest that some protection from influenza illness can be acquired through the use of the pneumococcal conjugate vaccine (PCV7), which is part of the Recommended Childhood Immunization Schedule. Researchers have observed that the PCV7 may serve to reduce the morbidity associated with influenza as well as other viral respiratory illnesses (Kalvaisits 2007). Researchers have also conjectured that people who have received seasonal influenza vaccines may also have some partial protection against a pandemic influenza. Such research makes a strong case for routine immunization in general, as well as suggests possible interdependencies between different vaccines. Further research is needed to quantify and further validate and explore such hypotheses.

VACCINE DISTRIBUTION AND PANDEMIC INFLUENZA RESPONSE

The United States healthcare delivery system provides the infrastructure to deliver routine pediatric vaccines to the (over) four million children born in the United States each year. This includes a comprehensive system of well-baby office and clinic visits during a child’s first two years of life. Support for such services is available not only through the private payer health insurance system, but also through government programs like the Vaccine for Children Program (see information available at the NIP website, http://www.cdc.gov/nip, which has a comprehensive collection of information and web links to all matters related to immunization and vaccination). Similar websites exist for EU countries (see, for example, the Health Protection Agency in the United Kingdom: http://www.hpa.org.uk/).

For nonroutine immunization, such as during a pandemic influenza outbreak, the public health infrastructure must serve as the first line of defense to efficiently and effectively collect, distribute, and deliver vaccines (and other medical supplies, like syringes, antivirals, facemasks, and latex gloves) to either targeted subgroups of the population (e.g., healthcare workers and critical infrastructure workers) or in the worst case, to the entire population. The United States Federal Government is working to provide guidelines to follow in the event of pandemic influenza outbreak. However, specific procedures are (appropriately) being planned at the local levels (states, counties, cities, towns).

Clearly, there is no “one size fits all” procedure that can be effectively applied across the wide spectrum of communities, ranging from those in large cities to those in small towns. Attempts at implementing a single uniform approach is destined to create a “Viral Katrina” in urban centers, where public health clinics are certain to be overwhelmed and vulnerable to collapse in the event of mass vaccination and treatment needs. On the other hand, short-term quarantining in very small rural communities may be feasible. Clearly, given the potential for misinformation being disseminated and limited avenues for transmitting accurate communications (and the possibility for widespread public panic), it is vital to have systems in place and well-defined procedures available that can analyze and create plans of attack for numerous likely (or even unlikely) scenarios.

Academic researchers have begun to meet this challenge by creating computer-based, real-time tools and systems to assist with this process. Aaby et al. (2006a 2006b) report the results of a simulation study to design and operate a public health clinic charged to stock and deliver or dispense vaccines and medication during a disease outbreak. Their simulation tool allows one to play “what-if” games with different disease outbreak scenarios, and hence, allows public health administrators to proactively have plans in place for a variety of such scenarios. Miller et al. (2006) use a simulation model to show how a public health community can design and effectively respond to a bioterrorism attack like smallpox. In such environments and
situations, mass vaccination may provide the best means to prevent widespread fatalities (Kaplan et al. 2002). Zhang et al. (2006) describe Flusurge, a computer software tool that estimates the number of hospital admissions and deaths that may arise during a pandemic influenza outbreak under a variety of scenarios. Based on a case study for the Atlanta metropolitan area, they suggest that the city’s hospital system will be severely taxed during such a crisis, largely due to the near-capacity utilization of hospital resources during normal operations. Hanfling (2006) provides a cost plan for supplies and personnel during an emergency.

The United States and the European Union are in the planning stage to stockpile pre-pandemic vaccines. For example, the United States plans to stockpile 20 million doses of a H5N1 vaccine. Several EU countries have preorders in for pandemic influenza vaccines, once or if they become available. Several EU countries are prepared to impose export limitations on vaccines manufactured within their borders. This means that in the event of a pandemic influenza outbreak, if a vaccine becomes available, its distribution may be limited by specific EU country export policies. This issue could lead to widespread panic in countries that do not have vaccine manufacturing plants within their borders, as well as to the propagation of counterfeit vaccines being offered on the Internet to those (desperate) populations. Note that this also provides incentives for countries to attract vaccine manufacturing facilities to locate within their borders. Given that a significant proportion of vaccine manufacturing capacity currently lies outside the United States, it will be interesting to observe how the United States Federal Government handles this situation in the coming decade.

A growing number of international organizations are making efforts to formulate plans in the event of a pandemic outbreak. Kieny et al. (2006) describes the results of a WHO meeting held 2–3 May 2006 in Geneva, Switzerland, that discussed the framework for a global response action plan in the event of a worldwide pandemic influenza outbreak, including the rapid production of vaccines and the surge capacity before and during the outbreak. On December 14, 2006, the United States Department of Health and Human Services issued a public request (through the Federal Register) soliciting ideas and comments on which subgroups of the population should have priority in being immunized with pre-pandemic and pandemic influenza vaccines (HHS 2006). The resulting prioritization policy is likely to have an enormous impact on vaccine distribution during a pandemic influenza outbreak, given the limited amount of vaccines that are likely to be available during the initial phase of such a crisis. Clearly, significant planning and coordination are needed in both the public health communities and the private health sectors to ensure that a country (or more accurately, the entire world) is adequately protected and treated before, during, and after a pandemic influenza outbreak, to ensure that each nation’s social and economic infrastructures are preserved during such a threat.

The population density in Europe (in particular, several densely populated urban areas), coupled with the challenges in coordinating efforts across several countries, make that region highly vulnerable to pandemic influenza outbreak. The social and economic consequences of a pandemic influenza outbreak are difficult to accurately predict. (For example, Page et al. [2006] consider a case study on managing tourism in Scotland in the event of a pandemic influenza outbreak.) ECDC leaders have expressed concerns that EU countries are not moving sufficiently fast in preparing for a pandemic influenza outbreak, and that the EU needs at least two years to be adequately prepared to respond to such an outbreak.

PANDEMIC INFLUENZA VACCINE PRODUCTION ISSUES

As the threat of a pandemic influenza outbreak grows, vaccine manufacturers are making important advances in creating new vaccines for such emerging viral threats. Given that such threats may surface both quickly and (to some degree) unexpectedly, the need for efficient and effective vaccine distribution systems is critical. In the best-case scenario, a DNA-based pandemic influenza vaccine can be created in six months, while a pandemic influenza vaccine that is manufactured using conventional (egg-based) methods can be available within 18–24 months.

Several issues must be considered to determine the optimal process of moving large amounts of vaccine from a vaccine manufacturing facility into the hands of healthcare personnel who are responsible for their delivery. First, the balance between depth and breadth of the vaccine supply chain must be addressed. In particular, is it better to have several manufacturing and/or distribution centers, each servicing a small population area, or a small number of manufacturing and/or distribution centers, each feeding vaccine supplies into a secondary and/or tertiary set of distribution centers?
Second, how many doses of a vaccine should be produced and be made available? Vaccine formulation and related manufacturing factors to be considered for this question include whether adjuvants will be used (see the next section, “Questions and Challenges: The Future” for further commentary on this issue); vaccine production yield and production process variability; start-up and set-up costs; as well as time to initialize a production run for a vaccine. Another related issue to consider is whether the vaccine will be cell-based or DNA-based, with each method having unique production, safety, efficacy, and delivery challenges that will need to be addressed. In addition, determining the optimal and minimal dosage requirements for a vaccine to achieve a specified efficacy, as well as the impact of the herd effect on determining what fraction of a population to immunize, are two factors that require further study and consideration.

Third, the need for and size of a vaccine stockpile are issues that require focused research. In particular, weighing the risk of a pandemic influenza outbreak versus the size of, the shelf life of pre-pandemic vaccines and antivirals (including cold-chain storage issues), and the cost of building and maintaining a vaccine supply and stockpile requires attention. All these issues, which are being discussed in both the United States and Europe, will become more urgent and complex as new pandemic influenza vaccines are being developed.

Given that infectious diseases cross borders and can strike people in any country at any time, there are numerous common practices being following in EU countries and the United States. The decentralized structure of the EU and each country’s public health system will facilitate a rapid response in the event of a pandemic outbreak. Interestingly, the ECDC has been effective in bridging communication between the EU countries, so that autonomy does not appear to correlate with disorganization and conflict. The United States has been promoting a decentralized pandemic response strategy, while providing national guidelines, although at present, it is not clear how successful this has been. This approach is consistent with EU procedures, and hence, will facilitate a unified worldwide response in the event of a global pandemic outbreak.

QUESTIONS AND CHALLENGES: THE FUTURE

The vaccine supply chain, which in the broadest sense, includes the development, testing, approval, manufacturing, distribution, and delivery phases, is long, thin, and fragile; it is only as strong as its weakest link. In the event of a pandemic influenza outbreak, assuming that a vaccine can be rapidly manufactured and made available in large quantities (which may still require several months, based on expert opinion), several challenges remain in moving the vaccines from manufacturers into the hands of healthcare professionals and public health clinic personnel responsible for delivering the product.

A key challenge is determining who should receive the vaccine, given that its availability and distribution will likely be limited by a ramp-up period. Clearly, this issue is highly sensitive and fraught with controversy. A plan is needed to quickly distribute the vaccines in a manner that allows priority populations to be immunized. The ability to rapidly adapt the existing public health infrastructure to achieve this will require further study and investigation. Moreover, the conflict between reaching people to deliver vaccines (and medicines) versus the need to keep people from closely congregating to limit disease transmission presents unique challenges to the public health communities. Research is needed to introduce novel, more flexible paradigms for the delivery of public health services that can accommodate both existing and pandemic influenza outbreak distribution requirements and needs.

The 1918 “Spanish flu” influenza pandemic outbreak resulted in entire communities quarantining themselves. This resulted in restrictions in how people moved around and through their communities, limiting the transmission of the disease. The value of this approach is to focus on disease prevention over disease treatment, which would be far more cost-effective, both in terms of morbidity and mortality. However, given the globalized world in which we live and the mobility of its citizens, the potential for rapid and widespread transmission of a virus is great, making quarantining a significant challenge. Communication technologies like the Internet provide an important avenue for maintaining social and economic networks during quarantine periods, which may impose an enormous strain on such systems. Novel approaches to quickly disseminate and update accurate information during such periods will be critical to maintaining social order and keeping people updated on travel restrictions, water and food safety warnings, and other primary life sustenance requirements. Research is needed to fully anticipate and provide the necessary support for the technology infrastructure to ensure that it remains reliable in the event of such overwhelming information demand surges.
Bringing a new vaccine to market often takes as much as one to two decades, with costs upwards of $1 billion. The creation and production of a malaria vaccine is a current example, where the hope is that such a vaccine will be available by 2025 (IDN 2007). Vaccine manufacturers are willing to make such investments if the profit potential is sufficient to overcome the risks of not succeeding. As new manufacturing processes are developed and refined, the time frame for the development process is likely to shrink, with an associated reduction in development costs. However, these costs will remain significant, and the risks will continue to exist (if not increase due to this collapsed time frame). A key challenge is creating new and appropriate incentives for such investments.

Building flexible manufacturing facilities, which allow for a smoother, seamless production transition across two or more vaccines, offers significant potential to mitigate risk. Distributed production, whereby modularized production plants are created and located around international population centers where the vaccines are needed, works to combine the manufacturing and distribution network. Such solutions can provide new paradigms for vaccine manufacturing and distribution that overcome the traditional economic model that separates manufacturing and logistics decisions currently employed by vaccine manufacturers.

During a pandemic influenza outbreak, if a vaccine can be rapidly developed, the ramp-up period from when production begins to when a sufficient amount of vaccine is available to protect the targeted populations may be prohibitively long. One approach to extending a vaccine supply is through the use of adjuvants, which can enhance and boost the immune response to the vaccine’s antigen. A recent study using adjuvant MF59 with an experimental H9N2 vaccine suggests that it may substantially increase the number of doses available for immunization (Pigliacelli 2007). At present, adjuvant MF59 is licensed for human use in Europe but not in the United States. The use of such adjuvants may be an important vehicle to immunize a wider group of people during the early phase of a pandemic influenza outbreak, when a limited vaccine supply is available. In the United States, the FDA has begun to recognize the reality of this situation and has begun to give more serious attention to their use, as is already the case in Europe.

As new vaccines become available, the types of delivery devices and ancillary medical supplies needed to safely and effectively deliver such vaccines will become challenging issues. For example, traditional syringe delivery devices may be inadequate for DNA vaccines. The volume of ancillary medical devices needed to deliver pandemic influenza vaccines or antiviral medication may be an unexpected bottleneck in the system. Determining ways to handle and dispose of the resulting biohazard waste materials presents a unique set of transient challenges. It may be possible to stockpile vaccination supplies before a pandemic influenza outbreak; at the same time, research is needed to design novel vaccine delivery systems that are safe, inexpensive, user-friendly, recyclable, and storable for long periods of time.

As the vaccine supply chain is unrolled and set into motion during a pandemic influenza outbreak, numerous economic and social issues will need to be considered to protect this supply chain’s effective and safe operation. At the national level, the Trust for America’s Health issued a report predicting in detail the impact of a pandemic influenza outbreak on the United States economy (Trust for America’s Health 2007). The report projects a $683 billion economic loss during such an outbreak, which represents over 5% of the goods and services produced in the United States; this would most likely move the United States, as well as the entire world, into an economic recession. States that rely most heavily on tourism, like Nevada and Hawaii, would feel the greatest impact of such an economic downturn.

Counterfeit pandemic influenza vaccines will be advertised on the Internet, increasing the volume of spam email being sent. Unwary and desperate people will pay large amounts of money for worthless products. Vaccine and antiviral shortages, particularly during the early phase of an outbreak, will likely create social panic, which could lead to violence and economic destruction in urban areas. Law enforcement may also be strained during such periods, due to manpower shortages and fears of contracting the virus. As was observed in New Orleans after Hurricane Katrina, a “Viral Katrina” effect (i.e., public panic coupled with socio-economic-based unequal distribution of services) may surface across several cities in the nation, depending on how information is disseminated and how people choose to receive, use, and act upon such information.

In conclusion, the economics of vaccine manufacturing provides enormous constraints and enormous incentives for maintaining the existing vaccine supply chain infrastructure, as well as for creating new manufacturing supply chain vectors. As biotechnology advances make it possible for vaccine manufacturers to rapidly produce and manufacture new vaccines, the economic drivers of cost and profit must be considered to push such potential into practice, particularly in the event of a pandemic influenza outbreak.
Moreover, innovative ways to distribute vaccines into the hands of healthcare providers is critical to realize the full benefit of such advances.

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APPENDIX A. PANELISTS’ BIOGRAPHIES

Joseph T. Bielitzki, MS, DVM (Panel Chair)

BS in Biological Sciences, University of Illinois at Chicago
BVS, MS, DVM, University of Illinois at Urbana-Champaign

Joe Bielitzki has a diverse background in science. His early experience was in the enteric infectious agents affecting nonhuman primates. He worked for 18 years in the National Primate Center System at both the University of Washington and the Yerkes Center of Emory University, supporting research across a wide range of biomedicine. In 1996, he became the Chief Veterinary Officer for NASA, where his efforts focused on coordinating the agency-wide animal care and use program, compliance issues, hardware design, and training. Joe served on the working group for safety issues surrounding sample-return missions from Martian environments. In 2000, he served as a government advisor on a task force for a Defense Science Board looking at defense against biological warfare. In 2001, Joe accepted a position as a program manager at the Defense Sciences Office at DARPA. At DARPA, he managed an extensive research portfolio in the life sciences, including Long Term Storage of Blood Products, Peak Soldier Performance, Rapid Vaccine Assessment, Surviving Blood Loss, Restorative Injury Repair, Biofilms for Defense, Pathogen Countermeasures, and Accelerated Anthrax Therapeutics. During this period, Joe interacted with a variety of Federal agencies in the area of biological warfare defense. After DARPA, he relocated to Orlando, where he now serves as the Chief Operating Officer at LensAR, Inc., in Winter Park, Florida. He also consults in the area of science and technology development for academia, industry, and government. Microbiology, pathogen evolution, and protective mechanisms of immunity are primary areas of interest for him. He is recognized for his expertise in the ethical issues surrounding the use of animals in research.

Stephen W. Drew, PhD

BS and MS in Food Science, the University of Illinois
PhD in Biochemical Engineering, Massachusetts Institute of Technology (MIT).

Stephen W. Drew is a former Distinguished Senior Scientist at Merck & Co., Inc., where his responsibilities encompassed the development of new process technologies for biologics and pharmaceutical manufacturing and technology transfer. Since retiring from Merck, he has founded two companies that support the biotechnology and pharmaceutical industries: Drew Solutions LLC, a direct consulting firm, and Science Partners LLC, an advocacy company for medicines and technologies. Prior to his retirement, he held the positions at Merck & Co., Inc., of Vice President of Vaccine Science and Technology, of Vaccine Operations, and of Technical Operations & Engineering. He joined Merck in 1980 to create the Department of Biochemical Engineering. At Merck, he contributed to the process development and manufacture of several conventional and recombinant microbial products ranging from antibiotics to vaccines. Dr. Drew has expertise in the following areas: manufacturing processes for human and animal vaccines; recombinant biologics; chemical, biological, and engineering technology for bulk manufacture of pharmaceuticals and biologics; capital project engineering; process engineering; and fermentation, cell culture, isolation, and purification processes for sterile products. He was elected to the National Academy of Engineering in 1993; has held offices in the American Institute of Chemical Engineers, the American Chemical Society, the American Society for Microbiology, and the Society for Industrial Microbiology; and is a Founding Fellow of the American Institute for Medical and Biological Engineering. He has served as Chairman of the advisory committee to the Engineering Directorate of the National Science Foundation. He is a member of two standing committees of the National Research Council (NRC) and has participated in many NRC studies.
Cyril Gerard Gay, D.V.M., PhD

BSc in Chemistry and DVM, Auburn University
PhD in Microbiology, The George Washington University

Dr. Gay has worked in the veterinary vaccine and animal health fields for the last 20 years, holding several positions of increasing responsibility in the Federal government and the pharmaceutical industry. As Chief, Biotechnology Section, Center for Veterinary Biologics (CVB), United States Department of Agriculture (USDA), Dr. Gay developed the procedures for licensing molecular vaccines that led to the first license for a live recombinant vectored vaccine worldwide. Dr. Gay has led several cross-functional teams in industry that developed veterinary vaccines. As Director, Global Product Development, Pfizer, Inc., he developed strategic and tactical plans that interfaced R&D, clinical development, manufacturing, marketing, and product life-cycle management. Dr. Gay is currently the National Program Leader, Animal Health, Agricultural Research Service (ARS), USDA. Dr. Gay provides program direction and national coordination for the department’s intramural Animal Health National Research Program, comprised of 124 scientists located in 11 research locations throughout the United States, including the National Animal Disease Center (Ames, IA); the Avian Diseases and Oncology Laboratory (East Lansing, MI); the Meat Animal Research Center (Clay Center, NE); the Southeast Poultry Research Laboratory (Athens, GA); the Plum Island Animal Disease Center (Orient Point, NY); the Animal and Natural Resources Institute (Beltsville, MD); the Arthropod-Borne Diseases Research Center (Laramie, WY); and the Poultry Research Unit (Mississippi State, MS). Vaccine discovery is a core component of the Animal Health National Research Program.

Sheldon H. Jacobson, PhD

BSc and MSc (both in Mathematics), McGill University
MS and PhD (both in Operations Research and Industrial Engineering), Cornell University.

Sheldon H. Jacobson is a Professor, Willett Faculty Scholar, and Director of the Simulation and Optimization Laboratory at the University of Illinois at Urbana-Champaign. Since 1996, he has been applying operation research methodologies to address healthcare problems associated with pediatric immunization and vaccination economics, pediatric vaccine pricing, and pediatric vaccine stockpile economics. He has received numerous awards for his research, including a Best Paper Award in IIE Transactions Focused Issue on Operations Engineering and a John Simon Guggenheim Memorial Foundation Fellowship. His healthcare research has been published in a wide spectrum of operations research and medical journals, including Health Care Management Science, Journal of the Operational Research Society, Pediatric Infectious Disease Journal, and Vaccine, among others. He has briefed the Advisory Committee on Immunization Practice (ACIP), the committee that provides guidance to the Secretary of the Department of Health and Human Services on issues related to immunization policy in the United States. He has also worked to transition his research into a publicly available website, http://www.vaccineselection.com, which has been widely used by both government and private sector organizations. He has received research funding from several government agencies and industrial partners, including the National Science Foundation and the Air Force Office of Scientific Research.
Appendix A. Panelists’ Biographies

Terrance J. Leighton

BS, Microbiology, Oregon State University, 1966
PhD, Microbiology, University of British Columbia, Vancouver, B.C., 1970
Postdoctoral Fellow, Biochemistry, University of California, Davis, 1972
Senior Staff Scientist, Children’s Hospital Oakland Research Institute

Synopsis of Relevant Interests

Molecular evolution of bacterial sporulation; AFM & NanoSIMS single bacterial or viral particle digital imaging under physiological conditions; Pathogen vaccines - Genomics of surface antigens, formulation and delivery technologies; Single antigen:antibody AFM imaging on native pathogen surfaces; Pathogen medical countermeasures – Genomics and structure-based drug design; DNA- and immuno-based threat agent detection technologies; Broad-band PCR detection of viral and bacterial pathogen signatures by mass spectrometry; Urban surveillance – viral and bacterial pathogen detection systems; Large-area chlorine dioxide pathogen decontamination technologies; Nontoxic personnel pathogen decontamination technologies.

Mary B. Ritchey, PhD

BA in Biology, Emmanuel College
PhD in Microbiology, Cornell University
Postdoctoral studies on influenza viruses, Mount Sinai School of Medicine

Dr. Ritchey, of Ritchey Associates, Inc., is currently engaged in consulting for the pharmaceutical industry with a focus in the vaccines area. Prior to taking on consulting assignments, Dr. Ritchey spent 29 years in the pharmaceutical industry working on the development, manufacturing, and quality aspects of vaccines and other sterile pharmaceutical products. She joined Lederle Laboratories in 1977, where her initial assignments involved developing processes for manufacturing influenza and poliovirus vaccines. During her tenure at Lederle and then at Wyeth Pharmaceuticals, she held positions of increasing responsibility in the areas of R&D, manufacturing, quality, and technical services. In 1992 she became Vice President of Operations for the Vaccines group and held additional vice president positions until her retirement in 2006. During her years at Lederle and Wyeth, she was involved in numerous product areas: vaccines for viral influenza and polio, including live attenuated and inactivated; diphtheria, tetanus, and pertussis, including acellular pertussis; Haemophilus influenzae, meningitis, and pneumonia, including polysaccharides and conjugates.
APPENDIX B. SITE REPORTS—EUROPE

Site: Baxter Vaccines AG
     Biomedical Research Center
     Uferstrasse 15
     A-2304 Orth/Donau, Austria
     http://www.baxtervaccines.com/

Date Visited: March 2, 2007

WTEC Attendees: M. Ritchey (report author), J. Bielitzki, T. Leighton, H. Ali

Hosts: R. Petermann, PhD, MBA, Global Marketing Manager
       Tel: +43 1 20 100-2895; Fax: +43 1 20 100-5073
       Email: Robert_Petermann@baxter.com

       D.I. Dr. W. Mundt, Vice President Process Development,
       Global R&D
       Tel: +01/20/ 100-3005; Fax: +01/20 100-3407
       Email: Wolfgang_mundt@baxter.com

       Denis Cavert, PhD, VP Marketing & Sales Europe
       Tel: +43 1 20 100-2815, Fax: +43 1 20 100-5073
       Email: denis_cavert@baxter.com

BACKGROUND

Baxter Vaccines, is a multinational company that provides healthcare professionals and their patients a variety of products for the treatment of complex medical conditions, including hemophilia, immune disorders, cancer, infectious diseases, kidney disease, trauma, and other conditions. Its products include medical devices, pharmaceuticals, vaccines, and other biotechnology products. Baxter Vaccines is comprised of the former Immuno, an Austrian company founded in 1956, which initially engaged in the development of a polio vaccine and later developed a vaccine against tick-borne encephalitis (TBE), and North American Vaccines, a U.S.-based company that developed a group C meningitis conjugate vaccine. In Europe, the Vaccines group currently has research, development, manufacturing, and testing facilities in Vienna and Orth, Austria. A cell culture facility for manufacturing influenza vaccine is located in the Czech Republic. Vaccines currently marketed in Europe include TBE and meningitis C. The cell-culture-based influenza vaccine is in late-stage development.

DISCUSSIONS WITH HOSTS

Host Perspectives on the Economic Climate for Vaccines

Historically, vaccines were prepared by family-owned enterprises for altruistic motives rather than income generation. As a result, the prices charged for vaccines are disproportionately low compared to the value that they provide in preventing disease. This has led to a declining number of vaccine suppliers over the years and has resulted in supply issues almost every year.

There is a high cost for entry into the vaccines business because of the need for specialized equipment and procedures and highly trained staff. This is also an impediment to making improvements that require different sets of process parameters and equipment. Improvements generally will not result in price increases, thus the return will not justify the investment. The fact that influenza vaccines are still made using embryonated eggs illustrates this issue. The technology exists to manufacture influenza vaccines using cell cultures, but the current cost of conversion is difficult to justify based on the return. (Cell culture offers many distinct advantages over egg-based production in terms of speed, reliability, and quality) Baxter is able to support a program for the production of influenza vaccines using cell culture because this is their initial entry into the influenza vaccine business and this initial investment will generate a totally new, additional revenue stream for the company.
To support innovation for current vaccines and rapid development of new vaccines, the mindset of governments, insurance companies and other stakeholders must change to recognize the high value that vaccines bring to the healthcare system.

With respect to providing vaccines for developing countries, Baxter participates in some programs, but needs to balance this with the need for the business to generate revenues. From Baxter management’s point of view, the GAVI Alliance\(^5\) is doing a good job of assisting developing countries with vaccination programs, and tiered pricing helps reduce vaccine costs to developing countries. This approach, however, is only suitable for traditional vaccines, not for innovation. Deploying innovative technologies for traditional or new vaccines in developing countries is complicated not only by the cost of producing these vaccines, but also control of intellectual property when transferring technology. Countries such as India and China are beginning to produce their own vaccines, and the quality is improving as experience is gained. Organizations in these countries can also provide supplies for other developing countries that lack vaccine production capability.

**Influenza Vaccine and Pandemic Preparedness**

Baxter has produced vaccine from the H5N1 strain in its cell-culture-based system and is the only manufacturer to have cell-culture-produced vaccine in the clinic. This technology offers a distinct advantage for preparing for a pandemic as Baxter’s system will allow for production using native virus strains, thus eliminating the time that it takes to develop an egg-adapted strain before production can begin; in addition, supply will not be dependent upon the ability to procure embryonated eggs from healthy chicken flocks.

The company has established agreements with Austria and other countries, including the UK, for providing vaccine supplies. Addressing issues of reimbursement and indemnity is also part of the planning process. Baxter is participating in the European plan to develop a license dossier based on currently available H5N1 strains as a means of making the approval process more rapid in a pandemic situation.

The European Vaccine Manufacturers Association is also focusing on devising strategies that should contribute to early control of a pandemic. One option is to stockpile vaccine in areas of the world that are likely to see a pandemic strain first and use immunization in those areas as a means of preventing spread to other parts of the globe.

In reviewing capacity issues for providing enough doses for mass immunizations, our hosts noted that in addition to finding or building the physical facilities, finding qualified staff to run the facilities would be challenging. With respect to using animal vaccine facilities for human vaccines, there may be some issue with the ability of these facilities to meet standards for human vaccines.

**Vero Cell Platform**

Our hosts presented a film that described the Vero-cell-culture technology that Baxter is using to produce the influenza H5N1 vaccine that is currently in the clinic. This system can potentially be used for a variety of other vaccines against viral pathogens.

The facility is built to GMP and BSL-3 standards, allowing it to work with wild viruses. Initial cell scale-up is processed in roller bottles, followed by bioreactors at the 150 L and 6000 L scale using microcarriers. Processes are automated to the extent possible, including robots to handle some of the roller bottle manipulations and closed systems with automated cleaning and sterilizing for the bioreactor and other downstream processing equipment. There is some use of disposable equipment, such as media bags, but most of the equipment is fixed in place.

Virus propagation is accomplished in 2 days with wild strains rather than egg-adapted strains, and downstream processing includes ultracentrifugation, filtration, and concentration. There are two inactivation steps, the first using formaldehyde and the second using ultraviolet (UV) irradiation. The process to scale up

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\(^5\) The GAVI Alliance is a public-private partnership launched in 2000, dedicated to improving child health in the poorest countries by extending the reach and quality of immunization coverage and strengthening local health services. Its partners include UNICEF, WHO, the Gates Foundation, the World Bank, developing country governments, donor country governments, the vaccine industry, civil society groups, and research and technical health institutes (http://www.gavialliance.org/).
the cells to the 6000L reactor, infect, and process the virus takes 11–12 weeks. The final vaccine is a whole-virus, inactivated preparation.

Phase I/II clinical studies with the H5N1 vaccine indicate that the safety is equivalent to vaccines produced in interpandemic years, and the presence of whole virus in the vaccine offers the potential of providing a more broad immunity than some of the vaccines that contain only the surface proteins (HA and NA) of the virus. Baxter is also working with U.S. government agencies and other partners to provide the cell-culture-based vaccine for further clinical testing in the United States.

In reviewing other technologies for potential enhancement of the Vero-based system, our hosts noted that disposable bioreactors at this point are not well suited for microcarrier systems. There is also the issue of waste disposal when working with wild viruses in these large, disposable culture systems. Lab–on-a-chip systems to aid in small-scale optimization studies can only be used when assays are available that can detect very small quantities of antigen. The standard flu potency assay that is currently used requires too much material.

Other Capabilities

The WTEC panel toured the Orth facility; our tour revealed that this location has R&D, development, and pilot-scale facilities in close proximity to production areas. Testing capability is also present on-site. This offers an ideal environment for developing and scaling-up new products.

A second Baxter platform is the Chicken Embryo Cell Aggregate System. Baxter researchers have worked out a means of aseptically collecting the embryos from the eggs and processing them to cell aggregates of uniform size in a closed automated system. The aggregates are ready for viral infection on the day of preparation. The TBE vaccine is produced using this system, which has potential for other products as well.

SUMMARY AND CONCLUSIONS

Baxter is the first company to clinically test a cell-culture-based H5N1 vaccine; results thus far are promising. This technology has the potential for allowing more rapid vaccine development in response to an influenza pandemic and to improve the overall process for making influenza vaccines.

Baxter has two platforms for vaccine manufacturing: Vero cells and chick cell aggregates. Because these systems are capable of being used for more than one type of vaccine, they offer a means of more rapid vaccine development against pathogens that can be grouped in these systems.

Baxter executives stressed that the economic climate is not optimal for innovation in the vaccine development area. They believe that governments and society need to be educated on the true value that these preventive vaccine products provide to the healthcare system so that innovation and improvements can be appropriately financed.

REFERENCES


Appendix B. Site Reports

Site: DECHEMA (Society for Chemical Engineering and Biotechnology)
Gesellschaft für Chemische Technik und Biotechnologie e.V.
Theodor-Heuss-Allee 25
D-60486 Frankfurt am Main, Germany
http://www.dechema.de

Date Visited: 28 February, 2007

WTEC Attendees: J. Bielitzki (report author), M. Ritchey, T. Leighton, H. Ali

Hosts: Dr.–Ing. Alexis Michael Bazzanella
Research and Project Coordination, DECHEMA
Tel: +49/ (0)69 75 64-343; Fax: +49/ (0) 69 75 64-117
Email: bazzanella@dechema.de

Dr. Thomas R. Dietrich
CEO, Mikroglas Chemtech GmbH
Galileo-Galilei-Str. 28
55129 Mainz, Germany
Tel: +49-61 31/55 55 0-50; Fax: +49-61 31/55 55 0-52
Email: t.dietrich@mikrogal.com
http://www.mikroglas.com

Dr. Reinhard Ditz
New Technologies Research and Development
Performance and Life Science Chemicals
Merck KGaA-Germany
Frankfurter Str. 250-64293 Darmstadt, Germany
Tel: +49 6151 72 2017; Fax: 49 6151 72912017
Email: reinhard.ditz@merck.de

Dr. Hanns Wurziger
Head of Department, Global Preclinical R&D – Medicinal Chemistry DA
Merck KGaA-Germany
Frankfurter Str. 250-64293 Darmstadt, Germany
Tel: +49 6151 72-7681; Fax: 49 6151 72-3129
Email: hanns.wurziger@merck.de

BACKGROUND
This site report covers a meeting between WTEC panelists and representatives of DECHEMA, Mikroglas, and Merck that took place at DECHEMA in Frankfurt. It was coordinated through the efforts of Dr. Alexis Bazzanella of DECHEMA and Dr. Thomas Dietrich of Mikroglas. Drs. Reinhard Ditz and Hanns Wurziger represented Merck KGaA-Germany.

DECHEMA (the Society for Chemical Engineering and Biotechnology) is a German nonprofit organization founded in 1926, located in Frankfurt on Main. The society is composed of 5000 private and institutional members with interest in chemical engineering, biotechnology, and environmental protection. The organization provides an interface between engineering, chemistry, and biology.

Mikroglas Chemtech GmbH is located in Mainz. Mikroglas is a spin-off company emerging from the Institute for Microtechnology Mainz (IMM) in 1996. Its primary focus has been on microreactor technology and three-dimensional microfabrication and MEMs processes. The CEO Dr. Dietrich has been instrumental in developing a consortium of organizations working in microreactors and microprocess engineering.

That consortium, MicroChemTec (http://www.microchemtec.de/), is comprised of 40 industrial groups. MicroChemTec has a catalog with more than 60 components that can be interfaced and combined to explore new concepts in microprocess engineering. Through these efforts the participating organizations have agreed
to certain standard methodologies and interfaces for their products that enable broader application of all microprocessing technologies. The ability to interface technologies from multiple manufacturers into a functional and integrated demonstration or pilot tests strengthens the industrial position of all of the stakeholders. Through cooperation this consortium has made the utilization of its members’ products cost-effective and broadly available while maintaining and respecting each player’s position in the overall market.

Merck KGaA is a leading global producer of pharmaceutical chemicals through Merck Serono. Its therapeutic products focus on five core areas: (1) reproductive health with an emphasis on infertility, (2) multiple sclerosis, (3) dermatology with psoriasis as a target for intervention, (4) growth and metabolism with a concentration on hormonal interventions, and (5) wasting syndromes and evolving therapeutic areas such as rheumatoid arthritis, osteoarthritis, systemic lupus, non-Hodgkin’s lymphoma, T cell lymphoma, breast and prostate cancer, acute myeloid leukemia, and hepatitis C. Other areas for Merck Serono are women’s health and hormonal therapies, oncology, cardio metabolic care, generics, and over-the-counter medications. Merck KGaA Darmstadt focuses on chemical production in the areas of liquid crystal displays, performance and life sciences, and new release chemistry. Within the performance and life science areas it has three key areas: (1) laboratory services that provide mobile analysis, reagents, and chemical databases; (2) life sciences solutions with an emphasis on products for skin care and sunscreen; and (2) pigments used by a broad cross-section of industry from plastics to autos.

DISCUSSION OF ISSUES RELATED TO VACCINE MANUFACTURING

WTEC panelists had a significant discussion with our hosts on the issues surrounding the incorporation of new technologies into the production of pharmaceutical chemicals. Dr. Ditz indicated that innovation is hampered by the regulatory process that requires significant revalidation of all processes that are new or validation of all downstream events arising from the insertion of new technologies. This seems to be a recurrent theme among industry representatives, who recognize the need for product safety and validated production methods on the one hand but on the other hand feel that it is difficult to incorporate change or innovation.

Another clear area of concern is the lack of interaction between the European Union and its counterparts in both the United States and Canada. There was a suggestion that collaborating on developing programs of mutual benefit might move progress in the area forward at a faster rate and not result in unnecessary duplication of effort or controversy over intellectual property.

Dr. Wurziger discussed the applications of microprocessing and modular components within Merck, concluding that company management sees significant benefit to doing process development on a micro level and then scaling afterwards. His second conclusion is that it is possible to do process design in less time and achieve the desired results.

Dr. Dietrich commented on the benefits that MicroChemTec has experienced as a result of collaboration. None of the individual companies in the consortium have a full catalog of all the components offered by the group, but through cooperation and the development of standard interfaces, a concept of modularity has evolved. This type of collaboration has indicated the need for a systems approach in process development. The end users of the components can in fact achieve both efficiency and economy by scaling and the rational
application of systems engineering. Such collaboration provides an opportunity for process validation to occur at both the modular and integrated level, and expenses can be shared by the participants to reduce costs. Integration of components from multiple producers also facilitates the development of standardized metrics for both performance and outcomes. A comment was made that funding is necessary for projects moving from development to commercialization. Many products die in this area because they are unable to establish proof of principle at this stage due to market competition and the inability to enter into the market with a modular product.

**ACTION ITEMS**

The proactive and innovative approach towards R&D strategies in vaccine manufacturing presented by the WTEC panel should be complemented by a joint R&D initiative between the United States, Canada, and Europe, reflecting the global relevance of the subject.

In parallel to a United States-Canada initiative, there is a EU Framework Programme 7 (FP7) joint call by the Health Technology Platform of the European Commission (TP1-Health) and the working group for Nanoscience, Nanotechnology, Materials, and Processes (TP4-NNMP) on “Innovative system-based development strategies for flexible vaccine manufacturing to effectively combat a pandemic accident.”

The MicroChemTec industry platform will propose this concept via the European Technology Platform for Sustainable Chemistry (SusChem) for submission into the FP7 planning schedule, emphasizing the need for an integrated R&D effort between Europe, the United States, and Canada in this matter.

In case this approach will be perceived positively, a strategic concept for cooperation and a first draft of a joint call, including objectives, goals, time line, and resources will be generated by closely interacting teams in Europe, the United States, and Canada. Such planning activities should already involve concepts for involvement of the regulatory authorities at the earliest possible stages of individual projects.

**SUMMARY AND CONCLUSIONS**

Dr. Wurziger summed up the reasons why a company might invest in microreactor technology with the following observations. In microreactor systems the surface-to-volume ratio is dramatically increased; heat transfer is efficient with improved control, increased selectivity, and increased yields; the stationary volume is small, thereby increasing safety and reducing the amount of material needed for the reaction; and the system has defined flow characteristics. His assessment was that it is cost-effective to do pilot and development work at this scale.

The efforts to incorporate modularity into process engineering by members of the microreactor/microprocessing community appear exceptional. Such a consortium increases collaboration, standardizes development, improves validation efforts, and provides the user community with a plug-and-play framework for process engineering. There is a recurrent theme for wanting to improve collaboration between academic, industry, and governmental partners within the EU and the United States and Canada.

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6 SusChem was jointly established in 2004 by Cefic (the European Chemical Industry Council), EuropaBio (the European biotechnology industry association), and the European Commission to support sustainable chemistry research and development in Europe to align with policies and issues high on the European Union agenda and the activities of its national platform organizations (http://www.suschem.org/).
Site: European Centre for Disease Prevention and Control
Tomtebodaskolan Building
171 83 Stockholm, Sweden
http://www.ecdc.eu.int/

Date Visited: February 26, 2007


Host: Professor Johan Giesecke, MD, Chief Scientist, Head of Unit for Scientific Advice
Tel: +46 8-5860-1210
Email: johan.giesecke@ecdc.eu.int

BACKGROUND
The European Centre for Disease Prevention and Control (ECDC) was established by European Parliament and Council Regulation 851/2004 of 21 April 2004. It became operational on 26 May 2005. Its mission is to help strengthen Europe’s defenses against infectious diseases such as influenza, SARS, and HIV/AIDS. It has a small core staff but an extended network of partners across the EU and the EEA/EFTA member states. The ECDC works in partnership with national health protection bodies to strengthen and develop continent-wide disease surveillance and early warning systems. Through such collaboration the ECDC pools Europe’s health knowledge in order to develop authoritative scientific opinions on risks posed by new and emerging infectious diseases. The centre’s tasks include:

- Providing expert advice to member states and their scientific advisors on issues of control and prevention of human disease
- Enhancing the capacity of the European Community generally and member states individually to protect human health through the prevention and control of human disease
- Acting on its own initiative when outbreaks of contagious illnesses of unknown origin are threatening the Community
- Ensuring complementary and coherent action in the field of public health by bridging together the tasks and the responsibilities of the member states, the EU institutions, and the relevant international organizations

Professor Giesecke’s broad experience includes ten years as an infectious disease clinician; over ten years as a State epidemiologist and professor, and a sabbatical year as a short-term consultant at the Communicable Diseases Cluster of the World Health Organization (WHO), prior to his present appointment at ECDC. His current research includes outbreak analyses; risk factors for infection with TB, Helicobacter pylori, Campylobacter, HIV, and other agents; role of networks for epidemic spread; and long-term effects of acute infections. Professor Giesecke offered a summary of the ECDC activities and its position within the EU public health infrastructure.

ACTIVITIES AND FINDINGS
The ECDC maintains no formal research laboratories but rather operates three separate divisions: Scientific Advice, Surveillance, and Preparedness & Response. It currently employs approximately 120 people in its office in Stockholm, Sweden, but is projected to grow to 400 by around 2012. The ECDC serves in an advisory capacity to each EU country’s public health departments and infrastructures. Each EU country is responsible for its own public health decisions and policies (each with its own autonomous public health departments), making such activities highly decentralized. The ECDC makes recommendations to such units, but it has no decision-making authority. For example, there are distinct paediatric vaccine immunization schedules for each country within the EU. Each country also has its own stockpile policy for paediatric vaccines and pandemic influenza supplies; for example, countries are stocking supplies of Tamiflu for between 10% (Sweden) to 100% (France) of their respective populations. This is in contrast to animal health issues and policies, which are centralized within the EU.
Appendix B. Site Reports

Professor Giesecke noted that several countries within the EU have preorders in for pandemic influenza vaccines once/if they become available. He noted that some countries may impose export limitations on vaccines manufactured within their borders. This means that in the event of a pandemic influenza, if a vaccine becomes available, its distribution may be limited by specific EU country export policies. In the event of a pandemic influenza, the ECDC will investigate the spread of the disease, study the epidemiology of the outbreak, and maintain surveillance as it progresses. However, each EU country will maintain full autonomy in how it addresses the situation in real time.

ECDC administrators and scientists are interested in learning more and assessing/predicting how a pre-pandemic vaccine for H5N1 will provide protection against the actual pandemic influenza virus, when/if it strikes. Along these lines, they are interested in determining if regular annual influenza vaccination will mitigate the morbidity of a pandemic influenza. They want to ensure that communication channels are intact during a pandemic influenza outbreak and that they are reliable and accurate; the Internet will be a valuable resource during such an event. A goal of the ECDC is that different phases of the vaccine approval process are streamlined so that as much as 80% of this process will be preapproved once a pandemic influenza vaccine becomes available during a pandemic influenza outbreak. This licensure is done by the “sister” agency, the European Medicines Agency (EMEA).

Professor Giesecke noted that the use of adjuvants will be particularly useful to stretch the vaccine supply during pandemic events. Also, the move from egg-based to cell-based influenza vaccines will be an important step in securing the public health. He observed that at present, the distribution of pandemic influenza vaccines and other treatments and devices (e.g., Tamiflu, facemasks) is not well conceived, especially given the fact that people may be quarantined during such events. Ensuring that the EU countries have well-designed plans in place prior to a pandemic influenza outbreak is critical, given the large population of the EU and the high density of this population around urban centres.

Professor Giesecke referenced the only approved cell-culture seasonal influenza vaccine on the market, MedImmune, but suggested that its pricing was too high to sustain deep penetration around the world. His description of the ECDC’s responsibilities and functions suggested to the WTEC team that, to some extent, the ECDC has assumed some of the traditional responsibilities of the World Health Organization for Western Europe, thus allowing the WHO to focus more of its resources on Eastern Europe.

**SUMMARY AND CONCLUSIONS**

The WTEC visiting team’s meeting with Professor Giesecke provided a clear and candid picture of the ECDC, its position within the EU, and its role in dealing with a pandemic influenza outbreak.
Virtual Site: European Collection of Cell Cultures  
Centre for Emergency Preparedness and Response  
The Health Protection Agency  
Porton Down, Salisbury  
SP4 0JG Wilts UK  
http://www.ecacc.org.uk

Date Visited: February 27, 2007

WTEC Attendees: S. Drew (report author), C. Gay, S. Jacobson, G. Lewison

Hosts: Dr. David Lewis, Head, HPA Culture Collections  
Tel: +44 (0)1980 612661; Fax: +44 (0)1980 611315,  
Email: david.lewis@hpa.org.uk  
Mr. Neil Grumbridge, Head of Quality  
Dr. Nigel Allison, Operational Manager, Developmental Production  
Dr. Andrew Gorringe, Head of Meningococcal Vaccine Group  
Mr. Ross Cameron, Business Development Manager

BACKGROUND

While visiting Dr. Alan Hay at the National Institute for Medical Research (please see separate report), WTEC panelists were able to engage Dr. Lewis and his staff in a brief teleconference to learn about the activities and roles of the European Collection of Cell Cultures (ECCC) in the development of vaccines. Dr. Lewis began our discussions with a brief description of ECCC’s four culture collections servicing the needs of the UK Health Protection Agency (HPA) and the European Union (Viral, Cells, Bacterial, and Fungal). Both the virus and cell culture collections are located at Porton Down, which maintains GLP (good laboratory practice) laboratories at a maximum containment level of BSL-3. The scale of culture capability is up to 2 L for both cell cultures and for viral cultures. The laboratories at Porton Down currently have the capacity to culture attachment-dependent cells in closed system roller bottles (the Roller Cell 40 apparatus). Their researchers have investigated rocking-bag disposable bioreactors as a way of scaling-up suspension culture and currently utilize techniques other than tube-welders for aseptic connection between closed systems. They do not currently have a pilot plant to support larger quantities of cell and viral materials, although there is a conventional pilot plant located at the site in Porton Down that has served the microbial scale-up needs of researchers for many years.

RESEARCH AND DEVELOPMENT AT THE ECACC AND THE PORTON DOWN PILOT PLANT

Dr. Lewis introduced Neil Grumbridge (Head of Quality), Nigel Allison (Developmental Production), Andrew Gorringe (Vaccine Design and Head, N. meningitidis vaccine development), and Ross Cameron (Business Development) associated with activities of the Porton Down production facilities and pilot plant.

The Porton Down Production Centre is designed for bacterial or fungal fermentation using bioreactors up to 30 L and can operate at cGMP and a containment level of BSL-3. It is able to provide seed expansion, fermentation, isolation and purification, and inactivation of components for bacterial vaccines. The pilot plant is chartered as part of the Emergency Preparedness & Response plan of the United Kingdom and the European Union. A separate part of the pilot plant facility can be rated and approved for manufacture of recombinant DNA materials and nontoxic microbial products up to the 750 L scale. It can also support 3 to 5 steps of downstream purification for its fermentation products. The plant supports academic and government research on bacterial vaccines and also provides contract manufacturing capability for industry.

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7 The Porton Down Centre does operate GLP laboratories, but the virus and cell culture laboratories are not GLP-certificated. All Porton Down laboratories operate in compliance with ISO / EN 9001 2000.
Appendix B. Site Reports

WTEC panelists asked the HPA team members to identify critical needs or gaps, which if resolved, could significantly improve their ability to speed the development of vaccines and fulfill their missions for emergency preparedness and response. They identified an urgent need for a cGMP pilot capability for animal cells and viruses. They have developed a proposal for a new facility conceptualized at the BSL-3 level of containment and based on an open internal architecture structure meeting these requirements. The envisioned facility would also be able to accommodate single-use, disposable systems for cell culture, viral culture, and viral vaccine production. Suspension cultures will utilize disposable, rocking-bag bioreactors (they mentioned the WAVE Biotech, Inc., bioreactor design as a candidate). Movable, flexible-film containment devices that can act as classified-air isolators will be used to house modular, flexible (often disposable) equipment configured and handled as closed systems up through inactivation of infectious agents. The overall facility environment will be at Class 100,000, while the individual isolators may be configured to as low as Class 100, as needed.

In addition to the needed cGMP facility and the disposable equipment paradigm for rapid response, the team also identified the need for an effective scale-down model of pilot-scale systems to support rapid process development at modest cost. With regard to vastly accelerated development of a vaccine to meet a pandemic threat or rapidly emerging infectious threat, they suggested that their clients might need a more robust understanding that scale-up to manufacturing would inevitably change the process to some degree and affect the risk-benefit decision to proceed with vaccine clinical development.

SUMMARY AND CONCLUSIONS

The European Collection of Cell Cultures (ECCC) is a repository for cell cultures and viral cultures that may be important in understanding the molecular biology, culture, and control of these important agents. The ECCC uses very modern techniques to prepare small quantities of cells and viruses (limited to 2 L operating volume) in unoptimized media and culture conditions. Although its researchers have the expertise to undertake optimization and scale-up, they currently do not have an appropriate facility available.

The Centre for Emergency Preparedness and Response has a cGMP containment facility (BSL-3) appropriate for handling pathogenic microbial cultures, including fungal systems, up to the 30 L operating level. The WTEC team did not see the facility, but the equipment described and discussed appears to be of broadly capable conventional design.

REFERENCES

BACKGROUND

GlaxoSmithKline Biologicals (GSK) occupies the historic Rixensart site of Recherche et Industrie Thérapeutiques (RIT), a famous early producer of antibiotics and vaccines in Belgium, which gradually expanded through acquisitions and mergers to the company that is known today, GSK is one of the premier vaccine R&D and manufacturing organizations in Europe, supplying about 25% of the world’s vaccines.

RESEARCH AND DEVELOPMENT AT GLAXOSMITHKLINE BIOLOGICALS

Dr. Hanon reviewed for the WTEC visiting team the history of vaccine development along what he called the “Pasteurian” path of seeking to induce a protective circulating antibody response, as this is regarded as a good correlate of protective immunity. He moved on to today’s understanding that the remaining difficult challenges involve many different aspects of immune response to infectious agents. Our discussion centered on vaccine manufacturing in general, although, given the interests of our hosts, much of our discussion focused around influenza vaccines, both seasonal and pandemic. When WTEC panelists asked about the existence of an H5N1 vaccine, our hosts noted that H5N3 vaccines currently exist for chickens and have been administered successfully for 15 years. They noted that the relative proximity between the H5N3 and H5N1 viruses has resulted in cross-protection with this vaccine. The concept of cross-protection and its amplification and maintenance became a central theme in the remainder of our discussions.

Drs. Hanon and Van Mechelen noted that the challenges in developing and marketing new vaccines are both fundamental (basic understanding of the diseases) and practical (identifying tools to induce appropriate and sufficient immunity). They felt that the first challenge is best left to academics and research scientists, while the second is one that they are well positioned to tackle.

Dr. Hanon identified gaps in (1) knowledge of the mechanisms of disease progression (e.g., HIV, influenza, cancer), (2) the natural mechanisms of immunologic response and protection against these diseases, and (3) the correlates of protection that best point towards suitable treatments that induce the “correct” immune response to protect against the disease. The GSK approach is to focus on elucidation of these areas to support design of vaccines against the most important virulence factors so that innate and adaptive immune responses directly attack the agent as opposed to causing unwanted immune responses such as a systemic allergic response.

Dr. Hanon quoted a February 2007 news article that claimed that one in ten Belgians will miss work that week because of influenza as evidence of the urgency of developing and providing effective vaccines against seasonal influenza. In his discussion he clearly identified the need for effective vaccines against seasonal influenza to raise protective antibody to viral hemagglutinin antigen and indicated that this is considered a minimum standard for effective annual influenza vaccines by the regulatory agencies. He also pointed out that other correlates of protection are important in establishing effective duration and the potential for cross-protection against variant viral, or “drifted,” strains.
A 2006 paper by Bresson and colleagues concluded that the minimum hemagglutinin (HA) antigen in a nonadjuvanted H5N1 inactivated split virus vaccine must be at least 90 µg administered in two doses for protective immunogenicity (Bresson et al. 2006). A study by GSK concluded that the presence of an oil-in-water adjuvant added to a split antigen preparation reduced the amount of HA antigen required to 3.8 µg per dose (two doses). Dr. Hanon concluded that modern vaccines for influenza would benefit by addition of an effective adjuvant that could minimize the effective dose of conventional vaccine, thereby extending the current manufacturing capacity and number of vaccine doses available.

**Preparing for the Threat of an Influenza Pandemic**

A GlaxoSmithKline press release (2006) says, “We believe that vaccinating populations with the appropriate H5N1 vaccine will help educate the body's immune system and reduce expected morbidity and mortality associated with a pandemic.” GSK has been closely involved with developing strategies to meet the challenge of a pandemic since the late 1990s. Dr. Hanon described the “mock-up” filing procedure defined by the EMEA in the late 1990s by which a manufacturer could preestablish the safety and efficacy of a platform for the preparation of potential pandemic influenza vaccines. The EMEA established updated criteria for mock-up filing procedures early in 2007, and GSK has aligned its efforts and developments of its first generation whole virus alum-adjuvanted vaccine to meet the new criteria. The EMEA granted a Mock-up File License to GSK for this first generation whole virus alum-adjuvanted vaccine in December 2006.

This license allows GSK to have a preapproved manufacturing process in place in the event of a pandemic influenza outbreak. The only factor that is uncertain is the specific strain of the virus (e.g., H5N1 versus a H7 variant). Drs. Hanon and Van Mechelen noted that such a license would allow GSK to be able to manufacture and have available a pandemic influenza vaccine within 3–4 months from the onset of the outbreak, using a specific standardized manufacturing process that is consistent with the small set of (preconceived) strains used in the mock-up file. They also recognized the challenges that would need to produce and distribute such a vaccine across the world during such a time period. In this regard, GSK is now pursuing a more promising strategy allowing vaccination of people much earlier after pandemic onset using a pre-pandemic vaccine.

Our hosts discussed the advantages of a pre-pandemic strategy whereby advance production and stock-pilling of such pre-pandemic vaccine using currently circulating avian strains would make vaccine available at the onset of a pandemic influenza outbreak. They cited studies using mathematical models indicating that the time from the first pandemic influenza case until the peak number of cases could be as short as 85 days. They also stated that using a pre-pandemic vaccine that is 30% effective, the time to the peak of the pandemic could be significantly lengthened. The outcome would be to effectively delay the onset of the disease for a large number of people, which in turn would provide such individuals an opportunity to be vaccinated with a “tailored” pandemic vaccine that contains the actual pandemic strain.

The GSK pre-pandemic vaccine uses a “split” antigen preparation in which the core of the influenza virus is removed by detergent precipitation, leaving the envelope antigens, in this second generation approach. Again, the “split” antigen vaccine will be prepared from egg-based systems, but production of vaccine antigen from cell culture methods is being considered for longer-term development. The split H5 antigen preparation has been tested in humans (with and without adjuvant) and the adjuvanted formulation was found to be safe and effective in raising immune response in humans to a level that correlates with protection against annual influenza. More importantly, this pre-pandemic candidate vaccine has been shown to induce strong cross-clade immunity which is a prerequisite for pre-pandemic vaccine effectiveness. GSK submitted a pre-pandemic vaccine file to EMEA in late 2006 that is still under evaluation by the EMEA.

The pace of pandemic progression, even slowed by a pre-pandemic vaccine, would still tax even the most aggressive vaccination program. Under pandemic conditions in the United States, the population would need to be vaccinated at a rate of 10,000,000 people per week, or almost 17 people per second, nonstop, over a 60-day period. To make it feasible to vaccinate the U.S. population (or in fact, the population of any country) with either a pre-pandemic or pandemic vaccine, Drs. Hanon and Van Mechelen believed that adjuvants would be needed.

They also noted that the logistics of moving such a large quantity of vaccines and supplies would be a significant challenge. Some of the issues that they discussed were whether a centralized or decentralized stockpile strategy should be used, how the large number of syringes would be secured, and how stockpiling
would affect the current supply chain environment where low inventory policies are the rule rather than the exception. All of these issues need to be further discussed and settled upon prior to the pandemic outbreak, rather than during the crisis.

GSK has championed the concept of combining effective influenza antigens, manufactured in the conventional egg-based process, with powerful adjuvants such as its proprietary oil-in-water adjuvant system as a vaccine strategy for pandemic influenza. Adjuvants can boost the immunization response of vaccines, and hence, allow lower doses of vaccines to be used to achieve a protective immune response. The use of adjuvants would effectively extend the supply of vaccines and make it possible to produce a larger number of vaccine doses in a shorter period of time.

Drs. Hanon and Van Mechelen believed that the vaccine antigen and proprietary adjuvant system (which when combined constitute the “pre-pandemic vaccine”) should be stored separately, which might require both refrigeration and systematic rotation to avoid expiration of the products. Separate storage could allow for the possibility of changing the influenza antigen to better reflect the currently circulating influenza strain as it mutates over time (genetic drift) without wasting the adjuvant needed to create the vaccine supply. In fact, Dr. Hanon believes, this will be the only way in which control of a pandemic can be achieved, because the speed of disease progression will outstrip more conventional strategies for vaccine manufacture and distribution. He indicated that stockpiled pre-pandemic vaccines would certainly evolve over time as greater knowledge of the likely pandemic clades of influenza become apparent. The clinical strategy of starting with a pre-pandemic vaccine and introducing subsequent vaccines typed to the actual clinical infections is a moving target that will require strategies to expand and replenish serotypes for direct and cross-protection. GSK’s strategy will be to manufacture its vaccine antigens in Germany and Canada (at the recently acquired IDB facility).

Dr. Hanon told us that GSK’s investment in understanding and developing novel adjuvant systems over the last decade has paid off handsomely with the ability to tailor the combination of adjuvants and vaccines to meet a wide range of immunologic needs and capabilities in vaccine development. He indicated that GSK has at least 25 adjuvant systems under study and development.

We asked if GSK would consider influenza vaccines prepared in cell culture. Dr. Hanon indicated that he and his colleagues are studying that possibility under an HHS grant from the United States government. While optimistic about the long-term potential for cell-culture-based influenza vaccine, he suggested that the time track to approval and commercial development might be long. He also indicated that GSK has a second HHS grant to study the ability of safe and effective adjuvant systems to lower the minimum effective dose requirement for protective immunity.

GSK’s work toward an improved pre-pandemic vaccine presumes that the most appropriate vaccine will deliver an H5 immune response, will utilize an appropriate adjuvant system, will be manufactured as a killed, “split” virus antigen from egg-based systems, and will lead to a better understanding of an appropriate immune response to genetic drift through studies of the correlates of cross-protection. Dr. Hanon alerted us to a publication on influenza that GSK was to release the following week at a meeting of clinicians in Hong Kong that analyzed cross-protection in an animal model (ferrets). Dr. Van Mechelen indicated that GSK had developed the analytic tools to study T-cell response, B-cell response, and peptide fractions in human blood samples obtained from clinical studies. She believed that these tools will facilitate the development of correlates of protection and cross-protection. We asked if GSK is able to use these kinds of insights to facilitate the molecular design of effective and safe influenza vaccines on the basis of correlates or first principles; she indicated that this is still a goal for the future.

**SUMMARY AND CONCLUSIONS**

GSK’s strategy for addressing a potential influenza pandemic relies on broad cross-protection from an adjuvanted pre-pandemic vaccine, while manufacturing will use classical technology. The success of this approach will rely primarily on clinical strategy rather than acceleration of technology.

**Observations**

GSK appears to operate on and apply the following beliefs and principles:
• Adjuvants are critically important components of modern vaccines against many diseases. Our hosts cited the company’s development of the only effective adjuvanted malaria vaccine for humans.
• Building on “tried and true” conventional vaccines for seasonal influenza will guarantee at least a minimum protective efficacy in evolving future seasonal influenza vaccines.
• Distributed geographic stockpiling of pre-pandemic vaccine and adjuvant will be required in the event of a pandemic to meet local needs within the infectious course of the disease. Company strategy calls for the separate stockpiling of vaccine antigen and adjuvant so that new epidemiological insights can refresh and expand the inactivated, egg-based vaccines over time.
• Manufacturing initiatives and geographical configurations are needed that allow stockpiling of pre-pandemic vaccine without interruption of the capabilities to prepare annual influenza vaccines.
• Key limiting factors in the rate of advancement of vaccine design and implementation are (1) lack of molecular-scale knowledge of the progression of disease and (2) lack of molecular-scale knowledge of the response of the human immune system to infectious agents.
• Cell culture systems (avoiding the use of eggs) are under development for second-generation vaccines.

REFERENCES


Site: Health Protection Agency, Centre for Infections
61 Colindale Avenue
London NW9 5EQ UK
http://www.hpa.org.uk/infections/

Date Visited: February 27, 2007

WTEC Attendees: S. Jacobson (report author), S. Drew, C. Gay, G. Lewison

Host: Professor S.P. Borriello, Executive Director
Tel: +020 8327 6838/6810; Fax: + 020 8205 1630
Email: peter.borriello@hpa.org.uk

Dr. Mary Ramsay, Consultant Epidemiologist, Immunisation Department
Dr. Robert George, Director, Respiratory and Systemic Infections Reference Laboratory
Dr. Maria Zambon, Head, Respiratory Viruses Unit, Virus Reference Department

BACKGROUND
The Health Protection Agency (HPA) is an independent body in the United Kingdom responsible for protecting people’s health and wellbeing. The Agency was established in April 2003 to provide better protection and advice against infectious diseases and other dangers to health, including biological, chemical, and radiological hazards. The HPA, which merged with the National Radiological Protection Board in April 2005, serves the population of the United Kingdom, providing a comprehensive health protection service. The key functions of the HPA fall into five categories:

1. Identify and respond to health hazards and emergencies
2. Anticipate and prepare for emerging and future threats
3. Alert and advise the public and government on health protection
4. Provide specialist health protection service
5. Support others in their health protection roles

HPA provides specialist support to the public, government, the National Health Service (NHS), and a wide range of other organizations within the UK and abroad.

Professor Peter Borriello welcomed and hosted the WTEC team’s visit to the Health Protection Agency Centre for Infections. Professor Borriello serves as the Centre’s Executive Director. The Centre for Infections draws together the Division of Specialist Microbiology and the Communicable Diseases Surveillance Centre. Our team met with Professor Borriello and three of his staff members: Dr. Mary Ramsay, Dr. Robert George, and Dr. Maria Zambon.

ACTIVITIES AND FINDINGS
An important responsibility of the HPA is to advise the UK government on vaccine needs (i.e., front-end recommendations and assessment), and to monitor vaccine adverse effects (i.e., back-end analysis). The HPA does not handle the physical distribution and delivery of vaccines, including stockpiling strategies; this is managed under the purview of the UK Department of Health. Phase IV studies that involve the evaluation and monitoring of vaccines is a critical role that the HPA plays within the UK, including tracking for vaccine safety, immunogenicity, and efficacy. Our hosts described their operational structure as a “bottom-up” organization, where scientists provide the motivation, inspiration, and impetus to conduct studies and follow various lines of research. This tactical approach provides an environment where important and practical problems are addressed. At the same time, they felt that the limited amount of “top-down” management directive pressure had the potential to create strategic voids that could limit coordination work across agencies that could be of significant benefit to the nation. For example, they cited processes in measuring adverse side effects that could require coordination across multiple agencies that could be executed more effectively with additional “top-down” attention.
Our hosts noted that there was a well-established system in place to measure the efficacy of routine pediatric vaccines. They cited good communication with local public health workers and epidemiologists across the UK in addressing issues associated with such vaccines. (The MMR vaccine issues were noted in particular, given the controversies that have surrounded it and the need to assuage public concerns over its use and its potential side effects). They also noted the lack of such a system in place for the annual influenza vaccine, due in part to the challenges involved in collecting the necessary information.

The vaccine decision-making environment in the UK is highly centralized. The HPA is the primary body that sets and modifies the recommended childhood immunization schedule—although their actions on this schedule serve only as recommendations. Pediatric vaccines are administered and tracked through general practitioners (physicians), who are required to maintain (and report) extensive immunization records. Such a controlled environment facilitates the tracking of any consequences associated with immunization, including adverse side effects and herd immunity statistics. In the event of a pandemic influenza, the HPA is prepared to use such a system to formally monitor the progress of the disease through the population and to retrospectively evaluate the effectiveness of any vaccine that may have been used during the outbreak.

The UK vaccination/immunization environment is driven by both clinical efficacy/effectiveness and cost-benefit/effectiveness, including herd immunity considerations. Unless a new vaccine that enters the market can meet specific criteria within both these domains, it is unlikely to be recommended for adoption. This makes for strong scientific, clinical, and economic analysis criteria in the vaccine evaluation process. In the event of a pandemic influenza and an associated vaccine, data would not be available to make any reasonable cost-benefit/effectiveness analysis, although the urgency of the situation would make the qualitative assessment of its value sufficient to pass such criteria. The HPA’s efforts in performing both clinical efficacy and cost-benefit analyses position it as a leader in the European Union (EU).

At present, there is no EU-licensed live, attenuated influenza vaccine. Our hosts noted that even if there were such a product available in the UK, cost-benefit/effectiveness studies suggest that it would not be recommended. (This is in contrast to the United States, where such a product was recently added to the Recommended Childhood Immunization Schedule, and is due in great part to the wide discrepancy in healthcare and hospitalization costs between the UK and the United States.) They noted that each country within the EU has its own specific population characteristics and immunization issues/practices, which is why each EU country maintains its own unique childhood immunization schedule. (There are, however, numerous common guidelines and features.) Our hosts noted that the biggest perceived infectious disease threats within the UK are influenza (annual and pandemic), hospital-acquired infections, HIV, and tuberculosis.

Their size and potential market make it difficult to move vaccine manufacturers to fill the HPA’s specific needs and requirements. However, the HPA’s specific requirements for the meningococcal C vaccine were responded to and ultimately met by a vaccine manufacturer. HPA staff members work with and contribute to the European Centre for Disease Prevention and Control (ECDC), and Professor Borriello holds a seat on the ECDC Advisory Forum. The UK is limited in its ability to assess new vaccine, something that the ECDC is well-positioned to undertake. Our hosts noted that the regulatory process in the EU for vaccines is different from the process followed by the U.S. FDA; the UK uses the EU regulatory agency to determine which vaccines to consider using.

The HPA group recognizes that delivering a pandemic influenza vaccine in the UK will pose significant logistical challenges (as would be the case in many countries). Our hosts suggested that the EU regulatory agency and the vaccine manufacturers must work together more harmoniously in the future—particularly in the event of a pandemic. They stressed the need for better communication across all stakeholder groups, including the World Health Organization, and observed that it may require a “top-down” effort within the UK to optimally coordinate local efforts. They did feel, however, that many of the regulatory process obstacles can and will be overcome in the event of a pandemic influenza. Their chief concern was the efficacy of a pandemic influenza vaccine and its ability to safely achieve protective immunity. They noted that the UK Department of Health has plans in place in the event of a pandemic influenza, although such plans are not yet being disseminated for public consumption. Under consideration are issues such as how many doses of such a vaccine will be needed, the order in which a vaccine will be administered, how a sickening population will be managed, monitoring the rate of such events, and the appearance and management of counterfeit drug treatments and vaccines. Our hosts underscored the important role of
effective communication with the public, particularly when misinformation on vaccines and disease outbreaks is reported in the press.

**SUMMARY AND CONCLUSIONS**

Our meeting with Professor Borriello and his staff clarified the role of the HPA within the UK, and how its services will be critical during a pandemic influenza outbreak. Their mission in support of public health within the UK will be vital during such an event.

**REFERENCES**

Appendix B. Site Reports

Site: Institut für Mikrotechnik Mainz GmbH (IMM)
Carl-Zeiss-Strasse 18-20
55129 Mainz Germany
Tel: +49 6131/990-0
Fax: +49 6131/990-205

Date Visited: 26 February, 2007

WTEC Attendees: J. Bielitzki (report author), M. Ritchey, T. Leighton, H. Ali

Hosts: Prof. Dr. Volker Hessel
Vice Director R&D
Head of Chemical Process Technology Department
Tel: +49 61 31 / 990-450; Fax: 49 61 31 / 990-305
Email: hessel@imm-mainz.de

Technische Universität Eindhoven
Department of Chemical Engineering and Chemistry
Laboratory of Chemical Reactor Engineering
Den Dolech 2, Helix, STW 1.41
P.O. Box 513
5600 MB Eindhoven, The Netherlands
Tel: +31 40 247 4959/2850; Fax: +31 40 244 6653
Email: v.hessel@tue.nl

Dr. Klaus Stefan Drese
Head of Fluidics and Simulation Department
Tel: +49 61 31 / 990-170; Fax: +49 61 31 / 990-205
Email: drese@imm-mainz.de

BACKGROUND
The Institut für Mikrotechnik Mainz GmbH (IMM) was founded in 1990 by the Rhineland-Palatinate Ministry of Economics as an interface organization to develop microdevices and microprocessing systems to support a broad spectrum of commercial activities. Recently, IMM has been moved under Germany’s Ministry of Science; it is a nonprofit organization receiving state support. Its activities have focused on meeting the needs of the chemical and pharmaceutical communities, as well as those for personal care, food industries, energy production, biotechnology, analytics, diagnostics, medical technology and sensor development. IMM employs an interdisciplinary approach to meeting the needs of its customers, focusing its research efforts on customer requirements and specific production requirements.

ACTIVITIES AND FINDINGS
IMM management believes that market pull must drive process engineering by providing cost-effective methods to increase yield and efficiency. As presented by Prof. Hessel, one of IMM’s core platform technologies is the evaluation of fine-chemical reactions, mixing, and final analysis; future activities will include bulk-chemical reactions. There is a strong emphasis on the insertion of the institute’s technologies into inline processing systems. Its product line includes mixers, reactors, heat exchangers, and complete laboratory and pilot plants (e.g., see Figure B.2). It provides services in mixing and reaction engineering, plant engineering and process automation, and heterogeneous catalysis and fuel processing.
Microprocessing components can be used both for process development and optimization at laboratory scale and for chemical production up to the bulk scale. There is potential and evidence for eliminating steps such as producing pilot-scale components and thus achieving a faster time to market. Chemical production can be intensified by using microreactors where the reaction environment, temperature, pressure, catalysis, and other factors can be controlled more effectively than in larger reaction vessels. Microreactor technology offers smaller reactor footprints at high process intensification, e.g., with orders of magnitude improvement in space-time yield. The concept of modularity in an integrated system allows for rapid adaptation and flexibility in the chemical process. Microcomponents are available for thermal control, mixing, heat exchange, chemical reaction, and catalysis.

A second core focus area relevant to vaccine manufacturing, presented by Dr. Drese, is laboratory–on-a-chip (Figure B.3). Microfluidic systems can be produced with embedded sampling and sensing capabilities to make the analytical portions of manufacture another inline process. A variety of microfluidic capabilities can be integrated into a single platform. IMM’s lab-on-a-chip incorporates modules designed for specific tasks but features fluidic control and valving, metering and mixing, amplification, and detection that is compatible with existing laboratory equipment and comes complete with computerized automation. The microreactor leads to continuous process technologies. The incorporation of microfluidic analytic systems leads to inline feedback loops for rapid adjustment of the reaction chambers. The core concept is to provide an interactive format that incorporates a variety of integrated processes. By using CAD/CAM technologies, designs can be developed without prototyping and then customized to meet the exact needs of the customer or end use. The plug-and-play format provides the flexibility needed for evaluating inline processes.

In the IMM approach, process flow will ultimately change the philosophy of operation. Modularity becomes normative for inline processes requiring standardization of modules and interfaces. Modules can be evaluated and validated prior to incorporation into the integrated process, allowing innovation and new methods to become routine. The chemical industry is pulling development in terms of cost-effective methodologies. Insertion of new technologies is limited by the replacement schedule and validation process. Modifications to methodologies are limited by costs and the impact on product intensification.
Manufacturing systems for vaccines must be scalable and validated. Production systems must be product-specific, requiring huge monetary investments in physical plants, equipment, and nonvariable processes. Validation itself makes vaccine manufacturing a rigid process having little flexibility to optimize beyond prescribed limits. Meanwhile, the bulk chemistry community is finding that modular design, especially in the pilot plant, provides significant benefit when scaling systems for commercialization.

Our visit to the Institut für Mikrotechnik Mainz provided the WTEC panel exposure to both practical concepts and new technologies that may be of benefit to accelerate and add new flexibility to vaccine manufacturing processes. Among the clear opportunities is to evaluate how or if microreactor technology may be incorporated into basic bioreactor technology. In the chemical arena, reaction rates can be accelerated by controlling the parameters of the reaction vessel. Can similar control systems improve fermentation and protein synthesis? Can the reactor environment be optimized to have a two-order-of-magnitude improvement in immunogen production beyond the current fermentation and roller bottle systems? A key rate-limiting step in vaccine production is the down time waiting for essential analyses to be completed to comply with validation requirements. By incorporating prefabricated and prevalidated microfluidic systems into the processing line, can the time required for analysis be reduced? IMM’s systems suggest that the fabrication of such essential test systems can be automated in vaccine manufacturing processes and serve both for validation and for continuous process monitoring with feedback to maintain optimal production.

SUMMARY AND CONCLUSIONS

Microreactor technology has significant promise to aid vaccine manufacturing, especially at the pilot developmental stage when basic synthetic systems are being evaluated. The modular and flexible designs provide significant benefit; by isolating and controlling hazardous materials in the system, reactions may occur in a solvent-free environment at increased concentrations. Microreactors provide the possibility of reactions at higher temperature and pressure, instant mixing of reagents and reactants, stable intermediates, and tight control of both exothermic and endothermic reactions in the system. Many of these concepts can be applied to bioreactor environments where cell growth and the production of immunogens might be optimized. The underlying philosophy in the microreactor community seems to be that the system should enable the chemistry rather than the needing to subdue the chemistry around the reaction vessel. Microfluidic concepts applied to lab-on-a-chip offer the possibility that routine and required analytic testing may be
incorporated into the inline process with intact feedback loops. These test systems can be lot-produced and fabricated in a validated system to provide a plug-and-play laboratory, thereby reducing production time while meeting the QA/QC needs of the production system.

REFERENCES


Appendix B. Site Reports

Site: Institute for Animal Health
Compton Laboratory
Newbury, Berkshire
RG20 7NN UK
http://www.iah.bbsrc.ac.uk/

Date Visited: February 28, 2007

WTEC Attendees: S. Drew (report author), C. Gay, S. Jacobson, G. Lewison

Hosts:
Professor Martin Shirley, Director of the IAH Compton Laboratory
Tel: +44 (0)1635 578411; Central Fax: +44 (0)1635 577237
Email: martin.shirley@bbsrc.ac.uk

Dr. Geraldine Taylor, Division of Immunology & Pathology
Tel: +44 (0) 1635 577259
Email: Geraldine.taylor@bbsrc.ac.uk

Dr. Colin Butter, Head, Avian Viral Immunology Group
Tel: +44 (0) 1635 577258
Email: colin.butter@bbsrc.ac.uk

BACKGROUND

The Institute for Animal Health (IAH) is a world-leading center of excellence, and the major center in the UK for research on infectious diseases of livestock. It has two sites, located at Compton in Berkshire and Pirbright in Surrey. Until 1 April 2007 it also included the Neuropathogenesis Unit in Edinburgh. The IAH is one of seven research institutes sponsored by the Biotechnology and Biological Sciences Research Council.

The mission of the IAH is “to deliver high quality fundamental, strategic and applied science into infectious animal disease and, from that knowledge, to advance veterinary and medical science, enhance the sustainability of livestock farming, improve animal welfare, safeguard the supply and safety of food, and protect public health and the environment” (http://www.iah.bbsrc.ac.uk/info/introduction.htm).

Professor Martin Shirley was appointed Director of the IAH Compton Laboratory in July 2006. He is an authority on the parasite *Eimeria* that causes the devastating and economically important disease coccidiosis in poultry. His research was instrumental in the development of Paracox vaccine (with Schering-Plough Animal Health, Ltd.); ~800 million doses of the vaccine are now sold annually worldwide. In addition to his research at IAH, Dr. Shirley is Visiting Professor in the Department of Veterinary Parasitology at the University of Liverpool. He has strong links with the UK and international poultry industries. He won the Research Medal of the Royal Agricultural Society of England in 2004.

RESEARCH AND DEVELOPMENT AT THE INSTITUTE FOR ANIMAL HEALTH

Professor Shirley and Drs. Taylor and Butter described the broad programs of the IAH in establishing animal health and controlling the spread of infectious disease. They noted that the IAH is charged with fundamental, strategic, and applied research. Its programs generally have a long time horizon and a strong basic research component with the goal of establishing an independent, expert assessment of animal health issues. In this capacity, its scientists interact closely with industry to share their expertise and visions for future programs for animal health. They expected scientists from Intervet to visit the Laboratory later on the day we visited, with visits scheduled from Pfizer on the next day and Elanco later in the week. They operate a Technology Transfer Office to facilitate the migration of new scientific opportunities from the laboratory bench to the commercial market. In this environment, market economics exert a strong controlling influence on commercial development of vaccines because the margins are so small for the animal food industry.

Preparing for the Threat of an Influenza Pandemic

Most of the WTEC panel’s discussion with our hosts at IAH centered on their work with viral diseases of animals, particularly avian species. They identified a broad range of programs that include understanding
animal system immune response, infectious agents such as influenza, and complex host-pathogen interactions in large populations. Several initiatives aim to better understand animal response to viral infection:

- Discovery of unique mechanisms of antigen processing and presentation in chickens, fundamentally important in vaccine design
- Generation of Major Histocompatibility Complex tetramers in three veterinary species, enabling precise analyses of T cell responses
- Development of methods to study isolated dendritic cells from cattle and chickens
- Participation in consortia for chicken genome sequencing and SNP (single nucleotide polymorphism) identification
- Leading the identification and functional study of avian cytokines and chemokines
- Identification and characterization of other molecules that may regulate responses

Dr Butter framed the challenges of developing an effective influenza vaccine for chickens. Each year ~40 billion chickens enter the human food chain system, the majority of which are meat birds whose total life span is 6 weeks. An effective vaccine in the prevention and control of avian influenza must generate a protective immune response very rapidly and be manufactured in a sterile format. The IAH has developed a recombinant Fowl Poxvirus (FPV) attenuated live virus vaccine. Our hosts shared their thinking on vaccine design. From their work with FPV and turkey herpes virus (HVT) they conclude that live virus vectors (attenuated, recombinant) are able to elicit rapid immune response. However, the degree to which one can expect cross-protection in the event of year-to-year genetic drift is unclear. Their data also suggest that a prime vaccination and a subsequent boost injection may be necessary to elicit cytotoxic T-cell lymphocyte involvement with broadened cross-protection. Further, they believe that adjuvants, including cytokines, will be important in establishing cross-protection. They quickly said that “depot” adjuvants such as alum would be acceptable, but that oil-in-water adjuvants would probably not be acceptable for broiler birds because they might trigger an “unfit for human consumption” ruling. (This was the first time that WTEC panelists had heard of this possible interference with the promising adjuvanted-attenuated-live-virus strategy and may signal an interface point for regulatory law and scientific development.)

Drs. Butter and Taylor briefly described their work with a prime-boost strategy in which the egg receives a prime with injection of an attenuated live virus followed by a booster with either live virus, DNA, or antigen. We asked if DNA could be used as the primer and were told that the half-life of DNA in the egg is short, and that this creates difficulties that are currently being addressed.

A brief discussion was held on the importance of developing challenge models for animal and human zoonotic diseases. Dr. Taylor identified the RSV (respiratory syncytial virus) challenge system in cattle and also told us of IAH’s collaboration with the Jenner Institute at Oxford on a challenge model for bovine TB (See the University of Oxford site report on our visit with Professor Adrian Hill).

**SUMMARY AND CONCLUSIONS**

Vaccines that recruit the chicken immune system rapidly enough to confer protection against a potential pandemic avian influenza of unknown serotype (1) will have to rely on cross-protection from preexisting influenza vaccines, (2) will likely require a prime-boost vaccination strategy and (3) may also require an adjuvant strategy to broaden cross-protection.

The logistical challenges of delivering a vaccine to 80 billion birds per year in response to an emerging avian pandemic influenza are daunting.

The development of challenge models in birds for human:avian zoonotic diseases is needed.
Appendix B. Site Reports

Site: Intervet and Nobilon International
Part of Schering-Plough (as of November 2007)
Wim de Koerverstraat 35
PO Box 31, 5830 AA
Boxmeer, The Netherlands
http://www.intervet.com

Date Visited: March 2, 2007

WTEC Attendees: Cyril Gay (report author), S. Drew, S. Jacobson, G. Lewison

Hosts:
- Dr. Danny Goovaerts, Director, R&D, Intervet International bv
  Tel: +31 485 58 7727; Email: danny.goovaerts@intervet.com
- Dr. Helmut Finkler, Director, Biologicals, Global Manufacturing, Intervet International
  Tel: +31 485 58 7279; Email: helmut.finkler@intervet.com
- Dr. Paul van Aarle, Director, Institutional Sales, Intervet International bv
  Tel: +31 485 58 5228; Email: paul.vanaarle@intervet.com
- Dr. Adriaan van Loon, Director, Manufacturing, Nobilon International bv
  P.O. Box 320, 5830 AH
  Tel: +31 (0) 485 585449; Email: adreaan.vanloon@nobilonvaccines.com

BACKGROUND

Schering-Plough owns as of November 2007 three additional business units:
- Organon – human prescription drugs
- Intervet – veterinary vaccines and pharmaceuticals
- Nobilon – human vaccines

The WTEC team had discussions in Boxmeer with both Intervet and Nobilon representatives.

Intervet International

Intervet has 14 R&D sites in The Netherlands, the UK, and the United States (DE and KS); 19 manufacturing sites, 54 subsidiaries worldwide, and a distribution network covering more than 100 countries; all told, Intervet and its subsidiaries employ more than 5,200 people and operate in over 50 countries.

Product range: poultry, ruminants, swine, horse, companion animals, aquaculture (salmon).

Intervet vaccine trade names: poultry: Nobilis; ruminants: Bovilis; swine: Porcilis; equine: Equilis.

Intervet’s equine Strep E. was the first recombinant bacterial live vaccine approved in Europe. Nobilis influenza vaccines H9, H7, and H5 are egg-based and oil-adjuvanted. Veterinary vaccines in the U.S are made on SPF (specific pathogen-free) embryonated eggs.

Nobilon International

Nobilon International is a company created in 2002 to produce human vaccines and capitalize on the knowledge of Intervet and Organon. It has a state-of-the-art manufacturing facility able to produce both human and animal vaccines.

ACTIVITIES AND FINDINGS

Emergency Preparedness for Veterinary Vaccines

The issue of vaccine development for emergency preparedness was discussed by the WTEC panel and our hosts as follows:

Key Points
- Suitable vaccines may exist but are not always available in the regions where they are needed
• The research and development of vaccines for emerging diseases will require public funding
• Market mechanisms are essential for success
• A close dialogue with government agencies is needed
  – What is the government strategy for in-sourcing?
  – Vaccines outside the U.S available or in development?
  – Other companies, tender or bilateral agreements
  – Need to define costs, conditions, and efforts for development for the U.S
  – Agree on development plan
  – Firm commitment on both sides on the development and establishment of stocks

_Broad Issues_

• Costs of developing a new vaccine:
  – From scratch to dossier (EU): 5–10 years, €5–10 million
  – Less costly (and shorter) in the United States
  – Emergency development: at least > 2 years
  – Scaling-up and stocks can only be justified if there is a market
• Reaction time in vaccine production is dependent on capacity, demand, and lead time:
  – Live vaccines: 3–4 months
  – Inactivated vaccines: 6–8 months
  – Dependent on what is currently being produced in the facilities, clash with other products, emergency procedures, specific requirements (e.g., labeling, extra tests, stocks)
  – Dependent on the relative surge in demand as compared to monthly routine output
• Vaccines for emerging diseases
  – The regulatory framework is generally insufficient
  – Trade politics seriously decrease responsiveness

_Issues for Development of Emergency Vaccines_

• Research and new technology needs investment
• Development of existing technology needs investment
• More important are market mechanisms:
  – Funding for production and maintenance of antigen stock
  – Funding for production and maintenance of vaccine stock
  – Funding for vaccine production capacity: the cost of an idle facility
  – Biosecurity standards are essential for containment, license in the country of production, license in the country of destination
• Compliance with international standards

_Vaccine Issues Vis à Vis European Technology Platforms_

• Diseases occur regionally and vaccines are usually available on a regional basis because countries demand that vaccines be produced with strains isolated from their own territory, regardless of whether there is scientific merit for this demand; e.g., vaccines against H5N1.
• Gap analysis
  – Disease prioritization
  – Will vaccination be an option?
  – Are there licensed vaccines in the region?
  – Are there other vaccines in other region?
  – Does a suitable vaccine exist?
  – Is there sufficient scale of operations in the region?
Technologies

- Egg-based antigen production
- Fermentor antigen production
- Roller bottles: suitable for small and medium-size quantities, labor-intensive
- Suspension cultures: require facilities and know-how, closed systems, scale-up potential
- Baculovirus system: suitable for large scale, for subunit antigen
- Vector vaccines, chimera vaccines, delivery of subunit antigens
- Choice of technology should be made on efficacy and safety and not on convenience for manufacturer; i.e., the correct vaccine for a specific disease

European Vaccines for the United States

There are some very good vaccines that exist in the EU but not in the United States. There are several barriers for transferring European vaccines to the U.S.:

- Acceptance of the dossier
- Transmissible spongiform encephalopathy compliance data
- Requirements for additional data
- Acceptance of manufacturing facilities
- Legal issues (e.g., intellectual property)
- Contract conditions: lead-time, packaging, distribution
- Feasibility: what is the level of commitment from the U.S.?
- Financial analysis: can we justify the investment in time and effort?

Overview of Nobilon International BV

Nobilon International BV was created “to bridge the gap between the animal and human vaccines” in learning how to best bring forward vaccines for both.

- Focus on zoonotic diseases
- Integrated biotechnology company

The Potential Use of Veterinary facilities for the Production of Human Vaccines; Three Issues

6. Liability
7. Transfer of technology (method of production)
8. Complex downstream processing (required for split and subunit method of production)

For emergency vaccines, Nobilon would treat with chloroform to get rid of potential contaminants (e.g., enveloped viruses such as NewCastle Disease) and inactivate with BEI (binary ethyleneimine).

Intervet representatives stated that poultry vaccines are much more efficacious than human vaccines because they use whole virus inactivated vaccines (which can be produced on embryonated eggs or cell lines) and they use an oil adjuvant emulsion. This provides excellent immunity, stops transmission, and provides good cross-protection within the same HA subtype. Human vaccines use either (1) split method (get rid of core) or (2) subunit (HA), neither of which is as efficacious.

Nobilon representatives stated that a study reported by Baxter suggests that whole virus is efficacious and that an adjuvant is not required. This is a strategy that Nobilon intends on pursuing long term. It is also pursuing a modified live vaccine (MLV) strategy. Russia has been producing safe MLV flu vaccines for the last 30 years.
Appendix B. Site Reports

Short term, to receive authorization for an EU “mock dossier,” Nobilon will pursue the licensure of an influenza vaccine for seasonal flu.

SUMMARY AND CONCLUSIONS

Issues

• Emergency veterinary vaccines are needed
• Cost of developing a new vaccine is significant
• Reaction time in vaccine production is dependent on capacity and lead time
• Development of vaccines for emerging diseases is slowed by the lack of effective regulatory direction and trade policies
• Research on new technology needs investment
• Development of existing technology needs investment
• Market mechanisms are needed
• Veterinary production of human vaccines has pros and cons:
  – Liability issues
  – Technology transfer benefits
  – Complex downstream processing required for human vaccines is an issue

Key Points

• Cross-talk between animal and human medical science can dramatically accelerate the development of effective and safe vaccines in response to a pandemic threat.
• The combination of out-of-the-box thinking and integration on process design, facilities design, and construction (using platforms of conventional engineering) can dramatically accelerate the build-out of BSL-3 contained facilities for multiple products for both human and animal vaccines. Intervet/Nobilon’s overall approach includes
  – Full segregation from product to product
  – EMEA inspection and approval
  – Pharma inspection and approval
  – Relatively low cost
  – World-class integration of engineering disciplines and knowledge of animal scientists and human medical scientists
• Intervet (Emergency Vaccines)
  – Suitable vaccines may exist but are not available in the region where it is needed
  – The R&D of vaccines for emerging diseases will require public funding
  – Market mechanisms are essential for success
  – Need close dialogue with government agencies
• Nobilon
  – Focus on zoonotic diseases
  – Animal challenge models/clinical studies knowledge
  – Integrated biotechnology company
Appendix B. Site Reports

Site: Karolinska Institute and
The Swedish Institute of Infectious Disease Control
Smittskyddsanstaltet (SMI)
Stockholm, Sweden
P.O. Box 280
Nobels Väg 16 (Karolinska Institutet)
Nobels Väg 18 (SMI)
S-171 77 Stockholm, Sweden

Date Visited: February 26, 2007

WTEC Attendees: S. Drew (report author), C. Gay, S. Jacobson, G. Lewison

Host: Professor Peter Liljeström, Department of Microbiology, Cell and Tumor Biology
Tel: +46 8 457 25 50; Fax: +46 8 37 08 48
Email: peter.liljestrom@ki.se

BACKGROUND

The Karolinska Institute’s research programs in vaccine design and development focus primarily on viral infectious diseases and cancer. Its activities are focused on developing recombinant vaccines against infectious diseases and cancer. Its researchers are characterizing innate and acquired immune responses after vaccination with various types of vaccines. While many projects are basic in nature, a number of vaccines are now moving into clinical trials. The most central vaccine technology utilizes the alphavirus replicon system, which is based on an infectious clone of Semliki Forest virus (SFV). Professor Peter Liljeström is an internationally recognized expert in the use of alphaviruses as vectors for vaccine development. His study of the molecular mechanisms of SFV replication established the potential of alphaviruses to act as recombinant vectors for the delivery of vaccine antigens. Professor Liljeström heads the Innate and Adaptive Immunity Study Group for HIV (a European Union 6th Framework Programme for Research and Development) and heads the EuroVac Consortium studying HIV vaccine platforms (a 5th EU Framework Programme effort funded at €35 million).

Prof. Liljeström is also the Head of Vaccine Research at the Swedish Institute of Infectious Disease Control, Smittskyddsanstaltet (SMI). The SMI is an expert authority with a mission to monitor the epidemiology of infectious diseases among Swedish citizens and promote control and prevention of these diseases. It supports research in the medical community and the academic community and interfaces with local, regional, and central authorities that have operational or political responsibilities for infectious disease control. The nature of this support ranges from conducting basic scientific research into the mechanisms of infectious disease and development of countermeasures, to establishing the standards for validation of analytic methods, including both standard measurements of biological activity and complex immunological methods (Professor Liljeström had recently secured a yearly €1.3 million to support this effort). SMI efforts also include diagnostic support for certain clinical needs (e.g., hospital clinician requests for assistance). Professor Liljeström described a broad set of supporting programs that constitute the SMI functions and commented that the analytical support function is fully modern, citing chip-based automated screening as an example of just one program of continuing advancement. SMI also participates in Sweden’s science council of its National Food Administration, the Preparedness Council of the National Board of Health and Welfare, and the AIDS council of the National Institute of Public Health.

RESEARCH AT THE KAROLINSSKA INSTITUTE AND THE SMI

Professor Liljeström described his work on the design and development of vaccine platforms for infectious disease (mostly for treatment of viral infectious agents) and for treatment of cancers. Scientists at the Karolinska Institute have a rich history of contribution in these areas.

Professor Liljeström’s research groups study and develop vaccine candidates using several vector platforms, including poxviruses such as the commercial NYVAC (highly attenuated poxvirus-based) vaccine for rabies, adenoviruses, and recombinant plasmid DNA carrying target antigen gene(s) from infectious viral agents.
These labs are perhaps most famous for their work on alphaviruses as vectors for the delivery and presentation of antigens from infectious viral diseases. Professor Liljestrom's early work on nonreplicating variants of Semliki Forest virus have been instrumental in guiding the development of alphavirus vectors in the United States, including work at the National Institutes of Health, Alphavax, Inc., and Novartis (formerly Chiron, Inc.).

Professor Liljestrom summarized the advantages of alphaviruses as delivery and antigen presentation vectors:

- Broad applicability and flexibility across a wide range of disease targets
- Natural, direct targeting of the body’s immune system
- Strong, effective, and consistent antibody and cellular immune responses involving strong CD-8 response (similar to that observed with other togaviruses such as rubella)
- Induction of a strong innate immune response (IFN-alpha and Toll-like receptor 3)
- Nonreplicating system with demonstrated safety (NIH studies with Eastern Equine Encephalitis virus and Venezuelan Equine Encephalitis virus) in human trials and animal models
- Remains and replicates solely in the cytoplasm, alleviating concerns with the possibility of integration into nuclear chromosomes
- High levels of disease target gene expression
- Virtually absent preexisting antivector immunity in human populations
- No or limited inhibition by antivector immunity, thereby enabling vaccine recipients to be treated with the same system multiple times or as part of prime/boost regimens

Professor Liljestrom's early work led to the development of Semliki Forest virus variants that were constructed without some of the structural genes necessary for the replication of the virus and incorporation of its progeny into infectious viral particles (“helper” vectors). These constructs, incapable of active replication in mammalian cells but able to replicate through addition of the “helper” vectors, became the backbone of the alphavirus replicon vector for the delivery and presentation of recombinant vaccine antigens. Professor Liljestrom terms this vector a “suicide” vector because although replication of the recombinant RNA replicon, containing a recombinant vaccine antigen, will take place in stable mammalian cells lines (e.g., BHK or Vero cells) to which the “helper” vectors have been added, the virus-like particles (VLPs) or “replicons” that form containing the recombinant antigen gene are not able to replicate themselves in normal mammalian cells. Ways in which the helper factors might be added to the host mammalian cells for expansion of the recombinant replicons include electroporation, chemical treatment of the cells to alter their membrane permeability, or creation of stable cell lines containing the recombinant genes for the helper factors.

Recent studies in Professor Liljestrom’s laboratories have developed a DNA analog of the replication-deficient SFV RNA replicon that, provided with appropriate helper factors, can generate immunologically effective recombinant replicons. Such constructs can be tailored with promoters (e.g., the cytomegalovirus promoter) that can drive recombinant antigen production to very high levels.

WTEC panelists asked Professor Liljestrom what characteristics of a successful vaccine vector platform would be required to warrant progression to human clinical trials. Aside from the more general characteristics of safety and efficacy, more specific characteristics were

- Preclinical demonstration of safety
- Demonstration of nonintegration into the host genome
- An understanding of the tissue distribution of the vaccine vector
- Prediction of appropriate dosage in two different mammals
- An understanding of the replication of the platform (or its absence) in animals
- Prediction of immunological response in humans

He indicated that studies in his laboratories have shown that the DNA analog of the alphavirus replicon is eliminated from animals within 9 days. The sensitivity of the assay was one molecule of DNA (although sensitivity of the assay for the resulting RNA is at best 1000 molecules). Professor Liljestrom was quick to say that this does not truly predict the longevity of the DNA replicon.
When asked what would be some of the components of an “adequate” clinical response in humans, he said,

- Polyfunctional response of cytokines
- Strong CD4 response
- Strong CD8 response
- Immunologic response maintained for at least one year
- Circulating antibody at “significant” titers

With regard to alphaviruses and the other classical togavirus, rubella, both are powerful recruiters of innate immune response.

We asked Professor Liljeström what gaps, if filled, could allow more rapid development of safe and effective vaccine platforms for viral vaccine development.

- He quickly responded that funding to carry meritorious candidates to human clinical trial was badly needed, particularly for meritorious academic and government laboratory leads.
- He also lamented that the tools and models to identify the single most appropriate candidate to move forward are not currently adequate or available; that in many cases there are no adequate challenge models or validated surrogates of protective immunity.
- He identified protection of the intellectual property to be paramount to achieving successful technology transfers to industry partners.
- He identified the need for academic/government researchers to secure an industrial “partner” to act as an engine to move forward the development of downstream processes, scale-up, and clinical testing.

Our host viewed intellectual property protection reform as critical to allowing academic research centers and small industrial research centers to maintain control of their research output and to prevent intrusion on their discoveries by large vaccine manufacturers. Legal issues (e.g., intellectual property) and economics (e.g., human clinical trials), not microbiology or virology, are in his opinion the greatest obstacles to the development and introduction of new vaccines into the marketplace. Until these systematic issues are fixed, it is unlikely that many new vaccines that have their roots in academic research centers will reach the market. History bears out this disturbing trend. A crisis event, like a pandemic influenza, may be the best avenue to break down such systems and remove the associated obstacles. One possible solution is to position and empower government agencies to facilitate such transitions. The enormous public health value of vaccines is far too great to not rethink the current system and consider alternative legal and economic guidelines.

**Prediction of New Platforms for Vaccine Development**

Professor Liljeström suggested that the alphavirus replicon platform might be achievable without the two-step process of adding helper factors to the expansion host cells and then using the resultant replicons as the vaccine delivery platform. Such strategies were originally applied due to safety concerns with recombinant vectored vaccines; however, research has shown that the genes that are deleted may impact efficacy of the final vector more than safety. A prudent pathway to development is to add back the genes that were deleted one-by-one to determine the most important virulent genes for proper attenuation without compromising efficacy. The concept that he suggested is the direct molecular establishment of a permissive attenuation of one or more structural genes. In early studies, this concept of molecular design of specific attenuation led to a permissive construct with severely limited replication (on the order of 3 or 4 replication cycles) in animal systems. Productivity of the system was impressive at $10^{10}$ replicons per mammalian cell during expansion.

Professor Liljeström believes that some degree of redundancy will be necessary in truly effective vaccines for viral diseases. He described several hybrid systems in which the immune systems of test animals were primed with one platform and then boosted with another to take advantage of the special capabilities of different, but complementary platforms. For example, he noted that poxviruses are particularly adept at recruiting CD4 response and interferon involvement. Alphaviruses elicit strong CD8 response and very strong interferon response. In one example, an alphavirus prime followed by a pox boost elicited an immune response that was 50 times greater than either alone. In another example, an initial vaccination by DNA suggested a 40% T-cell response (poxvirus priming alone resulted in a 50% T-cell response). However, when the test animals were primed with DNA and subsequently boosted with the poxvirus, 100% T-cell response
was detected, and the immune response was maintained for 2 years. Interestingly, additional experiments suggested that priming and boosting in the reverse order of poxvirus and DNA was not effective.

Our host provided a very informative example of the importance of kinetics of immune response in a discussion of the cross-talk between innate immunity and adaptive immunity. He showed data of the kinetics of immune response for SFV comparing prevaccination to postvaccination data using ELISPOT assays. The rate of the immune response was very steep and rapid, up to a peak at seven days and a decline to undetectable levels by 11 days. On boost with SFV at day 42, the rise was rapid after a delay of 2 days to a peak at day 47 of 3000 spots per assay and a gradual decline to ~500 spots at day 53. Since the rates of rise and the duration of peak values can be quite variable depending on the vector platform, it could be easy to miss the clinical potential of a particular regimen by too little reliance on the kinetics of immune response. Professor Liljesthröm speculated further that a much better understanding of the cross-talk between innate and adaptive immunity could lead to the next generation of molecular-designed vaccines.

Professor Liljesthröm spoke to the critical need of developing animal challenge models to test experimental vaccine formulations and identify correlates of efficacy. Studies in comparative immunology may provide critical information on mechanisms of immune evasion and protective immunity. He described Louping Ill Virus disease of sheep as an animal challenge model characterized as a predictive model of immune response for tick-borne encephalitis, the human disease counterpart.

Professor Liljesthröm identified the need to combine prime/boost strategies with molecular adjuvants for the induction of specific innate immune response mechanisms to achieve the desired level of protective immunity for difficult chronic diseases such as HIV and tuberculosis. He believes that this is an area of study that will generate many opportunities for improved vaccines for cross-protection.

CREATION OF A GLOBAL HIV VACCINE ENTERPRISE (GATES FOUNDATION PROPOSAL)

Professor Liljesthröm provided a brief overview of the need for a global vaccine enterprise funded by the Gates foundation (see Klausner 2003; Zinkernagel et al. 2004):

- Recognition that a vaccine will be required to control one of the most devastating epidemics the world is confronting
- Acknowledgement of the growing interest in HIV vaccines from political leaders and funders, arising from the increasingly accepted public health need for a vaccine
- Recognition that development of an HIV vaccine is one of the most difficult challenges confronting biomedical research today, but that scientific progress is creating new opportunities that should be harnessed through better organization of global efforts
- Acknowledgement that confronting these challenges and harnessing these new opportunities require an effort of a magnitude and intensity without precedent in biomedical research
- The growing consensus that this effort will require novel strategies based on a more systematic, collaborative, focused, and output-based approach (the “Global Enterprise” concept)

He believes that elements of this model of objective-based research and development can overcome some of the rate-limiting steps on the path from discovery to the clinical deployment of vaccines for emerging and developed infectious threats. He suggested that this general model might be needed to achieve the most rapid response to emerging infectious threat such as a pandemic influenza. Professor Liljesthröm reviewed the following points from the Gates Foundation Program, focusing particular attention on “Elements of the Scientific Plan” (see next page).

**Accelerating AIDS Vaccine Development R&D Challenges**

- Addressing the scientific challenges impeding progress
- Prioritizing the most promising candidates
- Ensuring capacity for multiple efficacy trials in high-incidence areas
- Ensuring capacity for process development and manufacturing of the leading candidates
- Accelerating licensure in developing world
What Can the Enterprise do?

- Advance the fundamental science of HIV vaccinology
- Provide a coherent basis for an iterative approach to HIV vaccine development
- Create informatics processes to accelerate information sharing and communication
- Create and/or augment HIV vaccine advanced development and production capability

A Model to Accelerate Vaccine Research

- A network of coordinated HIV development centers should be created, each of which would develop specific vaccine approaches
- Vaccine design consortia should be created in order to enable novel concepts to be translated rapidly into products appropriate for clinical trials; these consortia would also have better chances to solve the major scientific challenges in HIV vaccine development
- New dedicated facilities to manufacture HIV candidate vaccines must be developed
- A collaborative network of laboratories that use standardized assays to evaluate immunogens and specimens from clinical trials should be built
- An expanded global clinical trials system must be put into place

Six Working Groups charged with the initial development of the scientific plan of the Enterprise:

- Vaccine discovery
- Product development
- Manufacturing
- Laboratory standardization
- Clinical trials capacity
- Regulatory issues/Intellectual Property

Elements of the Scientific Plan

1. Vaccine discovery
   - Characterization of early infections
   - Immune correlates of protection in monkey models
   - Multi-approach “envelope” design
   - Comparative evaluation of T-cell immunogens
2. Laboratory standardization
   - Comprehensive system for decision making, standardization, reagent production and distribution, assays development, quality assurance
   - Neutralization Serotype Discovery Program
3. Product development and manufacturing
   - Dedicated HIV vaccine bioprocess and analytical development group
   - Bioprocess facility (at a later stage)
4. Clinical trials capacity
   - Increasing quantity and quality of human resources
   - Expanding access to appropriate trial populations
   - Establishing sustainable research facilities to support trials.
5. Regulatory considerations
   - Harmonization and facilitation of regulatory approval
   - Regulatory capacity building (including ethics)
   - Scientific issues on regulatory decision-making
6. Intellectual property issues
– Establish standards and mechanisms to promote sharing of data and reagents
– Enabling intellectual property environment that facilitates HIV vaccine R&D

**SUMMARY AND CONCLUSIONS**

Under Professor Liljeström’s guidance, the Karolinska Institute’s and the SMI’s focus on design and development of safe, effective vaccines for viral infections continues its leadership in the international community. The WTEC visiting team reviewed compelling data showing the ability of alphavirus vectors to recruit both innate and adaptive immune response to recombinant antigens. The SFV alphavirus vector is relatively well characterized, is well tolerated in animals, is scaleable to at least moderate volumes (used to provide materials for candidate Phase I trial), and elicits robust immune response. Proof of efficacy for influenza awaits an effective animal challenge model or human trial.

We asked Professor Liljeström to help us to draw conclusions about the factors that might limit the rate at which the world could respond to the threat of pandemic influenza or another emerging infectious threat. The broad conclusions from our discussions follow:

**Issues**

- We do not have adequate challenge models for most diseases.
- The manufacturing yield of suicidal recombinant viral vectors is an issue. This is true for alphavirus and poxviruses.
- Regulatory requirements are important but will need to be streamlined to achieve response in time to address a truly urgent infectious agent threat.
- The current timeline for developing a new vaccine can be as much as 10 years in the laboratory followed by an additional 10 years to progress through clinical trials establishing safety and efficacy. This is an impossibly long path for development of a vaccine to respond to a pandemic threat.

**Key Conclusions and Areas for Further Research**

- The scientific and medical communities need correlates and/or surrogates of protective immunity.
- Funds are needed to support research and clinical trials. The cost of Phase III clinical materials is, on the average, €1 million, which is beyond the resources of most academic or governmental laboratories.
- Animal models for zoonotic diseases are needed to better establish safety and efficacy profiles before human trials begin.
- The communities need validated immune assays for use in clinical trials.
- Prime-boost strategies need to be combined with adjuvant technologies for the induction of specific innate immune response mechanisms.
- Attenuated alphavirus offers a potentially safe (preclinical) way to strongly recruit CD8 or cell-mediated immunity.
- Defined media for alphavirus cultivation will accelerate scale-up.

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Site: National Institute for Medical Research  
Virology Division, Hay Group  
The Ridgeway, Mill Hill  
London NW7 1AA, UK  
http://www.nimr.mrc.ac.uk/  
http://www.nimr.mrc.ac.uk/virology/hay/

Date Visited: February 27 2007

WTEC Attendees: C. Gay (report author), S. Drew, S. Jacobson, G. Lewison

Host: Dr. Alan Hay, Head, Influenza Virus Ion Channels Research Group, Virology Division  
Tel: +44-(0)20-8816 2141; Email: ahay@nimr.mrc.ac.uk

BACKGROUND
The National Institute for Medical Research (NIMR) is a large facility situated in rural Mill Hill, England, on the outskirts of London. It is mainly funded by the UK’s Medical Research Council, or MRC, and is its largest establishment and the only one designated as an “Institute.” The NIMR has four main research foci:

- Genetics and Development  
- Infections and Immunity  
- Neurosciences  
- Structural Biology

There are 18 divisions, over 200 scientists, and at least 200 other trained personnel, including postgraduate students. The NIMR’s annual research budget is £25 million. NIMR is one of four WHO Collaborating Centers that collaborate on influenza surveillance. NIMR isolated the first flu virus four decades ago. There is a move to establish other collaborating centers, not to focus on seasonal flu, but for pandemic strains with the specific objective of catching new and emerging strains as early as possible.

Dr. Hay, who heads the NIMR Virology Department group that focuses on influenza virus ion channels, hosted the WTEC visiting team. The objective of Dr. Hay’s group is to monitor viral changes to determine the strains needed in influenza vaccines. Others in the Virology Department at NIMR and their focus areas are Rod Daniels (variation in HIV env-genes and their encoded proteins); John Doorbar (human papillomavirus); Mikhail Matrosovitch (influenza virus receptors); John McCauley (avian influenza viruses); John Skehel (influenza hemagglutinin); and Jonathan Stoye (retrovirus–host interactions).

DISCUSSION
Dr. Hay was of the strong opinion that there is not enough attention being paid to monitoring flu strains in swine and equine populations, which could evolve into pandemic strains. H3N8 is the equine strain that is currently circulating, and there is evidence suggesting that the virus may be changing. The World Organisation for Animal Health (OIE) is playing a critical role in monitoring animal flu viruses and working with its reference laboratories. H3N2 is a new virus that has been circulating in swine worldwide. It is a triple reassortant, but acquired its genes from human, not avian, flu strains.

Some thought was given a few years ago to let veterinary biologics manufacturers produce human flu vaccines for emergency use.

Dr. Hay informed the WTEC team that the Wellcome Trust has set up high-throughput sequencing centers. The community now expects full genome sequences. The goal is to determine the likelihood of a virus evolving virulence mechanisms or host range specificity. He noted that researchers are still dependent on animal challenge studies to determine phenotyping changes associated with genetic variations. His group is

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8 The other WHO collaborating centers are in the United States (CDC); Hong Kong, China; and Tokyo, Japan.

9 St. Jude Hospital, Tennessee, U.S, is the WHO Center for animal viruses: swine, avian, and equine.
interested in changes in key amino acids that may affect glycosylation and HA binding-receptor sites. Changes in neuraminidase structure/function have been critical to determine drug resistance. Unfortunately, classic mutations in the H3N2 amino acid 226 from leucine (human) to glutamine (avian) don’t hold up for H1N1. We are thus still dependent on conducting experiments in relevant animal challenges.

Issues

Sharing of samples. The flu surveillance community is dependent on National centers to provide samples to the WHO centers. The ethos of the WHO is to share viruses and samples. At the time of the WTEC visit, Indonesia had recently declared it would not share samples. NIMR had instituted material transfer agreements (MTAs) in the 6 months prior to the WTEC visit. It has placed no restrictions on what could be done with these viruses, including by vaccine manufacturers.

Cross-protection. At present, Dr. Hay mentioned, it is not possible to determine how new vaccine strains will protect against field strains. Conventional methods of production have not worked for H5N1 strains: at least two doses are required, giving poor cross-protection. Hemagglutination inhibition (HI) titers are fairly good correlates of protection for conventional vaccines, but H5N1 is more complicated. Research is needed to focus on determinants of host range. One of the most important issues is the lack of cross-protection provided by current vaccine platforms. There are currently two lineages of Influenza B that are circulating that are also of concern.

Manufacturing and regulation. The problem with manufacturing is not the ability to produce sufficient doses of flu vaccine but the problem is the time to produce them in the case of a pandemic. One outcome is that we are starting to see a softening in the regulatory requirements. Manufacturers want to produce platforms that have already been approved by regulatory authorities. They produce 500 million doses of human vaccines per year. These used to be alum-adjuvanted but there was no consistent effect. Human vaccines are currently not adjuvanted, resulting in marginal immune protection.

SUMMARY AND CONCLUSIONS

Dr. Hay stressed the need to differentiate what we can do now, what needs to be short term, and what needs to be done to achieve strategic goals 10 years from now:

- Short term: develop conventional platforms that can provide greater cross-protection and herd immunity
- Develop recombinant vector technologies for pandemic strains to produce highly effective vaccines to stop transmission

He also emphasized the need to understand the following:

- Correlates of efficacy
- Determinants of cross-protective immunity
- Levels of cross-protective immunity induced by natural infection
- Immune response in children
- Host range specificity

Key Issues

- Conventional production methods for human influenza vaccines have not worked for H5N1, resulting in a poorly immunogenic vaccine
- We need to enhance the surveillance and interest in equine influenza viruses as well as other mammalian influenza strains that are changing
- We need animal challenge models to determine mechanisms of cross-protective immunity and host range specificity
**Site:**  
Novartis Vaccines  
Via Fiorentina 1  
53100 Siena, Italy  
http://www.novartis-vaccines.com/  

**Date Visited:**  
February 27, 2007  

**WTEC Attendees:**  
T. Leighton (report author), J. Bielitzki, M. Ritchey, H. Ali  

**Hosts:**  
Rino Rappuoli, PhD, Global Head, Vaccines Research  
Tel: +39 0577 243414; Fax: +39 0577 278508-5073  
Email: rino.rappuoli@novartis.com  

Vittoria Pellegrini, Head of Primary TechOps  
Tel: +39 0577 243246; Fax: +39 0577 243262  
Email: vittoria.pellegrini@novartis.com  

Donatella Latocca, Head of Communications, Italy  
Tel: +39 0577 243256; Fax: +39 0577 243321  
Email: donatella.latocca@novartis.com  

![Figure B.4. Novartis Vaccines research campus in Siena, Italy.](image)

**BACKGROUND**

Novartis Vaccines, created in 2006 by the Novartis acquisition of Chiron Vaccines, is the world's fifth-largest vaccines business and the world's second-largest manufacturer of flu vaccines. Chiron had previously acquired the vaccine branch of Sclavo Institute (now Sclavo Diagnostics International) in 1992. Sclavo had produced vaccines at the Siena site since 1904. Novartis Vaccines produces approximately 400 million doses of vaccines per year that are sold in 70 countries. In addition to producing flu vaccines, Novartis Vaccines produces vaccines for meningitis, rabies, tick-borne encephalitis, *Haemophilus influenzae* B, and diphtheria vaccines for adult and pediatric markets. Novartis Vaccines utilizes both egg-based and cell-based vaccine production processes. Novartis vaccine research is located in Siena (Italy) and Emeryville (California), although Novartis has announced the transfer of its California-based research to Cambridge (Massachusetts). Novartis vaccines are produced in Siena and Rosia (Italy), Marburg (Germany), and Liverpool (United Kingdom). Cell-based vaccines are produced in Marburg, Germany, and other vaccines are produced at other locations around the world (Figure B.5). A new manufacturing plant to produce influenza in cell culture is being built in Holly Springs (North Carolina). Novartis plans to make Siena an international center of excellence for vaccine research and development, and the neighboring Rosia site a center for production excellence. Novartis collaborates with NIH and GAVI to improve access to vaccines in developing countries.

The Siena site is home to several biotechnology companies, including Alta, which is involved in HIV and TB vaccine research, and Sienabiotech and Toscana Life Sciences (TLS), and to programs of Siena University. The goal of TLS is to offer equipped buildings, services, and financing opportunities to facilitate the development of new biotech companies focused on pharmaceutical, biotech, diagnostic, and innovative biomedical technologies. TLS has close collaboration and synergy with the research labs of Novartis Vaccines, Sienabiotech, and Siena University, as well as international collaborations with centers of excellence in the life sciences.
The Novartis Sites

An associate of Dr. Latocca gave the WTEC team a tour of the Siena site (Figure B.6). All flu vaccine research and egg-based production are at that site. All polio, *Haemophilus*, and meningococcal vaccine research and production are also there. There is a long-term development plan to move all production to the Rosia site over the next few years. The Rosia site (Figure B.7) is currently used for vaccine formulation and filling operations. The Siena site, which is fully occupied, will become a research and biotechnology center; it is the largest biological production and employment enterprise in Toscana and also the only “full-cycle” biotech vaccine company in Italy, with research, development, manufacturing, and commercial operations. The Siena site is EU and WHO approved and produces over 500 million vaccines doses/year of flu, polio, Hib, and MenB OMV antigens. It includes development and GMP bacterial pilot facilities; a formulation development facility; chemistry, immunology, virology, and microbiology laboratories; clinical/manufacturing testing; and a research center. The Rosia site, approved by MOH, is the principle fill and finish facility for Novartis Vaccines' live virus secondary handling. It produces over 70 million units of secondary manufactured ampoules, vials (liquid and lyophilised), syringes, and oral dose dispensers. It includes warehouses, sterility testing laboratories, and new glyco-conjugate, fill/finish, and QC facilities.
Discussions with Dr. Rino Rappuoli

Dr. Rappuoli and his colleagues have been seminal thought- and technology leaders in vaccinology for the past several decades. For example, his group pioneered the application of genomics to antigen discovery. He has also been involved recently in a very deep and rich analysis of the current state of vaccinology science and technology. Dr. Rappuoli was preparing a major review of this topic but generously shared many of his pivotal conclusions and insights with the WTEC team prior to publication.

From a historical perspective, vaccinology has changed little over the past one hundred years (circa Pasteur). Most vaccines are produced by the principles that Pasteur articulated: *Isolate – Inactivate – Inject the Causative Agent*. These approaches have been very successful for pathogens that present stable antigenic structures to the immune system: diphtheria, pertussis and tetanus; polio, Hflu, HBV, and measles, mumps, and rubella. Vaccine development for pneumococcal and meningococcal diseases has been more challenging due to the much greater diversity of antigenic structures presented by these pathogens. These pathogens typically change major circulating antigen types once a decade. This rate of change is approximately the time currently required to develop a new vaccine, so vaccinology has been able to maintain protective immunity to emerging variants. Influenza changes antigenic variants on yearly cycles and is a stressing case for vaccine protection. HIV changes antigenic variants once a day and has confounded all attempts to develop vaccine protection; TB and malaria are similar cases.

Dr. Rappuoli ascribes the failure to develop successful HIV, TB, and malaria vaccines to lack of knowledge of how the immune system works. In particular, he noted, there are major scientific gaps in understanding:

- Mechanisms of mucosal immunity
- Mechanisms of nonantibody cellular protection
- The interplay between cell- and antibody-based protection
- Mechanisms of adjuvant immune system programming
- Strategies to reveal and defeat antigen cloaking

Dr. Rappuoli suggests that a new discipline of vaccinology, “Structural Immunology,” is required to elucidate the molecular and atomic interactions between target antigens and the immune system. He mentioned the recent success in determining the three-dimensional structure of the conserved neutralizing epitope of HIV gp120 (Zhou et. al. 2007) as providing a path forward to the genesis of a Structural Immunology discipline. These studies revealed that the gp120 surface is protected against antibody recognition by a dense array of carbohydrates and a shape-shifting mechanism that only exposes the conserved neutralizing epitope once it reaches the CD4 T-cell receptor (antigen cloaking).

When Novartis acquired Chiron Vaccines, its management and scientists carefully studied Chiron’s state of vaccinology science and technology (S&T) and advised Dr. Rappuoli that they considered it similar to pharma S&T in the 1950s. Vaccinology lacked knowledge of target-based mechanisms for protection and screening, the ability to elicit balanced T and B cell responses, availability of *in vitro* surrogate markers for protection, availability of single-dose oral or mucosal delivery systems, ability to produce life-long protection, and the ability to protect against all antigenic variants. They also emphasized that the current
economics of the vaccine market severely undervalues the long-term contributions of vaccines to reductions in morbidity and mortality and provide a disincentive for innovation. (The advent of emerging and pandemic infectious disease threats may substantially alter these market forces. Dr. Rappuoli also emphasized that vaccines offer a very attractive alternate strategy for enduring control of multidrug-resistant bacterial pathogens.) Dr. Rappuoli focused his first work at Novartis Vaccines on overcoming the deficiencies in the existing vaccinology know-how inherited from Chiron. Beyond these science gaps, Dr. Rappuoli next turned his attention to translational science and engineering gaps that affect the end-to-end process of vaccine production, which he has divided into three conceptual elements: (1) Conception, (2) Gestation and Incubation, and (3) Growth & Maturity. His concept is presented visually in Figure 3.2 (Chapter 3).

Conception, the “hot” basic discovery science front-end of vaccine development is well funded, has an attractive academic career path, but rarely produces products of human utility.

Gestation, the “boring and tedious” development engine of systematic discovery and optimization of vaccine candidates, production scale-up, establishment of GMP production and formulation conditions, safety and toxicology testing, and proof of concept in man is underappreciated in the academic community, resulting in all of the world’s education, training, and career development occurring in five major industrial settings. At the time of the WTEC panel’s visit, Dr. Rappuoli said there was only one, fifteen-day vaccinology course offered in the world, and it was continuously oversubscribed. He considered this a major gap in vaccine manufacturing education and training, particularly since there is no existing surge capacity to meet the challenges of a global pandemic. Gestation, he said, is the choke point for rapid vaccine manufacturing.

Growth & Maturity, which involves Phase II & III clinical studies, large-scale GMP manufacturing, licensing, lot release, and commercialization, follow well-established career paths and competencies in the pharmaceutical industry.

Influenza Vaccine and Pandemic Preparedness

Dr. Pellegrini described the Novartis development pathway for egg-based pandemic flu vaccines commencing with early clinical studies of H5N3 and H9N2 vaccines, and production and stockpiling of two types of H5N1 vaccines. Novartis Vaccines is the first company to receive EU “core approval” for a pandemic flu vaccine. Novartis is continuing to develop its MF-59 adjuvant for both cell- and egg-based vaccine formulations to reduce the required antigen dosage and to increase flu strain cross-protection.

Dr. Pellegrini discussed several gaps in pandemic flu manufacturing practice and/or knowledge:

- Inadequate surveillance or early identification of circulating strains that could require vaccine protection
- Need for earlier access to strains for testing and development of production processes (work outside of the flu season)
- Insufficiently precise predictive assays to identify strains and recombinants that could be problematic for large-scale production
- Lack of in vitro surrogate end points for protection

SUMMARY AND CONCLUSIONS

Novartis Vaccines provided the WTEC team with thought-provoking analyses of the state of vaccinology and made a pivotal contribution to our study. Dr. Rappuoli’s insights (that vaccinology has progressed little beyond Pasteur [or Pharma circa 1950]; that we do not understand the rules of immune system response, regulation, and adjuvant programming; and that there is an unmet need for rational selection and design of antibody responses [3D epitopes]), all integral to his concept of a path forward to structural vaccinology, suggest a number of high-reward investment opportunities for new funding initiatives.

The abstraction of the vaccine development pathway from conception-to-gestation-to-maturity is very powerful in illuminating the translational disconnections between these three essential elements. In the event of a pandemic, it will be extremely difficult to meet surge demand in the absence of an educational and training infrastructure that provides a pool of gestation practitioners beyond those already employed in industry. This is a critical area for investment. These concepts and analyses merit the attention of the highest levels of U.S. and European science funding and policy agencies.
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Site: Pfizer Animal Health s.a.  
Rue Laid Burniat, 1  
B-1348 Louvain-la-Neuve, Belgium

Date Visited: March 1, 2007

WTEC Attendees: M. Ritchey (report author), J. Bielitzki, T. Leighton, H. Ali

Hosts:  
Rudy Rosolen, Site Leader, Pfizer Global Manufacturing  
Tel: 32 10 47 02 11; Fax: 32 10 45 20 06  
Email: rudy.rosolen@pfizer.com

Luc Giorgi, Manager/Team Leader-Quality Operations, Pfizer Global Manufacturing  
Tel: 32 10 47 02 11, Fax: 32 10 47 03 45  
Email: luc.giorgi@pfizer.com

Christian Borgniet, Production Manager, Pfizer Global Manufacturing  
Tel: 32 10 47 02 11, Fax: 32 10 45 20 06  
Email: Christian.borgniet@pfizer.com

BACKGROUND

Pfizer, Inc., is a large, multinational research-based pharmaceutical company. The company’s principal products are pharmaceuticals for humans, but Pfizer also discovers, develops, and markets pharmaceuticals and vaccines for livestock and companion animals. In Belgium there are 7 Pfizer sites that employ around 3000 people. The site at Louvain-la-Neuve produces veterinary vaccines for 30 different markets around the world. The original site was developed by Syntex and then purchased by SmithKline-Beckman, which became SmithKline Beecham. In 1995 it was purchased by Pfizer, and today it is a state-of-the-art European veterinary biologicals facility.

Most of the Louvain-la-Neuve resources are used for formulation, filling and packaging, and testing veterinary vaccines. The site manufactures 6 of the antigens used in the products it produces. It receives 29 antigens from the Pfizer site in Lincoln, Nebraska. The site produces 39 formulations, 68 drug products, and more than 400 finished goods SKUs for its various markets. These products are used for companion animals and livestock. The site does not produce any avian vaccines. Some of the major products produced at the Louvain-la-Neuve site are Stellamune Mycoplasma® vaccine against the most predominant respiratory disease in pigs; Rispoval®, a full line of modified live respiratory vaccines for cattle; Vanguard®, a full line of canine distemper combination vaccines; and Leukocell®, which protects against feline leukemia.

TECHNOLOGIES

The site is a center of excellence for aseptic processing for formulation, filling, and freeze drying. Class 100 A/B areas are available for this work. Air pressure and non-viable particulates are continuously and automatically monitored. Closed systems with automated CIP and SIP are available to ensure equipment is clean and sterile (Figure B.8). Key infrastructure elements include oil-free air compressors, clean-steam generators, and WFI generation and distribution to ensure the air and steam used in processing and water in formulation are suitable for sterile products (Figure B.9). There is a SCADA system in place to collect monitoring data on air quality and manage the interlocking management system for cleanrooms.

Viral antigen manufacturing is accomplished using roller bottles, cell factories, and closed systems for inactivation and concentration. The SCADA system is available for data collection and control for a number of these operations. Disposable bags are also used in the media and viral harvest areas. Both live attenuated and killed inactivated antigens are prepared.

Aseptic formulations capabilities are extensive (see Figures B.10 and B.11), with the ability to prepare simple mixtures just prior to filling or to complete in a closed system, a complex formulation with many components including adjuvants. Emulsions are also prepared for some of the formulations. In-process controls exist for pH, mixing, etc., and use of disposable bags in the formulation process is also under deployment.
Figure B.8. Example of closed, automated systems.

Figure B.9. Formulation tank.

Filling capabilities include single and multidose lines with the ability to freeze-dry if needed. The lines are highly efficient, automated, and integrated, with speeds up to 40,000 vials per hour. Vials to be filled are passed through a washer, depyrogenation tunnel, and a contained area for filling and automatic stoppering. For freeze-drying operations, (Figure B.12) the vials are diverted to a freeze dryer with fully automatic loading and unloading under aseptic conditions prior to capping. Automatic monitoring of the freeze-drying cycle parameters is built into the system. Most of the products are presented in glass vials. There is one filling line capable of processing plastic vials.

Procedures are in place in the aseptic formulation, fill, and processing areas to clean and fumigate, thus allowing a change-over from one vaccine to another without the risk of intermixing. The site works with antigens that include live, modified or attenuated, and killed, inactivated from both viral and bacterial sources.
Figure B.10. Formulation.

Figure B.11. Filling.
The packaging lines are highly automated, integrated, and are used to produce 407 different packages. The site has the capability to produce artwork for boxes and labels. It prepares packaging materials in 43 different languages, including items with multiple languages on a single piece. Bar codes are in use for identification. The Optical Control Vision System allows for 100% control of variable information on the labels, leaflet presence and identity, vial presence in the boxes, and caps and color verification. Robots are used for boxing and palletization (Figure B.13). The computerized in-process controls allow identification according to the International Federation for Animal Heath standard to achieve drug traceability from manufacturer to final user. Louvain-la-Neuve within the Pfizer network was the first site to implement this directive.
The warehouse is semiautomated and fully integrated for reduced labor and efficiency. Materials are bar-coded, and their movement is controlled by a computer system that also directs the fork-truck movements. Hand picking of materials is controlled by using laser bar code readers. Cold storage capabilities include 2-8°C, -20°C, -40°C, -70°C, and -196°C (liquid nitrogen). Rapid freezing capability is also present. Extensive experience and validated procedures exist for shipping at controlled, cold temperatures for antigens received from Nebraska and products sold to Pfizer’s customers. Temperature monitoring is performed for all shipments.

Analytical capabilities include the performance of a variety of biological in vivo and in vitro assays for potency, safety, and other product characteristics in addition to in-process testing, water, steam, and environmental monitoring. Each batch of product must be tested in animals before it is released for sale. Testing groups on-site include Virology and Immunology and Bacteriology. Isolator technology is used for sterility testing (Figure B.14). Capabilities for physical and chemical testing include UV, HPLC, atomic adsorption spectroscopy, IR, optical rotation, and others.

Annex 16 of the EU regulations is in place. This requires confirmatory testing for imported materials and the release and certification of each batch of finished product by the Qualified Person who resides on-site.

Figure B.14. Sterility Testing.
IMPROVEMENT PROJECTS
The Louvain-la-Neuve site has a number of programs going on that invest in people, facilities, and processes to continue to advance excellence at the site. A Six Sigma quality program is in place wherein a number of individuals have achieved “Green Belt” status. Projects in this area include such items as annual product review optimization and improved deviation report analysis. Efficiency projects and quality systems reviews are also in progress. This site is also using Process Analytical Technology to investigate ways to increase the robustness and control of processes. Projects include a study of conjugation reactions, inline monitoring of virus inactivation, and rapid microbiological monitoring methods, among others. There is also ongoing work on control of emulsion preparations during formulation. Real-time, round-the-clock, remote monitoring of the freeze-dryer equipment from employees’ homes is implemented through use of portable computers. The site is in the process of establishing a pilot plant that can be used to scale-up and improve technologies and prepare clinical supplies. This will accelerate the development of vaccines for Europe.

ISSUES RELATED TO RAPID VACCINE DEVELOPMENT AND MANUFACTURING
Once a manufacturing process has been established, it requires about 10–12 months to prepare and release product. More than half of this time is consumed in testing, and a large number of tests must be done in animals. Key to more rapid vaccine development and testing is to develop robust formulations and potency tests. A more in-depth understanding of the vaccine is needed to allow for replacement of in vivo tests with tests that do not require animals and are shorter in duration.

Veterinary vaccines in Europe fall under the same directive for GMPs as human vaccines (Directive 2003/94/EC of 8 October, 2003). Thus the possibility of using veterinary facilities for human vaccine production for EU markets exists. This directive also allows products to be released for multiple countries using a common release protocol and test regime, although countries may have some individual requirements. (Regulations between the United States and Europe, however, are dissimilar. In the United States, human vaccines fall under the FDA standards and veterinary vaccines falling under USDA standards.) Because of the complexity of manufacturing and control of vaccines, a highly educated staff is required to run the facilities. At this site, all workers have a least a high school education, 38 percent have a bachelor’s degree in a technical area, and 23 percent hold at least one university degree. Thus, the ability to increase manufacturing capacity also depends on the ability to recruit qualified staff.

SUMMARY AND CONCLUSIONS
The Pfizer site at Louvain-la-Neuve is a state-of-the-art veterinary vaccine manufacturing plant with extensive expertise in antigen production, formulation, filling, and freeze-drying under aseptic conditions. Procedures in place allow for flexibility in manufacturing a variety of vaccines in the same areas and on the same lines within the plant. Investment in continuous improvement and excellence is an ongoing process.

There are common GMP regulations within Europe for both human and veterinary vaccines, making it potentially feasible to use this Pfizer site for production of human vaccines for Europe. Because U.S. veterinary vaccines and human vaccines are under the control of different government agencies, use of this facility for human vaccines for the U.S. market is more problematic. Rapid vaccine development could be enhanced by investigating methods to develop more robust formulations and test methods that are shorter in duration, more precise, and do not require animals.

REFERENCES
EMEA Good Manufacturing Practices (GMP) directives
Appendix B. Site Reports

Site: PowderMed, Ltd.
The Oxford Science Park
Oxford OX4 4GA UK
http://www.powdermed.com/

Date Visited: February 28, 2007

WTEC Attendees: S. Drew (report author), C. Gay, S. Jacobson, G. Lewison

Hosts:
Dr. John Beadle, Chief Medical Officer & CEO
Tel: +44 (0)1865 501 532; Fax: +44 (0)1865 501 501
Email: john.beadle@powdermed.com

Dr. Robin Marriott, Executive Vice President, Pharmaceutical Development
Tel: +44 (0)1865 501 530; Email: robin.marriott@powdermed.com

Dr. Peter Loudon, Research Alliance Manager, Tel: +44 (0)1865 501 538,
Email: peter.loudon@powdermed.com

Dr. Hamish A. I. McArthur, Pfizer Global Manufacturing
Director of BioProcess Technology, Pfizer, Inc.
235 East 42nd Street, 685/6/11, New York, NY 10017
Tel: 1-212-733-3674,
Email: hamish.mcarthur@pfizer.com

BACKGROUND
PowderMed is an immunotherapeutic product company located in Oxford, UK, with six lead programs targeting influenza, chronic viral diseases, and cancer. These products are all based on proprietary DNA particle-mediated epidermal delivery (PMED™) technology. This technology has been tested for vaccine delivery in human trials and found safe and able to stimulate immunological response.

The company was founded in May 2004 based on ballistic delivery of gold particles developed by its predecessor company, PowderJect. Although a young company, PowderMed was launched with six mature clinical and preclinical programs and a management team with many years experience in the pharmaceutical industry. In its first year of activity, PowderMed announced its first clinical results in the influenza program and launched three further Phase I trials in its therapeutic vaccine programs. PowderMed has a subsidiary called PowderMed Vaccines, Inc., a U.S.-based commercial entity in Frederick, Maryland, that works with the National Biodefense Analysis and Counterterrorist Countermeasures (NBACC) centre at Fort Detrick.

PowderMed has produced an H5N1 avian influenza vaccine that is delivered by a fully developed and patented PMED™ system in which gold particles carrying adsorbed vaccine DNA are propelled into the skin using high-pressure helium. In this way, vaccine DNA is delivered directly to antigen presenting cells of the immune network in the skin, leading to an immune response. This approach provides a rapid route to vaccine development that could be applied to existing or emerging influenza strains, including for example, the threat of a pandemic influenza strain emerging. PowderMed is now a wholly-owned subsidiary of Pfizer, Inc.

RESEARCH AND DEVELOPMENT AT POWDERMED, LTD.
PowderMed’s primary operations focus around the company’s PMED™ technology. This device (Figure B.15), designed and manufactured for the delivery of DNA vaccines, is a single-use, disposable system that involves a mechanism powered by high-pressure helium gas to deliver gold particles coated with the DNA vaccine to the dendritic cells in the skins. PMED is conceptualized as a safe and efficient platform for rapid delivery of any DNA vaccine. The amount of gold used with each dose administered is small (one milligram of gold per dose), and PowderMed estimates that the cost of DNA delivery with this approach would be comparable with other DNA vaccine delivery method. Dr. McArthur estimated that preparation of recombinant DNA supercoiled plasmids would reach 40 grams of recovered plasmid per 300 L fermentation batch.
PMED™ DNA vaccination is well tolerated and produces generally mild, transient local reactions; no significant findings of systemic toxicity or treatment-emergent serious adverse events have been reported.

Several issues were raised by the WTEC visiting team, including the feasibility of stockpiling such a device (given its current size), the logistical challenges of moving a large number of these units in the event of a rapid vaccination program, the amount of helium that would be needed in the event of a rapid vaccination program, and the disposability and “green manufacturing” issues surrounding the individual delivery devices. The company indicated that all of these items were being considered and solutions developed in the context of a potential pandemic threat.

**Delivery of Recombinant DNA Vaccines to the Immune System**

PowderMed presented an elegant introduction to the PMED technology. Supercoiled recombinant plasmid DNA encoding an antigen gene is adsorbed to crystallized gold particles at 1 to 4 micrograms per milligram of gold (the clinical dose configuration; 1 mg of micron-sized gold particles delivered to a skin surface area of ~ 50 square millimeters). A single dose of the DNA-gold particles is placed in a plastic cylindrical cassette whose ends are capped with a flexible membrane. The particles are discharged to the epidermis by explosive decompression of helium that ruptures the upper membrane, entrains the micron-sized gold particles, ruptures the lower membrane and develops particle velocities approaching supersonic speeds. The excess helium vents to atmosphere before reaching the skin, and the gold particles impact the skin, penetrating through the stratum corneum to the epidermis.

This system has been used to administer 1500 deliveries of DNA to human patients across 10 human trials. Three influenza studies in humans are ongoing as of February, 2007, with administration of 2 to 4 μg of DNA per exposure. An H5N1 vaccine challenge trial in mice, with and without an encoded heat-labile *E. coli* enterotoxin as an auto-adjuvant, showed 100% survival after two doses.

The concept and rationale for delivery of DNA to dendritic cells in the epithelium (Langerhans Cells) is well developed and may prove to be a uniquely effective way of stimulating an immune response. The ballistics of gold powder delivery to the epithelium is understood and predictable. A combination of energetic source, cassette technology for presentation of DNA on microcrystalline gold particles, and a velocity development bell (to establish uniform distribution of gold particles at the stratum corneum/epithelium) has been developed.
A single-use delivery device has been developed and optimized. It can be manufactured from several fairly simple components into three subassemblies. Aseptic filling of the cassette has been demonstrated at small-scale manufacturing, and the entire process is amenable to automation. The process and manufacturing operations are straightforward, amenable to scale-up, and much of the manufacturing could be out-sourced, if needed, to speed development and distribution. Tooling for manufacturing of component parts is the likely rate-limiting step for expansion.

Gold particle ballistic delivery of DNA, proteins, or drugs to the epithelium seems limited to a few micrograms per discharge event across approximately 50 mm$^2$ of skin surface. The value of gold particle delivery of DNA as a vaccine is dependent on precise delivery into dendritic (Langerhans) cells that are concentrated in the epithelium. Too low an impact of the ballistic gold particles may result in failure to penetrate the *stratum corneum*; too high an impact may result in undesired injury to the surrounding tissues. PowderMed has established this process such that 1mg of gold delivered to 50 mm$^2$ is optimum to reach the dendritic cells and minimize tissue damage. The data presented for immune response to selected antigens was very impressive, showing response at least equivalent to a conventional antigen vaccine. The kinetics of DNA adsorption and ballistic delivery with gold may establish a narrow window for the application of this technology for multivalent vaccines or those that require higher doses of DNA to achieve acceptable immunological response; so far, the data show strong immune response.

PowderMed’s ability to respond to a pandemic threat (influenza or other viral agent) in record time seems plausible and awaits proof of safety and efficacy in clinical trial, and the company reports strong immunogenic response from a first Phase 1 study, and it is undertaking an influenza challenge study in humans (83 people in three groups).

The WTEC panel’s hosts at PowderMed alluded to the ballistic delivery of proteins to the epithelium. When queried about this, they claim cellular and immunological response to protein delivered without gold particle carriers. Ballistic delivery to a specific tissue through various resistances is proportional to the density, velocity, and radius of the particle. If this report could be validated, it might suggest a way to overcome the limitations on the mass of DNA delivered on gold particles. It is not clear whether gold and other materials might be delivered in a single application even though the physics of delivery is dependent on the density of the particles.

The minimum dose configuration can be thought of as just the cassette containing DNA adsorbed onto gold particles. This suggests that alternative delivery methods (including gas-powered ballistic delivery using a single injector with a replaceable per-patient splash shroud and dosing cassette) could drive the cost of vaccination lower and extend the ability to provide emergency delivery in the face of a pandemic.

**SUMMARY AND CONCLUSIONS**

PowderMed has demonstrated DNA vaccines safety and immunogenicity in humans (Phase I) across 10 completed trials and will add to that in three more influenza trials. PowderMed’s delivery device is effective in accessing dendritic cell mediated innate and adaptive immunity and could form the basis of very rapid emergency scale-up and delivery of influenza vaccine for pandemic. With regard to the special conditions of an emerging infectious threat or pandemic influenza threat, the following conclusions have been reached by the study team. These conclusions are based on the assumption that clinical data for delivery of safe and efficacious DNA vaccines for therapeutic or preventative use will continue to show the impressive merit demonstrated in Phase I studies as PowderMed progresses through Phase III studies.

1. The time to respond to a pandemic threat could be short for a DNA-based vaccine developed from a typed and characterized influenza strain. PowderMed researchers believe that their technology could mount an effective response, assuming that DNA vaccines can be shown to be at least as effective as conventional vaccine therapy and that the safety data gathered to date could allow immediate deployment of a recombinant DNA vaccine. Our hosts estimated that the time to carry out the molecular biology and initial development and scale-up of a commercial-scale process based on their existing delivery system could be as short as 3 months. Production could then start; they alluded to a nominal manufacturing capacity of 40 million doses per year, but no details were presented.

2. Our hosts indicated that the cost per dose at a level of 40 million doses per year would be competitive with other conventional protein-based vaccines such as Inactivated Seasonal Influenza vaccines.
3. The current configuration of the delivery system for DNA adsorbed on gold is elegant, high-tech, and amenable to administration by nonmedical personnel. It is currently configured as a single-dose, disposable unit for high-end vaccines. Relative to needs for a pandemic threat, the delivery system is bulky, likely to be perceived as a storage and handling burden by medical professionals, and might generate a large amount of waste material per patient. PowderMed is investigating a multidose configuration.

4. The stability of DNA on gold particles may afford real advantage by allowing > 4-year stability under ambient conditions. (For example, delivery of a pandemic influenza vaccine to developing nations might require ambient distribution and storage conditions.)

5. It is evident that the epithelial delivery of DNA by this method is at least as effective as the traditional trivalent influenza vaccine in terms of raising immunogenic response in human trials. The data to date suggest that this methodology might be as effective as conventional methods, but the numbers of patients are small to support this assertion. The clinical trials ongoing will add important data.

REFERENCES


Site: Scientific Institute of Public Health
Juliette Wytsman Street 16
B-1050 Brussels, Belgium
http://www.iph.fgov.be/

Date Visited: March 1, 2007

WTEC Attendees: M. Ritchey (report author), J. Bielitzki, T. Leighton, H. Ali

Hosts: D. Roland Dobbelaer Dr. Sc., Head, Biological Standardization
Tel: +32 (0)2.642.50.50, Fax: +32 (0)2.642.52.10
Email: Roland.Dobbelaer@iph.fgov.be

M. Mustapha Chafai, Vaccine responsible, Section of Biological Standardization
Tel: +32 (0)2.624.52.80, Fax: +32 (0)2.642.52.10
Email: Mustapha.Chafai@iph.fgov.be

M. Mathias Janssen, Vaccine responsible, Section of Biological Standardization
Email: Mathias.Janssen@iph.fgov.be

BACKGROUND
The Scientific Institute of Public Health (IPH) in Belgium is a part of the Ministry of Public Health. Its main mission is scientific research in support of health policy, but it also provides expertise and public service in the field of public health. The IPH plays an important role as part of Belgium’s representation in functions of the European Union and international organizations such as WHO when scientific/technical aspects of public health are involved. The IPH is also a part of the network of laboratories and organizations within the EU that participate in the centralized procedures for review and approval of vaccine dossiers and lot release for marketed products. The European Medicines Agency (EMEA), which directs the centralized procedures, is a decentralized body of the European Union with headquarters in London. The EMEA is responsible for the scientific evaluation of applications for medicinal products (centralized procedure). Under this procedure, companies submit one application. This procedure applies to medicinal products for both human and animal use. There is a network of European experts that are made available to the EMEA from the competent authorities of members of the EU. These experts can serve as members of EMEA scientific committees, of working parties, or of scientific assessment teams.

Dr. Roland Dobbelaer serves as head of the Biological Standardization group within the IPH in Belgium and also as one of the EMEA experts. He is a member and the Chair of the EMEA Vaccine Working Party. He and the members of his team at IPH perform two main functions:

1. Lot release of vaccines and blood products as part of the European network of labs that form the Official Medicines Control Laboratories (OMCL). Every lot that is produced is released according to a protocol that has been agreed upon within the OMCL Network. A selection of the release tests is performed, and the manufacturer’s test results and information are reviewed as part of the release process.

2. Scientific evaluation of CTD Module 3 of applications for European marketing authorization of biological medicinal products according to the EMEA centralized procedure.

CONTROL OF PANDEMICS
In the case of influenza, procedures in the European system allow for a core dossier to be developed using a model or mock-up strain that may not be the one selected for vaccine manufacturing in a given pandemic situation. Submission of the core dossier can allow for a more rapid approval of vaccine products during a pandemic because common elements between the processes described in the dossier and the pandemic vaccine would have already been reviewed. There are currently three core dossiers within Europe that are either approved or close to being approved.

Within Europe there is a legal provision that allows for use of a vaccine directly from the manufacturer’s shelf in a pandemic situation. The public health ministry within each country in the European Union makes its own decision on whether to invoke the legal provision or not for the various population groups in its
country. This provision could also be applied to other pathogens besides influenza viruses, for example, the SARS virus.

Concerns with these procedures for handling pandemics include the fact that vaccination may proceed in the absence of clinical data that would normally be used to assess vaccine efficacy and safety. For example, there may not be data on safety and efficacy in children. Often, purified antigens do not stimulate immunity as well in children as in adults. The level of cross-protection with other influenza strains may not be known. There may be questions as to whether the level of antibody raised is sufficient for protection. Results thus far suggest that the H5N1 strains are not as immunogenic as other (e.g., seasonal) influenza viruses.

In a pandemic situation, production capacity can be an issue for making enough doses of vaccine quickly enough for mass immunization. A possible mitigation factor is the prequalification of animal vaccine facilities to produce and package human vaccines. In Europe, there is a common set of GMP guidelines for control of manufacturing facilities for both animal and human vaccines. There is also a common group of inspectors for both types of facilities. It is therefore feasible to consider use of animal vaccine facilities for production of human vaccines if the need arises.

Applications for license review and lot release can also be bottlenecks for rapid availability of vaccine in a pandemic situation. Europe has the advantage of a network of 27 states that could pool resources to accomplish these tasks more efficiently; testing in parallel by the manufacturer and control lab is possible and can speed up lot release. In addition, a system for electronic data review is under development that would save the time spent in performing some of these operations using a paper system.

GENERAL COMMENTS ON OBSTACLES AND OPPORTUNITIES FOR RAPID VACCINE DEVELOPMENT

Science
A key issue for vaccine development is the diversity of pathogens that exist, making it difficult to find common production platforms for manufacturing the active ingredients for a vaccine. There is often a lack of understanding of which product attributes influence safety, potency, and effectiveness. It is difficult to measure output, because assays for potency are typically very broad in terms of the attributes they measure, and not very precise. There is also a lack in assay standardization, making it difficult to compare results generated by different groups.

Additional areas for further research efforts include better and more relevant animal models, use of in vitro correlates rather than animal potency tests, and more genetic studies to look for common characteristics among organisms that could lead to common design elements and manufacturing platforms for vaccines.

Control of adjuvants, commonly used with vaccines, presents other challenges. In Europe, there are no biological master files for these compounds, and thus typically, no individual assessment of the value of the adjuvant itself. Typically, a vaccine manufacturer such as GSK will buy the adjuvant company and evaluate the adjuvant on the basis of combination with its own vaccines. A more methodical approach to dealing with adjuvants would be helpful; for example, a set of common standards could be established for gathering data on adjuvants as a group, and regulatory input could be given to guide the process. EMEA has a guideline (EMEA 2005) on the use of adjuvants in vaccines.

Regulation
The funding systems for regulatory authorities are generally poor, which can impede the rapid approval of new vaccines. The solution is not to minimize regulatory intervention but to provide adequate support so that competent, experienced personnel can be recruited. Investment in human competence is key to providing an appropriate match in education and experience between the regulators and those in industry being regulated. Incentives are needed to make public service more attractive.

On the training side, there is a system among the network of control laboratories within the European community to provide training. There has also been EMEA training on conducting influenza vaccine assessments. Collaboration with industry exists when establishing tests to be performed in the control labs. The link with universities is generally poor. Often discovery and early development programs do not take regulations into account. A better understanding of regulatory requirements from the beginning could facilitate a more rapid overall process for vaccine development.
Harmonization of regulations between countries has been a practice for many years within the European community. Currently there are centralized processes for both review of license applications and lot releases. There are also harmonization and networking efforts going on in Latin America and Asia. As an example, the Pan American Health Organization (PAHO) stimulated concertation among Latin American countries in the process of licensing rotavirus vaccines.

**Intercompany Cooperation**

More rapid vaccine development may be facilitated if there were better sharing/collaboration among companies of information gathered in the early stages of working with candidate vaccine strains. Currently, the regulators can work through the European Manufacturers Association via both closed and open sessions to resolve problems common to more than one manufacturer. However, there does not seem to be sufficient collaboration for developing initial data on potential pandemic influenza vaccines. Each company relies on its individual data. More rapid progress could be made if sharing information on candidate influenza virus vaccine strains were made a routine part of the process.

**SUMMARY AND CONCLUSIONS**

The EMEA has a plan in place for regulatory management of potential influenza pandemics. The plan includes preapproval of a model influenza vaccine dossier that would facilitate more rapid approval of a vaccine if a pandemic should arise. Currently, three companies have submitted dossiers. European law also allows individual EU member states to authorize use of vaccine with less than typical preapproval testing.

There are 27 EU member states that could contribute to a resource pool to handle vaccine review/testing/lot release in a pandemic situation. To ensure that appropriate expertise and stature is maintained within the EU’s regulatory groups, more incentives are needed to attract and retain public service employees.

Companies working on influenza vaccines do not typically share early data that they derive on potential influenza vaccine strains. If a mechanism could be developed to facilitate the sharing, more rapid vaccine development in a given instance would be possible.

Rapid vaccine development could be facilitated by a better system for collecting and reviewing information on adjuvants. EMEA is working toward a system for this purpose. Better animal models and a better understanding of how vaccines stimulate immunity are areas for future research that can advance our ability to develop common platforms for the design and manufacturing of vaccines.

**REFERENCES**


Appendix B. Site Reports

Site: University of Oxford
Centre for Clinical Vaccinology and Tropical Medicine
Churchill Hospital
Oxford, United Kingdom

Date Visited: Wednesday, February 28, 2007

WTEC Attendees: C. Gay (report author), S. Drew, S. Jacobson, G. Lewison

Host: Professor Adrian Hill, Director, The Jenner Institute, and Professor of Human Genetics, Wellcome Trust Centre for Human Genetics
Roosevelt Drive, Oxford OX3 78N, UK
Tel: +44 (0) 1865 287686, Email: Adrian.hill@well.ox.ac.uk
Dr. Sarah C. Gilbert, Reader in Vaccinology
Wellcome Trust Centre for Human Genetics
Tel: +44 (0) 1865 287575, Email: sarah.gilbert@well.ox.ac.uk

BACKGROUND

University of Oxford Vaccinology Activities

- Center for Clinical Vaccinology and Tropical Medicine
- Wellcome Trust Centre for Human Genetics
- Weatherall Institute of Molecular Medicine
- John Radcliffe Hospital
- Sir William Dunn School of Pathology
- Jenner Building, Compton
- Medawar Building for Pathogen Research

As of late 2007, University of Oxford is currently the only institution with new vaccines in field trials for AIDS, malaria, and tuberculosis. This reflects a renewed emphasis on clinical and translational research in the medical school.

Strengths include:

- Critical Mass and Facilities (cGMP with containment capability)
- Basic vaccinology
- Immunomonitoring
- Small scale challenge studies
- Field sites in developing countries

THE JENNER INSTITUTE

- Formerly the Edward Jenner Institute for Vaccine Research
  - Founded as the national vaccine research institute in 1995
  - Then based solely at Compton, Berkshire
  - Funded for 10 years by the UK’s Medical Research Council (MRC), Biotechnology and Biological Sciences Research Council (BBSRC), GlaxoSmithKline (formerly GlaxoWellcome), and the Department of Health
- From November 2005 a new partnership was formed between Oxford University and the Institute for Animal Health to form the Jenner Institute, incorporating scientists from the former Edward Jenner Institute for vaccine research and the leading vaccine research and development groups from Oxford University and the Institute for Animal Health. The new institute (http://www.jenner.ac.uk) emphasizes links between human and veterinary vaccine development, translational research, and diseases of developing countries. The leading programs at the time of the WTEC visit were as follows:
influenza: human and avian
foot and mouth disease
tuberculosis: human and bovine

Basic Immunology
A major program in vaccine research is funded by the Gates Foundation through the Foundation for NIH. This program is part of the Gates Grand Challenge in Global Health programs. The project is entitled “Improving the immunogenicity and efficacy of vectored vaccines.” It encompasses the following steps:

- Generate vaccine vectors with increased immunogenicity
- Use molecular adjuvants eliciting innate host responses, especially TLR pathways signalling and co-stimulatory molecules
- Demonstrate improved protection in animal models of malaria and tuberculosis
- Conduct Phase I/II clinical trials with leading candidates
- Design improved viral vectors
  - Human genetics shows that TLR pathway genes are pivotal in influencing resistance to pathogens
  - Vaccine design can now manipulate these pathways by adding “internal” adjuvants to vectored vaccines
  - Adjuvants are now being incorporated into leading vectors, e.g., simian adenoviruses, poxviruses, plasmid DNA

Immunomonitoring
R&D in this area is a major focus. Assays have changed markedly in a decade.
1997
- Lymphoproliferation (tritium isotopes)
- Cytotoxicity (radiolabeled chromium release)

2007
- ELISPOT
- Flow cytometry (cytokines, surface markers, tetramers)
- RNA immunomonitoring (RT-PCR and microarrays)

OXFORD UNIVERSITY CENTRE FOR CLINICAL VACCINOLOGY AND TROPICAL MEDICINE
The Centre for Clinical Vaccinology and Tropical Medicine was opened in 2003 at the Churchill Hospital. This new center received capital funding from the Wellcome Trust and the government-funded Joint Infrastructure Fund. It provides a strong focus on the interface between research and clinical studies in the international effort to reduce the global burden of major infectious diseases in developing countries in the 21st century. Priority diseases are

- Malaria
- Tuberculosis
- HIV
- Influenza

Although clinical trials of vaccines have traditionally often been undertaken by vaccine companies themselves, it is now recognized that there is an urgent need to provide academic centers of excellence for the rigorous evaluation of vaccine candidates under circumstances that are free from marketing bias. This is particularly important for diseases of developing countries where proof-of-concept trials may need to be conducted in humans, often to Phase II development, in order to encourage interest by commercial companies. Research activities of the Oxford Vaccine Group, which will form part of the new Centre, include design and conduct of clinical vaccine trials, other vaccination-related research, and infectious disease
epidemiology and surveillance. The new Centre has established Phase 1 volunteer vaccine trials programs testing vaccines for malaria, tuberculosis, and HIV, and a new influenza program. The Centre has undertaken eleven Phase IIa sporozoite challenge studies to test malaria vaccines as well as a recent blood-stage parasite challenge study.

Oxford-based programs and their three permanently staffed and longstanding overseas units in Thailand, Vietnam, and Kenya pay close attention to vaccines to control major infections where deaths from these diseases are most prevalent. The work of the Centre for Tropical Medicine has been recognized by the Royal Anniversary Trust, which awarded the Centre a Queen's Anniversary Prize in 2000.

Professor Hill described a clever approach to adenovirus vector vaccine development that minimizes the effect of preexisting immunity to adenoviruses in humans (particularly in developing countries) while capturing the ability of adenoviruses to strongly stimulate CD8 response. More than 400,000 American soldiers have been exposed to oral adenovirus vaccines to control disease when housed in bivouac conditions (AdHu4 and AdHu7).

Chimpanzees also host adenoviruses, but there is little or no cross-reactivity with human adenovirus serotypes. Hill has obtained chimp adenoviruses (ChAVs) from Pennsylvania State University and from Okaiors, a Merck spin-off company based in Italy, and is studying these vectors for vaccine development in humans. Professor Hill is undertaking a Phase I clinical trial in humans with Okaiors to assess the safety and immunogenicity of a chimpanzee adenovirus (AdCh63) vector encoding a malaria antigen at 10⁸ to 5 x 10¹⁰ viral particles. Professor Hill expects that the most effective dose will likely be near 1.5 x 10¹⁰ vp virus.

WTEC panelists asked Professor Hill to identify areas that currently limit the rate of development of safe and effective vaccines. He suggested the following areas of current interest:

1. Increased funding is needed for the most critical disease conditions. The conditions of funding and its continuation should be milestone-driven rather than driven to the endpoint of a licensed product. This will allow much more meaningful development of understanding that will ultimately support more and better licensed vaccines.

2. Modern high-throughput genetics now looks at changes in literally thousands of genes as a result of clinical trial of new vaccines. This has driven the size and cost of even the simplest of clinical trials to very large numbers. The field needs to find additional people to participate in these trials and ways of constraining the overall cost.

3. There are disparities in funding for vaccine research between the greater funding in the United States (~$2.5 billion) and the United Kingdom. The cost of early developmental studies are lower in the UK; for example, the toxicology requirements for clinical trials are less in the UK than under the U.S. system.

4. Much more understanding is needed about the immunogenicity of candidate viral vaccines; for example, two adenoviruses may trigger immune response in two different pathways. We are beginning to understand some of the pathways, including (1) Toll-like receptors, (2) RIG-like receptors, and (3) nucleotide:oligonucleotide receptors. More focused work is needed (see concept 8 below). Further we need to understand the factors involved in maintaining T-cell memory over longer times. Dr. Hill believes that signaling pathways are central to these questions and suggests that those studying this “translational” area should be rewarded to keep them focused on this application in the face of pressure to move to more basic research. It seems possible that the work of groups such as Lauffenberger and colleagues at MIT might have insights to offer vaccine immunology and vaccine design.

5. Improved in vitro assays for immunogenicity are needed to support the design and development of new vaccines.

6. Researchers must look beyond simple interferon gamma-secreting CD4 and CD8 T cells to define the range of T cells necessary for robust, reproducible, and long-lasting immune response. In particular the role of regulatory T cells and the polyfunctional character of the T cell responses appear important.

7. Most current studies look at peak immune responses as measures of immunologic potential. Dr. Hill suggests that the relationships of components of vaccines (including adjuvants) and how they are administered will define the conditions of maximum response over time, and better early markers of durability of protection need to be identified. He suggests complex relationships exist between the many
systems involved in natural and vaccine-induced immune response, and vaccine-inducible protection may often differ from naturally evoked protection.

8. Many biotechnology companies form to study the mechanisms of immune/inflammation pathways with the goal of down-regulating inflammation in autoimmune disease; for vaccine applications should also study the pathways with a goal of up-regulating immune response to control/trigger/maintain immune response.

9. One of the panel members wondered if genetic variation in clinical populations might not confound the understanding of these issues in conventional clinical trials. Professor Hill agreed that this is certainly a factor to consider.

SUMMARY AND CONCLUSIONS

Issues

• Increased funding is needed for the most critical diseases
• We need better correlates of immune protection
• We need to understand much more about the immunogenicity of candidate viral vaccines
• We need to understand the factors involved in maintaining T-cell memory over longer times
• The lack of in vitro assays for immunogenicity hampers the design and development of new vaccines

Key Points

• Chimpanzee adenoviruses offer the potential for strong recruitment of CD4 and CD8 without facing preexisting immunity to common human adenoviruses
• Renewed emphasis on clinical and translational research
• A new partnership between Oxford University and the Institute for Animal Health
• Need to take advantage of animal models to demonstrate improved protection of new vaccine platforms

REFERENCES


Wellcome Trust Centre for Human Genetics, Oxford University. The Hill Group website: http://www.well.ox.ac.uk/hill/.
Site: University of Siena
53100 Siena, Italy

Date Visited: February 27, 2007

WTEC Attendees: T. Leighton (report author), J. Bielitzki, M. Ritchey, H. Ali

Hosts: Emanuele Montomoli, PhD, Professor of Public Health
Department of Physiopathology, Experimental Medicine, and Public Health
Via Aldo Moro, 53100 Siena, Italy
Tel: +39 0577 2341344, Fax: +39 0577 234090
Email: montomoli@unisi.it

Maria Grazia Cusi, PhD, Associate Professor of Microbiology
Department of Molecular Biology, Virology Section, Policlinico “Le Scotte”
Viale Bracci, 53100 Siena, Italy
Tel: +30-0577 233850, Fax: +39-0577 233870
Email: cusi@unisi.it

BACKGROUND

The University of Siena (Università degli Studi di Siena, UNISI) is one of the oldest universities in Italy. Founded in the 13th century, it has an enrollment of approximately 20,000 students and is particularly known for its schools of Law and Medicine. There is no delimited university campus: Siena, population 50,000, is the campus (Figure B.16).

Figure B.16. Town center, Siena, Italy.
Discussions with Dr. Emanuele Montomoli

Prof. Montomoli has a research group of six scientists who work in the area of vaccinology, with a particular focus on viral respiratory diseases (influenza, metapneumovirus, adenovirus, RSV). His laboratory is a Toscana Regional and WHO Reference Center for these diseases. It has three areas of primary research:

1. **Influenza surveillance.** Using classical serotyping, ELISA, and molecular methods including real-time PCR. A recent collaboration has been established with NIH and TIGR (The Institute for Genomic Research, now part of the J. Craig Venter Institute) to provide viral samples for genome sequence determination.

2. **Vaccine clinical trials.** Dr. Montomoli has been involved in approximately 40 phase II and phase III vaccine clinical trials with Novartis, Sanofi, and Baxter. The focus of these clinical trials is to evaluate vaccine immunogenicity and reactogenicity.

3. **Standardization of immunological assays for detection of viral antibodies in sera.** The assays include single radial hemolysis (SRH), Hemagglutinin Inhibition (HI), virus neutralization, ELISA, and other methods.

The research laboratories include a BLS-3 lab, a BLS-1 lab, a molecular biology lab and three portable BSL-2 laboratories. The laboratory also has a very interesting repository of approximately 50,000 sera collected in Italy from 1987 to the present, which are randomized for age and gender. There are also archival sera dating to 1919.

During the influenza season the laboratory is responsible for epidemiological and virological surveillance of respiratory viruses circulating in the Region of Tuscany. This research is conducted in collaboration with the Superior Institute of Health and consists of viral identification through molecular biology techniques (nested RT-PCR) and viral isolation and typing in chicken egg embryos and cell cultures. Dr. Montomoli has carried out sero-epidemiological surveillance of the immune status of the population of Siena for influenza viruses since 1994.

The laboratory also coordinates and performs epidemiological and sero-epidemiological studies for monitoring other infectious diseases that are preventable with vaccination (tetanus, hepatitis B, diphtheria, meningitis, etc.).

Dr. Montomoli finds that the SRH assays are unreliable and that there are no standardized protocols for specific SRH assays. In spite of these shortcomings, SRH assays are highly correlated with virus neutralization assays and appear to measure both HA and NA viral antigens. There is an unmet need for either greater standardization of the SRH assay or its replacement with more reproducible, reliable, and specific assays.

Discussions with Dr. Maria Grazia Cusi

Dr. Cusi’s laboratory is studying a new emerging virus, Toscana virus, associated with clinical cases of acute central nervous system disease, which is transmitted by sand flies and has spread throughout the Mediterranean region. Research in Dr. Cusi’s laboratory focuses on

1. Etiological agent discovery and development of animal models for Toscana virus
2. Delivery systems for DNA vaccines to dendritic cells using influenza virosomes
3. Intranasal immunization with mumps virus DNA using influenza virosomes
4. Influenza virosomes as a delivery system for RSV-F antigen
5. Study and development of new recombinant vaccines against viral diseases
6. Diagnosis and epidemiology of new emerging respiratory viruses

Discussions with Dr. Cusi focused on her views of major needs, gaps, and trends in vaccinology. Dr. Cusi believes that there are unmet needs for

- Improved animal models for viral diseases for which humans are natural hosts, i.e. measles, mumps, and rubella
- Better methods to identify immunodominant antigens that are protective
- Research that focuses on aligning immunization routes with the natural routes of disease infection
Dr. Cusi’s experience with virosomes suggests that these DNA vaccine and antigen delivery systems have considerable promise. Virosomes can contain cationic lipids, fusion-active influenza hemagglutinins. They are unilamellar vesicles with diameters of 120–150 nm that can be thought of as reconstituted influenza envelopes without RNA or nucleocapsid proteins. Dr. Cusi has demonstrated that intranasal administration of virosome DNA vaccines can induce IgA mucosal antibodies and circulating IgG antibodies. Virosomes are capable of targeting DNA delivery to dendritic cells. In the case of virosome-mediated intranasal delivery of the RSV-F antigen, both humoral and cell-mediated immune responses are induced. Virosomes are promising as an adjuvant and carrier system; it is approved by regulatory authorities and is the only approved adjuvant that has carrier capabilities (e.g., DNA).

Other facets of Dr. Cusi’s work include the following:

- She has developed an animal model for studying the pathogenesis of Toscana virus and is developing a vaccine able to protect animals from viral challenge.
- She is studying the immune response, in particular the cell-mediated response, in adults reinfected by respiratory viruses such as RSV. This could be useful for designing a safe and efficacious vaccine to be administered to infants and possibly to adults.
- During the influenza season, the laboratory is responsible for the virological surveillance of respiratory viruses circulating in the Region of Tuscany.
- Classical and molecular virology are practiced in her lab. The research laboratories include a BLS3 lab, two molecular biology labs and six BSL 2 laboratories. An animal facility for mice is also available.
- Prof. Cusi collaborates with European laboratories (Max Planck Institute, Pasteur Institute, NIBSC) and private companies (Novartis, Berna Biotech, Pevion Biotech).

**SUMMARY AND CONCLUSIONS**

Drs. Montomoli and Cusi provided interesting insights into the academic research enterprise in Italy. Very little research support is available through Italian sources. The majority of their research support comes from collaborative EU programs and private corporation funding. They both felt that they are isolated and are very interested in collaborating with U.S. scientists and research agencies. These opportunities should be seriously explored, because there are unique scientific and clinical resources at the University of Siena that are not well represented in the United States.

**REFERENCES**

**Montomoli**


Appendix B. Site Reports

Site: University of Vienna
Department of Medicinal Chemistry
Althanstrasse 14, A-1090, Vienna, Austria
http://www.univie.ac.at/
and Bird-C GmbH&CoKEG
http://www.bird-c.at

Date Visited: March 2, 2007

WTEC Attendees: M. Ritchey (report author), J. Bielitzki, T. Leighton, H. Ali

Hosts: Univ.-Prof. Dr. Werner Lubitz
Dept. of Medicinal Chemistry
Tel: + 43 4377 55115 Fax: +43 4277 55120
Email: Werner.lubitz@univie.ac.at
CEO, Bird-C GmbH&CoKEG
Email: werner.lubitz@bird-c.at

Dr. Mayr Ulrike Beate, Chief Production Officer, Bird-C GmbH&CoKEG
BIRD-C Laboratory, Althanstr. 14, UZA2 2B522, A-1090, Vienna, Austria
Tel: +43 1 4277 55115; Fax: +43 1 4277 55120
Email: beate.mayr@bird-c.at

Dr. Lubitz Petra, Chief Operation Officer, BIRD-C GmbH&CoKEG
Hauptstr. 88, A-3420 Kritzendorf
Tel: +43 2243 28491; Fax: +43 2243 28514
Email: petra.lubitz@bird-c.at

BACKGROUND

The University of Vienna in Vienna, Austria, was founded in 1365 by Duke Rudolf IV and is one of the oldest universities in Europe. There are 63,000 students from 130 countries who are enrolled in a broad range of scientific disciplines and the humanities. There are more than 135 Bachelor’s, Master’s, Diploma, and Doctoral degree programs. The university has been the home of a number of Nobel Laureates.

The 5,400 members of the academic staff of the University of Vienna engage in teaching and research in the following scientific areas: Theology, Law, Business, Economics and Statistics, Computer Science, History, Philosophy, Education, Psychology, Social Sciences, Mathematics, Physics, Chemistry, Earth Sciences, Geography, Astronomy, Life Sciences, Translation Studies, and Molecular Biology. Professor Doctor Werner Lubitz is a member of the faculty in the Department of Medicinal Chemistry, which is located at the Vienna Pharmacenter on Althanstrasse.

Bird-C (Biotech Innovation Research Development Company) is a biotechnology company that Professor Lubitz founded in 1998 in Vienna. The mission of the company is to translate the knowledge and technologies accumulated during more than a decade of academic research in microbiology, genetics, and biotechnology into applications for the life sciences marketplace. The company is working in two areas:

1. Vaccine Development
2. Carrier and Targeting Vehicles

Bird-C plans to develop its product candidates through Phase I clinical studies and partner with larger companies for further product development. The company’s current product pipeline is based on the bacterial ghost (BG) technology platform. All of Bird-C’s technology is well protected by patents.

BACTERIAL GHOST PLATFORM

Bacterial Ghosts are nonliving Gram-negative cell envelopes that are produced by controlled expression of the lysis gene E from bacteriophage PhiX174. Activation of this gene allows the formation of a tunnel through the cell wall, which allows the cytoplasmic contents to escape due to the high internal osmotic
pressure of bacterial cells. The evacuated bacteria retain their cell wall and outer cell surface structure (see Figure 3.1 in Chapter 3).

These ghosts provide a facile system for the presentation and delivery of antigens, DNA, drugs, proteins, and small molecules. BGs are particularly promising vehicles for vaccines because of their ability to carry multiple antigens while retaining the intrinsic adjuvant and particulate properties of Gram-negative bacterial cell envelopes. Advantages of the system include the following: they are non-living and thus pose no pathogenic threat, they are free of nucleic acids and toxic substances, they are easy to produce in large quantities, there are several compartments (inner and outer cell envelope membranes) in which antigens or other drug components can be carried, and they are stable at ambient temperatures. The bacterial ghost may be engineered to include novel epitopes that are not usually found on the carrier ghost. The system has potential for use in cancer therapy or other drug delivery treatments in addition to vaccines. Routes of administration can include oral, nasal, subcutaneous, or aerosol.

BGs have been prepared using a wide variety of Gram-negative microorganisms with a number of different target antigens, including HIV proteins, *Vibrio cholerae* antigens, and *Helicobacter pylori* antigens. Heterologous antigens such as *B. anthracis* protective antigen and *Chlamydia trachomatis* outer membrane antigens have been incorporated into the BG system. A variety of animal studies have been performed that demonstrate immune response to the target antigen and the safety of BG preparations.

**TRANSLATION OF THE TECHNOLOGY**

The current major obstacle to further development of this system is the large investment required to prepare and validate clinical materials for Phase I studies. Dr. Lubitz is working with several organizations to bridge this “Valley of Death.” His company has a strategic alliance with Vital Probes, Inc., and is actively seeking development agreements with companies in the United States and Germany. Collaborations have been established with Johns Hopkins University for clinical studies in India or China and with Walter Reed Army Institute of Research for production of clinical supplies. DARPA has also provided some funding. If appropriate to the target use and product profile, it may also be possible to commercialize some of these bacterial ghosts containing vaccine antigens or other treatment compounds as food additives, thus lowering the barriers to market entry.

**SUMMARY AND CONCLUSIONS**

The bacterial ghost platform is a novel delivery system that has broad application for vaccines and other drug therapies. The range of potential uses includes DNA delivery, multiple antigen delivery, and drug delivery for prevention and/or treatment of a variety of infectious or noninfectious diseases.

The principle choke point for further development of this system is the absence of EU translation (commercialization) funding mechanisms to advance meritorious technologies into clinical studies. We strongly recommend EU-U.S. initiatives to overcome these barriers to early-stage technology development. The partnerships that Dr. Lubitz has established are exemplars of how collaboration between universities, small companies, and governments can facilitate product development.

**REFERENCES**


BACKGROUND

The World Health Organization (WHO) is an international agency governed by its 193 member nations through the World Health Assembly. Founded in 1948, it is the United Nations’ specialized health organization chartered for the attainment by all peoples of the highest possible level of health. The WHO Initiative for Vaccine Research (IVR) is focused on the need to streamline vaccine research and development. The program is looking for ways to more rapidly develop vaccines against diseases with significant public health importance, the improvement of existing vaccine technologies and to move these technologies forward for broad industry applications. IVR employs a three-pronged approach to achieve its goals, including the generation of partnerships to facilitate innovation resulting in improved vaccines and vaccine related technologies, research support for priority vaccines, and the development of implementation research and tools to support optimal use and benefit from vaccines.

ISSUES

In the case of pandemic influenza, public expectations may need to be mitigated as production is scaled to meet demands once the immunogenic strains have been identified. Dr. Marie-Paule Kieny stated that it could be 4 to 6 months between the index case for the disease and availability of the first doses of vaccine. Such delays need to be clearly explained to all stakeholders to keep expectations linked to realistic production goals. Vaccines might be prepared in advance of a pandemic for specific hemaglutinins and key immunogenic epitopes. Vaccine types that need to be considered are H5N1, H7, H9, and other H5 types.

A second issue concerns what vaccine format will have the shortest time to delivery. Live attenuated vaccines would have a significantly higher yield of viral particles than killed products. Dr. Kieny estimated that a live attenuated vaccine in eggs would yield at least 30 times more doses than an inactivated vaccine. The WTEC panel’s experience suggests that this could be as much as a 100-fold advantage in vaccine dose yield. Alternative vaccine delivery systems are just starting to mature based on molecular genetics, DNA vaccines, virus-like particles, viral vectors, and DNA electroporation; these will take many years to come to the market. New adjuvants are urgently needed to enhance immunogenicity of subunit or killed products, as the ones that have proven effective so far have restricted access due to intellectual property rights. New immunogen production systems such as baculovirus and other recombinant methods could provide interesting approaches in the future.

Stockpiles and prepositioning of vaccines could reduce some of the need for increased surge capacity during a pandemic, but such stockpiling requires broad cross-protection or new predictive mechanisms for looking at potential strains and clades. Dr. Kieny suggested that, building on experience with other vaccines, some
human vaccines might be produced in veterinary vaccine facilities in case of an emergency. She suggested that those facilities could begin to prepare their standard operating procedures for this application.

SUMMARY AND CONCLUSIONS

The WHO has a plan for rapidly responding to a pandemic. It has continuous interest in innovation and its application of the production of quality vaccines for the international marketplace.

REFERENCES


APPENDIX C. GLOSSARY OF ACRONYMS

ACIP Advisory Committee on Immunization Practices (U.S.)
AAFP American Academy of Family Physicians
NIP National Immunization Program
AIDS acquired immune deficiency syndrome
AIS (human) artificial immune system (Vax Design)
APC antigen-presenting cell
APHIS Animal and Plant Health Inspection Service (USDA)
ARS Agricultural Research Service (USDA)
BLA Biologics License Application (U.S.)
BSL Biosafety Level
CAD/CAM Computer-assisted design/manufacturing
CBER Center for Biologics Evaluation and Research division (U.S./FDA)
CDC Centers for Disease Control and Prevention (U.S.)
CE capillary electrophoresis
CFR Code of Federal Regulations
cGMP current good manufacturing practice (manufacturing facility)
CHMP Committee on Medicinal Products for Humans (EU/EMEA)
CIP clean-in-place
CMC Chemistry, Manufacturing, and Controls, i.e., quality control sections of biologics licensing applications
CTD Common Technical Document (EU/EMEA)
CVB Center for Veterinary Biologics (USDA)
CVMP Committee for Medicinal Products for Veterinary Use (EU/EMEA)
DC dendritic cell
DHS Department of Homeland Security (U.S.)
DNA deoxyribonucleic acid
DOD Department of Defense (U.S.)
ECDC European Centres for Disease Prevention and Control
EEA European Economic Area
EEC European Economic Community
EFTA European Free Trade Association
ELISA enzyme-linked immunosorbent assay
ELISPOT enzyme-linked immunospot (assay)
EMEA European Agency for the Evaluation of Medicinal Products
EU European Union
ESI-MS electrospray ionization mass spectrometry
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>EUA</td>
<td>Emergency Use Authorization (U.S.)</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration (U.S.)</td>
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<tr>
<td>GAO</td>
<td>General Accounting Office (U.S.)</td>
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<tr>
<td>GAVI</td>
<td>the GAVI Alliance, formerly known as the Global Alliance for Vaccines and Immunisation; only the acronym is used now</td>
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<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>GLP</td>
<td>Good laboratory practices designation</td>
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<tr>
<td>GMP</td>
<td>Good manufacturing practices designation</td>
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<tr>
<td>GP or gp</td>
<td>glycoprotein</td>
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<tr>
<td>GSK</td>
<td>GlaxoSmithKline Biologicals</td>
</tr>
<tr>
<td>HA</td>
<td>hemagglutinin (or haemagglutinin)</td>
</tr>
<tr>
<td>HBV</td>
<td>Hepatitis B virus</td>
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<tr>
<td>HCD</td>
<td>high cell density (bioreactor)</td>
</tr>
<tr>
<td>HHS</td>
<td>Department of Health and Human Services (U.S.)</td>
</tr>
<tr>
<td>HI</td>
<td>hemagglutination inhibition</td>
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<tr>
<td>Hib or Hflu</td>
<td>Haemophilus influenzae type b</td>
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<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
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<tr>
<td>HPA</td>
<td>Health Protection Agency (U.K.)</td>
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<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
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<tr>
<td>IABS</td>
<td>International Association for Biological Standardization</td>
</tr>
<tr>
<td>ICH</td>
<td>International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use</td>
</tr>
<tr>
<td>IND</td>
<td>Investigational New Drug application process (U.S./FDA/CBER)</td>
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<tr>
<td>μg</td>
<td>microgram(s) Note: the medical and veterinary professions often use mcg to avoid confusion, especially when writing prescriptions</td>
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<tr>
<td>MAA</td>
<td>Marketing Authorization Application (EU)</td>
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<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
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<tr>
<td>NA</td>
<td>neuraminidase</td>
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<tr>
<td>NDA</td>
<td>New Drug Application (U.S. FDA)</td>
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<tr>
<td>NIBIB</td>
<td>National Institute of Biomedical Imaging and Bioengineering of NIH (U.S.)</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health (U.S.)</td>
</tr>
<tr>
<td>NIP</td>
<td>National Immunization Program (U.S.)</td>
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<tr>
<td>NRC</td>
<td>National Research Council (part of the U.S. National Academies)</td>
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<tr>
<td>NSF</td>
<td>National Science Foundation (U.S.)</td>
</tr>
<tr>
<td>OIE</td>
<td>World Organisation for Animal Health (founded in 1924 as the Office International des Epizooties; the name changed in 2003 but the historical acronym OIE was retained).</td>
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<tr>
<td>ORA</td>
<td>Office of Regulatory Affairs (U.S./FDA)</td>
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<tr>
<td>PAMP</td>
<td>pathogen-associated molecular patterns</td>
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<tr>
<td>PAT</td>
<td>process analytical technology</td>
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<td>Acronym</td>
<td>Description</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PMED™</td>
<td>Particle Mediated Epidermal Delivery device (PowderMed, Ltd.)</td>
</tr>
<tr>
<td>RIG</td>
<td>intracellular retinoic-acid-inducible-gene-like (receptors)</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RSV</td>
<td>respiratory syncytial virus</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>reverse transcription polymerase chain reaction</td>
</tr>
<tr>
<td>S&amp;T</td>
<td>science and technology</td>
</tr>
<tr>
<td>SARS</td>
<td>severe acute respiratory syndrome</td>
</tr>
<tr>
<td>SCADA</td>
<td>Supervisory Control and Data Acquisition</td>
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<tr>
<td>SEC</td>
<td>Securities and Exchange Commission (U.S.)</td>
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<td>SFV</td>
<td>Semliki Forest virus</td>
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<tr>
<td>SIP</td>
<td>sterilization in place</td>
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<td>SME</td>
<td>small- and medium-sized enterprises (EU/designation or office of the EMEA)</td>
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<tr>
<td>SMI</td>
<td>Swedish Institute of Infectious Disease Control, Smittskyddsinstitutet</td>
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<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
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<tr>
<td>SPF</td>
<td>specific pathogen-free</td>
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<tr>
<td>SRH</td>
<td>single radial hemolysis (assay)</td>
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<td>TB</td>
<td>tuberculosis</td>
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<tr>
<td>TBE</td>
<td>tick-borne encephalitis</td>
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<tr>
<td>TLR</td>
<td>Toll-like receptors</td>
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<tr>
<td>USDA</td>
<td>U.S. Department of Agriculture</td>
</tr>
<tr>
<td>VICH</td>
<td>International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products</td>
</tr>
<tr>
<td>VLP</td>
<td>virus-like particle or pseudovirion</td>
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<tr>
<td>WFI</td>
<td>Water for Injection Generation System</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>WTEC</td>
<td>World Technology Evaluation Center, Inc.</td>
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APPENDIX D. DEFINITIONS OF TERMS

Note: Definitions here are largely derived from open online sources Wikipedia (http://en.wikipedia.org) and/or Dictionary.com (http://dictionary.reference.com), chiefly its Merriam-Webster’s Medical Dictionary entries.

adaptive immunity the ability of the vertebrate immune system to generate immunity to and also recognize and remember specific pathogens in order to prepare itself to mount a stronger attack should the pathogen be encountered again.

adjuvant a vaccine ingredient that is mixed with an immunogen in order to facilitate or enhance immune response.

antigen any molecule or portion of a molecule that can induce an immune response (such as of a toxin).

attenuated rendered less virulent.

B cell(s) lymphocytes produced in the bone marrow of most mammals that play a large role in the humoral [bodily fluids] immune response; their principal function is to make antibodies against soluble antigens; they are an essential component of the adaptive immune system.

bacterial ghosts nonliving bacterial cells that are useful as vaccines because they retain the morphology and structural integrity of their living counterparts without their properties, and they have intrinsic adjuvant properties.

BHK baby hamster kidney cells, an immortalized cell line often used in molecular genetics.

CD4 (cluster of differentiation 4) a cellular surface glycoprotein expressed by mature T_h (helper) cells, regulatory T cells, monocytes, macrophages, and dendritic cells. On T cells, CD4 is the co-receptor for the T cell receptor and amplifies the signal generated by the TCR.

CD8 (cluster of differentiation 8) a transmembrane glycoprotein that serves as a co-receptor for the TCR. Like the TCR, CD8 binds to a major histocompatibility complex (MHC) molecule, but is specific for the class I MHC protein; it is predominantly expressed on the surface of cytotoxic T cells, but can also be found on natural killer cells.

cytokine(s) a group of proteins and peptides that function in organisms as signaling compounds and are important in both innate and adaptive immune responses; due to their key role in the immune system, they are involved in a variety of immunological, inflammatory and infectious diseases.

ELISPOT (enzyme-linked immunospot) assay, a flexible, sensitive tool for analyzing the immunological secretions of peripheral blood and lymphoid cell populations in order to detect, enumerate, and characterize individual cytokine-secreting cells within cultured cell populations.

epitope a region on the surface of an antigen capable of eliciting an immune response and of combining with the specific antibody produced by such a response.

H3N2 a subtype of the influenza A virus that contains the proteins hemagglutinin (H) and neuraminidase (N) on its surface coating; H3N2 viruses infect humans and pigs, and in each species, the virus has mutated into many strains.

H5N1 a subtype of the influenza A virus that can cause illness in humans and many other animal species; a bird-adapted strain of H5N1 is the causative agent of what is commonly known as "avian influenza" or "bird flu"; it is endemic in many bird populations, especially in SE Asia.

humoral the part of the immune response that involves antibodies secreted by B cells and circulating in bodily fluids.
Appendix D. Definitions of Terms

**immunogen**
any substance, cell, or organism introduced into the human body in order to provoke an immune response

**MCH**
major histocompatibility complex, a large genomic region or gene family found in most vertebrates that plays an important role in the immune system, autoimmunity, and reproduction; the proteins encoded by the MHC are expressed on the surface of cells to T cells that have the capacity to kill or coordinate the killing of pathogens or infected or malfunctioning cells

**morbidity**
the symptoms of or rate of sickness and spread of a disease

**NFκB**
a protein complex found in all cell types that helps regulate the cell’s response to bacterial or viral antigens, thus helping orchestrate its immune response

**split/subunit vaccine**
a vaccine prepared by growing a virus strain in the conventional egg-based process then treating the recovered virus to remove the core, leaving only the outer antigenic determinants; most egg-based vaccines today are split or subunit vaccines; they are less likely to cause adverse reactions than vaccines containing the whole virion

**Tamiflu**
the trade name of an oral antiviral drug Oseltamivir marketed by Hoffmann-La Roche (Roche) and generally available by prescription only that is used in the treatment and prophylaxis of both Influenza virus A and B; it acts as a transition-state analogue inhibitor of influenza neuraminidase, preventing new viruses from emerging from infected cells

**TCR**
T-cell receptor

**T helper (Th)**
(also known as effector T cells) cells that are a subgroup of lymphocytes that play an important role in establishing and maximizing the capabilities of the immune system; although they cannot themselves kill infected host cells or pathogens, they are actively involved in activating and directing other immune cells

**T cell(s)**
a subgroup of white blood cells known as lymphocytes, produced by the thymus, that play a central role in cell-mediated immune response; they can be distinguished from other lymphocyte types, such as B cells and NK cells, by the presence of a special receptor on their cell surface that is called the T-cell receptor (TCR).

**Toll**
a key component of the innate immune system of the fruit fly; in the mid-1990s, the mammalian equivalent, Toll-like receptors (TLR), were identified

**tumorigenicity**
agents capable of causing tumors

**variolation**
the deliberate inoculation of an uninfected person with the smallpox virus (as by contact with pus) to protect against severe forms of smallpox, widely practiced before the era of vaccination

**Vero cell(s)**
lineages of cells used primarily in virus cell cultures/replication that were isolated from kidney epithelial cells extracted from African green monkeys; they constitute a continuous cell lineage that can be replicated through many cycles of division and not become senescent

**virion**
a complete virus particle that consists of an RNA or DNA core with a protein coating and that is the extracellular infective form of a virus

**whole-virus vaccine**
a vaccine produced from the intact virus that has been rendered inactive or attenuated, including killed and split virus preparations; a vaccine for pandemic influenza is likely to be a whole-virus vaccine

**zoonotic**
pertaining to or describing an infectious disease that has originated in animals and/or is able to be transmitted from animals to humans