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The

BRIDGE

LINKING ENGINEERING AND SOCIETY

Planning for Pandemics of Infectious Diseases

Roy M. Anderson

Pharmaceutical Preparedness for a Pandemic

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Egg-Based Production of Influenza Vaccine: 30 Years of Commercial Experience

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Adapting Industry Practices for the Rapid, Large-Scale Manufacture of Pharmaceutical Proteins

David Estell

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The

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BRIDGE

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Editor's Note

- 3 **Engineering and Vaccine Production for an Influenza Pandemic**
Arthur H. Heuer

Features

- 5 **Planning for Pandemics of Infectious Diseases**
Roy M. Anderson
Health care providers and public health officials can use proven mathematical models to plan responses to a pandemic.
- 10 **Pharmaceutical Preparedness for a Pandemic**
Patrick J. Scannon
H5N1 may be the first real test of a rapid pharmaceutical response to a pandemic.
- 17 **Egg-Based Production of Influenza Vaccine: 30 Years of Commercial Experience**
James T. Matthews
Despite improvements in technology and increased automation, egg-based vaccine production still has a long timeline.
- 25 **Cell-Culture-Based Vaccine Production: Technological Options**
Rino Rappuoli
The next generation of influenza vaccines will be cell-culture-based for seasonal influenza and for pandemics.
- 31 **Alternative Methods of Making Influenza Vaccines**
Alan Shaw
The keys to survival of a pandemic will be surveillance, public health planning, and a publicly agreed strategy for behavior.
- 39 **Adapting Industry Practices for the Rapid, Large-Scale Manufacture of Pharmaceutical Proteins**
David Estell
The tools and methods of industrial biotechnology could be adapted to produce pharmaceutical proteins for a pandemic influenza vaccine.

NAE News and Notes

- 45 NAE Newsmakers

(continued on next page)

46	2006 German-American Frontiers of Engineering Symposium
47	Christine Mirzayan Science and Technology Policy Graduate Fellows
48	NAE Report on Assessing Technological Literacy
48	NAE and Royal Swedish Academy Launch Parallel Studies on Noise Control
49	Calendar of Meetings and Events
50	NAE Members Continue the Culture of Philanthropy
51	In Memoriam
52	Publications of Interest

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Editor's Note



Arthur H. Heuer is University Professor and Kyocera Professor of Ceramics, Case Western Reserve University, and a member of NAE.

Engineering and Vaccine Production for an Influenza Pandemic

Every year, influenza A and B cause epidemics that lead to an average of 36,000 deaths and 114,000 hospitalizations in the United States alone. Periodically, new influenza strains evolve with the capacity to cause pandemics (epidemics that spread around the globe). The recent spread of avian influenza (H5N1) among

birds and more than 200 cases of transmission to humans, indicating the potential evolution of a pandemic influenza virus, have stimulated a great deal of discussion of measures to combat it.

Pharmaceutical companies have been very successful at producing safe, effective vaccines to protect against seasonal influenza epidemics, but current methods may not be sufficient to produce large amounts of vaccine rapidly enough to combat a pandemic. Despite ongoing efforts to predict the nature of the next pandemic strain, correct identification cannot be assured in advance; thus, advance stockpiling of vaccine may not be helpful. In addition, the efficacy of available antiviral drugs, such as amantadine (Symmetrel™) and oseltamavir (Tamiflu™), is limited by resistant strains and a short window of opportunity for effective therapy.

Thus, the options for treating pandemic influenza are limited, and the main defense is still prophylactic vaccination. However, once a pandemic strain has been identified, it is estimated it will take at least five to six months using current production methods to produce enough vaccine to protect a substantial proportion of the population. Depending on many variables, including the seasonal timing of onset, a pandemic could spread and resolve within six months, leading to the grim prospect that vaccine would be available mainly for survivors.

Current vaccine production involves numerous time-consuming steps: reassortant virus must be generated and then grown in embryonated eggs (trivalent

vaccines, the usual vaccine for seasonal [epidemic] influenza, require separate growth campaigns for the three different viruses); the virus must be purified and inactivated; the virus must then be split and the antigenic components enriched via biochemical procedures; the vaccine must be formulated to combine the three antigen preparations (for a trivalent vaccine) plus adjuvant. The last three steps—filling, packaging, and distribution—involve many engineering considerations that may not have been given enough attention. In addition, numerous quality-control tests must be done along the way (many of them time consuming and expensive) to ensure the safety and efficacy of the vaccine.

In a pandemic, resources and production capacity will be focused on producing a monovalent vaccine targeted for the pandemic virus, rather than a trivalent vaccine. Current worldwide production capacity for trivalent influenza vaccine is 300 million doses per year; a theoretical equivalent of monovalent vaccine would be 900 million doses, enough to vaccinate only 10 to 15 percent of the world's population with a single dose. Decreasing the dose per person might be one way to stretch vaccine supplies, but this would have only a marginal impact on the time to delivery, and the effectiveness of reduced doses would have to be carefully tested.

Currently, vaccine producers have little excess production capacity because, under current conditions, industry would incur significant costs in building standby production capacity and maintaining standby readiness.

With these distressing facts in mind, a recent meeting, "Vaccine Production: Potential Engineering Approaches to a Pandemic," sponsored by the National Academy of Engineering (NAE) and the Institute of Medicine (IOM) and hosted by Case Western Reserve University (CWRU), was held to explore issues related to vaccine production and alternative manufacturing processes. The meeting provided a venue for a "community" of academic and industrial engineers, government regulators, and other scientists and physicians to bring an engineering perspective to bear on critical issues in vaccine production. The meeting was attended by 275 participants representing 6 countries, 60 companies, and 44 universities. There were also about 1,000 web-cast viewers.

The papers included in this issue of *The Bridge* cover the major issues discussed at the meeting. The first article by Roy Anderson, a noted epidemiologist from Imperial College, London, is based on his keynote lecture, "Scientific Methods Underpinning Policy Formulation for an Influenza Pandemic," which helped set the stage for the meeting. The next article, "Pharmaceutical Preparedness for a Pandemic," is by Pat Scannon, chief biotechnology officer of XOMA, a small pharmaceutical company. He explains why it takes so long to introduce vaccines and other pharmaceuticals, identifies "choke points" inherent in current methods of pharmaceutical development, and makes an eloquent appeal to the engineering profession for help in meeting the challenges of an influenza pandemic. James Matthews of Sanofi Pasteur, a major vaccine manufacturer, describes "Egg-Based Production of Influenza Vaccine: 30 Years of Commercial Experience."

The fourth article, "Cell-Culture-Based Vaccine Production: Technological Options," by Rino Rappuoli of Novartis (formerly Chiron Vaccine), argues that cell-culture-based vaccine production is likely to obtain FDA approval in the future and to replace egg-based methods. Alan Shaw of VaxInnate, a small biotech company, then reviews "Alternative Methods of Vaccine Production," all of which are still in the R&D phase (mostly R). At this point, it is difficult to predict which of these alternative technologies, if any, will make it to market.

The final article, "Adapting Industry Practices for Rapid, Large-Scale Manufacture of Pharmaceutical Proteins," is by David Estell of Genencor, a medium-sized biotech company. He describes the biotechnology of industrial protein production (e.g., for commercial-grade enzymes), which produces materials that sell for ~\$1/gm active protein; this is compared to vaccine production costs of ~\$1,000/gm active protein. Estell argues that there are no obvious technological barriers to adapting industrial protein-engineering technologies to the production of vaccines and other pharmaceuticals. However, a major unanswered question is whether regulatory and other constraints will permit these commercial technologies to be used in vaccine production.

Based on the discussions at the meeting summarized in this issue, the real possibility of an influenza pandemic clearly raises enormous engineering challenges. I urge NAE and IOM to use their resources to promote more discussions and critical analyses to meet these challenges.



Note: I gratefully acknowledge the help of Clifford Harding and John Angus (both of Case Western) in preparing this editorial. Archived presentations from the workshop are available at <http://www.vaccine2006.org>. The website also identifies 21 academic, government, and industry co-sponsors.

Health care providers and public health officials can use proven mathematical models to plan responses to a pandemic.

Planning for Pandemics of Infectious Diseases



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Roy M. Anderson

In the past decade, major changes have been made in the way governments and international agencies plan for the management and control of epidemics. These changes have been brought about by the growing realization that mathematical models can provide accurate descriptions and analytical tools for predicting and interpreting the typical course of an epidemic of a given aetiological agent and the consequences of various combinations of control options (Anderson and May, 1991). Just as physicists and engineers have done for much of the past century, health care providers and public health officials are beginning to use well defined mathematical templates to help them understand natural phenomena.

The mathematical theory of epidemics has a long history, dating back at least to the beginning of the twentieth century—and perhaps much earlier, as illustrated by the work of Bernouilli published in 1761 on the mortality caused by smallpox epidemics. Until relatively recently, however, most of this research has been published in specialized technical journals and has not been on the everyday reading lists of physicians and public health workers—let alone policy makers.

A number of recent events have led to changes in this pattern. First, there is a growing appreciation among scientists of the power of mathematics as a tool for dissecting the causal factors that underlie complex dynamic behaviors in biological systems, ranging from ecosystems to the human immune system.

Second, and perhaps more important, mathematical models have recently been used successfully to inform responses to epidemics that were covered heavily in the media: (1) an epidemic of foot and mouth disease, a viral disease in cattle and sheep, in Great Britain in 2001; and (2) the 2003 epidemic of SARS, a lethal respiratory tract virus that spread out of China to many other countries at alarming speed.

Thankfully, although both epidemics caused suffering and mortality and much anxiety, they were brought under control relatively quickly. In both cases, mathematical models, constructed and analyzed as events unfolded, provided insight and guidance to veterinary and public health authorities on optimal control policies and the expected course of the epidemics under different intervention scenarios (Ferguson et al., 2001; Lipsitch et al., 2003; Riley et al., 2003). Mathematical models also provided estimates of key epidemiological parameters: typical incubation periods (the average time from infection to the appearance of symptoms); the duration of infectiousness; and the basic reproductive number, R_0 , the average number of secondary infections generated by one primary infection in a susceptible population.

We are now facing a serious threat of a highly pathogenic strain of avian influenza, H5N1, mutating or reassorting with a human strain to permit sustained transmission within human communities. This threat has stimulated a great deal of research on mathematical models of an influenza A pandemic and the best ways to control its spread and limit consequent morbidity and mortality before the event occurs. Both government departments of health (e.g., in the United Kingdom and the United States) and the World Health Organisation have encouraged this research and are actively participating in the development of quantitative tools to assist in planning for a pandemic.

In the past, such planning would have been based on the consensus opinion of expert committees, consisting largely of specialists in medicine, infectious diseases, biology, and public health. Today, however, calculation and simulation are replacing opinion and consensus—although not yet in all countries. This article includes a brief review of recent work on the development of a mathematical model for an influenza pandemic and offers conclusions based on this research to date.

Simple and Complex Models

Simple mathematical models, which permit a degree of analytical exploration, can improve the general

understanding of determinants in the course of an epidemic and the impact of intervention. Even the simplest models are nonlinear and include analyses of once-simple heterogeneities, which are integral to disease spread (Anderson and May, 1991). However, because only a small number of parameters can be estimated from observed trends, the advantages of simple models are limited.

At the other end of the spectrum, complex individual-based stochastic frameworks can simulate the behavior and disease state of all individuals in a defined population, and, given sufficient computational power, can explore the efficacy of various interventions. However, because of the large number of parameters involved, there is always a degree of uncertainty about the validity of simulated patterns.

Ideally, different approaches with varying degrees of complexity should be adopted. After comparing the results, attention should then be focused on differences in model predictions that could influence policy making.

Both simple and complex formulations require a subset of key parameters, such as incubation and infectious periods and the basic R_0 . Complex models require much more information, such as the details of demography, the movement of people, mixing patterns (at household, local community, and larger scales), and the spatial distribution of the population in a defined area. Ideally, international travel patterns would also be mirrored in these models.

The simplest models view the human population as consisting of individuals classified by disease state—such as susceptible, X ; infected but not yet infectious (i.e., incubating), H ; infectious, Y ; and recovered, Z . The key equations follow:

$$\begin{aligned} dX/dt &= -\beta XY - \mu X \\ dH/dt &= \beta XY - (\mu + \sigma)H \\ dY/dt &= \sigma H - (\mu + \alpha + \gamma)Y \\ dZ/dt &= \gamma Y - \mu Z \end{aligned} \quad (\text{eqn. 1})$$

Here, β is the transmission coefficient, $1/\mu$ represents life expectancy, $1/(\mu + \gamma)$ is the average incubation period, $1/(\mu + \alpha + \gamma)$ is the average duration of infectiousness, and α is the disease-induced death rate. Given the very short time scale of influenza epidemics, which are typically over in a given country within six months, births are not accounted for. In some circumstances, an epidemic may

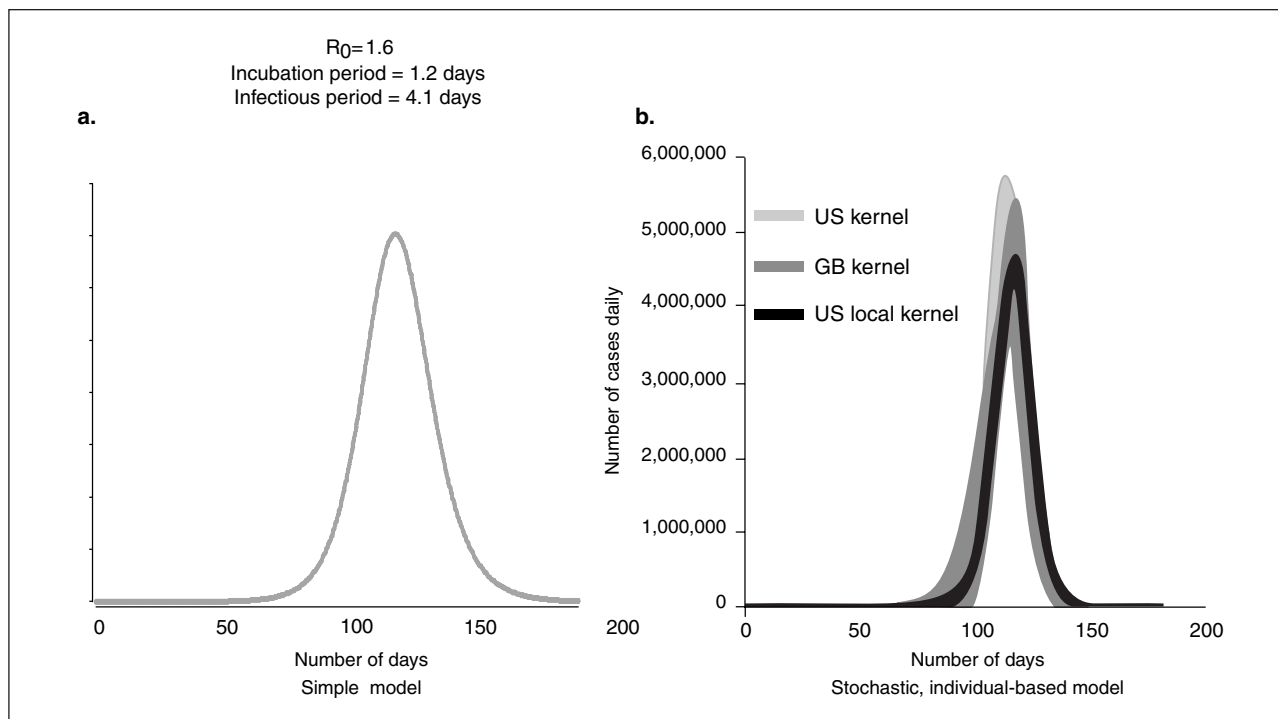


FIGURE 1 Comparison of the predictions of (a) a simple epidemic model and (b) an individual-based stochastic model. The predictions of the complex model are shown as averages from sets of simulations and with spatial movement kernels derived from data sources in Great Britain and two sources in the United States. Source: adapted from Ferguson et al., 2006.

last for two influenza seasons (a season usually lasts from October to February in the northern hemisphere).

Ferguson et al. (2001) have described more complex models, in which the fine details of age structure and spatial distribution of population density are accounted for, as is the capability of embedding distributions for incubation and infectious periods, mixing patterns, and spatial kernels for the probability that someone in a given location will move a certain distance in a defined period of time. The day of the week, spatial location (i.e., city or remote rural area), and time of year are important parameters in these probability distributions.

Measurements of movements and mixing patterns can be made in a variety of ways, including using population-based anonymized samples of tracking data based on mobile phone use, census and survey records of travel patterns, and questionnaires. Work to date suggests that the probability distributions of movement are not scale-free except for a middle section of distance movement between local and long distance. The vast majority of people move mostly locally. A few, so-called “super-travelers,” often contribute most to the early stages of epidemic spread. This was well illustrated by the SARS epidemic in March 2003 (Hollingsworth et al., 2006).

If, as Ferguson et al. (2006) suggest, reasonable parameterization is possible for complex models, they would have the great advantage of including a great amount of detail for possible control options. For influenza A, the options are focused on: increasing “social distance” (i.e., restricting travel or encouraging people to stay at home and not attend social, entertainment, or sporting events); therapeutic or prophylactic treatment with anti-influenza drugs (such as oseltamivir); contact tracing and treatment; and vaccination, either before or after the start of an epidemic. Complex, individual-based, stochastic simulation structures can mirror the logistics of drug and vaccine delivery, as well as delays in the diagnosis, treatment, and isolation of infectious patients.

Despite the differences in the level of detail in simple and complex models, their predictions of overall patterns of disease spread through time are surprisingly similar. Figure 1a shows outcomes for the simple equations defined above (eqn. 1). Figure 1b shows the outcome from the individual-based stochastic model for the United Kingdom and United States. Note the similar shape and timing of the overall epidemic for a typical influenza A virus.

This simple comparison shows that much can be gained by using both simple and complex models.

Where their predictions differ, we can take those opportunities to further our understanding of the key determinants of observed patterns.

Control of an Influenza Pandemic

The rapid control of the SARS epidemic in 2003 left some with the impression that “we have done it once, and we can do it again for the next emerging pathogen.” However, a sense of complacency is misplaced for one particular reason—the typical course of the SARS infection in the infected patient. Detailed clinical virological studies showed that peak viraemia (i.e., peak infectiousness to susceptible contacts) in nasal or faecal secretions and excretions occurred many days after the onset of clinical symptoms of disease, often as long as 10 days after the end of the incubation period. Therefore, if a patient was isolated a few days after the onset of illness, the duration of the infectious period was significantly decreased. In other words, contact tracing and isolation were very effective public health control measures for SARS.

In the case of influenza A, the clinical pattern of infection is very different. First, the average incubation period is very short—on the order of two days—roughly half the incubation period for SARS. Thus, the generation time for influenza (the time from infection to transmission to a susceptible contact) is only three to five days. An epidemic of influenza A, therefore, can develop very quickly in dense, highly mixed populations, with the vast majority of cases occurring within the first 200 days. More important, peak viraemia, hence peak infectiousness, is synchronous with the onset of clinical symptoms (e.g., see Figure 2). Thus, contact tracing and isolation will be much less effective because a good part of the infectious period occurs before the onset of clinical symptoms. Even with the rapid diagnosis and isolation of contacts, these simple public health measures are very unlikely to have a substantial impact on the course of the epidemic.

Simple and complex models both suggest that the best option for suppressing an epidemic significantly is via prevaccination of the population with a vaccine that has some antigenic similarity to the emerging strain. The development of such a vaccine will require making a guess about (1) the antigenic composition arising from mutations in the H5N1 virus that would make it transmissible in human communities or (2) the reassortment of two viral genomes, one human and one the H5N1 bird virus.

Some countries have chosen to go this route, with the

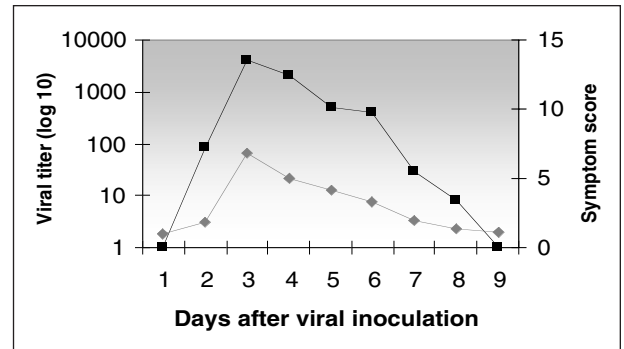


FIGURE 2 The typical course of influenza A infection in a patient in terms of viraemia (◆) and the onset of clinical symptoms (■). Source: adapted from Hayden et al., 1998.

key antigens related to the H5 haemagglutinin and the N1 neuraminidase of the bird virus. If the guess about the vaccine antigens is close to what actually emerges, this is clearly a good option. If not—then the vaccine will not be effective. Thus, this approach is a high-risk, costly strategy—but one that could work well.

To prevent an epidemic, the fraction of the population that must be immunized, p , is shown in the following simple expression:

$$p > [1 - 1/R_0] / \epsilon \quad (\text{eqn 2})$$

Here, p represents vaccine efficacy (unity represents perfect protection and zero represents no protection). R_0 , the basic reproductive number, typically has a value between 1 and 2 for new strains of influenza A in human communities. With perfect efficacy and an R_0 value of 1.6, 37.5 percent of the population would have to be pre-immunized to prevent an epidemic. The percentage would be lower for the total population if vaccination were targeted to 5 to 15 year olds (where most transmission typically occurs in respiratory tract infectious-disease epidemics).

If vaccine development is delayed until after the emergence of a new strain, the vaccine will, of course, be precisely targeted on the correct antigens. But the formulation and scale-up of production will take time, perhaps more than the 200 days of a typical epidemic.

The conclusions of the very detailed studies done by Ferguson et al. (2006) exploring a wide range of options other than vaccination alone or in combination can be summed up very simply. Unless delivery systems and logistics of the supply of antiviral drugs are extremely efficient, it will be very difficult to control the spread of the epidemic. Prophylactic drug treatment can inhibit

infection, and, if the infection still occurs, it can limit infectiousness by lowering viraemia. Even after the onset of clinical symptoms, it can reduce morbidity and mortality. However, the timing of drug delivery is key. Optimally, it must be given one or two days after the onset of symptoms.

Ferguson et al. show that a combination of (1) treatment of sick patients; (2) contact tracing; and (3) prophylactic treatment of household contacts plus restrictions on travel (i.e., increasing social distance) can very significantly reduce the scale of a potential epidemic. This is encouraging and gives us a target to aim for. However, these options must be delivered optimally in terms of very rapid tracing (the first day after a sick patient is seen in a primary or secondary care setting because that patient will probably come in one or two days after the onset of symptoms); very rapid delivery of treatment; and excellent compliance with treatment regimens and “stay at home” requests. To be successful, simulations suggest that logistics and delivery must be efficient and uniform across districts, counties, and states.

Conclusions

Both simple and complex models suggest that controlling the spread of a highly pathogenic influenza A strain will be very difficult. Although the emergence of such a virus is all but certain in the coming decades, the timing and antigenic nature of the virus are very uncertain. Restrictions on international travel and closing borders will have little effect on the rate of spread unless more than 99.5 percent of entries are stopped (Hollingsworth et al., 2006). In practice, this seems impossible to achieve. Although some time might be gained by these restrictions, the gains would be measured in weeks, not months.

Substantial reductions in morbidity and mortality are possible via drug treatment and increasing social distance, but only if the availability, logistics, and delivery of the drug are extremely efficient and compliance with treatment regimens is strictly observed. Prevacination, by far the best option, will require significant expenditures and good guesswork about the nature of the novel strain that will emerge.

The key message of analyses based on models to date is that logistics and delivery must be carefully planned and efficiently carried out. Once an epidemic starts, health authorities must be able to get drugs and care to

sick individuals quickly—in all areas of the country. Rehearsals are essential—as is uniform performance across county, district, or state boundaries.

The worst option is to delegate authority to the lower tiers of local governments, which are likely to adopt diverse responses. The authority to deliver drugs and care can be delegated, but, as simulation studies show, the fine details of contingency plans must be decided at the national level, communicated to everyone, and applied uniformly. The present need is for national contingency planning based on extensive simulations and calculations rather than on qualitative opinions.

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H5N1 may be the first real test of a rapid pharmaceutical response to a pandemic.

Pharmaceutical Preparedness for a Pandemic



Patrick J. Scannon is chief biotechnology officer at XOMA Ltd.

Patrick J. Scannon

As H5N1 spreads around the world, the requirements for pandemic preparedness are being discussed on an unprecedented international scale. Despite an increasing awareness of need, the question remains: is the pharmaceutical industry prepared to meet this challenge and others like it? From a pharmaceutical perspective, the answer to the question is a resounding NO. The follow-on question should then be, why aren't we ready? Or, more constructively, what is the baseline from which we can build toward readiness?

A central purpose of the NAE/Institute of Medicine (IOM) conference was to bring together engineers, scientists, and global health care leaders to discuss opportunities for improving pharmaceutical preparedness. This is not a theoretical exercise—H5N1 influenza virus may become the first real challenge to the innovations and interventions catalyzed by this initiative. And if the H5N1 challenge should be averted, it is only a matter of time before another virus with pandemic potential arises.

The Threat of Pandemic Influenza: Are We Ready? Workshop Summary, an IOM report published in 2005, addressed the question of pharmaceutical preparedness and summarized strategies for readiness in the United States and around the world (IOM, 2005):

1. Vaccines are the single most important intervention for preventing associated morbidity and mortality during both seasonal epidemics and pandemics.

2. No country will have adequate supplies of vaccine at the start of a pandemic. At least 4 to 6 months will be needed to produce the first doses of vaccine following isolation of a new pandemic virus. The subsequent augmentation of supplies will be progressive. Stockpiling in advance is not an option.

3. Antivirals are expected to be effective against human illness caused by avian influenza and human pandemic strains. Pending the availability of vaccines, they will be the only influenza-specific medical intervention for use in a pandemic.

4. Inadequate supplies are a major constraint. Supplies are presently extremely limited, and manufacturing capacity could not be augmented during the course of a pandemic. At current capacity, several years would be needed to increase supplies appreciably.

One approach to assessing the state of pharmaceutical preparedness is to understand how the seasonal influenza vaccine is made and to determine if such a process is applicable for an H5N1 (pandemic) vaccine. After all, H5N1, a strain of avian influenza, and seasonal influenza, are both influenza viruses (even though they are potentially very different diseases), and the seasonal influenza vaccine is made by a proven production process that uses fertilized chicken eggs.

Once a year (for each hemisphere), a group of scientists, epidemiologists, and other public health specialists assemble to decide upon the virus composition for that year. Prior to that meeting, egg production in approved sites has already begun, so that when the vaccine strains are selected hundreds of millions of eggs are ready. Over a period of nine to ten months, 60 to 100 million doses of vaccine are produced for the U.S. market (~300 million doses worldwide). Some of the vaccine is released as early as six or seven months after production begins (Figure 1).

With that as a “standard” for vaccine production, one of the most significant pandemic-related concerns is the life-and-death

competition between the time it takes to make and release a useful vaccine and the time it takes a contagious virus to spread around the world. In “The Next Killer Flu,” an article in the October 2005 issue of *National Geographic* by Tim Appenzeller, the rate of spread of a pandemic (such as H5N1) is compared with vaccine responsiveness. The mathematical model used in the article predicts that a human pandemic would circle the globe within 180 days (roughly half the time it took the last pandemic infection in 1968), but the first vaccine would become available only after day 250 (i.e., about nine months after the start of production). This model assumes that scientists would identify the exact H5N1 isolate only after the pandemic had begun and that current egg-production methods would be used to produce a vaccine. These same assumptions were used in the dire IOM conclusions.

Drug-Development Process

To understand current pharmaceutical responsiveness, we first need a basic understanding of the drug-development process in general and, more specifically, the development process for vaccines. A common lay perception of drug development is that discovery research and product identification are the “gating” steps for drug approval (Figure 2). Looked at another way, the common misperception is that critical post-research processes, such as manufacturing, somehow just happen. In fact, the manufacture of drugs and vaccines involves very complex scientific and engineering challenges, quite separate from discovery research.

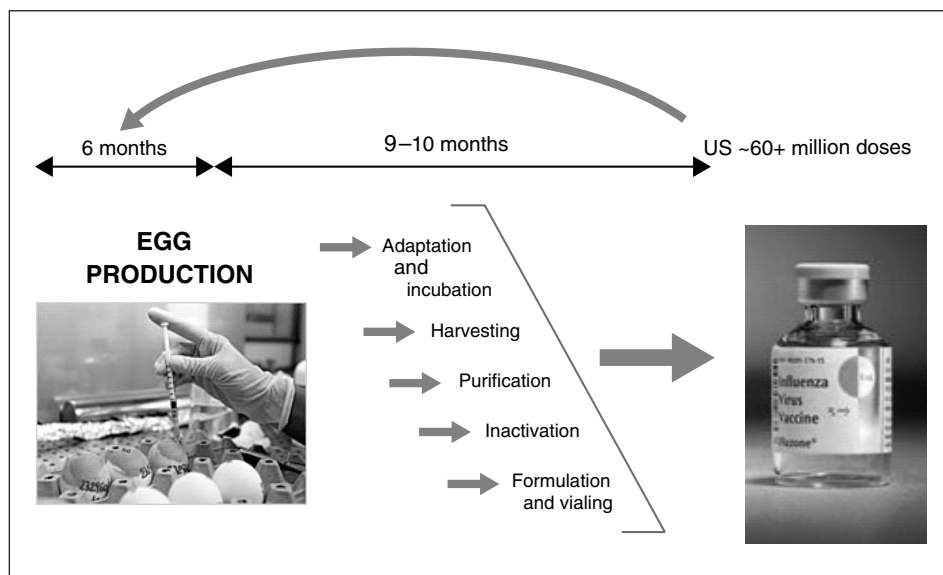


FIGURE 1 Seasonal influenza annual vaccine production in eggs, a proven cyclic process. Source: IOM, 2005.

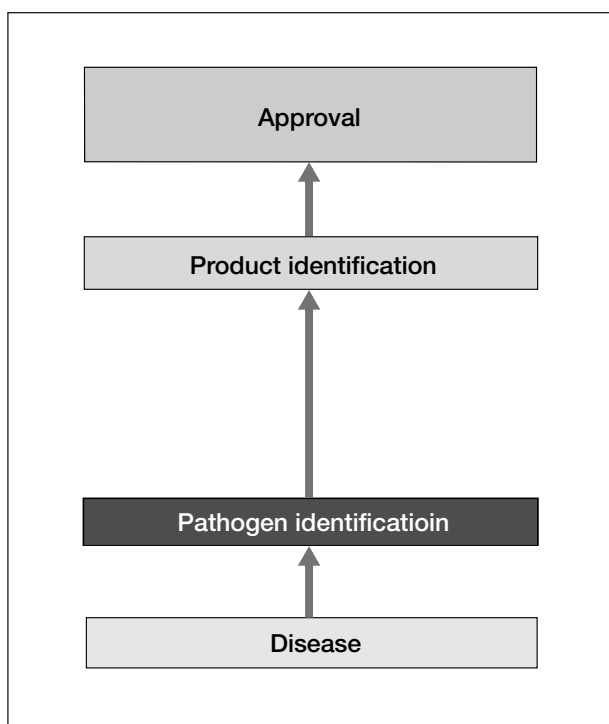


FIGURE 2 A common (non-pharmaceutical view) of drug development with the emphasis on discovery and product identification.

Another misconception is that the pharmaceutical industry is a single industry. In fact, it is a series of industries with many regulatory steps in common but also with independent technologies, depending on the type of product being made: biological drugs resulting from the biotech revolution include both recombinant proteins and antibodies; the targets of vaccine development, a separate business, are toxins and viruses; synthetic small molecules, a very important third class of drugs, are used across the spectrum of human diseases.

These three sub-industries are not at parity. The biggest revenue generator, by far, is synthetic small molecules. Vaccine revenues are a distant third. The three classes of pharmaceuticals, by and large, also use different manufacturing technologies.

In recent years, new discovery technologies have accelerated the pace of vaccine discovery, but vaccine manufacturing processes have essentially remained unchanged, in large measure because there has been little or no incentive to change post-discovery downstream processes. Thus, even though a vaccine might be selected much more rapidly today than ever before, the impact on time-to-patient availability (especially in a pandemic setting) is minimal, because the manufacturing and testing steps have not kept pace. In contrast,

the processes to make a synthetic molecule like Tamiflu[®], an important antiviral drug against H5N1, are completely different from those used to make vaccines, even though the regulatory (e.g., Food and Drug Administration [FDA]) steps are similar.

To ensure drug safety and effectiveness, the pharmaceutical industry is highly regulated. Drug development and regulatory requirements must be integrated with each technology to create an acceptable pathway to marketing approval. Even within the vaccine world, technologies besides eggs have been developed for making human vaccines—and each of these is separately regulated.

“Knowns” and “Unknowns” in Drug Development

During the drug-development process, some components are unavoidably “unknown” (meaning new discoveries are expected to be made); other components are kept, as much as possible, as “knowns,” to minimize complexity, time, and expense. Figure 3 shows examples of new-product “unknowns,” such as human safety, efficacy, potency, dosage, drug kinetics, drug metabolism, and drug interactions. In both drug and vaccine development, it is highly desirable to limit the “unknown” variables to the product itself, because new downstream manufacturing processes are very costly, both in dollars (tens to hundreds of millions) and time (months to years). To the extent that downstream manufacturing processes can be kept as “knowns,” the overall development program is greatly simplified.

Changing even minor manufacturing methods requires validating the new methods to meet FDA regulatory guidelines, such as the current Good Manufacturing Practices (cGMPs). New manufacturing methods, which may offer major improvements, are useless until they are validated (i.e., meet cGMPs) and are thus carefully considered before changes are made. The risk of a new manufacturing process—which could set a program back by years if it fails—provides considerable motivation for retaining the “old way” of doing things. In the pharmaceutical world, one must not only be highly motivated, but also willing to take multimillion dollar risks and be well financed to change “known” manufacturing processes that work, however inefficiently by modern standards. Hence, most of the time, drug-development risks fall primarily on human testing of the product (“unknowns”). Only if absolutely necessary, is manufacturing risk included.

Egg Production for Pandemic Vaccine Production

Egg production certainly has some advantages. First, this decades-old technology is an established, validated process, and regulated egg production facilities are in place. The only significant parameter that changes from year to year is the influenza virus selected for insertion into the eggs. However, this stable, predictable process may not work in a pandemic setting for many reasons:

1. The pandemic could occur off cycle when not enough eggs are available.
2. The virus could mutate sufficiently to nullify the potency (immunogenicity) of a vaccine already prepared and stockpiled (i.e., start over).
3. H5N1 may not be the ultimate cause of the pandemic (i.e., start over).
4. If the pandemic is rapidly contagious, there may not be enough time to produce an adequate supply of vaccine (e.g., difficult to start over).
5. Increased worldwide demand from a pandemic, combined with the requirement for seasonal influenza vaccine, would completely overwhelm the programmed seasonal influenza vaccine manufacturing capacity.
6. If an avian influenza infected the population of chickens that produce eggs for seasonal influenza vaccine, egg production would cease.

Considering these plausible possibilities, what can be done to ensure vaccine manufacturing preparedness? And how fast can it be done?

The Regulatory Process

A good place to start is with the FDA, the federal body that regulates all pharmaceutical manufacturing for the United States. A tendency in discussions of preparedness is to assume that the FDA imposes excessive, burdensome regulations that interfere with rapid change. From this author’s perspective, despite the strict regulations for manufacturing, the FDA is not the problem.

cGMPs, which have been in place for 30 years, establish compliance for drug products, require reproducibility of manufacturing, and ensure drug safety for the American public (Figure 4a). Although expensive and time consuming for companies to implement, cGMPs have established a gold standard for the entire world for the production of pharmaceutical products. In fact, cGMPs are essential.

However, the FDA also has recognized that in times of emergency, routine requirements may not be implementable. In June 2005, the FDA put out a draft guidance document, “Emergency Use Authorization of Medical Products,” that addresses emergency situations (Figure 4b). The FDA is committed to working with industry on preparedness and related problems and has addressed the regulatory spectrum for product availabilities according to the actual need. So, if the FDA is not the problem, then what (or who) is?

Engineering Challenges in a Pharmaceutical Context

Many pharmaceutical rapid-response issues are, in fact, engineering challenges. But engineering solutions, however potentially beneficial, must fit into pharmaceutical business practices. Like all other successful industries, the pharmaceutical business is driven by profitability. Unlike most other industries, however, manufacturing expenses (the cost of goods sold) are typically only a small component of the total selling price. In this setting, even major savings in manufacturing are likely to have a minimal impact on a drug’s profitability. Because impact on profitability is a key driver in

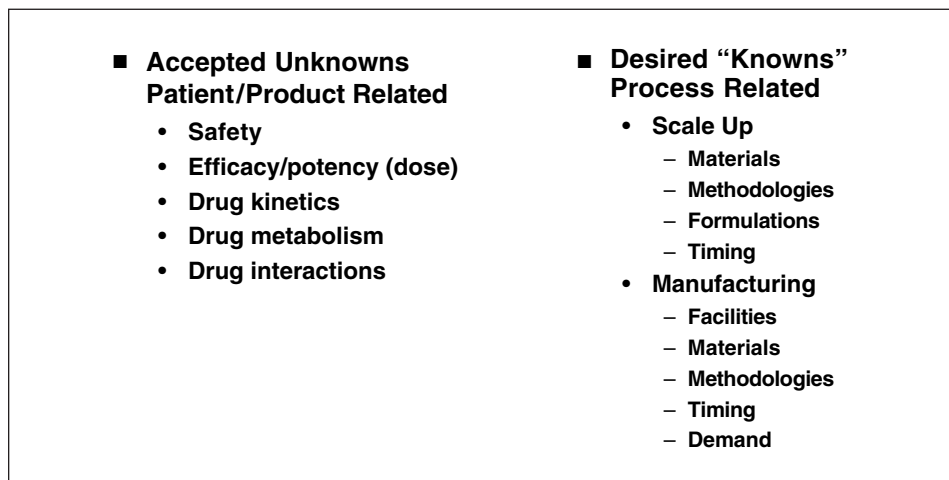


FIGURE 3 “Idealized” pharmaceutical perspective in any drug development.

evaluating change, pharmaceutical companies will have to be sold on the value (financial or otherwise) of novel engineering solutions that might replace regulated, established manufacturing processes.

Pandemics, however, are not routine, and pharmaceutical companies are beginning to address industry responses to emergencies. Examples of engineering challenges that might impact pharmaceutical pandemic responsiveness include: capacity, yield and potency, timing, and dosage.

Capacity

Egg Industry

Increasing capacity in the egg industry might be feasible if chickens could be made to lay more (or much bigger) eggs. Short of that, the challenges of increasing the supply from 300 million eggs to one billion or more eggs on demand are staggering. The logistics alone of providing feed, facilities, and routine care would be monumental. Although the egg method may yet prove useful for production of a pandemic vaccine, we need new, more flexible manufacturing approaches.

Mammalian Cell-Culture Systems

Mammalian cell-culture systems (essentially fermentation systems that produce protein instead of alcohol) are being developed as alternatives to egg-based vaccine systems. Although cell-culture systems are not dependent on the vagaries of egg availability and have greater flexibilities, worldwide mammalian cell-fermentation capacity is limited.

Right now, approximately 1.5 million liters of pharmaceutical mammalian cell-culture capacity exist between contract manufacturers and pharmaceutical companies (additional capacity is also under construction). Contract manufacturers own a total installed capacity of ~400,000L; the rest is owned by product-development companies. However, only a very small percentage of this fermentation capacity is currently available or fully dedicated to producing vaccines; most of it is routinely used

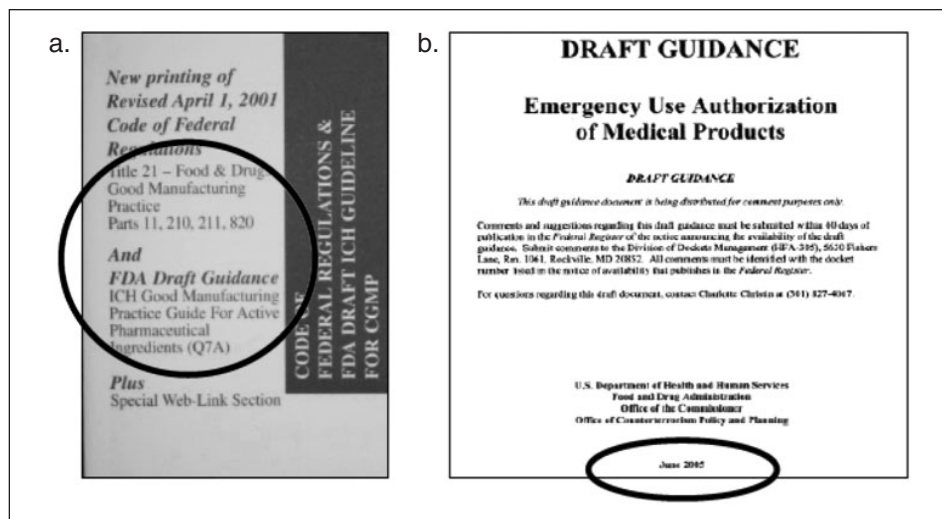


FIGURE 4 FDA—the established and the new: spectrum for product availability. Source: FDA, 2000, 2005.

to make other essential biological drugs.

In a recent analysis, forecasted demand was compared with forecasted supplies across the biopharmaceutical industry for the next five years (Levine, 2005). Figure 5 shows that after 2006, forecasted demands will closely match forecasted supplies, which means there is no meaningful surge or idle capacity to meet a sudden demand for a pandemic vaccine. The reason is simple—it is too expensive to allow multimillion dollar facilities to sit idle. Therefore, even in the most extreme emergency, companies with existent facilities would have few alternatives to halting production of otherwise needed biological drugs.

Thus, the promise of cell-culture vaccine production appears to be limited, not by technical feasibility, but by the current lack of expansion capacity. Even if construction were begun today to make such capacity available, worldwide capabilities would remain essentially fixed for the next five years, because it takes that long (and upwards of a billion dollars) to construct and validate a *single* large biological production facility.

Even interim alternatives, such as upgrading or expanding existing facilities, would cause major interruptions until revalidation and recertification could be completed. In addition, lead times for large manufacturing components are very long; for example, it can take up to two years for the order and delivery of a large stainless steel fermenter. There are no easy answers.

Yield and Potency

For a pandemic, the more vaccine available, the better. By increasing the potency of the vaccine, if it can

be accomplished safely, the dose per patient could be reduced, thus allowing more people to be vaccinated with the same amount of vaccine. Conversely, if the vaccine is less potent than expected, fewer people could be vaccinated, or more vaccine would have to be manufactured. Unfortunately, vaccine potency (and dosage) is not predictable until the vaccine is tested in humans—and in a true pandemic, it may not be feasible to look for a vaccine with optimal potency.

Maximizing the vaccine yield would help mitigate against this vagary. And because many variables can affect the final yield of a vaccine, there are many different kinds of engineering opportunities for increasing yield. Increasing yield would have the added advantage of increasing the productivity of existent facilities.

In the egg system, for example, increases in the vaccine virus yield per egg (sometimes, but not always, possible) and downstream virus recovery from the egg could greatly increase overall vaccine yield. In cell-culture systems, numerous process components are targets for increasing yield. These include, for example, cell-line genetics and selection, growth media, and other feed variables, such as temperature, gases, and pH. Exploring these areas to find meaningful yield improvements would require, at a minimum, input from cellular/genetic engineers, biochemical engineers, and mechanical engineers.

Independent of a global pandemic, improving cell-culture manufacturing parameters to increase yield is currently an area of intense study throughout the biopharmaceutical industry, in large part to get more out of existing manufacturing facilities. Downstream improve-

ments after fermentation, such as minimizing purification losses or increasing shelf life, are examples of engineering opportunities that could increase the availability of vaccine to the patient.

Time Scales

For each new product, the manufacturing development program includes both upfront times to develop the process and standardized cycle times thereafter. Even though a “known” manufacturing process may be used, each new product brought into an established process has specific variables (e.g., cell division time, temperature, media development requirements) that must be determined.

The typical ramp-up time for bringing a new product online in an *established* cell-culture system is 10 to 12 months (Figure 6). It often takes six months just to establish a new cell bank (clone) because mammalian cells only divide at a certain (slow) rate. In evaluating the potential of mammalian cell-culture systems for pandemics, it will be important to look for technological opportunities to compress this time frame. For example, if it were biologically possible to establish a cell bank more rapidly (not at all certain), this would speed the entire process. The incorporation of systems management into plant operations (a more certain type of improvement) has already been shown to reduce both initiation and cycle times in other settings; this would also probably be helpful in a pandemic response, especially with prior planning.

Dosage

The smallest amount of vaccine per patient and the fewest injections per patient are highly desirable goals, especially for global administration of a pandemic vaccine. Today, vaccine dosages and schedules are empirically determined through careful human clinical trials that take many months. Might there be technologies to determine dosages more quickly or even to find ways of reducing effective dosages safely? Rapid screens to

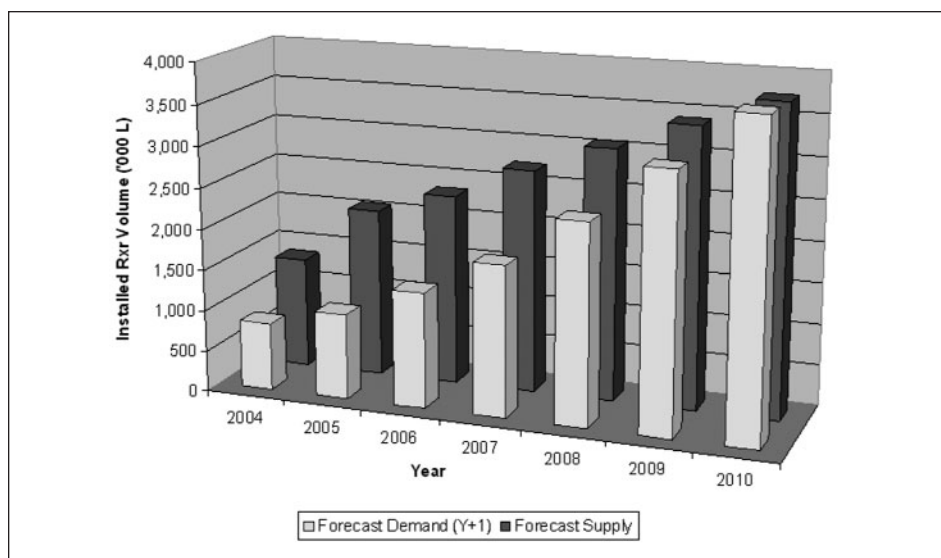


FIGURE 5 Forecasted demand and forecasted supplies for the biopharmaceutical industry through 2010. Source: Levine, 2005.

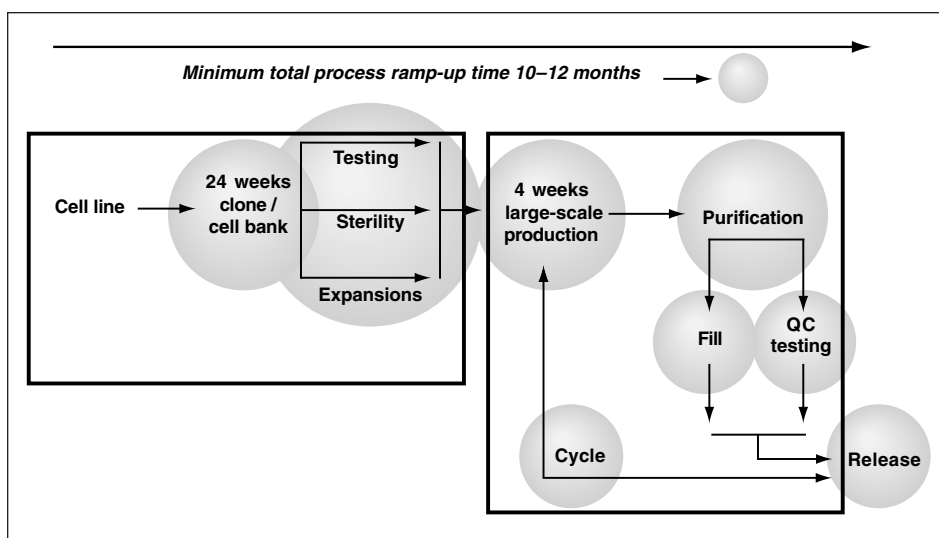


FIGURE 6 Ramp-up period for protein production.

find more immunogenic (potent) vaccines and novel/safe adjuvants (given with vaccines to boost their immunogenicity) are two possibilities already being evaluated. Other possibilities are novel systems of administration to minimize losses and speed delivery in the field. In short, any technology that leads to fewer doses and/or smaller total doses per patient will make vaccine available to more people.

Take-Home Message

The pharmaceutical industry has never before been called upon to solve rapid response issues on a global scale. Although during World War II the production of penicillin was truly an unprecedented engineering feat, it cannot be compared with meeting worldwide pandemic vaccine requirements. Right now is the time for novel solutions. Yet, the pharmaceutical industry, even with its huge research capacity, cannot solve these rapid response issues alone.

Meeting the needs of the global community for vaccine during a pandemic outbreak will require exponential improvements in today's capabilities. It is likely that such advances will have to be made across many fronts to meet the time and volume requirements. Depending on which estimates are used, up to a 1,000-fold composite increase in yield, shorter time, and smaller dosage, as well as other process innovations and enhancements, will all be necessary to have a meaningful impact on a truly global pandemic. We need unprecedented and as yet unknown solutions, which may only be possible through previously unheard of scientific and engineering collaborations.

Conclusion

The technological solutions necessary to meet the rapid-response requirements of the pharmaceutical industry will require input from many engineering disciplines—mechanical, structural, civil, chemical/biochemical, genetic, materials, and systems engineering. The sum of such innovations will require integration to meet both pharmaceutical and regulatory requirements, but (and importantly) these are technologically surmountable

obstacles. With this conference, we have created a unique opportunity to examine, perhaps for the first time, “potential engineering approaches to a pandemic,” if not for H5N1, then for an unknown virus that may threaten our world in the future.

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Despite improvements in technology and increased automation, egg-based vaccine production still has a long timeline.

Egg-Based Production of Influenza Vaccine: 30 Years of Commercial Experience



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James T. Matthews

The two influenza viruses that are medically and clinically important to the human population, known as Type A and Type B, are segmented, negative-strand genomes with an envelope that contains two major antigens, hemagglutinin (HA) and neuraminidase (NA), and a minor component designated M2. Figure 1 shows the virus envelope with the HA and NA surface antigens. Throughout the vaccine manufacturing process— inactivation, extraction of the virus envelope, and purification—both the quantity and inherent antigenicity, or immunogenicity, of the HA and NA antigens must be preserved.

The HA and NA antigens undergo both mutation and reassortment, and, most significant, both antigens are subject to antigenic drift, subtle mutations that occur continually. Therefore, formulation of the influenza vaccine must be changed on a yearly basis. If two viruses co-infect a single host, the separate gene segments shown in Figure 1 can reassort to produce novel and unexpected combinations, some of which are extremely virulent in humans.

The efficacy of vaccines for Type A and Type B influenza is measured primarily by their ability to stimulate antibodies to the HA antigen. Antibody titers are often expressed in terms of hemagglutinin inhibition (HAI), which becomes a correlate of protection or immunity for influenza vaccines. M2, NP, and other antigenic components, although they probably play a role in cellular immunity, are not part of the yearly evaluation or licensing process for seasonal influenza vaccines.

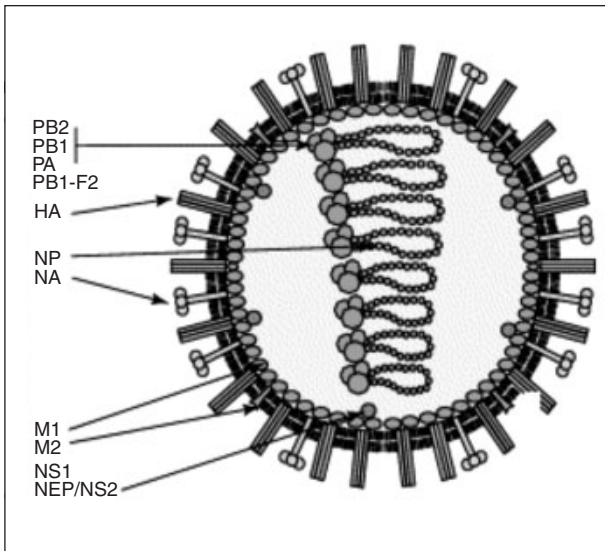


FIGURE 1 Schematic representation of an influenza virus particle. Source: adapted from Ludwig et al., 2003.

Types of Vaccine

There are generally two types or classes of influenza vaccines: (1) inactive vaccines and (2) live attenuated vaccines. Inactivated vaccines, which account for more than 90 percent of vaccine sales worldwide, include vaccines made from (1) whole viruses; (2) viruses subject to two different splitting procedures (a simple detergent split with enrichment or a subvirion highly enriched for HA and NA); (3) virosomal vaccines, which are primarily licensed in the European Union (EU); and (4) influenza vaccines formulated with adjuvant to increase their potency. The vaccine primarily discussed in this paper is Fluzone[®], a split (subvirion) vaccine manufactured by Sanofi Pasteur.

Live attenuated influenza vaccines represent only a small portion of the licensed influenza vaccines.

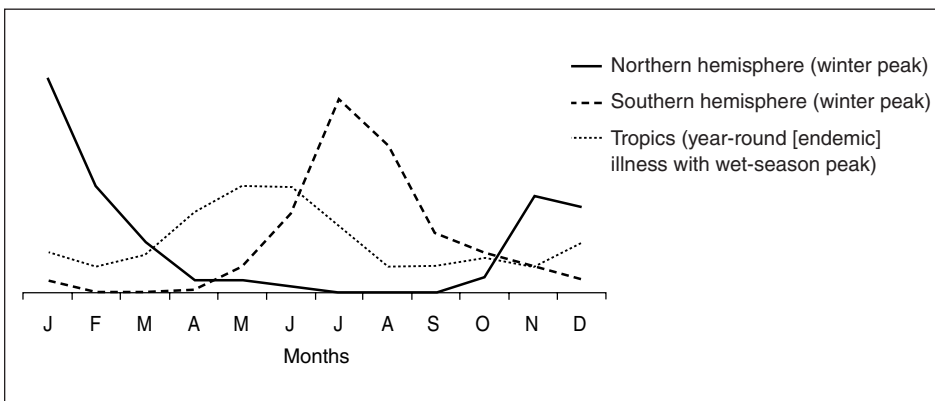


FIGURE 2 Seasonal occurrences of influenza. Source: adapted from Reichelderfer et al., 1988.

Examples include FluMist[®], made by MedImmune Vaccines Inc., and a similar formulation produced in St. Petersburg for use in Russia.

Seasonal Influenza

Seasonal influenza is the seventh leading cause of death in the United States and the leading cause of death in children ages 1 to 4 years. Ninety percent of deaths in people 65 and older are the result of influenza with associated pneumonia. Every year in the United States, approximately 36,000 people die, 114,000 are hospitalized, \$600 million are spent in direct costs to the health care system, and the country incurs \$1 billion in economic costs.

Figure 2 shows the seasonal occurrence of influenza; Figure 3 shows the absolute numbers of strains isolated in each season, as well as the proportion of strains isolated over the flu season. Figure 3 also shows that the number of strains isolated varies dramatically from season to season. Therefore, discussions about modifying formulations for the seasonal influenza vaccine by eliminating one strain must include careful considerations of the numbers and types of co-circulating strains. A bivalent vaccine produced for a flu season with three co-circulating subtypes would offer limited protection at best.

Figure 3 also shows that the peak incidence varies from season to season. The 2002–2003 season peaked during the week of February 23, 2003; the 2003–2004 season peaked during the week of November 29, 2003. Because it takes two to four weeks after immunization for an adequate immune response, vaccines must be manufactured and distributed well in advance of November to be effective in protecting against seasonal or pandemic influenza.

History of Influenza Vaccines

The seasonal influenza vaccine of the 1940s was a monovalent preparation; that is, the vaccine was formulated from a single strain of influenza. From the 1960s to early 1970s, seasonal vaccines were bivalent. Since 1978, vaccines have been trivalent with a strain of influenza B

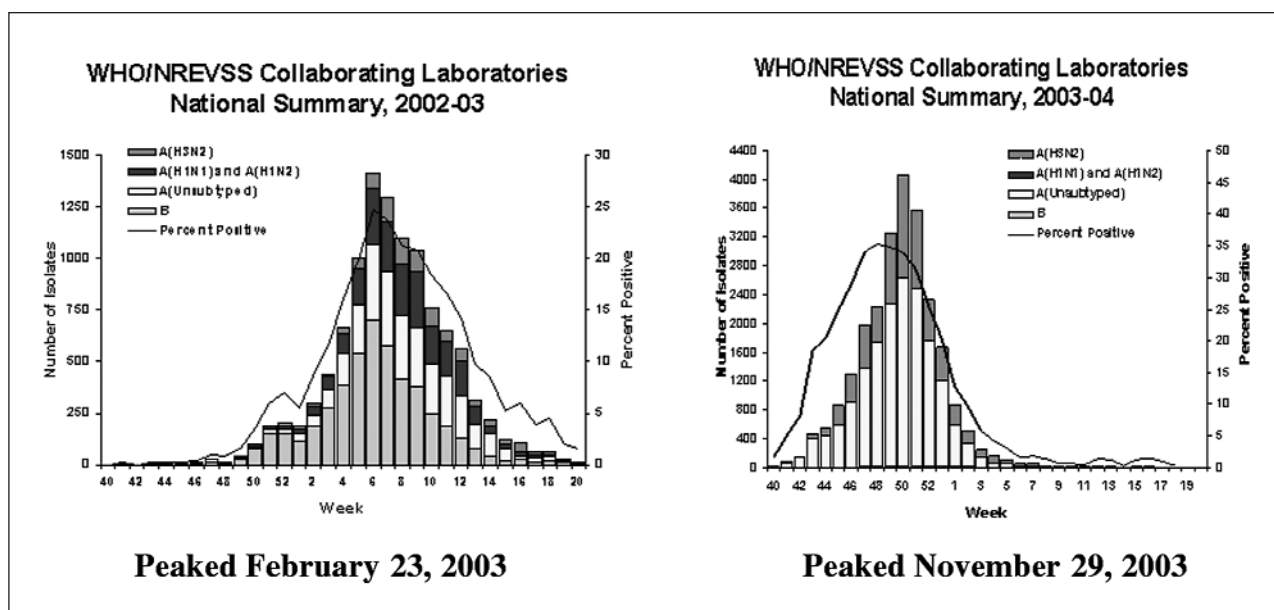


FIGURE 3 Weekly incidences of influenza for two seasons. Source: DHHS, 2006.

and two strains, H1 and H3, of influenza A in each formulation. From 1970 to 2004, the formulation changed approximately 40 times for one strain of the trivalent vaccine. Requests to change two of the strains in the vaccine were made eight times, and additional monovalent preparations to supplement the trivalent vaccine were made several times. On one occasion, all three strains in the vaccine were changed.

Even though the seasonal influenza vaccine is considered a conventional vaccine by the industry, new challenges with respect to timing and availability of strains and the composition of the influenza vaccine are the rule. As we say at Sanofi Pasteur, “when you’ve seen one influenza season, you’ve seen one influenza season.”

Typical Influenza Campaign

Eight discrete activities are associated with a seasonal vaccine campaign; these activities occur concurrently in the northern and southern hemispheres.

Surveillance, supervised by the World Health Organization (WHO), is continuous on a global scale for every month of the year. *Strain selection* is done twice a year, once for each hemisphere. *Reassortant preparation*, a step not necessarily apparent to the general public, involves co-infection of the wild-type and egg-adapted strain (PR/8) that have been developed to support egg-based production. The next step, *potency reagent preparation*, takes place after the strains to be used in a vaccine have been identified. This step involves the

development of a release test for any new strain included in the vaccine. Antibodies to the new virus strain must be developed that can be used in a single radial immunodiffusion (SRID) assay for release testing by manufacturers.

The *production of vaccines* for both the northern and southern hemispheres takes place almost year round. Production is followed by *release of the vaccine*, which can be a lengthy process (discussed further below). *Vaccine distribution* must occur early enough for the vaccine to be available well before the flu season. *Administration of the vaccine* must be done early enough for individuals to accumulate enough antibodies to fight the disease.

The participants in annual influenza campaigns include WHO, national reference centers and contract laboratories responsible for surveillance, the development of reference strains, and reassortants; vaccine manufacturers, who are responsible for preparing the monovalent bulk production and formulation, as well as for filling and distribution; and regulatory agencies/national standardization laboratories, which review yearly license amendments when vaccine formulations change. Regulatory agencies are also responsible for reviewing changes in product labeling, labeling for new products, and changes in packaging and product inserts for new virus strains. In the United States, monovalent bulk vaccine requires a regulatory review and lot release. Standardization laboratories are responsible for the calibration of potency reagents.

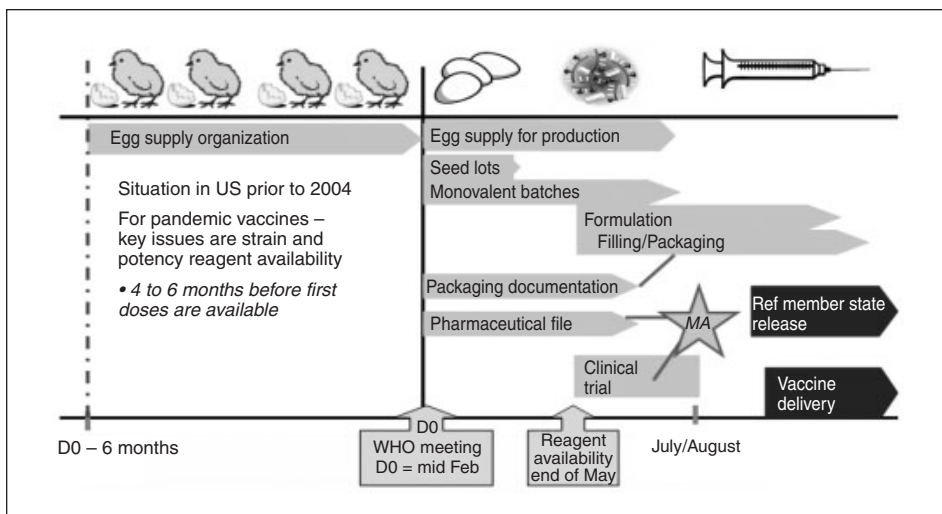


FIGURE 4 Generalized timetable for influenza vaccine production.

Timetable

Figure 4 shows one cycle in the production of a monovalent lot of influenza vaccine. Note that the egg supply must be prepared well in advance of vaccine production dates. Once the egg supply is in hand and the seed is developed from the reassortant, “in-house” seed lots can be developed; this requires passing and expanding the seed lot a number of times until it reaches a level that can support commercial production. Once the seed lot is released, production can begin on a monovalent concentrate bulk batch for one strain to be included in the vaccine. Figure 4 shows one campaign for one strain (e.g., an H1 strain). Additional campaigns are required for the H3 and Type B strains to be included in the

particular formulation. Such studies are required in the EU, as are market authorization and releases by member states, before the vaccine can be delivered and the immunization campaign begun.

Egg Supply

Until recently, the egg supply was organized to support seasonal vaccine production, and there were often gaps of three or four months when eggs were not readily available. Clearly, this is an unsatisfactory situation in preparing for a pandemic. Working on pandemic preparedness with manufacturers, the U.S. government awarded Sanofi Pasteur a contract to improve the egg supply and develop new technologies

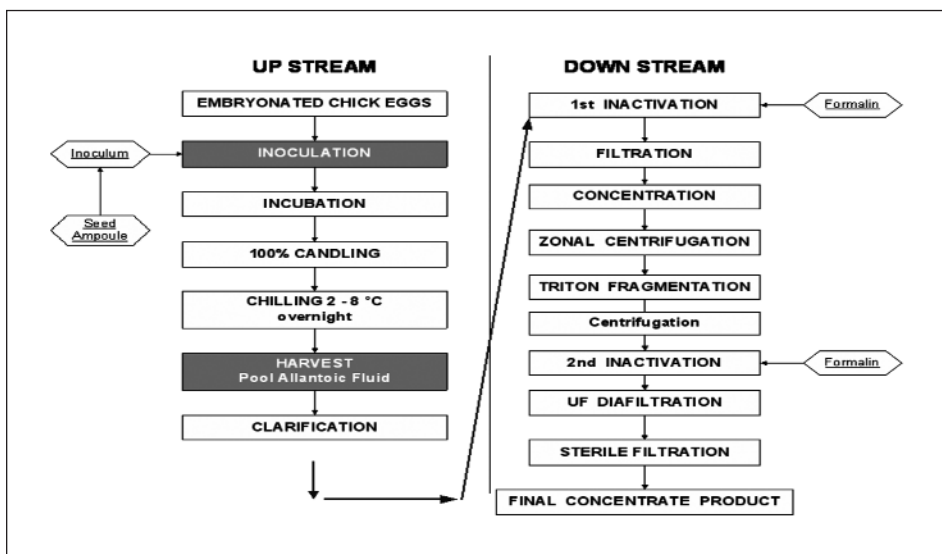


FIGURE 5 Processes in vaccine production.

trivalent vaccine. After each monovalent batch has been prepared, the formulation stage and filling and packaging stages can move forward.

Figure 4 also shows some of the common constraints to the regulatory pathway, such as clinical studies that must be done with hundreds of healthy volunteers, both young people and elderly adults, to assess the safety of the vaccine and the strength of the HA titers expected from that

and has asked manufacturers to provide expert knowledge and solutions to specific problems in vaccine manufacturing.

To address the variability in the egg supply, Sanofi Pasteur restructured its flock management so that embryonated eggs would be available to support vaccine production at full capacity throughout the year. Because embryonated eggs are themselves potentially susceptible to avian influenza, flocks associated with vaccine production

are under strict contract and must be completely housed, monitored by veterinarians, and raised under biosecurity regulations. With government support, Sanofi Pasteur has also established contingency flocks as a backup against avian influenza and other risks.

Automated Process

The typical automated vaccine manufacturing process shown in Figure 5 takes approximately seven days for completion. The upstream process, which lasts for most of the seven days, begins with embryonated eggs brought in on a daily basis from biosecure flocks. A seed ampoule is used to inoculate the chick eggs during the inoculation phase. This is followed by a mandatory three-day incubation period during which the virus grows to ensure that sufficient quantities can support further manufacturing. After three days, all of the eggs are candled to make sure there are no cracks or contamination; the eggs are then chilled to 2° to 8°C to constrict vessels and make harvesting easier. The allantoic fluid is then harvested; a low-speed clarification process follows.

The first step in the downstream process is inactivation, which involves the addition of formalin to inactivate the virus. Extensive filtration and concentration steps yield a concentrate, which is then loaded onto zonal centrifugation equipment. The first purified bulk virus, which is recovered from the centrifugation process in a sucrose band, is split in a fragmentation step by treatment with Triton detergent. The material is then clarified by centrifugation to remove large particulates and treated with

formalin in a second inactivation step. An ultrafiltration (UF) step is followed by terminal sterile filtration to generate one monovalent bulk concentrate.

The inoculation and harvesting steps are now 100 percent automated. In addition, although overall this is not a closed system, two distinct operations in the upstream process are closed systems: the harvesting and clarification steps. The first three downstream steps, first inactivation, filtration, and concentration, are also closed; and the final downstream steps: second inactivation, UF diafiltration, and sterile filtration, are closed. Material transported from one step to another in the closed-system portions of the processes is pumped, rather than poured from open containers, to prevent contamination by processing aids and viable or nonviable particulates.

Broad-Scale Manufacturing

The 28-week timeline shown in Figure 6 represents a worst-case scenario, in which one of the strains to be included in the vaccine is changed, making it necessary to create a new reassortant. Preparing high-growth reassortant seed requires a seven-week period to accommodate the preparation of new reagents and antibodies.

A large portion of the time in this timeline is focused not on manufacturing but on regulatory concerns, either regulatory review and release or internal testing, so that when seed lots, monovalent bulk concentrates, or pooled bulks are moved along, the quality of the material is assessed for sterility and potency.

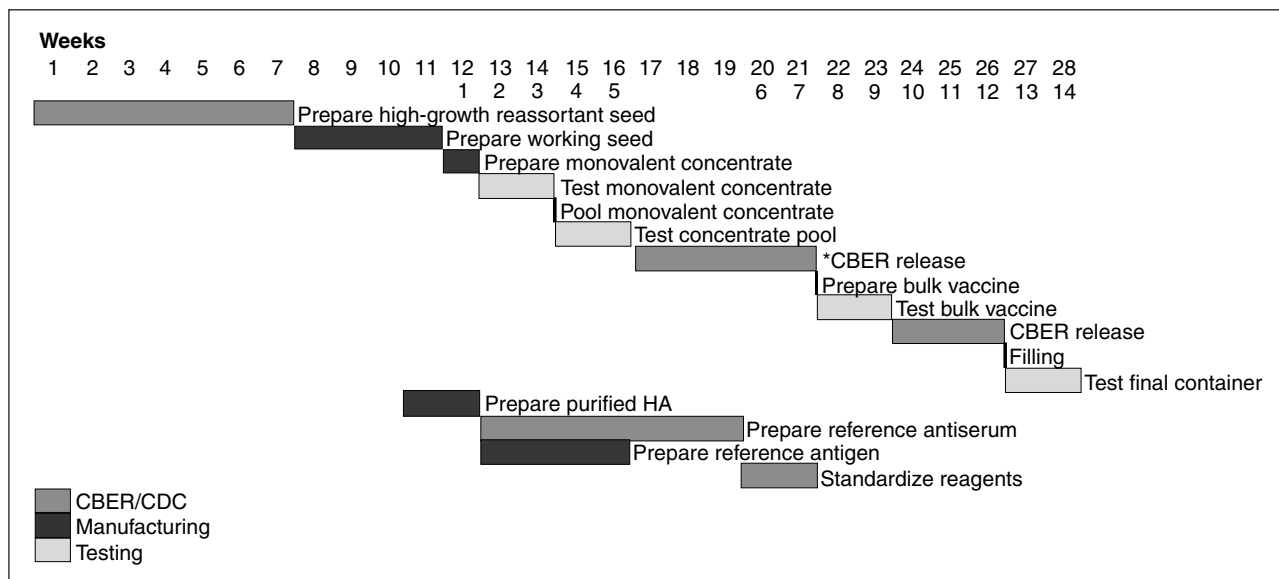


FIGURE 6 Timeline for broad-scale vaccine manufacturing.

Formulation, Filling, and Labeling and Packaging

Formulation is a manual process consisting of the addition of diluent to achieve the required 15 microgram antigenic component per strain (H1, H3, and B strain) for each dose of vaccine. Quality-control testing of the final container comprises several labor-intensive testing regimens to ensure that all release requirements have been met. Sterile *filling* of vials or syringes and subsequent inspection of the filled vials or syringes are completely automated. The *labeling and packaging* process is also automated; individually labeled vials are placed into cartons along with relevant product inserts, leaflets, and syringes. The *packing* of individual cartons into shipping packs, however, is another labor-intensive manual process primarily because of customer-specific or special orders, which are very difficult to automate.

A considerable amount of time is spent on steps beyond the manufacturer's control.

Rate-Limiting Steps in the Seasonal Vaccine Process

One of the most time-consuming steps in the seasonal process is the selection of updated reference strains each year. Delays are often justified because the epidemiology or transmission of a particular strain of influenza can change during the course of a flu season, and it is crucial that the best match for all three virus strains be included in the vaccine. Sometimes, one of the strains is not available as an egg-derived isolate. Current licensing requirements stipulate that isolates be passed exclusively in embryonated chicken eggs or cell cultures derived from embryonated chicken cells. If that strain is missing from the vaccine and is widespread during the flu season, the “mismatch” of vaccine and virus can substantially decrease the effectiveness of the vaccine.

A considerable amount of time is spent on steps beyond the manufacturer's control, such as the creation of reassortants through the WHO Global Influenza Program. Furthermore, the collaborative, highly regulated process of standardizing the antiserum for each strain takes about eight weeks. In addition, a good deal of time is devoted to product-quality assurance or conformance

to finished product requirements, as regulatory groups confirm potency values reported by the manufacturer.

Unique Aspects of Manufacturing Influenza Vaccine

The manufacture of influenza vaccine, although considered conventional, is unique in a number of ways. First, it is a global enterprise. The vaccine manufacturer is just one participant in a consortium of regulatory agencies, surveillance laboratories, certified laboratories, hospitals, and clinicians, as well as providers of embryonated eggs, components, equipment, raw materials, and transport agencies.

Second, the composition of the trivalent vaccine changes almost yearly. The vaccine must be licensed yearly (for every amendment), and the license is granted for one year only (July 1 to June 30). This has significant implications for the manufacturer in planning a production campaign:

- Unused formulated and filled vaccine is usually discarded.
- Monovalent concentrates are not typically used after 12 months from pool.
- Yields of the strains are variable and are not known by the manufacturer until commercial scale-up production.

Third, formulations are updated twice a year to reflect the epidemiologies in the northern and southern hemispheres. The manufacturer thus has a very short window of opportunity to respond to changes. If the release of reassortants is delayed because of epidemiological changes during the course of the flu season, this window can be very, very short. The currently licensed influenza vaccine is trivalent; thus if one of the strains cannot be produced, there is no vaccine. Balancing production schedules to provide vaccine in the northern hemisphere for the October 1 immunization campaign can be challenging.

Producing Vaccine for a Pandemic

Seasonal and pandemic influenza vaccines are clearly interrelated. The capacity available for the production of a pandemic influenza vaccine is largely based on current seasonal capacity. Even though a pandemic vaccine is expected to be a monovalent formulation (e.g., H5N1 or H7N7), it will still require using existing processes and capacity because there

will be no time either to develop new processes or to expand capacity.

Some 300 million doses of trivalent vaccine are available worldwide. Theoretically, assuming a monovalent pandemic vaccine, 900 million doses could be produced worldwide. However, manufacturing data indicate that the number of doses per egg would be fewer than during typical seasonal production. Clinical data reported on experience

to date indicate that significant amounts of antigen, more than for seasonal vaccines, will be necessary to protect even healthy young adults.

Stockpiling is a key element of pandemic preparedness. However, the strain(s) in stockpile vaccines for possible pandemic influenza must be continually updated. Emerging epidemiology indicates that there are two distinct clades in the H5N1 influenza virus (a clade change is indicated when there is a change in the HA gene tree phylogeny). In other words, the ancestral relationship among H5 hemagglutinin (HA) genes from H5N1 avian influenza viruses collected in a specified region has changed (WHO Global Influenza Program Surveillance Network, 2005)

Seasonal influenza vaccination rates are still too low (MMWR, 2001). In the U.S. population, vaccination rates for healthy young adults and children are significantly below the 90 percent target. Figure 7 shows that the vaccination rates for people aged 65 and older are higher but are still below 90 percent. Therefore, meeting the 2010 target rate of 90 percent for each age group might provide an incentive and rationale for expanding manufacturing capacities. This expanded capacity might then be available to meet the demand in the event of a pandemic.

Keep in mind that capacity expansion is a time-consuming process and should not be considered a rapid solution for the vaccine industry. At best, expansion will take four to five years from concept design to validation and licensure.

Engineering Opportunities

Egg-based vaccine technology has been used to

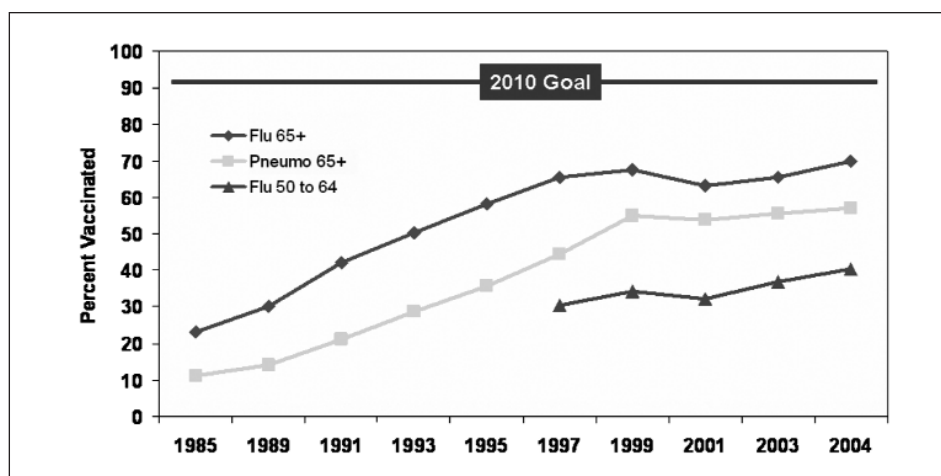


FIGURE 7 Influenza and pneumococcal vaccination rates. Source: MMWR, 2001.

produce seasonal vaccine for more than 30 years. Recently, egg-based vaccine technology has also been used to produce more than 30 million conventional doses of H5N1 vaccine and pilot lots of H7N7 vaccine. The technology has been steadily improving since it was introduced and is becoming increasingly automated. However, technological improvements in alternate release and quality assurance assays would be beneficial. In addition, increasing immunization rates could lead to an expansion of manufacturing capacity.

Cell-culture technology is likely to supplement egg-based technology. However, cell-culture technology will have to be very robust and will still focus on the same physical parameters for vaccine purification, extraction, and HA enrichment. In the near term, constraints related to the availability of strains and potency reagents will present constraints for both egg-based and cell-culture technologies.

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The next generation of influenza vaccines will be cell-culture-based for seasonal influenza and for pandemics.

Cell-Culture-Based Vaccine Production: Technological Options



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Rino Rappuoli

Leading manufacturers of vaccines and antiviral drugs are working hard to develop new and novel methods of preparing seasonal influenza vaccines, as well as pandemic vaccine candidates. Until recently, most efforts have been focused on improving currently licensed egg-based vaccines. Manufacturers have been racing to increase production capacities and automate portions of largely manual steps in egg-based vaccine technology to meet the demands of the next seasonal campaign and to generate prototypes of pandemic vaccines for clinical trials. At least 30 clinical trials of avian pandemic prototype vaccines are in progress, and manufacturers are working with international agencies, such as the World Health Organization, European Medicines Agency, and the National Institutes of Health, on the development, licensing, and production of pandemic vaccines on a global scale (IFPMA, 2006).

Currently, about 25 of these projects are based on classical egg-based technology, and six are based on cell-culture systems (Chiron has one cell-culture vaccine in the final stages of development and approval by regulatory agencies). Cell-culture technologies may offer distinct advantages over egg-based manufacturing methods.

- They eliminate the need for embryonated chicken eggs from managed, biosecure flocks.

- They combine and automate upstream and downstream processes.
- They reduce the potential for contamination by viable and nonviable particulates.
- They eliminate the four- to six-month lead times for the organization of egg supplies.
- They have faster, high-volume start-up times for production.
- They have higher initial purity.
- They could supplement seasonal vaccine supplies when multiple strain changes are necessary.
- They would substantially increase global stockpiles of pandemic influenza vaccines.

The cell-culture vaccine process is suitable for large-scale manufacture.

Cell-Culture-Based Vaccine Production

Cell-culture-based technology is robust and reliable and could become a practical alternative for the pharmaceutical industry in vaccine production. Once the virus is propagated and harvested, the downstream processing parameters for purification, filling, and packaging of the vaccine are similar to current pharmaceutical methodologies and egg-based methodologies. However, there are no lead times involved, because typical cell-culture processes use cell lines; once a cell line is infected with the seed virus in a fermenter, the process can begin. The critical step is the availability of the seed virus. The substrates or media for cell-line propagation are not susceptible to virulent virus strains as embryonated chicken eggs are.

The cell-culture vaccine process is suitable for large-scale manufacture, and the process parameters can be ramped up and run routinely and cost effectively. The typical cell-culture production process can be run in batch sizes of practical scale, sufficient to provide vaccine quantities for interpandemic periods and pandemics. However, to date, no vaccines have been licensed using this technology.

Chiron has already submitted “mock-up” dossiers to

European Union (EU) regulatory authorities for review and approval of an avian influenza vaccine (currently in clinical trials) and a license application for a cell-culture-based vaccine. This paper is focused on the cell-culture vaccine manufacturing process used by Chiron, as well as the use of adjuvants to enhance immunogenicity and reduce dosage size.

Biochemical Challenges of the Haemagglutinin (HA) Protein

Besides the engineering issues associated with establishing a new vaccine manufacturing process, biochemical challenges unique to the influenza haemagglutinin (HA) protein must be met for manufacture by any process. The three-dimensional HA protein structure is both stable and functional. However, if during purification steps, the pH drops to less than 5.5, which is a common approach to improving purification recovery processes, the conformation of the HA protein changes. The resulting conformations provide the same microequivalence of protein, but the folding of the HA protein no longer provides antigenic properties for the vaccine. Because this change is irreversible, the entire lot must be discarded. Clearly, the biochemical properties of the HA molecule must be maintained.

Production Steps

Bulk production begins with the cultivation of the virus in a fermenter equipped with numerous process parameters to control temperature, pH, dissolved oxygen, and other factors. Two methods of mass cultivation of cells are recognized in the industry today, *microcarrier cultures* and *free-cell suspension cultures*. Both systems begin cultivation of the cell line in a fermenter, which can be scaled up to thousands of liters.

In *microcarrier systems*, the cells are first anchored to microbeads. In the presence of nutrient media, the cells grow and proliferate covering the beads uniformly. Microbeads provide a high surface-area-to-volume ratio, which can lead to high cell densities. Once a bead is covered, the cells are dislodged, dispersed, and allowed to reattach to achieve another round of cell growth on the surface of the bead.

Larger volumes of media are required to achieve the same results with *free-cell suspension* because the cell line proliferates while growing freely suspended in the nutrient medium. However, the scaling up of the system is easier, and there is no limit to the volume.

Formulation is the process of mixing the product

setting dose requirements, concentrations, and final volumes, and establishing secondary process parameters, such as materials, vials, syringes, caps, plugs, incubators, and so on. *Filling*, often referred to as sterile filling, is part of formulation. Filling involves bringing together sterile vials or syringes with sterile filtered vaccine solution. The syringe or vial is filled in a controlled environment to ensure that the final product is sterile. *Packaging* is also an important step for ensuring the integrity of the product for the duration of its shelf life.

Cell Line Selection

The cell line used to cultivate the virus must be able to propagate the virus in large quantities, must be rapid and efficient in expressing the desired virus, and must be suitable for a wide variety of flu strains. It is desirable that the cell line be able to grow in a chemically defined synthetic medium that does not contain animal-derived components. It should also be scalable for industrial processes. Equally important, if the cell line has not been previously approved by regulatory agencies, the requirements for licensing should be known and validated.

Serum-Based Media

Serum-based media have some disadvantages. First, typical acceptance criteria for serum can vary as much as ±20 percent, which could contribute to batch-to-batch variations during fermentation. Second, contamination of serum with adventitious agents is always possible. Third, if the target protein is functionally, biochemically, or physically related to a serum protein, it can be difficult to separate the target protein from the serum protein during purification. Finally, a serum-based medium is not always available, especially for large-scale cell-culture use.

Synthetic Media

Synthetic, serum-free media have some important advantages. First they are much better defined than

serum-based media. Second, the potential source of infectious agents has been removed. Third, there is much less lot-to-lot variability than for serum-based media. Fourth, the purification of the desired protein is easier, requires fewer steps, and costs less. Fifth, serum-free media contain readily available components that are usually non-animal derived and have relatively easy storage requirements. Finally, shortages are unlikely.

Removal of Residual DNA

A critical step in selecting a cell line for cell-culture vaccine production is the removal of residual DNA from the final product. Regulatory agencies provide guidance for specific data for continuous cell lines (as they do for new cell lines). Continuous cell lines must have a well documented “clean” history with no tolerance for adventitious agents or other contaminants. The removal and/or inactivation of DNA must be much more thorough than for therapeutics. Testing paradigms have been defined to assess potential risk and to ensure safe use by the public.

There are three stringent regulatory requirements for validating a new cell line for use as a substrate in cell-culture formulations. First, there must be documentation to support the complete removal of the cells from the final product and documentation to show that the cell line does not bring any transforming agent

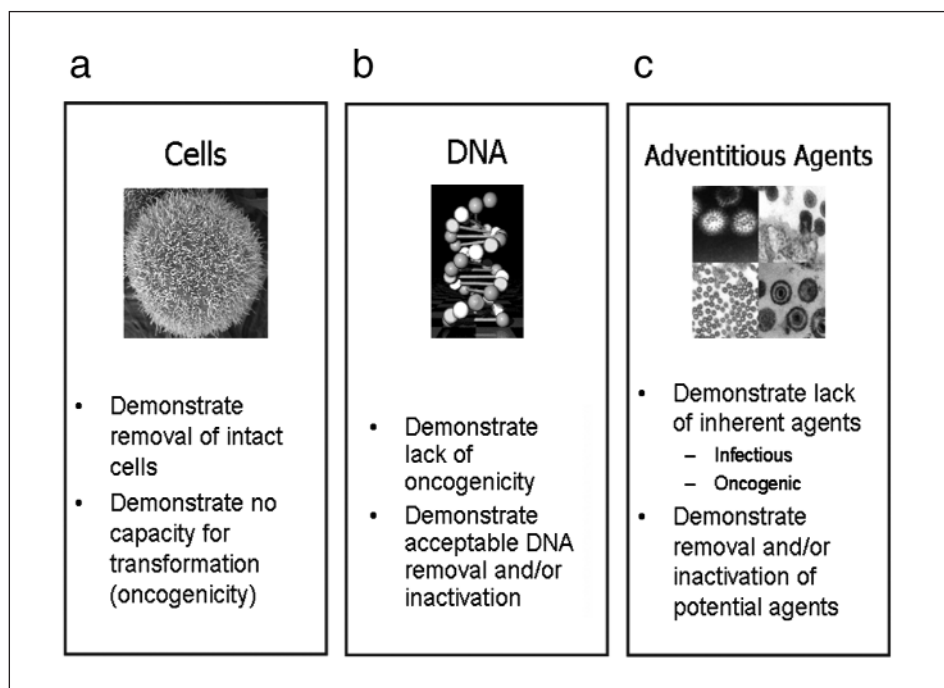


FIGURE 1 Regulatory requirements for new cell lines.

TABLE 1 Biomedical Engineering Challenges for Cell Cultivation Methods

Microcarriers	Free Cell Suspension
Cells are anchorage dependent and grow on solid or macroporous microcarriers. Cell expansion often occurs in roller-flasks.	Cells are suspended and grow freely.
Major Challenges	Culture is homogenous and sampling is easy. Process is simple, scalable, robust. No additional factors or proteins are necessary for adhesion or spreading. Costs are lower.
<ul style="list-style-type: none"> • Shear stress and mixing adaptation of stirrer speed for attachment. • Subpassages. • Continuous perfusion of fresh culture media. • Longer lag phase. • Microcarrier preparation (swelling and autoclaving). • Trypsin for cell-passaging. 	Major Challenges <ul style="list-style-type: none"> • Increase volume. • Lengthen cell-culture lead time.

(oncogenic transformation) into the final product (Figure 1a). Second, documentation must show that no genetic material is left from the cell line in the final product that can cause tumors to be formed. All residual DNA must be removed or inactivated so it cannot give rise to tumors in animal models (Figure 1b).

Finally, documentation must show the removal and/or inactivation of infectious and/or oncogenic agents from the final product, regardless of whether they originated in the media or the cell line (Figure 1c). This requirement was developed in response to contamination of dura mater grafts (e.g., the outermost layer of the meninges surrounding the brain and spinal cord) in Creutzfeldt-Jakob disease (mad cow disease). These stringent requirements are intended to protect the public from changes in our immune systems caused by foreign DNA.

A complete characterization of the cell line is required to meet licensing requirements in any country, and selecting the most appropriate approach for a cell-culture vaccine process must be based on growth rates, yields, and regulatory obstacles. Current cell lines being used to express the

influenza virus are: PerC.6[®], a proprietary formulation by Crucell; EBx[™], a stem cell line derived from chicken embryos by a member of the Sigma-Aldrich Group; VERO, a kidney cell from the African green monkey; and Madin-Darby canine kidney (MDCK) cells.

MDCK cells are known to produce large quantities of virus and require easy downstream purification. Although this cell line has not yet been approved by regulatory agencies, it would be a considerable

biochemical-engineering accomplishment if an influenza vaccine candidate used cell-culture manufacturing that includes MDCK cells growing in suspension in a synthetic medium. VERO is currently licensed with regulatory agencies but does not express large quantities of virus.

Manufacturing and Formulation

Assuming that a cell line can propagate the virus and that regulatory agencies will approve it, the selection criterion then becomes whether the cell line can be industrialized. Can it be grown in a fermenter, and should a free-cell suspension culture or microcarrier

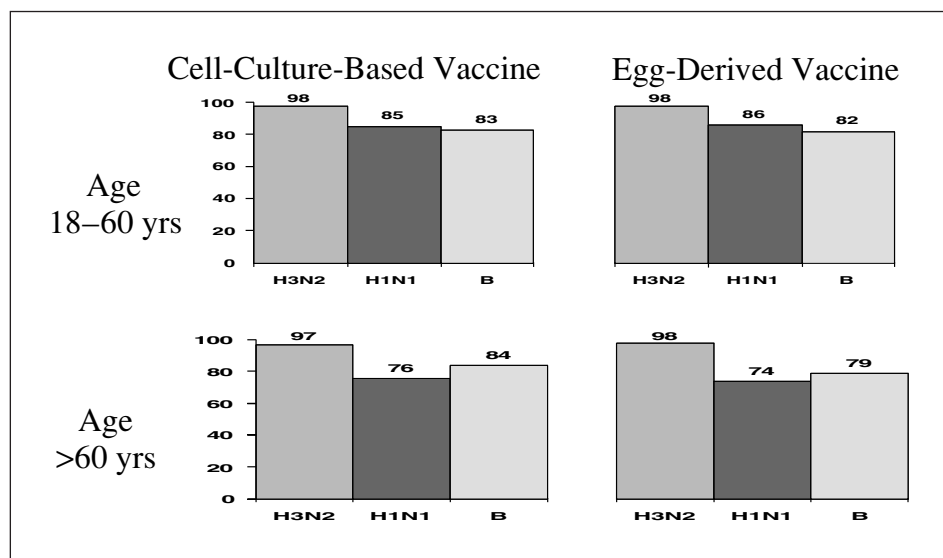


FIGURE 2 Results of clinical trials with cell-culture and egg-based vaccines.

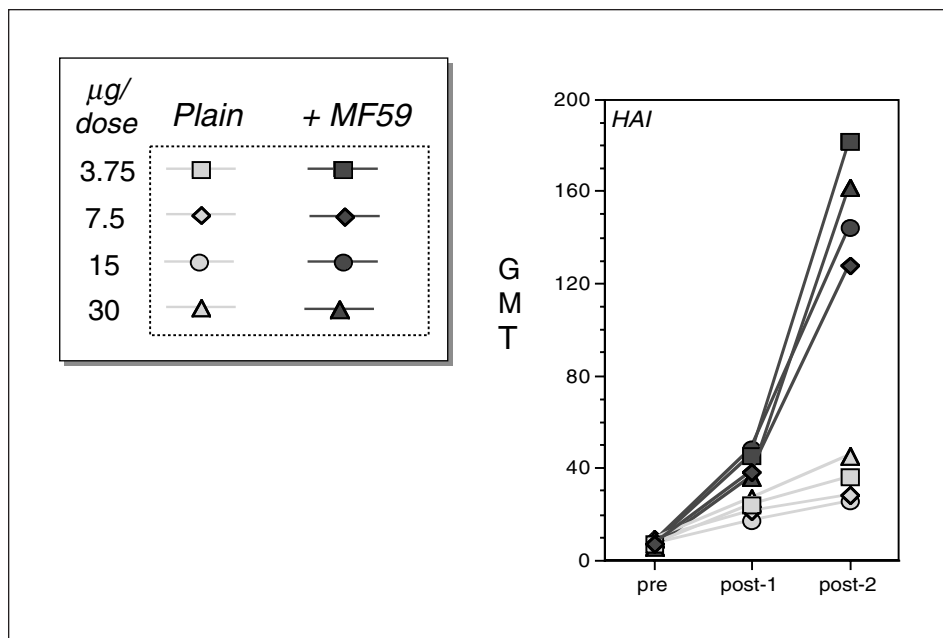


FIGURE 3 Comparison of results of trials in mice of HA vaccine and MF59 HA vaccine.

culture be used? There are engineering challenges associated with both methods (Table 1).

Regardless of the cell-cultivation method, the cell line must be grown in a nutrient medium. A medium is a solution of either synthetic (serum-free) nutrient components or a complex substance of animal-derived protein or serum. There is less risk associated with synthetic media, provided they promote the growth of the cell line. The use of serum-free synthetic media has increased significantly, particularly when using serum presents a safety hazard and a potential source of unwanted contamination.

During the formulation and manufacturing stages of the cell-culture process, the decision of whether to make a whole-virus vaccine, a split vaccine, or a subunit vaccine must be finalized, qualified, and validated. Today, most egg-based vaccines are split or subunit vaccines. A vaccine for pandemic influenza is most likely to be a whole-virus vaccine.

Preparation of a cell line for propagation begins with the thawing of the cell line “seed” lot (e.g., PerC.6®, EBx™, VERO, or MDCK). (In contrast, it can take up to six months to organize the egg supply for initial inoculation.) “First-pass” cell line propagation begins with the small-scale pre-culture propagation of seed cells after thawing. The cells are then introduced to the fermenter vessel with the selected nutrient medium. When the cell line reaches a predetermined cell density, the virus

is introduced and begins to propagate in the cell line; after approximately three days the virus is harvested. After treatment of the infected cell line, the virus is released into the supernatant, and the cellular debris is centrifuged away. This occurs in a clean, closed environment, whereas harvesting of an egg-based virus is largely a manual process that requires extracting infected cells, breaking down cell walls, and then collecting the virus.

After inactivation, the whole virus can be purified, split, and ultrapurified as a “subunit.” Initial chromatography with ultrafiltration is often followed by treatment with beta-propiolactone, which deactivates the virus; final splitting of the virus is followed by ultracentrifugation. This ultrapurification technology is basically similar to the egg-based vaccine ultrapurification process, and the resulting purified subunit vaccine is identical in composition to egg-based vaccine.

At this point, the development phase of an influenza cell-culture vaccine is complete (Figure 2). All that remains is to complete the licensing process. Phase III

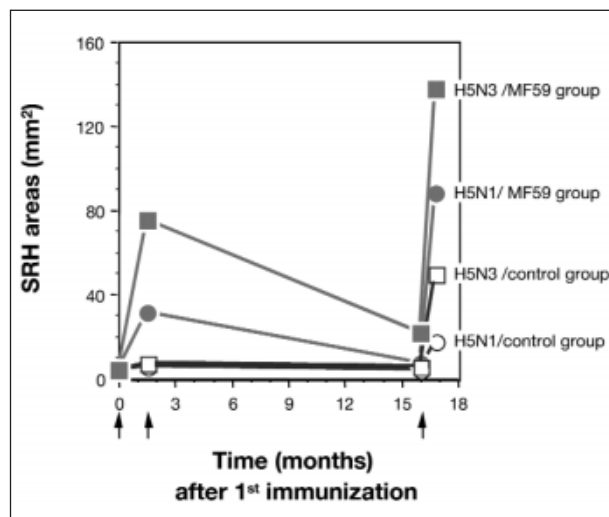


FIGURE 4 Data showing cross protection provided by MF59 adjuvanted vaccines.

clinical trials in Europe have shown equivalent safety and immunogenicity of cell-culture influenza vaccine strains and established egg-based vaccines. The protective line is well above the minimum limit for both formulations. Phase I and II studies were completed in the United States in 2005.

Adjuvants

Adjuvants are substances added to vaccines to improve antibody production and the immune response of the recipient or to decrease the amount of antigen (dose size) required in the vaccine. The latter is the most effective way to increase global vaccine manufacturing capacity. So far, the only two adjuvants that have met regulatory standards for safety are aluminum compounds (which have been used safely for many years) and M59 (licensed in most European countries). More than 25 million doses with M59 have been administered since commercial operations began.

Chiron selected MF59 adjuvant because in mouse model experiments, older mice challenged with an influenza virus after they had been vaccinated with the HA protein plus MF59 had a better immune response than younger mice vaccinated with the HA protein without the adjuvant. In addition, clinical data on the use of MF59 adjuvant with H9N2 in a new trial in 2004 confirms the potency of MF59 against avian influenza (Figure 3). The most recent work on M59 in influenza

vaccines shows that persistent antibodies against different influenza virus strains are boosted by a third immunization. Thus, MF59 adjuvant also offers protection from antigenic drift in influenza strains. This cross protection is only afforded when the adjuvant is present (Figure 4).

Conclusion

The efficacy of cell-culture-based influenza vaccine production has been demonstrated to have many advantages over egg-based vaccine production and should be licensed by regulatory agencies in the near future. Adjuvants have been shown to be not only effective, but also to provide a method of increasing global vaccine manufacturing capacities through antigen sparing.

I believe we could initiate immunization campaigns without waiting for the latest virus variant to be identified and before a pandemic occurs. Cell-culture vaccines can be manufactured over a longer period of time, with the assurance that they can be administered months before a pandemic occurs and remain effective in creating an effective immune response.

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The keys to survival of a pandemic will be surveillance, public health planning, and a publicly agreed strategy for behavior.

Alternative Methods of Making Influenza Vaccines



Alan Shaw is president and CEO of VaxInnate.

Alan Shaw

When talking about the development of vaccines to avert an influenza pandemic, we must keep in mind a sobering fact. A very well run vaccine development program, from early development to finished product launch and including all regulatory filings and product requirements, currently takes about 13 years to complete. A good example is the human papilloma virus (HPV) vaccine. The program started in 1993 and was licensed in the summer of 2006 in the United States. This was a full-throttle, fully funded project that did not run into major problems and did not have to cut any corners. It is important to keep in mind that 13 years or more is the norm.

Before reviewing some of the ways of making an influenza vaccine, it is important to consider the structure of the influenza virus and to review the primary targets for the vaccine. Figure 1 is a schematic drawing of the influenza virus showing the primary target the vaccine industry has focused on for the last 60 years, the hemagglutinin (HA) protein (spikes). HA is the major ingredient assayed in classic egg-based vaccine production, and it is the component upon which a dose is based. The neuraminidase (NA) protein on the virus surface (pegs) is another target. HA and NA denote different subtypes of influenza viruses (e.g., H5N1). Finally, there is the M2 ion channel, which is discussed in detail below.

Current methods of making vaccines for influenza involve growing the virus in a bioreactor, either a fermenter containing VERO, Madin-Darby

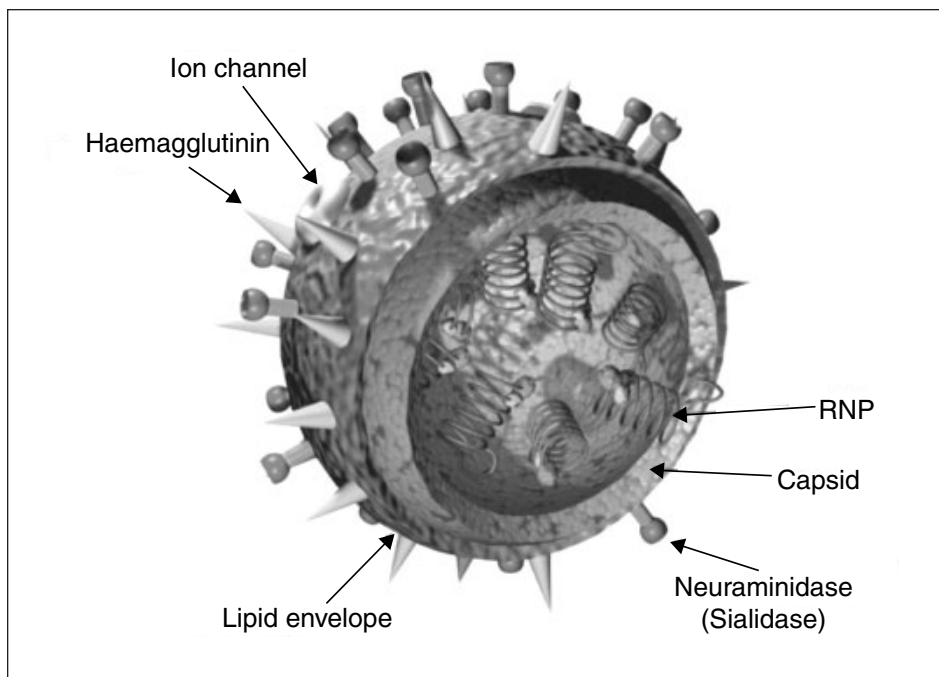


FIGURE 1 Schematic drawing of the influenza virus. Source: Smith, 2003.

canine kidney (MDCK) cells (WHO, 2005), PerC.6 cells, or embryonated eggs. Once the virus is grown, it can be inactivated and taken apart; the parts are then used to make the vaccine.

Figure 2 shows a ribbon model of the HA protein, the structural feature of the influenza virus targeted by most influenza vaccines. HA is a trimer of a glycoprotein with a long skinny stalk under a globular head. The trans-membrane region at the bottom of the stalk interacts with the matrix protein that holds the virus together.

Recombinant DNA-Based Approaches

HA protein can be synthesized by a variety of recombinant DNA (rDNA)-based approaches: insect cells; plants; yeast; bacteria; chemically synthesized peptides; naked or enhanced DNA; and vectored gene-delivery systems. Most of these approaches are in various stages of development, but it is not clear whether any of them will make it to market.

Every rDNA HA production system has a promoter section, an HA coding sequence, and a terminator section; this rDNA HA protein “cassette” can be inserted into a plasmid or some other type of genetic element and placed inside any number of systems (e.g., brine shrimp, bacteria, yeast, or plants). The biggest variables are the size and nature of the bioreactor and the process for extracting the HA from the producing organism.

Insect Cells

Several companies (Novavax and Protein Sciences are two of the main players) are working on using insect cells as vectors for making HA protein. Insect cell-culture systems grow at relatively low temperatures ($\sim 22^{\circ}\text{C}$), and incubators must be equipped with both cooling and heating capacity to maintain a constant temperature. The devices in which insect cells are grown are very similar to the equipment used in blood banks. In fact, insect cells can be incubated in large blood bag type containers (wave reactors) that con-

stantly rock back and forth in rocker trays.

One of the most common methods of inserting the rDNA HA into insect cells is via a baculovirus (BV) expression system (Kost et al., 2005). The natural BV virus makes an abundant protein, polyhedrin, and the virus contains a very strong promoter for this polyhedrin gene. The gene of interest, in this case rDNA HA protein, is inserted into the BV insect virus, and the promoter for the viral polyhedrin drives the product synthesis (rDNA HA) in cells infected with recombinant BV.

There are two alternative ways to use this system. One is to make the HA by itself (the Protein-Sciences approach); the other is to co-express HA with NA and the matrix protein that underlies the membrane and make a virus-like particle (the Novavax approach). The virus-like particles apparently have a high propensity for being taken up by the immune system; an effective dose of a virus-like particle vaccine in a mouse model is about one-tenth the dose necessary for HA itself. The potential advantage here is the reduction in the amount of vaccine necessary to elicit an effective immune response.

Plants

Work on rDNA protein production in plants has been done at the Boyce Thompson Institute (BTI) for Plant Research, Cornell University, and at Arizona State

University. One of BTI's first forays into vaccine production was a hepatitis B surface antigen expressed in potatoes. Previously established immunity could be boosted after the ingestion of the raw potato with its expressed hepatitis B surface antigen. One of the difficulties with this approach was that the potatoes had to be eaten raw because cooking or heating them denatures the protein. BTI as well as Monash University in Melbourne, Australia, are now working on tomatoes and other plants that are eaten raw and plants from which the vaccine can be extracted. However, because of societal resistance to genetically modified organisms and foods, this type of vaccine production may meet with opposition.

Yeast

Yeast is one of the workhorses of the vaccine industry. Vaccines currently made in yeast include the recombinant proteins hepatitis B surface antigen and the newly approved HPV vaccine. Yeast has the capability of secreting and assembling large proteins, which are then glycosylated, but not exactly the way human cells operate. GlycoFi Inc. reported early this year that they have engineered the human enzymology for glycosylation side chain addition into yeast, so a more human-looking sugar additive is produced as proteins are formed (Li et al., 2006). Yeast should be a good system for making HA.



FIGURE 2 The HA protein. Source: Isin et al., 2002.

Bacteria

Avant Immunotherapeutics Inc. is making innovative bacterial-vectored vaccines from bacteria that normally invade the lining of the human gut (e.g., salmonella, shigella, cholera). Because the genetics of these bacteria are now understood, their metabolic processes can be used to advantage. First, knock-out mutations are made in various metabolic pathways so the bacteria cannot survive outside of an artificial environment. After ingestion, they travel to the gut, invade the mucosa, and stop growing because they are missing a critical element for growth.

The plasmids in the bacteria have been genetically engineered to express the protein-coding gene fragment that has been loaded into them (i.e., the HA antigen). The plasmid is a double-stranded, typically circular DNA molecule, not part of the cell nucleus with its chromosomal DNA, but capable of autonomous DNA replication. Plasmids in genetic engineering are called vectors. Once released into the mucosa, the protein is picked up and processed by the immune system of the person, elicits a response, and establishes immunity. Immunogenicity of the HA surface antigen is currently being evaluated in mouse and rabbit models.

Chemically Synthesized Peptides

Another attractive possibility for vaccine production is fabrication of peptides-reductive immunology taken to its end state. The process for making selected peptide-containing antigenic epitopes by chemical synthesis is outlined in Figure 3. Figure 3a shows a solid support resin to which amino acids are added one by one by synthetic chemistry in a controlled sequence. In the most preferred application for vaccines, an array is fabricated with the appropriate density of these epitopes to elicit an immune response. For example, Figure 3b shows how multi-armed lysine trees can be made and the peptides of choice attached in multiple copies in a very dense array.

Many companies are working with synthetic chemistry peptide technology. The challenge is to pick the right conserved epitopes out of the 600 amino acid sequence of the HA protein.

Naked or Enhanced DNA

The principle of using naked DNA is to select a plasmid vector into which the gene of interest can be inserted with the right controlling sequences around it. The plasmid is then propagated in *E. coli*, which can be engineered to make profuse amounts of the DNA

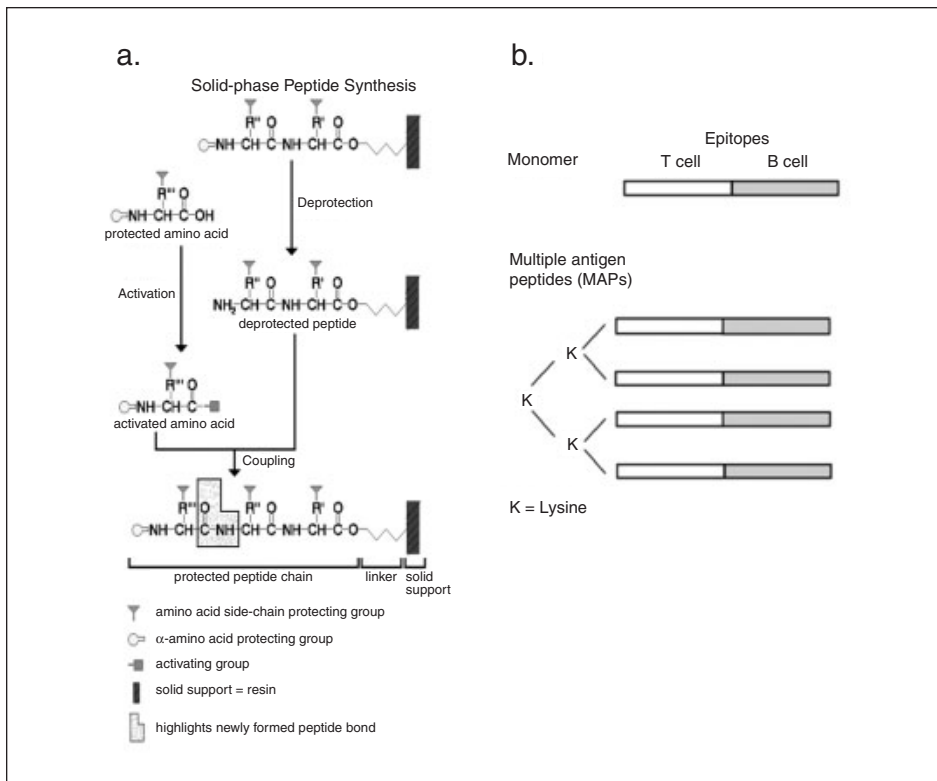


FIGURE 3 Peptides made by (a) synthetic chemistry and (b) selected epitopes. Sources: UIUC, 2004; SciELO, 1997.

containing the gene of interest. The plasmids are then purified and injected directly into muscle tissue or are coated onto gold beads that can be injected under the skin with a gene gun. The DNA is taken up by the human host cell, carried to the nucleus, and expressed, thus allowing the host to make the active antigen.

The limitation so far in this approach is the inefficiency of DNA transfer. The human body contains many nucleases and other enzymes that destroy “free” DNA, simply because DNA is not supposed to be free. Thus, a large dose of free DNA (about 5 mg) is necessary in humans because most of it is broken down before it reaches the nucleus. Although very little DNA makes it to the nucleus, the DNA that does get there is highly effective in expressing protein and eliciting an immune response. A variety of vehicles for enhancing the uptake of DNA have been applied to this technology, largely by Vical of San Diego, the originator of this technology.

Adjuvants

Adjuvants are agents or drugs that have few or no antigenic effects or properties but may increase the efficacy or potency of vaccines or other drugs when given at the same time. Some of the most common adjuvants

are aluminum salts and monophosphoryl lipid (MPL)/QS21 cocktails (being developed by Glaxo Smith Kline). Adjuvants and enhancements make it possible for larger fragments of naked DNA to reach the intended target. Although poorly understood scientifically, the successful development of adjuvants is important because they can greatly increase the efficacy of existing vaccine supplies. This technology is currently being used for vaccines against malaria and HIV, as well as for influenza.

Virus-Vectored Gene Delivery

Adenoviruses

Adenoviruses, which infect both humans and animals, can be rendered nonpathogenic by taking out part of their genomes, so that the virus cannot replicate on its own unless the host cell provides the missing portion of the genome (usually the E1 genes); this vector is then taken up by the host cells (Figure 4). Adenoviruses have a propensity for infecting dendritic cells, which are the antigen-presenting cells that can carry antigens to the lymph nodes. This is advantageous because proteins expressed by an adenovirus are fairly immunogenic; in fact, the leading candidate for an HIV vaccine is a viral-vector system.

Most humans have antibodies against adenoviruses; in fact the virus was first isolated from the human adenoids (tonsils), from which the name is derived. Thus, the effectiveness of an adenovirus-vector system could be limited because of pre-existing immunity to the vector. Nevertheless, these systems are being used in the development of vaccines.

Alpha Viruses

The alphavirus Venezuelan Equine Encephalitis virus, which is normally pathogenic for horses, has an unusual property. Its genome is expressed in two parts (i.e., separate systems for expressing early genes and late

genes). The RNA for the late genes can be commandeered to express the protein of interest; Alphavax is the leader in alphavirus-vector technology (Schultz-Cherry et al., 2000). Alphaviruses are not normally pathogens for humans, so immunity against most alphaviruses does not exist in the human population.

Alternative Delivery of Antigens

Vaccines can be delivered in ways other than by direct intramuscular (IM) injection. Any method can be used to introduce the vaccine directly to dendritic cells (the cells that carry antigen to the lymph nodes) located in the intradermal compartment of the skin, whence they will be effectively taken into the immune system. Thus, vaccines can be delivered through the skin using patches, micro-abrasion, or micro-tines coated with antigen. They can also be delivered through the intranasal system; Flu-Mist® administers a live, attenuated vaccine by spray into the intranasal compartment to create local mucosal immunity.

Alternative Influenza Antigens

The influenza virus (Figure 1) has ten gene products: HA and NA on the surface; the M2 ion channel and a variety of intraviral proteins; the M1 matrix proteins; and various RNA packaging proteins that make up the viron. HA and NA, which are on the surface of the virus, are available for attack by the

immune system, but they are subject to immune pressures and antigenic drift (mutations) in their genetic material over time.

Antigens other than HA might be good candidates for making a flu vaccine. The neuraminidase (NA) protein is one possible candidate, although it is susceptible

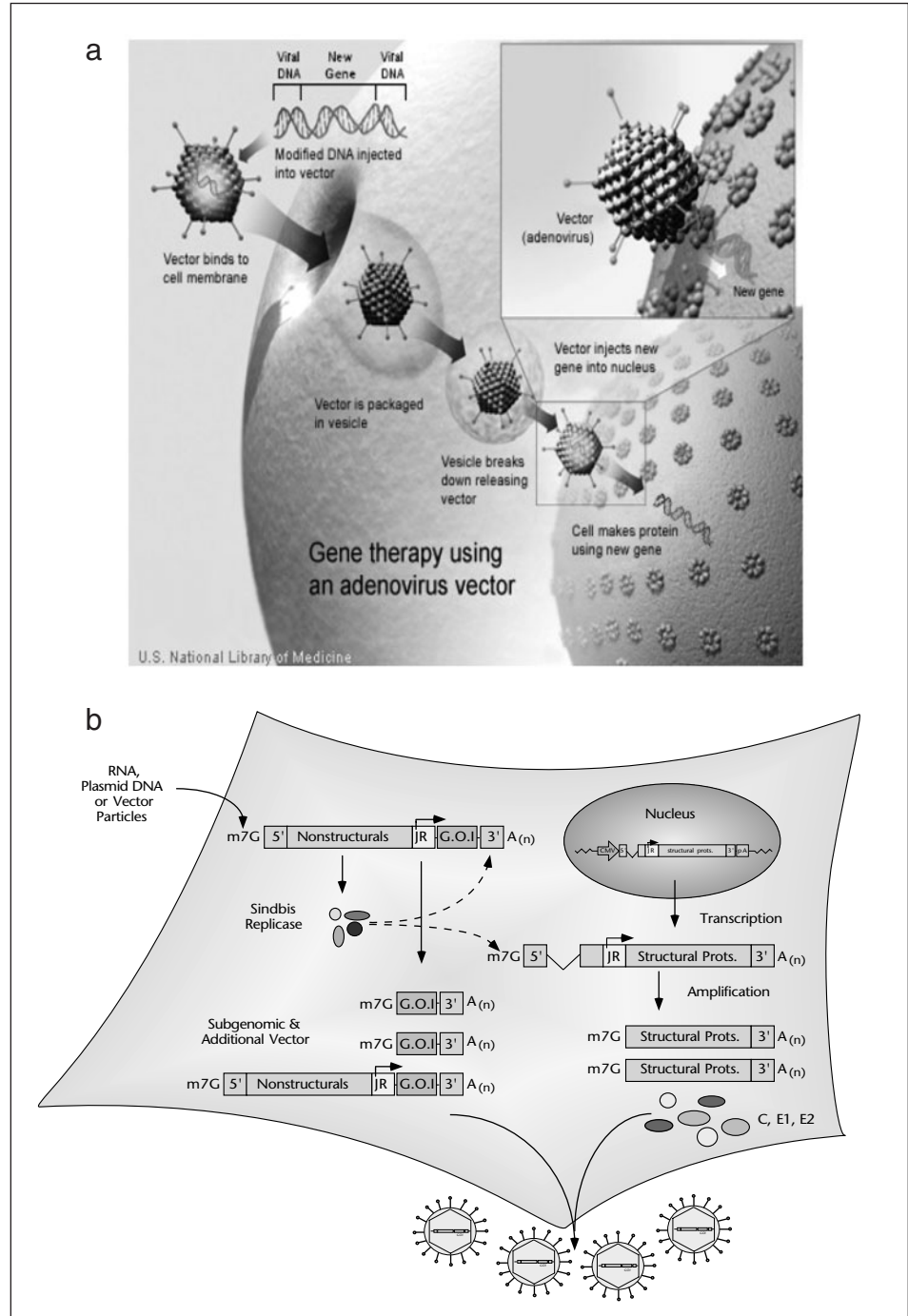


FIGURE 4 Viral-vectored gene delivery. a. Adenovirus vector. b. Alphavirus two-part genome. Sources: NLM, 2006; Dubensky et al., 1998.

to antigenic drift and immune pressure, so it may not have any advantages over HA other than as another surface target. NA is not a strong neutralizing antigen; rather it is an enzyme that cleaves progeny virus away from the cellular debris after an infection is complete, so that the progeny can then carry out the next round of infection. Blocking this enzyme activity with antibody may confer some benefit. Other possible conserved antigens are the NS1 protein (a nonstructural nuclear protein), M1 (the matrix protein), NP, and M2 (which is the ion channel).

Innate and Adaptive Immunity

Analysis of the immune system to identify its smallest critical element has stripped it of some of its power. We have spent decades identifying epitopes, purifying the essential antigens, and focusing on the details of antigen processing and presentation. In the late 1990s it was discovered that another arm of the immune system, the innate immune system, has a limited repertoire of receptors, which are called “toll”-like receptors (TLRs). (Toll was a gene found in the fruit fly [*Drosophila*] that involves body-part segmentation and orientation.)

As nature has a propensity for recycling motifs, the toll gene has been duplicated dozens of times and has mutated and been adapted by mammalian cells to serve as antennae on the outside of antigen-presenting cells. TLR antennae specifically recognize unique elements of pathogens that are not part of mammalian biology; they actually provide the first line of defense, an inflammatory response. TLRs also control the initiation of adaptive immune response, which is the T-cell and B-cell response that immunologists have been working with for 60 years.

The adaptive immune system has a random, highly dispersed repertoire of antigen receptors that reshuffle and adapt over time to antigens through a series of clonal selections and expansions. There are limitations to immunological memory, however, and the system

cannot distinguish between what is dangerous and what is not without the support of the very effective innate immune system.

Figure 5 shows a series of pathogen-associated molecular patterns (PAMPs), which are elements of pathogens belonging to bacteria or viruses (e.g., porins, lipopolysaccharides, lipoproteins, peptidoglycans, and flagella) that are not found in mammalian cells. Because these are all elements or pathogenic components specific to bacteria or viruses, they are recognized by TLRs as being foreign. TLRs also recognize the DNA sequence CpG (cytosine and guanine separated by a phosphate) as being foreign.

In classical antigen processing and presentation (Figure 6), that is, without TLRs, extracellular antigens are taken into the cell in an endocytic vesicle, where protease containing lysosomes break up the antigen into peptides by partial protein degradation. The major histocompatibility complex (MHC) class II presentation system comes in through the Golgi apparatus, a small organelle inside the cell, picks up these peptides, and carries them back to the surface of the cell for presentation. The MHC class II presentation system allows cells to tell surrounding cells if they are healthy or infected.

Figure 7 shows the antigen-presenting system with TLRs present. The antigen now is coupled to a pathogen pattern, or is part of a pathogen, and is picked up by a TLR and carried into an endocytic vesicle. The TLR

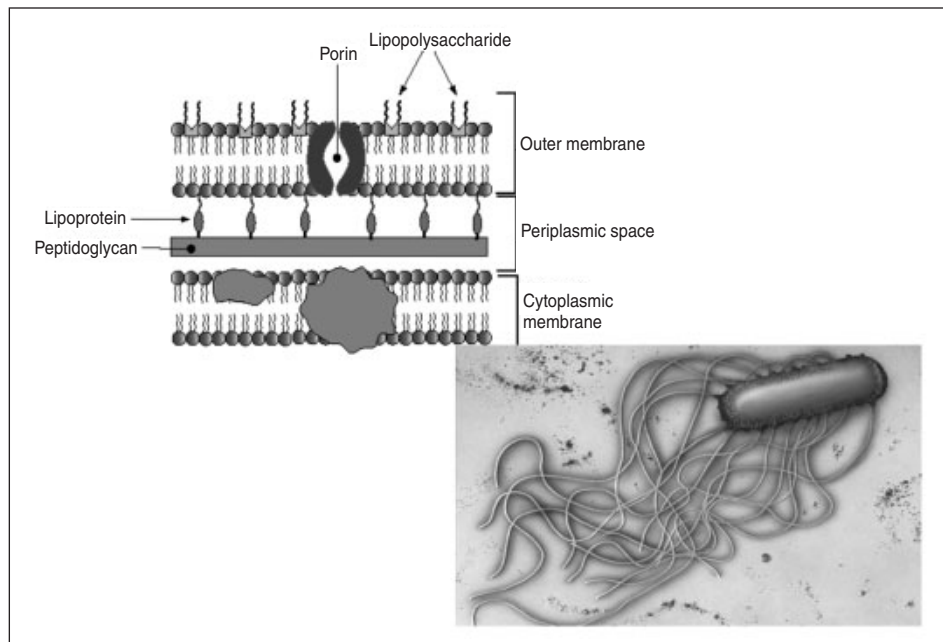


FIGURE 5 Pathogen-associated molecular patterns. Source: ARCHES, 2000.

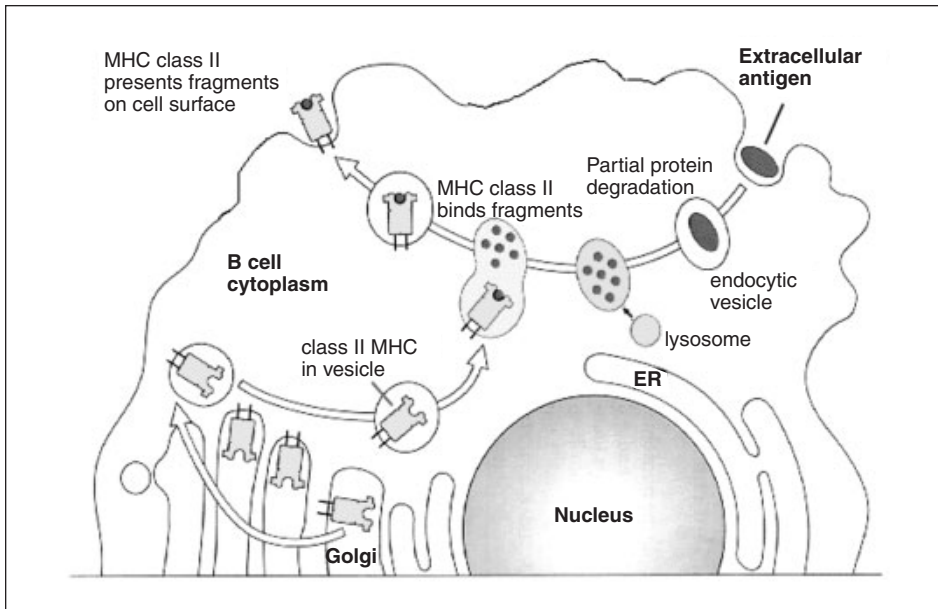


FIGURE 6 Antigen processing and presentation with no TLRs present. Source: Gilbert, 2006.

then sends signals to the nucleus to create a battery of cytokines that stimulate the neighboring T and B cells. These signals also cause the antigen-presenting cell to express special structures (CD80/86) that interact with T cells to drive an immune response. These PAMPs are recognized as components of bacteria or viruses and, in the presence of a TLR, send signals to the nucleus of the dendritic cell to trigger the synthesis of cytokines, which activate the adaptive immune system.

The M2 Ion Channel Protein

In the context of the influenza virus, the M2 ion channel protein is particularly important. There are only about 20 copies of M2 ion channel on the surface of the virus, many fewer than of HA or NA. The ion channel is important in virus replication, because during the uncoating of the virus a pH change must take place before it can start its infection. The M2 ion channel is actually a hole that protons go through during the pH change.

VaxInnate has explored two methods of making the influenza M2e vaccine: (1) hooking four copies of the 24 amino acid M2 protein to flagellin produced in *E. coli*; and (2) hooking a copy of M2e to Pam3-Cys (e.g., tri-palmitoyl-cysteine, a synthetic adjuvant linked to M2e, the antigenic protein) to make Pam3Cys.M2e. The flagellin fusion is thought to be the better method, because the M2e 24 amino acid peptide is almost invisible to the immune system and arouses almost no immune response.

Only when the antigen is coupled with a PAMP does something meaningful happen in terms of immunity.

Human Influenza Multivalent Vaccine Strategy

An advantage of driving the immune response with the TLR coupling system is that the M2e peptide appears to be relatively conserved and is a relatively stable target that should not change year after year. If we look at historical data for the 24 amino acid sequences in H1, H2, and H3 influenza subtypes over

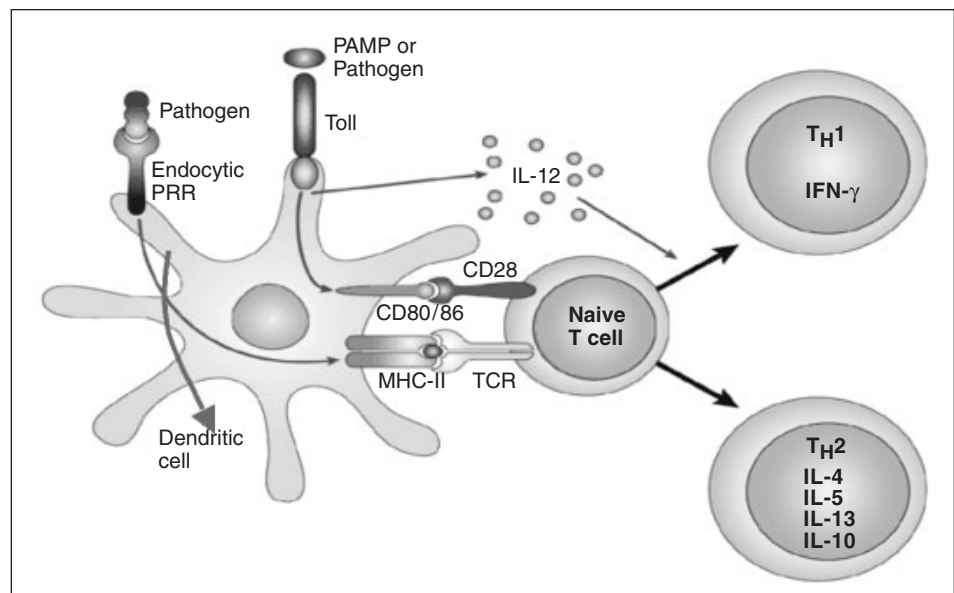


FIGURE 7 Antigen presentation with TLRs present. Source: Medzhitov, 2001.

time, it appears that the protein is indeed relatively conserved and relatively stable. In the VaxInnate consensus vaccine, there is a very nice cross-reactivity with H1, H2, and H3 human subtypes.

Knowing that the H5, H7, and H9 strains currently show a number of mutations in the M2e 24 amino acid sequence in avian subtypes, three separate consequence vaccines would be required to develop a multivalent (quadravalent) vaccine with four different versions of the 24 amino acid peptide. This could confer immunity for H1, H2, and H3, as well as H5, H7, and H9 strains. Once developed, the vaccine could be made in advance and stockpiled for use in pandemic situations. In animal models this seems to work well.

Prospects for the Immediate Future

Refinements in egg-based systems and cell-culture systems look like promising second-generation product advancements. Just using a new cell-culture system will not be enough, but it would get us away from eggs, which would have some advantages in terms of speed and yield. But the process would still be very slow. The approaches for rDNA-based vaccines and new models for M2e vaccines I've just described may not be available in time to counter a pandemic. Thus, the key to survival in the next few years will be good surveillance, good public health planning, and a publicly agreed strategy for how to behave when a pandemic appears.

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The tools and methods of industrial biotechnology could be adapted to produce pharmaceutical proteins for a pandemic influenza vaccine.

Adapting Industry Practices for the Rapid, Large-Scale Manufacture of Pharmaceutical Proteins



David Estell is vice president of technology for Genencor International.

David Estell

At Genencor, an industrial biotechnology company, full-scale industrial manufacturing of proteins at volumes of 30,000,000 gram-active proteins per month is possible within weeks of the creation of a final protein-based molecule, and prices are less than \$1 per gram-active protein. By comparison, pharmaceutical production volumes are routinely less than 10,000 gram-active proteins per month, selling prices are higher than \$1,000 per gram-active protein, and full-scale manufacturing timelines are more than a year from creation of a final protein-based molecule.

An important aspect of industrial protein manufacturing is the development of the production process before the creation of the final product molecule. This is essentially the opposite of the classical pharmaceutical approach in which the product is created, and the manufacturing process is then developed from the research chemistry bench. In industrial protein manufacturing, an initial robust process is developed for a scaffold protein that has many of the desired properties; then a few changes are made in this protein to create the final molecule. The production and purification processes developed for the scaffold protein can then be modified for the final molecule. Industrial protein manufacturing can supply a protein-based product at yields, volumes, and cost levels not possible with the classical pharmaceutical approach.

The tools and methods of industrial biotechnology could be adapted to produce several million doses of pharmaceutical proteins for a pandemic

influenza vaccine, all within a fairly rigid time frame for an effective vaccination campaign. The technology is available, and industrial costs and volumes would provide incentives. Industrial production costs and volumes could also enable alternative delivery systems that are less protein efficient, but have simpler logistics, such as topical, oral, and inhaled delivery systems. The prerequisites are in place for the pharmaceutical industry to take advantage of these incentives, and the time to act is now.

Protein Production

Protein engineering begins with the identification and assembly of a *protein scaffold*. In the case of influenza vaccines, protein scaffolds (the influenza proteins that contain the epitopes) are already identified, because influenza strain selections are made and formulation cultures prepared from the Center for Biologics Evaluation and Research/Centers for Disease Control and Prevention (CBER/CDC) reassortant “seed lots.” Every year, the scaffold is modified as regulatory agencies add different epitopes for the strains that have changed. Insofar as the production process is already in place for the scaffold proteins, which are modified to have new epitope sequences every year, the production of influenza vaccines already follows the general principal of industrial protein production.

It is important that the *gene/host system* be able to produce a desired protein in abundance with a minimum of unwanted by-products. It is also necessary that the *high-yield fermentation process* maximize end-product output from the *gene/host system*. From the same protein scaffold and basic protein, a robust, rapid, and efficient *recovery process* can then be developed. The fermentation and recovery processes are designed to be completed within hours or days to achieve the high throughput necessary for industrial manufacturing.

Formulation and delivery systems must allow flexibility and end-product stability. Protein-based end products that are transported and stored around the world must be able to withstand the high temperatures and humidity of tropical locations without losing potency, strength, or efficacy and must remain stable for years with no refrigeration. Finally, the protein scaffold must be engineered to provide the *desired properties*. In the case of the influenza vaccine, epitopes for the new strain are added to the protein scaffold, which is then introduced to the protein production pathway.

Protein Scaffolds

The protein scaffolds listed here have been created and engineered by Genencor; for each scaffold, a high-yield fermentation process and recovery or purification

process has been developed. Multi-domain fusion proteins, monoclonal antibodies, viral or bacterial coat proteins, protein-based inhibitors, and enzymes have been used in an array of industrial formulations, many expanding the application and use of genetically engineered processes to new domains as varied as treating textiles, processing milk to make cheese or yogurt, cleaning contact lenses, processing paper, brewing low-calorie beer, boosting the nutritional value of food, and converting plants such as corn to chemicals such as ethanol.

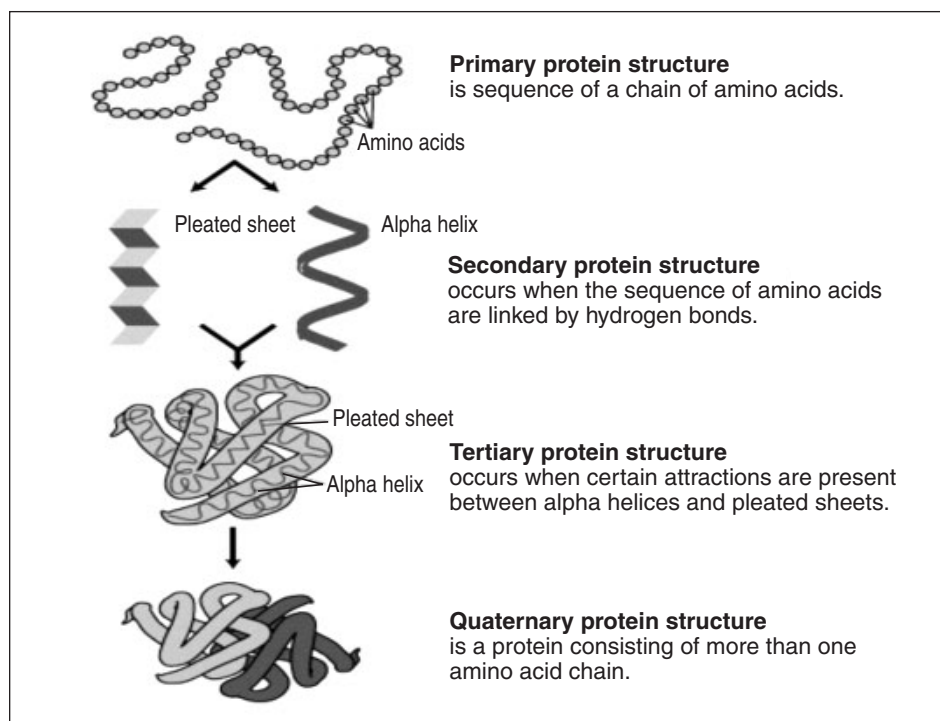


FIGURE 1 Schematic representation of protein structures. Source: Genencor, 2006.

Multi-Domain Fusion-Protein Scaffolds

It is most desirable to express the protein of interest properly folded, with no refolding, and in a clean medium. A properly folded protein structure has a tertiary or quaternary three-dimensional shape in a functional, stable conformation (Figure 1). In general, proteins expressed using *E. coli* as the host production organism do not express the properly folded protein with great efficiency. Other bacterial/fungal systems are better equipped to act as the host production organisms.

The desired foreign protein can be produced/secreted at very high levels using the host's own expression system simply by linking the foreign protein to a protein already secreted in abundance by the host organism. The "fused" protein goes through the production/secretion machinery of the host to produce properly folded product protein in very high yields.

Aspergillus niger, one of the most commonly used host production organisms, secretes very high levels of glucoamylases. Yields of 30 grams per liter, at rather fast fermentation rates and at very low production costs, makes this an extremely efficient microbial expression process (Figure 2). Glucoamylase, the fusion protein partner for foreign protein (e.g., vaccine or antibody) production, is an enzyme (a protein that contains amino acids), that is a multi-domain protein. Glucoamylase has a starch-binding domain separated from the catalytic domain by a linker. The glucoamylase pulls the starch-binding domain through the secretion machinery. The foreign protein is then expressed in the properly folded structure, regardless of the complexity of the protein. Thus, this is the system of choice for the expression of any antibody or vaccine candidate.

Gene/Host Systems

A multitude of systems have been used for large-scale, efficient expression of proteins or enzymes. The gene/host production organisms listed here all secrete fused proteins through the secretion machinery of the host to provide properly folded, secreted, product

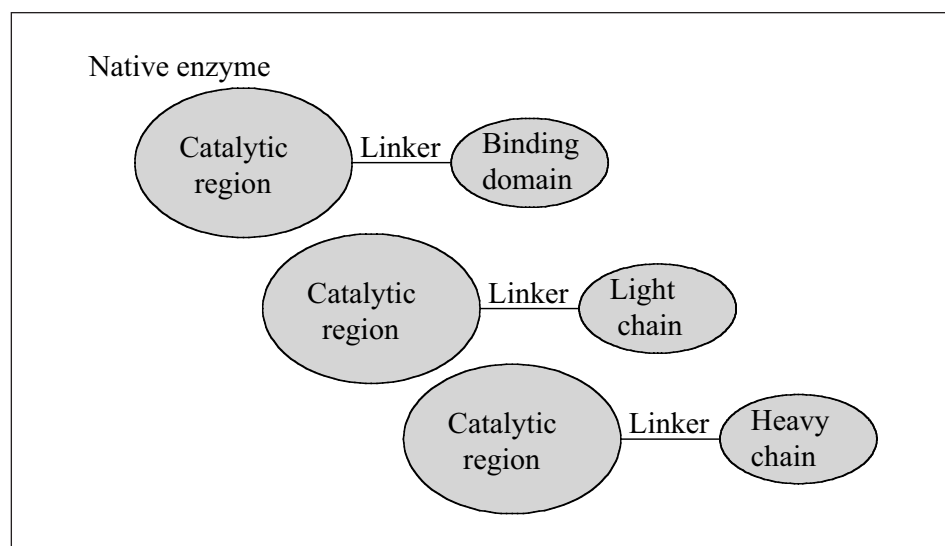


FIGURE 2 Schematic drawing of fusion protein antibody expression.

proteins in high-level yields. Bacterial examples include, *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus lentis*. Fungal host expression systems include *Aspergillus niger* and *Trichoderma reesei*.

Each gene/host production organism has been characterized, most of the genomes sequenced, and the expression systems modified to enable the system to express non-native genes with high efficiency. Knock-out strains have been developed and metabolic pathways altered so that the host utilizes simple carbon sources in manufacturing the desired protein in a process called metabolic pathway engineering. The fermentation processes are rapid, and the downstream processing maximized and efficient. These are robust, commercially viable formulations that are easy to scale up and transfer to a customer, and costs are competitive.

Microbial/Fungal System

One advantage of microbial or fungal systems is the speed at which stable host production strains can be constructed. Instead of six months, it takes less than two weeks for bacterial systems, and approximately four weeks for fungal systems, to prepare a seed bank and make it available for high-yield fermentation processes. Another advantage is that screening for improvements in the manufacturing process can be accomplished using the host organism. This translates into higher efficiency and higher yield. Fermentation times are shorter than for cell-culture systems, the processes are robust, and the capital expenditures are lower than for cell-culture systems. As a result, the cost of goods sold is also lower.

Fermentation Systems

In bacterial systems, the typical fermentation time or process turnaround time—from the time the seed is started until the time the production fermenter is removed from operation—is three days; the tank is ready to be placed into operation again on the third day (Figure 3). In fungal systems, the turnaround times can be as long as 20 days, with an average of 10 days. Production fermenters generally hold 30,000 liters to 300,000 liters; a few recovery pilot plants use 3,000 liter fermenters for smaller volume products.

Protein-Recovery Processes

The recovery or purification process accounts for a large portion of pharmaceutical production expenses. In industrial conditions, expenses can be limited, as long as clean media are used during production recovery, and as long as the processes have been maximized to produce high yields. In industrial applications, large-scale filtration and extraction are used to recover bulk protein. Chromatography, which can also be used, is scalable to recover the bulk of large-molecule proteins. As in some small-molecule applications, crystallization can be used to purify the protein. Initial purification by crystallization, which is also scalable, often yields a product of greater than 95 percent purity. All of the recovery systems already being used for injectable antibiotics produced through fungal fermentation can also be used for proteins produced at the same yields in industrial production.

Formulation and Delivery Systems

The technology is available to provide stable products in delivery systems that can withstand extreme conditions (Figure 4). In the past, entire shipments of frozen pharmaceutical products were lost during shipping delays or power outages, not to mention the lack of storage facilities in remote locations. Genencor integrates final formulation of the protein as part of the process-development strategy, so protein formulations can be

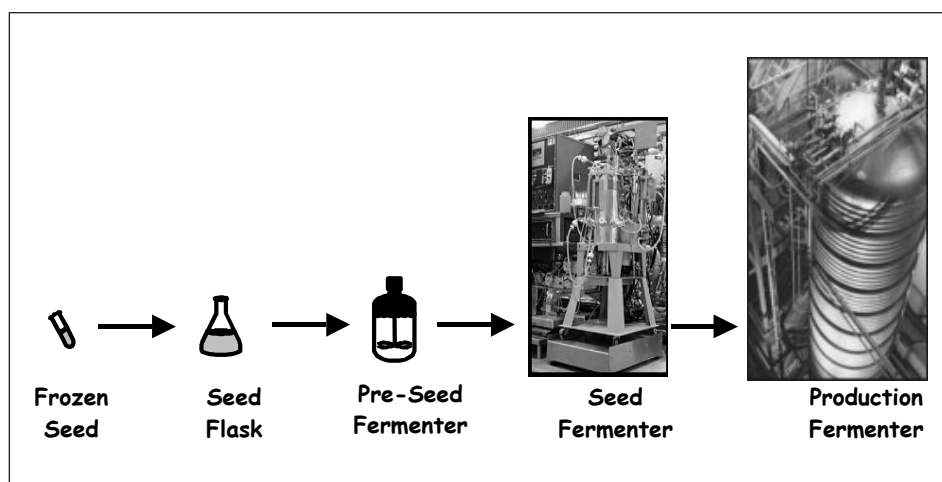


FIGURE 3 Typical fermentation train with total time of 3 to 20 days.

shipped around the globe under extreme conditions. Temperatures during shipment can be anywhere from below freezing to higher than 40°C, but the performance and stability of the active ingredients remains efficacious for the life of the product, including during shipment and storage, in either solid or liquid form. These protein formulations do not require refrigeration or freezing.

Unique properties of solid, multilayer, granular formulations can control the release of active ingredients and delay degradation by humidity, temperature, and other environmental influences. Compartmentalizing the active ingredient against other components of the product may also help maintain its effectiveness and control the release of active ingredients. An example everyone knows is the “tiny little time pills” in Contac® cold formulations.

Formulation and delivery systems are currently engineered to meet strict quality and regulatory requirements for food-grade products. The specific needs of the customer dictate the level of stability and sophistication of the final formulation.

Typical Microbial Production Process

The production of a vaccine by typical microbial processes requires two to four weeks to create the production host (two weeks for a bacterial system and up to four weeks for a fungal system), at which point the seed bank can be created. The fermentation process can take anywhere from three days in a bacterial system to 20 days in a fungal system. Recovery and formulation can take from two to ten days. This is a routine timetable using 30,000 to 300,000 liter vessels and producing tremendous amounts of protein.

Rapid Protein Drug Production

The first step in applying industrial techniques to the production of a vaccine is to ensure that a gene/host expression system is in place for the protein scaffold; the base scaffold should be part of the drug. For an influenza vaccine, the scaffold should be the epitope containing proteins from the influenza virus strains for that year, engineered so that the basic properties are in place. The engineering of proteins for the desired properties can also include a predictive assay for immunogenicity (Stickler et al., 2000) and a process by which the immunogenicity can be increased or decreased for the desired protein. Other properties include required pharmacokinetics. All of these properties are engineered into the basic protein scaffold. Next, a high-yield fermentation process is developed for the scaffold. Finally, formulation and delivery processes are engineered.

When a request is made for several million doses of a product with certain properties, the designed molecule can be linked to the multi-domain protein and produced with the pre-existing high-yield fermentation system. In a protein drug, for example, only a few changes in the sequence of a protein scaffold would be necessary to make a revised protein drug to meet a new need. For example, if there is an existing antibody, then only the variable regions (i.e., the binding sites of the antibody on the antibody scaffold) would have to be changed to make a new antibody that can recognize the very specific new antigen. If a new vaccine must be made to counter a pathogen with different peptide sequences (antigenic drift), the new sequences can be added in much the same way as they are in current flu vaccine development; then the epitopes (three-dimensional surface features of the

antigenic molecule) can be linked to the same scaffold and introduced to the fermentation system.

The same is true of a new drug with new target binding sites to act as inhibitors in a metabolic or chemical reaction. The new target binding site or receptor binding site is engineered into the protein scaffold and introduced to the fermentation system, which is already in place for the protein scaffold.

These are all variations of the same biotechnology platform, that is, protein engineering of “biomachines” to manufacture desired product proteins or enzymes and formulated to remain stable and effective over the shelf life of the product. Manufacturing platforms could be established now for known pathogens and licensed pharmaceutical drug products. Regulatory groups could review and approve gene/host systems, fermentation and recovery processes, as well as analytical testing processes and release criteria. If these steps were completed now for pharmaceutical drug products already established in the market, when new drug products are identified (e.g., new viruses, new pathogens, new target binding sites), the process could be modified and the regulatory filings revised. This process would be similar to the current yearly process for influenza vaccine production. The FDA would then be reviewing familiar processes, and approval time would probably be shorter.

Glycosylated Protein Production

Glycosylation is the process by which polysaccharides (complex carbohydrates, such as starch, glycogen, and cellulose) are added to proteins. Most protein drugs on the market today do not depend on glycosylation for their activity. Currently, proteins for which glycosylation is not required for efficacy of the protein

can be produced by existing engineered pathways. The preferred way to produce these proteins would be as non-glycosylated versions. However, proteins for which glycosylation is important may require additional gene/host engineering or post-production modification.

If a rare protein requires an absolute replication of the sugar sequence in order to be efficacious, either the

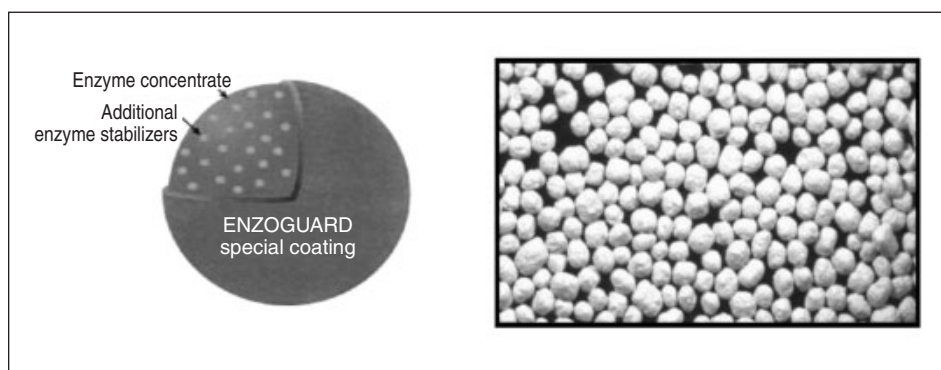


FIGURE 4 Stable formulation delivery systems. Left: schematic representation of patented multilayer granular formulation with both internal stabilizers and special exterior coating. Right: spray-coated granular formulation for stable, controlled release of active ingredient. Source: Genencor, 2006.

host would have to be engineered to duplicate the exact sequence of polysaccharides, or the polysaccharide unit would have to be added post translationally, which presents a separate problem.

Dose-Related Protein Drug Production

Vaccines and other products with individual dose sizes of < 100 mg can be made with existing technology and capacity; yields of 1 gram per liter are all that is required. Products that require dose sizes of >100 mg (e.g., monoclonal antibodies), which are rare in the pharmaceutical industry, may require initial improvements in the host system for the scaffold protein to ensure that yields are high enough to provide enough protein for several million doses of vaccine.

Conclusion

Current industrial biotechnology production processes are capable of producing 100,000,000 grams of protein in less than 12 weeks. However, to appropriate the use of biotechnology capacities, all aspects of protein manufacturing processes must be in place, including protein scaffolds for the gene/host systems, high-yield fermentation systems from which robust recoveries can be made, and final formulations that allow transportation and storage under extreme conditions. Yields must be at least 1 gram per liter to meet timelines. The industry has the capacity and the incentives to do this.

The same is true for capacities in the injectable and oral antibiotics industry, which currently manufactures product through fungal fermentation. This industry works closely with the FDA to meet regulatory requirements and improve antibiotic processes continually to reduce the necessary capacity. This is a \$13 billion per year industry with sales averaging \$1 to \$100 per gram product.

The antibiotic fermenter capacities available in this industry or the biotech industry would more than meet the needs for manufacturing proteins by the pathways discussed in this article. The fermentation processes for production of antibiotics or industrial proteins and enzymes are comparable to those anticipated for protein production pathways. The same is true for recovery and purification processes. The tools are available, and the time is now.

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NAE News and Notes

NAE Newsmakers

Linda M. Abriola, dean, Tufts University School of Engineering, is one of 200 scholars, scientists, artists, and world leaders to be named to the **American Academy of Arts and Sciences**. Dr. Abriola, a specialist in groundwater contamination, was honored for her investigations of chlorinated solvents, degreasing agents, and dry cleaning fluids.

Anjan Bose, Regents Professor and Distinguished Professor of Electric Power Engineering, Washington State University, is the recipient of the **2006 IEEE Herman Halperin Electric Transmission and Distribution Award**. Dr. Bose received the award for the “enhancement of transmission system operation through the development of real-time operator training simulators.”

Van C. Mow, Stanley Dicker Professor and chair, Department of Biomedical Engineering, Columbia University, was awarded the **Clarence E. Davies Medal for Engineering Achievement**, the highest award for an alumnus of the School of Engineering of Rensselaer Polytechnic Institute (RPI). President

Shirley A. Jackson (NAE) presented the award at a ceremony on April 7. The award was endowed by J. Erik Jonsson, RPI '16, founder of Texas Instruments.

Robert M. Nerem, professor and director of the Institute for Bioengineering and Bioscience, Georgia Institute of Technology, was elected a **foreign member of the Royal Swedish Academy of Engineering Science (IVA)** on March 29, 2006. His election into this prestigious academy is a tribute to his dedication to the bioengineering field, his extensive career in research, and his overall influence on academic and government policy. Established in 1919, IVA is the oldest engineering academy in the world. The institution has approximately 1,000 members, 250 of whom are non-Swedes.

Choon Fong Shih, president, National University of Singapore, has been elected a **Foreign Honorary Member** of the **American Academy of Arts and Sciences (AAAS)**. Dr. Shih was cited for his pioneering work in nonlinear fracture mechanics and computational

methods for fracture analyses. A formal induction ceremony will be held at the AAAS headquarters in Cambridge, Massachusetts, on October 7, 2006.

C.P. Wong, Regents' Professor of Materials Science and Engineering, School of Materials Science and Engineering, Georgia Institute of Technology, received the **2006 IEEE Components Packaging and Manufacturing Technology Award**. The award, which is given in recognition of contributions to the advancement of components, electronic packaging, or manufacturing technologies, was presented on June 1, 2006, at the 56th IEEE Electronic Components and Technology Conference in San Diego, California.

Wm. A. Wulf, president, National Academy of Engineering, was inducted into the **Chinese Academy of Engineering (CAE)** at the 8th General Assembly on June 5, 2006. Dr. Wulf also had the honor of addressing the CAE General Assembly during the ceremonies.

2006 German-American Frontiers of Engineering Symposium



On May 4–6, the 2006 German-American Frontiers of Engineering (GAFOE) Symposium was held at Bell Labs, Lucent Technologies, in Murray Hill, New Jersey. **Elsa Reichmanis**, director of the Materials Research Department at Bell Labs, Lucent Technologies, and U.S. co-chair of the 2006 GAFOE meeting, was instrumental in arranging the hosting of this event. **Jeong Kim**, president of Bell Labs, the site of many historical breakthroughs, such as transistors, lasers, and digital encryption, welcomed the group of 60 German and U.S. engineers. Co-chairing the symposium with Dr. Reichmanis was Theodor Doll, professor of microstructure physics at the University of Mainz.

The four topics covered at the meeting were managing technological risk, security and privacy

implications of connected products, emerging applications of nanotechnologies, and oil and gas exploration and production. Presentations by two Germans and two Americans in each of the four areas covered a wide range of topics, including the benefits and risks of increasing autonomy in air and space systems, compliance with legal requirements related to information security, nanotechnology in cardiovascular medicine, and modeling to improve petroleum reservoir development and production.

On Thursday evening, **David Billington**, Gordon Y.S. Wu Professor of Engineering in the Department of Civil and Environmental Engineering at Princeton University, gave a fascinating talk about engineering innovations and innovators that transformed America in

the twentieth century. Other highlights of the meeting were poster sessions, where all participants had an opportunity to share information about their research or technical work, and tours of Lucent laboratories, where research projects in areas such as telesensing and nanotechnology were described.

Funding for the meeting was provided by the National Science Foundation, the NAE Fund, and the Alexander von Humboldt Foundation, which is also a co-organizer of GAFOE symposia. The tenth GAFOE meeting is planned for April 26–28, 2007, in Hamburg, Germany.

NAE has hosted annual U.S. FOE symposia since 1995, and GAFOE symposia since 1998. NAE also has bilateral programs with Japan and India. FOE symposia bring together

emerging engineering leaders (ages 30 to 45) from industry, academe, and government to learn about cutting-edge developments, techniques, and approaches in fields other than their own. Exchanges of information made possible by FOE

meetings have become increasingly important as engineering has become increasingly interdisciplinary. GAFOE and the other bilateral programs also encourage the development of cooperative networks of younger engineers across

national boundaries.

For more information about GAFOE or other FOE activities, contact Janet Hunziker in the NAE Program Office at 202/334-1571 or by e-mail at jhunziker@nae.edu.

Christine Mirzayan Science and Technology Policy Graduate Fellows



Lily Tong

Lily Tong is completing her Ph.D. in chemical engineering in the Bioinformatics and Metabolic Engineering Laboratory at MIT. In her research, funded by an NSF graduate fellowship, she uses gas chromatography-mass spectrometry to measure and identify patterning in blood metabolite concentrations in disease and non-disease states. Her focus is on patients with end-stage renal dialysis and gamma-hydroxybutyrate toxicity in rats.

Lily's interests range beyond the research lab. After completing her undergraduate degree at Georgia Tech, she spent nine months learning Chinese in Beijing and volunteering at the Boys and Girls Club in Atlanta. At MIT, she hosts a radio show that provides an opportunity for guests from the MIT community



Yvonne Bennett

to chat about themselves, often translating abstruse scientific concepts into everyday language. In addition, she recently performed in a theatrical interpretation of the impact of women in science and the challenges they face. In her spare time, Lily enjoys reading, cooking, traveling, and knitting.

Lily's work at CASEE reinforced her longstanding interest in education. Her goal was to learn the process of writing policy and how scientists can improve the public understanding of science and engineering.

Yvonne Szymko Bennett received her Ph.D. in bioengineering from the University of Pennsylvania and her undergraduate degree in electrical engineering from Union College. Her doctoral thesis was focused

on several projects involving inner ear hair cell micromechanics and transduction in the chick cochlea. After receiving her doctorate, she continued to work in the area of hair cell electrophysiology as a research associate at the Institute for Sensory Research at Syracuse University. To bridge the clinical and basic science areas of hearing research, she earned a second master's degree in audiology and became a licensed audiologist.

From 1998 to 2002, as a research fellow at the National Institute on Deafness and Other Communication Disorders (NIDCD) at NIH, Yvonne published several papers in the area of hereditary hearing loss and auditory electrophysiology. Also at NIH, Yvonne was the NIDCD representative and, later, clinical co-chair of the NIH Fellows' Committee. This experience aroused her interest in science policy and administration.

As a policy fellow with the NAE Engineering, the Economy, and Society Program, Yvonne worked on a project to explore the efficacy of noise-control technologies to reduce the harmful effects of noise in our advanced society. In her spare time, Yvonne enjoys Ashtanga yoga, reading, and boating.

New NAE Report on Assessing Technological Literacy

In a broad sense, technology is any modification of the natural world made to fulfill human needs or desires. From computers and genetically modified foods to super-highways and satellites, technology is pervasive. To be an informed citizen requires knowing something of the nature of technology: how it is created, how it shapes society, and how people influence its development. In other words, it requires technological literacy. However, very little has been done to determine the level of technological literacy in the United States.

A new NAE report, *Tech Tally: Approaches to Assessing Technological Literacy*, explores methods and opportunities for assessing technological literacy in K–12 students, K–12 teachers, and out-of-school adults. Prepared in collaboration with the National Research Council Board on Testing and Assessment, this report suggests how scientifically valid, broadly applicable assessments might be developed for the three target populations. Findings and related recommendations are provided in five critical areas: instrument development;

research on learning; computer-based assessment methods; framework development; and public perceptions of technology.

The report adds to previous studies focused on the issue of technological literacy, including the 2002 NAE publication, *Technically Speaking: Why All Americans Need to Know More About Technology*. The 16-person Committee on Assessing Technological Literacy was chaired by NAE member **Elsa Garmire**, Dartmouth College. The project was funded by the National Science Foundation.

NAE and Royal Swedish Academy Launch Parallel Studies on Noise Control

The NAE Council recently expressed its intention to strengthen the ties among engineering and science academies worldwide. The council placed a high priority on studies of common interest with international engineering organizations, particularly with member academies of the international Council of Academies of Engineering and Technological Sciences (CAETS).

NAE and the Royal Swedish Academy of Engineering Sciences (IVA), both founding members of CAETS, have embarked on parallel studies of issues related to noise control in the United States and Sweden. An unwanted by-product of practically all human activities that involve the use of mechanical devices, noise adversely affects the quality of life of millions throughout the world. Excessive noise can

cause deafness, stress, sleep disturbances, interference with communication and learning, heart disease, and other health and psychological problems. As urban centers grow larger, the effects become more severe. Noise is also an important trade issue, and many governments are setting limits on noise from domestic and imported products.

Over the past two decades in the United States, more than a dozen federal departments and agencies have funded noise-abatement projects. The amount of their expenditures is not known, but no uniform policies or coordinated programs have been instituted. In the same time period, the European Union (EU), of which Sweden is a member, has issued directives to institute a uniform noise policy throughout Europe. There too, although millions of euros have been spent on noise

research, progress has been slow.

If the United States and EU continue on their present course, neither America nor Europe is likely to be noticeably quieter in the foreseeable future. Progress to date has been hampered by industry indifference and a lack of knowledge by politicians. Significant improvements in the control of noise emissions will require new policies—and the involvement of engineers. As advisors to their respective governments on matters of engineering and technology policy, NAE and IVA can assume leadership roles in initiating change.

The 30-month NAE study on the development and deployment of technologies to alleviate the negative effects of noise in workplaces, communities, and homes was described briefly in the last issue of *The Bridge*. The Technology for a

Quieter America Project, chaired by **Dr. George Maling**, will examine existing and potential solutions and recommend policies for their development and implementation. The objectives of the study are: (1) to summarize the current state of practice in noise control engineering; (2) to develop a research and education agenda that promotes the generation of new knowledge to benefit all sectors of society (employees, corporations and manufacturers, and individuals); and (3) to recommend policies to improve the American soundscape.

This is not the first time IVA has taken up the problem of excessive noise. A study was undertaken as far back as 1958, and a study of noise source emissions, "Machinery Noise," was published in 1963 (a year before NAE was founded). Encouraged by the present NAE initiative, in 2005 IVA appointed an ad hoc committee, chaired by Dr. Tor Kihlman, to conduct a parallel study. Support is now in place for a technology study of road vehicle noise and detailed proposals for reducing it, such as new requirements for tires, vehicles, and road surfaces. Although narrower in scope

than the NAE project, the IVA study will address a persistent problem in European cities and towns, and the results may be applicable to the American soundscape.

Excessive noise is a universal problem, and several countries that are now members of the EU have formulated long-term goals for limiting noise levels from traffic. Nevertheless reducing traffic noise appears to be a more distant goal now than it was several decades ago. Thus, other CAETS member academies may be encouraged to undertake studies of this important issue.

Calendar of Meetings and Events

September 21–23	12th U.S. Frontiers of Engineering Symposium Dearborn, Michigan	October 14	NAE Peer Committee Meeting	November 9–11	Japan-America Frontiers of Engineering Symposium Tsukuba, Japan
October 4	Workshop on News and Terrorism: Communicating in a Crisis Seattle, Washington	October 15–16	NAE Annual Meeting	December 1–2	2007 NAE Election Committee on Membership Meeting Irvine, California
October 12	NRC Executive Committee Meeting	October 23	2007 Draper Prize Final Committee Meeting		
October 13	NAE Finance and Budget Committee Meeting	October 24–25	Offshoring of Engineering Workshop		
October 13–14	NAE Council Meeting	October 26	2007 Russ Prize Final Committee Meeting		
		November 8	NAE Regional Meeting of Foreign Associates NTT Atsugi R&D Center, Atsugi, Japan		

All meetings are held in the National Academies buildings, Washington, D.C., unless otherwise noted. For information about regional meetings, contact Sonja Atkinson at satkinsa@nae.edu or (202) 334-3677.

NAE Members Continue the Culture of Philanthropy

Whether you are a new member, new donor, or long-time supporter of the NAE, your contributions make a difference in the academy's ability to guide policy in areas of national import, support engineering education and professional advancement, and continue the dissemination of life-improving information.

The Center for the Advancement of the Study of Engineering Education (CASEE), Frontiers of Engineering, and many other programs and publications focused on engineering education and the ethical practice of engineering all benefit from members' contributions.

Gifts to the NAE Annual Fund provide independent resources that enable the academy to fulfill its mission. Federal grants typically require 350 days from request to receipt—almost an entire year before a project can even begin. With unrestricted contributions, however, we can address urgent issues swiftly with reports such as the 2005 report, *Rising Above the Gathering Storm*, which outlined the critical importance of science and engineering research and education in maintaining U.S. economic and technological leadership.

In 2005, unrestricted contributions to the Annual Fund totaled more than \$702,000, with a member participation rate of 30 percent. Thirty-eight of the donors were newly elected members, whose gifts have reinforced the academy's culture of philanthropy.

In a recent statement, new NAE vice president **Maxine Savitz**, retired General Manager for Technology Partnerships, Honeywell Inc., said, "I look forward to working closely with colleagues dedicated to helping the academy strengthen our profession and sustain the welfare of our nation through communication, education, and expert guidance in engineering and technology. The number of newly elected members who have chosen to support the academy speaks volumes about our membership and the importance of private philanthropy."

Each and every gift that supports NAE's existing programs and future endeavors is greatly appreciated. We especially want to recognize the contributions of our newer members, listed below, who have made generous contributions to NAE since September 2005. All of them were elected since 2002.

Linda M. Abriola
 Kenneth E. Arnold
 Ilan Asriel Blech
 Mark T. Bohr
 Jeffrey P. Buzen
 Joe C. Campbell
 Chau-Chyun Chen
 Edmund M. Clark
 Joseph M. DeSimone
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 S. P. Shah
 Neil G. Siegel
 George Tchobanoglous
 Spencer R. Titley
 Ali G. Ulsoy
 T. L. Youd

In Memoriam

HOLT ASHLEY, 83, Professor Emeritus, Departments of Aero/Astronautics and Mechanical Engineering, Stanford University, died on May 3, 2006. Dr. Ashley was elected to NAE in 1970 for "contributions to the field of aeroelastic structures and unsteady aerodynamics, aiding in the solution of problems in vibration and gust loading."

STANLEY HILLER, JR., 81, founder/president, Hiller Aviation Museum, died on April 20, 2006. Mr. Hiller was elected to NAE in 1999 for "leadership in helicopter development with great value to human life, safety, and quality."

JOHN E. KUNZLER, 82, retired director, Future Device Research Center, AT&T Bell Laboratories, died on January 11, 2006. Dr. Kunzler was elected to NAE in 1982 for "practical realization of high-field superconducting solenoids."

I. HARRY MANDIL, 86, founding partner and board member, MPR Associates, Inc., died on April 27, 2006. Dr. Mandil was elected to NAE in 1998 for "engineering design and development of materials for naval and commercial nuclear reactors."

ROBERT W. MANN, 81, Whitaker Professor Emeritus of Biomedical Engineering, Department of Mechanical Engineering, Massachusetts Institute of Technology, died on June 16, 2006. Dr. Mann was elected

to NAE in 1973 for "contributions to design education and to the advancement of biomedical engineering." Dr. Mann was also a member of the National Academy of Sciences (since 1982) and the Institute of Medicine (since 1971).

RUBEN F. METTLER, 82, retired chairman and CEO, TRW Inc., died June 1, 2006. Dr. Mettler was elected to NAE in 1965 as an "outstanding creative missile and systems engineer."

ARTHUR B. METZNER, 79, H. Fletcher Brown Professor, Emeritus, Chemical Engineering Department, University of Delaware, died on May 4, 2006. Professor Metzner was elected to NAE in 1979 for "research in the fluid mechanics of viscoelastic and polymeric materials."

FREDERIC C.E. ODER, 86, retired executive vice president, Lockheed Missiles & Space Company, Inc., died on May 11, 2006. Dr. Oder was elected to NAE in 1980 for "leadership in conceiving and developing civil and military satellites."

ROBERT C. REID, 81, Professor Emeritus of Chemical Engineering, Massachusetts Institute of Technology, died on May 18, 2006. Dr. Reid was elected to NAE in 1980 for "contributions to methods of computing physical properties and the understanding of boiling heat transfer between immiscible liquids."

JAMES E. ROBERTS, 75, consulting bridge engineer, Imbsen & Associates, Inc., died on July 6, 2006. Mr. Roberts was elected to NAE in 1996 for "the development and implementation of innovative bridge retrofit concepts and criteria under seismic conditions."

THOMAS B. ROBINSON, 88, retired, Black & Veatch, Engineers-Architects, died on January 16, 2006. Mr. Robinson was elected to NAE in 1979 for "contributions as a practicing engineer and manager in water supply and collection and disposal of liquid and solid wastes."

HERMAN E. SHEETS, 97, Professor Emeritus, Ocean Engineering, University of Rhode Island, and consultant, died on April 22, 2006. Dr. Sheets was elected to NAE in 1967 for "ship and submarine design."

JEROME J. TIEMANN, 74, consultant, GE Corporate Research and Development, died on April 25, 2006. Dr. Tiemann was elected to NAE in 1984 for his "creativity and leadership in developing advanced electronics for communications, medical diagnostics, radar, and video information processing."

JACK H. WERNICK, 82, retired executive director, Network, Environment, Materials, and Component Reliability Research, Telcordia Technologies, died on March 3, 2006. Dr. Wernick was elected to NAE in 1979 for "contributions to the synthesis of new electronic materials and their use in devices."

Publications of Interest

The following reports have been published recently by the National Academy of Engineering or the National Research Council. Unless otherwise noted, all publications are for sale (prepaid) from the National Academies Press (NAP), 500 Fifth Street, N.W., Lockbox 285, Washington, DC 20055. For more information or to place an order, contact NAP online at <http://www.nap.edu> or by phone at (888) 624-8373. (Note: Prices quoted are subject to change without notice. Online orders receive a 20 percent discount. Please add \$4.50 for shipping and handling for the first book and \$0.95 for each additional book. Add applicable sales tax or GST if you live in CA, DC, FL, MD, MO, TX, or Canada.)

ERRATUM In the summer issue, **Meyer J. Benzakein** was mistakenly listed as chair of the committee that produced *Aeronautics Innovation: NASA's Challenges and Opportunities*. Although Dr. Benzakein was a member of the study committee, **Alan Schriesheim** chaired the study. Our apologies for the error.

A Risk Reduction Strategy for Human Exploration of Space: A Review of NASA's Bioastronautics Roadmap. The National Aeronautics and Space Administration (NASA) Bioastronautics Roadmap is "the framework used to identify and assess the risks of crew exposure to the hazardous environments of space." The road map was created to facilitate and support three design reference missions: (1) a one-year mission to the International Space Station; (2) a

month-long stay on the lunar surface; and (3) a 30-month round-trip journey to Mars. In 2003, NASA asked the National Academies to provide a comprehensive assessment of the road map and identify the unique challenges to the design reference missions. After assessing the content of the road map, the processes used to develop and update it, and the context in which it was developed and will be used, the study committee concluded that the current version is a useful first step but is not adequate to achieve its stated goals. The committee recommended changes to the document, as well as changes in the thinking and actions of NASA management.

NAE member **James P. Bagian**, director, National Center for Patient Safety, Veterans Health Administration, was a member of the study committee. Paper, \$31.75.

Going the Distance? The Safe Transport of Spent Nuclear Fuel and High-Level Radioactive Waste in the United States.

This independent, objective, and authoritative analysis of the transportation of spent nuclear fuel and radioactive waste in the United States examines risks and identifies current and potential technical and societal concerns. *Going the Distance?* provides comparisons of the health and safety risks of transporting spent fuel and radioactive waste to other kinds of risks, such as the routine transport of radiological wastes, which can cause chronic radiation exposures and latent cancer, and the risk from accidents,

which can cause acute radiation sickness and death, as well as latent cancer.

NAE member **Thomas B. Deen**, consultant and former executive director, Transportation Research Board, was vice chair of the study committee. **Melvin F. Kanninen**, principal, MFK Consulting Services, was a committee member. Paper, \$39.00.

Improving the Quality of Health Care for Mental and Substance-Use Conditions: Quality Chasm Series.

Every year, more than 33 million Americans receive health care for mental or substance-use conditions, or both. Together, mental and substance-use illnesses are the leading cause of death and disability in women and men ages 15 to 44, and the second highest cause of death for all men. Although effective treatments exist, services are frequently fragmented, and many people do not receive these treatments as designed, or at all. This report examines the distinctive characteristics of health care for mental and substance-use conditions, including payment, benefit coverage, and regulatory issues, and health care organization and delivery issues and puts forth an agenda for improving the quality of care.

NAE member **Seth Bonder**, Bonder Group, was a member of the study committee. Hardback, \$59.95.

State and Federal Standards for Mobile Source Emissions.

Mobile sources, such as cars and light- and heavy-duty trucks, diesel-powered cranes,

bulldozers, and tractors, and equipment powered by small gasoline engines (e.g., lawnmowers), contribute significantly to air pollution in the United States. However, it is not clear whether state or federal government is responsible for establishing mobile-source emissions standards. To clarify the issue, Congress called on the Environmental Protection Agency (EPA) to commission an independent study of the practices and procedures used by California to develop separate emissions standards and other states to adopt the California standards. This report provides a comparison of the scientific and technical procedures used by states with those used by EPA and an assessment of the impacts of state emissions standards on compliance costs and emissions. The report concludes that, despite substantial progress in reducing emissions from mobile sources nationwide, federal air-quality standards are badly needed in many parts of the country. The report also concludes that California should continue its pioneering role in setting emissions standards for cars, trucks, and off-road equipment.

NAE member **Karl J. Springer**, retired vice president, Automotive Products and Emissions Research, Southwest Research Institute, was a member of the study committee. Paper, \$69.00.

Linking Knowledge with Action for Sustainable Development: The Role of Program Management: Summary of a Workshop. This report summarizes a workshop organized by the National Academies Roundtable on Science and Technology for Sustainability. The workshop brought together a select group of program managers from the public and private sectors

to discuss specific cases in which knowledge and action were linked in various observation, assessment, and decision support systems. Workshop discussions explored a wide variety of experiments in harnessing science and technology to promote development and conserve the environment. Participants reflected on significant challenges they faced in implementing programs and the strategies they used to address them. The report summarizes workshop discussions and identifies common themes in the process of linking knowledge with actions for sustainable development in a wide range of cases.

NAE member **Lawrence T. Papay**, retired sector vice president for integrated solutions, Science Applications International Corporation, is a member of the roundtable. NAE member **Arden L. Bement Jr.**, director, National Science Foundation, is an ex officio member of the roundtable. Paper, \$30.25.

Is That Real? Identification and Assessment of the Counterfeiting Threat for U.S. Banknotes. A key mission of the Bureau of Engraving and Printing (BEP) of the U.S. Department of the Treasury is the design and printing of U.S. banknotes. BEP is responsible for producing easily recognizable currency that is difficult to counterfeit. In recent years, the bureau has requested a number of studies by the National Research Council (NRC) to assess evolving threats made possible by modern information technology. NRC was asked to identify and evaluate significant emerging counterfeiting threats and assess technologically feasible counterfeit-deterrent features for new designs. This first

report provides an assessment of emerging threats including a wide range of digital imaging and printing techniques and an analysis of a systems approach to the counterfeiting threat. The second report will provide an evaluation of features of new banknotes to address these threats.

NAE members on the study committee were **Charles B. Duke**, retired vice president and senior research fellow, Xerox Corporation; **Pradeep K. Khosla**, dean, College of Engineering, Carnegie Mellon University; **Stephen M. Pollock**, Herrick Emeritus Professor of Manufacturing, University of Michigan; and **Gary K. Starkweather**, retired architect, Microsoft Corporation. Paper, \$18.00.

An Assessment of Balance in NASA's Science Programs. When the space exploration initiative was announced, Congress asked the National Research Council (NRC) to review the science proposed by NASA to carry out the initiative. The NRC was also asked to assess whether this program would provide for balanced scientific research in the established disciplines supported by NASA. In 2005, NRC released three studies, but the last phase was postponed because of changes at NASA. This final report provides an assessment of the status of NASA's scientific disciplines under the budget requests imposed by the exploration initiative. The report also provides an analysis of how well NASA's science budget reflects cross-disciplinary scientific priorities.

NAE members on the study committee were **Ronald F. Probst**, Ford Professor of Engineering Emeritus, Massachusetts Institute of Technology; and **A. Thomas Young**, retired executive vice president,

Lockheed Martin Corporation. Paper, \$18.00

Globalization, Biosecurity, and the Future of the Life Sciences.

Bioterrorism and the proliferation of biological weapons capabilities have increased concerns that advances in genetic engineering and biotechnology might enable the production of biological weapons with unique and unpredictable characteristics. This report identifies current trends and future objectives for research in public health, life sciences, and biomedical sciences that could be relevant to the development of biological weapons in the next 5 to 10 years. The report proposes measures that can be taken to anticipate, identify, and mitigate these dangers.

NAE member **C. Kumar N. Patel**, chairman, Pranalytica, Inc., was a member of the study committee. Paper, \$56.00.

Terrorism and the Chemical Infrastructure: Protecting People and Reducing Vulnerabilities.

The chemical sector is a key component of the national economy and has been designated by the U.S. Department of Homeland Security (DHS) as one of 17 sectors in the nation's critical infrastructure. Although chemical products represent only 2 percent of the U.S. gross domestic product, they are essential to most manufactured goods. The report identifies vulnerabilities; identifies points of weakness in supply chains; assesses the likely impacts of significant disruptions in supply chains; identifies actions to prevent disruptions and mitigate losses and injuries; identifies incentives and disincentives to preventive and mitigating actions; and recommends areas of scientific, engineering, and economic research

and development. The report concludes that the consequences of a deliberate attack on the chemical infrastructure would be similar to the consequences of accidents we have already experienced. Under limited circumstances, such an attack could cause catastrophic casualties and loss of life, but it would take several simultaneous events to cause catastrophic economic consequences. Overall, the recommendations in this report emphasize the benefits of investing to improve emergency preparedness and response capabilities to chemical events and the development of cost-effective processes that reduce the volume, toxicity, and hazardous conditions under which chemicals are processed.

NAE member **Barry M. Horowitz**, professor of systems engineering, University of Virginia, was a member of the study committee. Paper, \$33.00.

Toward a New Advanced Hydrologic Prediction Service (AHPS).

The National Weather Service (NWS), which is responsible for providing flood forecasts and warnings in the United States, established the Advanced Hydrologic Prediction Services (AHPS) Program in 1997 to advance technology for hydrologic services, and, specifically, to provide accurate forecasts that support timely warnings. The goal of AHPS is to provide information at the right time to facilitate responses to mitigate damage to life and property. AHPS is slated to be fully implemented nationwide in 2013. With seven years remaining on the development and implementation timeline. This report provides an assessment of AHPS' research on hydrologic science and technology,

river routing and mechanics, systems engineering, and implementation. Overall, the report finds that AHPS is an ambitious program that could provide timely and necessary services and products. The report calls for AHPS (1) to develop a detailed, comprehensive, multiyear implementation plan and (2) to bring the program's goals and budget into closer alignment.

NAE member **Soroosh Sorooshian**, UCI Distinguished Professor and director, Center for Hydrometeorology and Remote Sensing, University of California, Irvine, chaired the study committee. Paper, \$25.25.

Managing Coal Combustion Residues in Mines.

Burning coal in electric utility plants produces, in addition to power, residues that contain constituents harmful to the environment. The management of large volumes of coal-combustion residues (CCRs) is a challenge for utilities, because CCRs must either be placed in landfills, surface impoundments, or mines, or alternative uses must be found for them. This study focuses on the placement of CCRs in active and abandoned coal mines. The committee concludes that placement of CCRs in mines as part of the reclamation process may be a viable option as long as the placement is properly planned and is carried out in a way that does not have significant adverse environmental and health impacts. This report discusses a variety of steps in planning and managing the use of CCRs as minefills, including an integrated process of CCR characterization and site characterization, management and engineering design of placement activities, and design and implementation of monitoring to reduce the risk of contamination

moving from the mine site to the ambient environment. Enforceable federal standards are needed for the disposal of CCRs in minefills to ensure that states have adequate, explicit authority and that minimum safeguards are implemented.

NAE member **Thomas J. O'Neil**, retired president and chief operating officer, Cleveland Cliffs Inc., was a member of the study committee. Paper, \$50.00.

To Recruit and Advance: Women Students and Faculty in U.S. Science and Engineering. Although more women than men participate in higher education in the United States, the numbers are reversed in careers in science and engineering. This report identifies and discusses practices for better recruitment, retention, and promotion of women scientists and engineers in academia. Rather than a catalogue of challenges to the advancement of women, this report describes actions taken by universities to improve the situation, including ways to improve the recruitment of female undergraduates and graduate students, reduce attrition in science and engineering degree programs in the early undergraduate years, improve retention rates of

women at critical transition points, improve the recruitment of women for tenure-track positions, increase the tenure rate for female faculty, and increase the number of women in administrative positions.

NAE members on the study committee were **Uma Chowdhry**, vice president, Central Research and Development, DuPont Company Experimental Station, and **Julia R. Weertman**, Professor Emerita, Department of Materials Science and Engineering, Northwestern University. Paper, \$29.95.

CLEANER and NSF's Environmental Observatories. Degradation of the nation's water resources threatens the health of humans and the functioning of natural ecosystems. To understand the causes of these adverse impacts and how they might be mitigated, especially in urban and human-stressed aquatic systems, the National Science Foundation (NSF) has proposed the establishment of the Collaborative Large-scale Engineering Analysis Network for Environmental Research (CLEANER). This program would provide a platform for the near-real-time and conventional collection and analysis of data; improve

understanding and predictions of processes that control large-scale environmental and hydrologic systems; explain human-induced impacts on the environment; and identify adaptive management approaches to mitigate the adverse impacts of human activities on water and land resources. At NSF's request, the National Academies undertook a review of this proposed program. The report concludes that an environmental observation network could transform the environmental engineering profession and increase its contributions to society and recommends that NSF proceed with planning, implementation, and intra- and interagency coordination activities for the program.

NAE member **Daniel P. Loucks**, professor, School of Civil and Environmental Engineering, Cornell University, chaired the study. Other NAE members on the study committee were **Richard A. Conway**, retired senior corporate fellow, Union Carbide Corporation, and **Charles R. O'Melia**, Abel Wolman Professor of Environmental Engineering, Johns Hopkins University. Paper, \$18.00.

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