Reducing Timelines for Vaccine Potency Testing

By Martin Smith
at Synbiosis

Shortening the vaccine development cycle by reducing the need for costly, time-consuming manual analyses of vaccine potency is allowing pharma and biotech companies to increase their throughput. Using this strategy, automation can help to ensure that the most effective vaccines reach the market more rapidly.

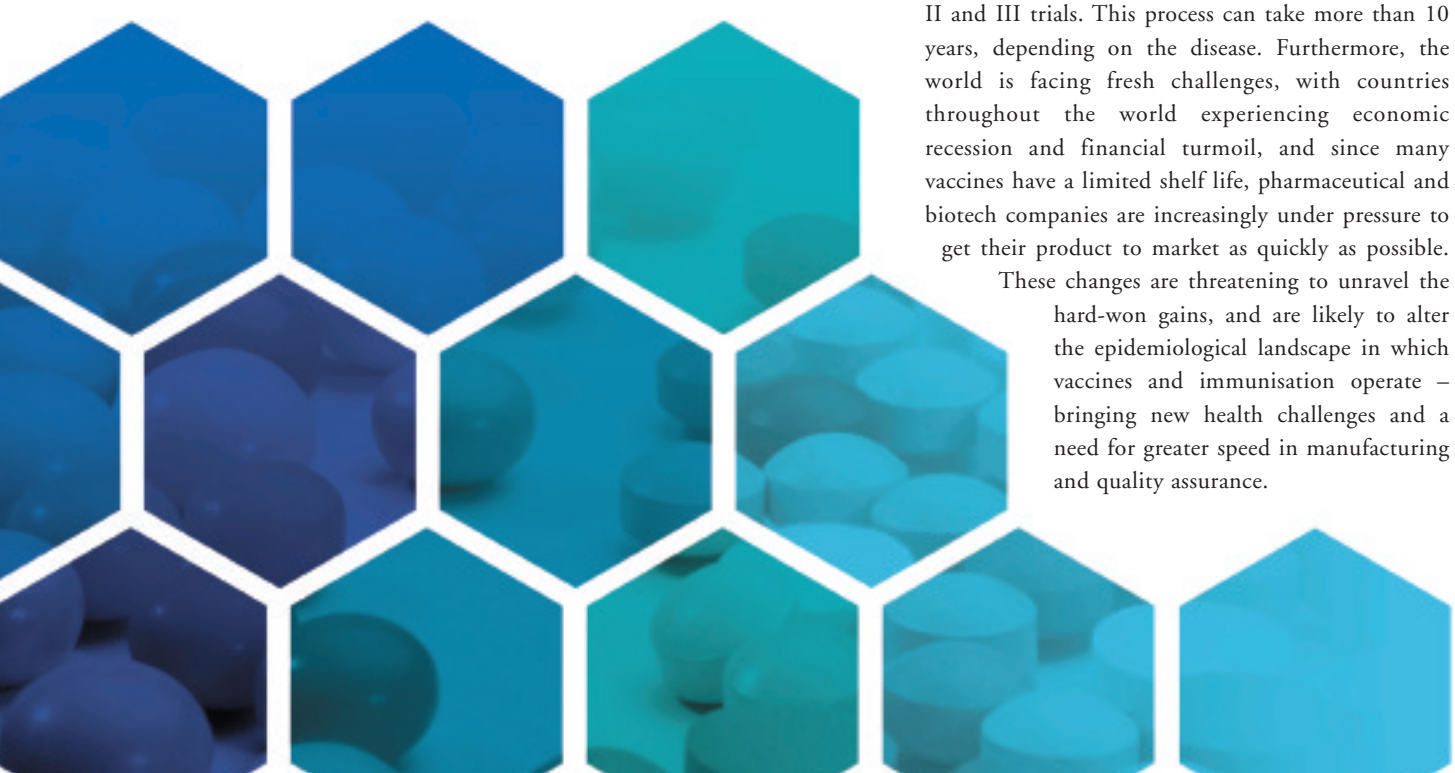
Infectious diseases and illnesses – such as rotavirus diarrhoea, influenza and pneumococcal disease – are prominent public health problems in developing countries, killing more than one million people each year. Prevention of these infections by vaccination is one of the main approaches in controlling the high rates of morbidity and mortality. The wide availability of vaccines for public use has had a tremendous impact on reducing the disease burden by preventing deaths, and vaccines have emerged as one of the most profitable sectors in the global pharmaceutical industry. The global vaccine market has almost tripled during the past nine years, reaching over €15 billion per annum in 2008, and is forecast to be €33 billion by 2018 (1). By the end of 2008, the total number of vaccine products had reached a record of over 120, making the first decade of this century the most productive in the history of vaccine development.

VACCINE DEVELOPMENT

There is an extensive range of vaccines in the development pipeline undergoing various stages of testing and clinical evaluation, and the stimulation of private industry to engage in these markets is of paramount importance. Paediatric vaccines – such as measles, mumps and rubella – presently dominate the global vaccine market; however, it is thought that adult vaccines – such as influenza, meningococcal and pneumococcal vaccines – will define future growth directions and become a profitable area for vaccine manufacturers, although these vaccines are generally not anticipated to become licensed before 2012. In addition to new vaccine development, research into novel technologies to improve the delivery of vaccines is in progress so that administration can be faster, safer and more effective.

The timeline horizon of vaccines for the developing world is complex and influenced by many technical, programmatic and market conditions. Traditionally, the introduction of a new vaccine into the developing world – following market entry into the industrialised world – is a very slow trajectory, as vaccine development proceeds through discovery, process engineering, toxicology and animal studies to Phase I, II and III trials. This process can take more than 10 years, depending on the disease. Furthermore, the world is facing fresh challenges, with countries throughout the world experiencing economic recession and financial turmoil, and since many vaccines have a limited shelf life, pharmaceutical and biotech companies are increasingly under pressure to get their product to market as quickly as possible.

These changes are threatening to unravel the hard-won gains, and are likely to alter the epidemiological landscape in which vaccines and immunisation operate – bringing new health challenges and a need for greater speed in manufacturing and quality assurance.



POTENCY TESTING

A major concern regarding vaccine quality control is the requirement that each individual batch of vaccine is tested before use for safety and effectiveness. For this reason, the potency test was designed to measure the ability of a vaccine to protect against subsequent challenge from the active component responsible for pathogenicity, and represents a valuable tool for testing the actual relative strength of manufactured batches of vaccine, as well as being essential to the pre-clinical and clinical development of a vaccine. Biological-based manufacturing methods are inherently variable, and potency testing provides a tool to ensure lot-to-lot consistency of commercial vaccines. Once established, the potency test provides the batch release data required by the relevant regulatory authorities to enable the subsequent licensing of a novel vaccine.

TESTING BACTERIAL VACCINES

To evaluate novel bacterial vaccines, such as pneumococcal vaccines, an enzyme-linked immunosorbent assay (ELISA) is commonly used alongside a modified *in vitro* opsonophagocytic-killing assay (OPKA). However, although the ELISA method allows antibody quantification, it cannot distinguish between functional and non-functional antibodies. Thus, the OPKA is useful as an additional test for measuring antibody function and is a good surrogate assay for immune protection (2). Patient blood samples are taken before and after vaccination, serially diluted and tested by OPKA and plated out onto Todd-Hewitt agar plates. An agar overlay containing antibiotics and 2,3,5-triphenyl tetrazolium chloride dye can be added (3), and the resulting red bacterial colonies are counted the next day to determine the vaccine's effectiveness in terms of antibody induced following vaccination (see Figure 1).

Pneumonia is the world's leading cause of death in young children under the age of five; it also poses a serious risk to the elderly. There are more than 90 strains of pneumococcus, which is the major cause of this infection, as well as the cause of meningitis, sepsis and other complications. Thus, new vaccines against this bacterium are required because conventional antibiotics are becoming less effective due to the increasing numbers of multi drug-resistant *Streptococcus pneumoniae*. In a typical clinical trial of a new pneumococcal vaccine, anything up to 11,000 colonies can be generated from every patient. Since colony enumeration provides the data on which vaccine efficacy is based, it is essential to obtain the most precise colony counts possible, so rapid and accurate counting of these colonies is critical to this testing method.

The most commonly used counting process is manual counting, and requires microbiologists to use a light box and pen, and then to key in the results manually into a computer. Colony counting is widely recognised as a tedious, repetitive task that is both time-consuming and exhausting; as a result, samples can take days to process. Furthermore, manual counting can lead to reading and transcription errors, especially when counting such large numbers of *S pneumoniae* colonies, many of which can be less than 1mm in diameter. Additionally, because this method does not produce any digital images of the plate alongside the colony

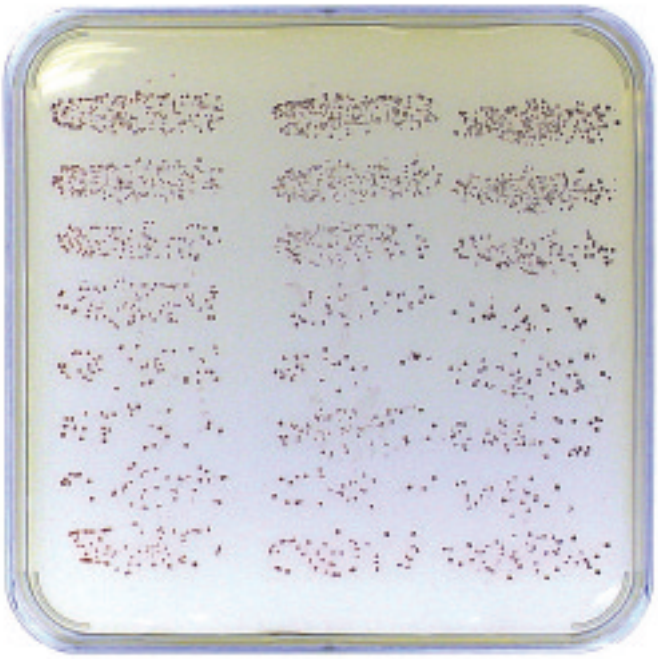


Figure 1: *S pneumoniae* on a Todd-Hewitt agar-yeast extract plate with an overlay containing TTC and antibiotic

Images: Synbiosis

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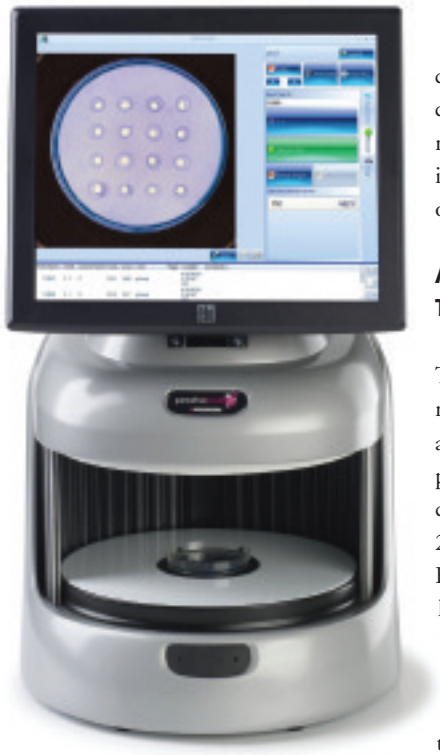

Figure 2: ProtoCOL 2 System for Automated Colony Counting and Inhibition Zone Measurement

count, an independent audit cannot be carried out by regulatory authorities – which is compulsory for the approval of new vaccines.

AUTOMATED COUNTING TECHNOLOGY

Thus, it is not surprising that researchers would benefit from a system that automated the process; an automated colony counter (such as the ProtoCOL 2 system from Synbiosis – see Figure 2) provides a novel, high-throughput technique for such purposes. The system, complete with a high-resolution camera integrated to a computer, uses a unique combination of red, green and blue light (rather than just white light) to image the plates. The images produced are overlaid by the system's software to produce an accurate life-like colour image, making it possible to easily distinguish and count the red colonies on the Todd Hewitt agar. The system can also evaluate colonies on OPKA plates because its software automatically compensates for different agar thickness and artefacts, such as bubbles or debris that can occur during overlaying, and can even enumerate touching colonies and colonies of different sizes. The results produced from OPKA assays are compliant with Good Clinical Practice (GCP) guidelines and so can be presented to regulatory authorities, such as the European Medicines Agency (EMEA) and US Food and Drug Administration (FDA). For example, the colony count results can be automatically transferred via a single button click into programmes for statistical analysis to produce ED50 calculations as well as parallel line, slope ratio, Probit, 4-parameter logistic curve and single dose models.

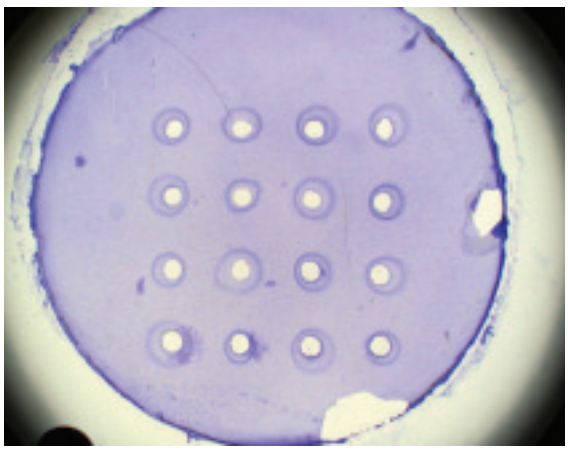

Figure 3: SRD assay plate stained with Coomassie Blue

The software is password protected, thus ensuring data security and integrity. Every detail of the sample – including pictures of the OPKA plates, system configuration, members of staff that read the plate, date and time – are recorded in a professional report. The ProtoCOL 2 user interface has also been designed to include provisions for electronic signatures and different permission levels for secure access to the data. This ensures that it can be integrated into any 21 Code of Federal Regulations (CFR), Part 11 environment, and so can be used in any GMP (Good Manufacturing Practice) compliant facility.

Automated colony counting technology is being successfully used for assessing the results of OPKA assays at a number of prestigious companies and research institutes; one example is the Danish biotech company, ACE BioSciences which is using a ProtoCOL automated colony counter in pre-clinical trials to significantly speed up potency testing of its novel pneumococcal vaccines. This is saving researchers at ACE hours of repetitive counting, as well as improving the accuracy of results by eliminating errors that can occur when having to enumerate large numbers of colonies and manually key-in results.

TESTING VIRAL VACCINES

In vitro potency testing is also widely used for evaluating the potency of some viral vaccines, such as influenza viruses. The influenza vaccines licensed in the EU consist of killed influenza virus and contain three different strains of influenza – A (H1N1), which includes the new pandemic strain known as swine flu, A (H3N2) and B. Large quantities of virus are required to prepare the hundreds of millions of doses of seasonal flu vaccine manufactured every year, selecting the most common circulating flu strains around the world. The method currently recommended by the World Health Organisation (WHO) to the pharmaceutical industry for determining influenza vaccine potency is known as the single radial immunodiffusion (SRD) assay (4,5). This *in vitro* test provides a quantitative method for determining the concentration of antigen present in the test sample.

SRD assays have been used to determine the potency of all human inactivated influenza virus vaccines licensed by the FDA for use in the US since 1978, and similar

SRD assays have been used experimentally to determine the potency of inactivated polio and rabies vaccines. In each case, the assays are based on the diffusion of viral antigen into an agarose gel containing specific antibodies to the antigen being measured. The interaction between antigen and antibody produces a zone of precipitation whose size is directly proportional to the amount of antigen applied (see Figure 3). A potency value for unknowns is obtained by comparing the sizes of zones produced by unknown preparations, to the sizes of zones obtained with a calibrated reference of known antigen content. Once the specific reference antigens and antibodies are prepared and the test standardised, it is an extremely simple technique. Unlike agglutination assays, it is very reproducible and is relatively unaffected by minor variations in test conditions; it is also far less time consuming and cumbersome than *in vivo* assays for potency. More importantly, clinical studies demonstrate that standardisation of influenza vaccines by SRD provides a better correlate of human immunogenicity than previous methods.

One of the world's largest WHO pre-qualified vaccine suppliers, PT Bio Farma (Bandung, Indonesia) is using a ProtoCOL system in the Quality Control Department to automate and speed up the quality testing of its seasonal flu vaccines. Scientists originally tested influenza vaccine potency on SRD plates by measuring zones using the error-prone manual techniques of measuring with a ruler. After consulting scientists at the UK's National Institute of Biological Standards (NIBSC), one of the global leaders in the field of biological standardisation, Bio Farma, purchased a ProtoCOL system in 2008. Using its unique zone measurement technology, Bio Farma is now capable of rapidly measuring inhibition zones on 16-zone SRD plates of flu vaccines – analysing 10 SRD plates in the time it would take manually to perform one analysis, thus producing a more accurate and reproducible indication of vaccine potency.

CONCLUSION

The market for vaccines, such as for pneumococcal and seasonal flu, is very competitive and although there is a large product demand, it lasts for only a short period of time. In 2009, for example, health officials prepared for one of the biggest winter flu vaccination campaigns in almost 50 years, with the UK Government ordering enough vaccine to cover the entire population.

Pharma and biotech companies that can have their vaccines available first have a clear advantage over their competitors. Since it is unrealistic and virtually impossible to test each vaccine lot manually prior to release for general use, the use of automation has become invaluable for testing the actual relative strength of manufactured assembly lots of vaccine. The use of automated technologies can help to achieve this because automated analysis of SRD plates and colony counting means a vaccine's potency can be determined in minutes, rather than hours. This improvement in vaccine testing throughput has demonstrated how automated technology can really benefit pharma and biotech companies looking to make vaccines available more rapidly. By ensuring that companies deliver their vaccines to market sooner, and to healthcare professionals as and when they are needed, automated counting technology means that such products will reach the market more rapidly in the future.

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