Preventing pandemics

Over the years, influenza has taken the lives of millions. While vaccines are helping to reduce the damage caused by the virus, there is still a major capacity shortfall. Could access to US and European expertise and quality training improve capacity and prevent the next pandemic?

By Dr Nathaniel Hentz

When new and deadly strains of influenza appear, their effects can be devastating, since a lack of immunity among the population can lead to pandemic disease. There were three pandemics in the 20th century – in 1918, 1957 and 1968 – which killed millions of people around the world. While flu shots have been available since the 1940s, when a virulent new strain appears there is a real scramble to get sufficient doses of the vaccine ready to protect the population.

A significant problem derives from the fact that the vaccine required to give protection changes every year. Unlike the situation with viruses such as measles, the antigen required to give protection varies depending on the predominant circulating strains of the influenza virus in any given year, as it constantly mutates to form new strains. Vaccination remains the only realistic weapon against a flu pandemic. The antiviral drugs oseltamivir (Tamiflu) and zanamivir (Relenza) that were stockpiled by governments in response are not particularly effective[1], whereas vaccination can be efficacious two-thirds of the time, according to a large meta-analysis published in Lancet Infectious Diseases in 2012[2]. While it is clearly less than perfect – and the protection figures are even worse for the elderly – vaccination is still the best weapon we have against influenza.

H1N1 pandemic underlines capacity challenge

The H1N1 'swine flu' strain was first identified in April 2009, and by June its geographic spread was so vast that the World Health Organization (WHO) declared it to be the first pandemic of the 21st century. By this time, the seasonal flu vaccine for the year was already in production. (The annual make-up of the vaccination is determined by WHO's Influenza Surveillance Network, which monitors the circulating influenza strains. It usually contains three antigens, two of which are influenza A and one influenza B, and while it may be the same from one year to the next, more commonly one or two antigens are changed.) An add-on vaccine was hastily prepared to provide protection against the new strain – but the twin problems of time and capacity meant there were never going to be sufficient doses in time for everyone who wanted one, despite the pandemic preparedness activities that were already in place at the vaccine manufacturers (**Figure 1**).

Influenza vaccines have historically been egg-based, with the antigen for each dose requiring one egg to grow, and the whole process taking several months. Capacity is finite, and when the pandemic was announced most of this capacity was already committed to producing the normal seasonal vaccine[3]. Cell culture is now starting to be used to produce influenza vaccines, with the first cell culture-derived vaccine, from Novartis, having been approved by the FDA in November. This will significantly reduce the time it takes to make the vaccine and increase capacity. Cells can be banked and stored for long periods of time, ultimately allowing manufacturing capacity to surpass that for egg-based influenza vaccines. Furthermore, viruses do not have to be adapted as they currently are for egg-based vaccine product. Finally, vaccines produced in cells do not have the same immunogenicity profile as with egg-based, potentially allowing a wider patient population to be immunised.

If sufficient vaccine is ever going to be available for the entire global population in the case of an influenza pandemic, then the global manufacture of influenza vaccine will also have to increase. In the US alone, more than 130 million doses were

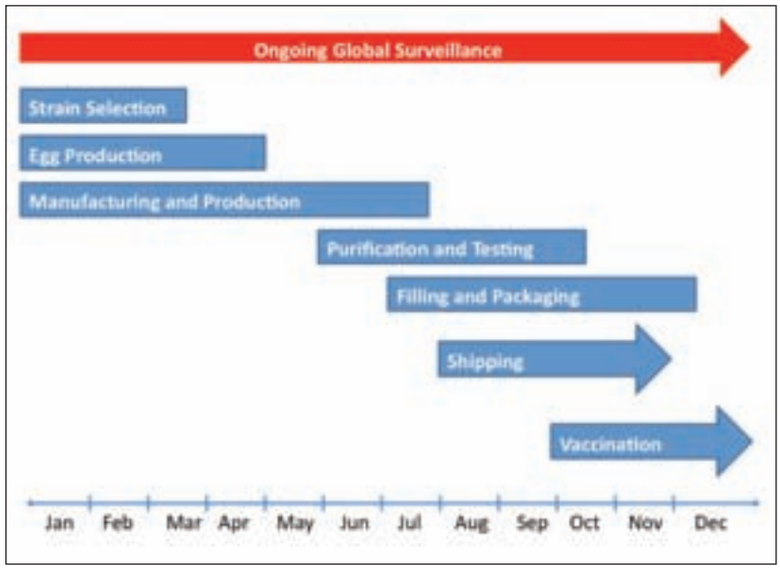

Figure 1
Annual influenza production timeline

distributed for the 2011-2012 influenza season. Globally, manufacturers produced 620 million doses of seasonal trivalent influenza vaccine in 2011[4]. At the time of the 2009 H1N1 pandemic, the best estimate was that maybe one billion doses of a pandemic vaccine could be made. More could be made if adjuvants that enable smaller amounts of antigen to be used in each dose were more widely accepted. With a global population now almost seven times the maximum non-adjuvanted capacity, manufacturing capacity was clearly insufficient.

Quality challenges put capacity at risk

While there may be sufficient capacity in a normal, non-pandemic year to meet demand for seasonal vaccine doses, if one of the manufacturers has a production problem, this would adversely affect availability. This was brought into sharp focus in October, when several countries suspended the distribution of Novartis's seasonal flu vaccine after protein aggregates of predominantly haemagglutinin protein were found in doses. Rapid evaluation showed that the doses were effective and the doses were released to patients. Dutch vaccine manufacturer Crucell – now part of Johnson & Johnson – has also been hit by manufacturing issues, withdrawing its Inflexal V vaccine after two of 32 batches failed quality control tests.

Issues like this highlight the potential problems that can result from a quality problem at a manufacturing site, and thus the importance of ensuring product quality throughout the manufacturing process. The majority of flu vaccines are currently manufactured in the US and Europe, where quality systems are embedded into the manufacturing culture – and yet quality problems still occur. There is a real drive to expand the global reach of influenza vaccine manufacture as this will increase both manufacturing capacity and responsiveness in the case of a pandemic. But what impact might this have on quality? And what is being done to ensure that quality standards are maintained, and that quality control processes ensure that any sub-standard batches are not released to patients?

What is being done about vaccine capacity?

To address these capacity issues, WHO instituted a global action plan for influenza vaccines in 2006. One of its main objectives is to increase production capacity for pandemic vaccines without altering the capacity for normal seasonal vaccines. The aim is that, by 2015, enough vaccine for two billion people should be available six months after the vaccine prototype strain is transferred to manufacturers. In the longer term, the hope is that sufficient vaccine for the entire world's population could be prepared. This is to be done by expanding vaccine capacity, particularly in those regions where capacity is lacking or absent, and the development of high yield technologies to enable surge capacity in the event of a pandemic.

The project has already had some success – since 2006, global seasonal influenza vaccine capacity had increased from less than half a billion doses to nearly a billion doses by 2010. In addition, more than a dozen developing countries have been awarded grants to establish their own influenza vaccine capacity, including Brazil, China, Egypt, India, Indonesia, Romania, Russia, South Korea and Thailand. Several of these now have their own vaccines on the market, with the rest in the late stages of development. WHO has also set up technology transfer hubs in the Netherlands for the production of inactivated influenza vaccine in eggs, and Switzerland for adjuvant production.

With much of the capacity and technology in the US and Europe, pharmaceutical manufacturers, academic groups and health authorities in those regions are working with WHO to help achieve increased production. For example, the US Department of Health and Human Services' Biomedical Advanced Research and Development Authority, or BARDA, has awarded several multi-million dollar grants to WHO to assist developing countries to foster pandemic influenza vaccine manufacturing infrastructure, train staff about influenza vaccine manufacturing and establish and

distribute technologies that will be useful in the event a pandemic vaccine is required.

As part of the BARDA-WHO partnership, North Carolina State University's Biomanufacturing Training and Education Center (BTEC) was awarded a significant grant to assist in the training of personnel from vaccine manufacturing or research organisations in WHO member countries. The goal is to provide key staff from these institutions with hands-on instruction in the latest FDA good manufacturing practices in vaccine production technology, so that these can be implemented back home to improve quality systems and quality control within the facility.

The unique role of BTEC

The BTEC training programme started in 2010, where three cohorts of 12 students each were trained in the first year. At first, managers from some of these vaccine-producing institutions attended a three-week long course on the fundamentals of vaccine manufacture. The course provides an A-Z on how to work with eggs and cells, right through the whole processing of the influenza vaccine from production to purification, analysis and the final aseptic fill. The idea was that they would be able to provide their own people with some training in vaccine manufacture. While this will certainly be helpful, it was clear from the outset that it would not be sufficient, particularly as some of these institutions were more based in research than manufacture, and thus did not have the background in working under GMP conditions.

Now, more advanced courses are being run, and instead of managers the attendees are people who are actively involved in the production of vaccines. In addition to the first course on the fundamentals of cGMP influenza vaccine manufacturing, two further courses are now being run on advanced processes for influenza vaccine manufacturing, one looking at upstream aspects and the second downstream. The objective is to get detailed knowledge of best practices from the US into the hands of operators in places without a history of vaccine manufacture. If the number of countries where good quality vaccines are made is increased, then WHO's goal of raising the global capacity and spread of influenza vaccine manufacture is more likely to become a reality.

In addition to training scientists from around the world the process of vaccine manufacturing under GMP conditions, BTEC also focuses on QC fundamentals such as basic statistics, data analysis, troubleshooting, instrument calibrations and even pipetting, while using state-of-the-art instrumentation. The trainees can alter their pipetting technique and measure the effect on accuracy and precision. The underlying importance of this programme is that emphasis on quality permeates through all levels of scientists within vaccine manufacturing process. Training normally occurs in the specific area that the scientist works. For example, QC training is typically reserved for QC scientists, but through this unique training programme at BTEC, scientists that normally work upstream (virus production) or downstream (virus processing and purification) have an opportunity to learn the QC side. By using this approach the importance of quality is instilled into all manufacturing areas, with the assumption that the quality of the product will ultimately improve.

Quality control in vaccine manufacture

One of the key issues that the BTEC courses cover is quality control. Fundamentally, QC ensures that a product is what it says it is, and meets all quality and purity standards. In terms of influenza vaccine manufacture, while the assays that are currently required by the regulators are fairly unsophisticated, they are labour-intensive and require care.

SRID Assay

Potency is measured using the single radial immunodiffusion, or SRID, assay[5-8]. The antigen is introduced on to the centre of a gel containing an antibody to that antigen. The antigen diffuses through the gel, creating a precipitin ring that continues to grow until it reaches equilibrium, a process that can take hours to overnight. The

May 2012 – Trainees learn SDS-PAGE for assessing influenza vaccine purity and identity in the QC/analytical lab of the BARDA-sponsored Fundamentals of cGMP Influenza Vaccine Manufacturing course at BTEC

May 10, 2011 – Trainees participate in a hands-on activity in the intermediate scale upstream processing lab during the BARDA-sponsored Fundamentals of cGMP Influenza Vaccine Manufacturing course at BTEC

diameter of the precipitin rings are proportional to the concentration of hemagglutinin (HA) on the surface of the antigen presenting vaccine product. To estimate potency, the samples rings are compared to those created by reference standards (provided by approved agencies such as CBER and NIBSC) and whether this meets required specifications. Although ring diameter measurement is automated through imaging software, the whole testing process is manual, and there is little opportunity for automation. The gels are prepared as needed at the time of the analysis. Furthermore, the gels are prepared with antibodies to the particular influenza strains announced each year. Finally, this assay requires an overnight incubation, followed by staining, destaining and drying steps. While it does give an indication of the potency of the antigen, the variability of the assay is quite high and could use improvement. However, because SRID measures the effectiveness of a vaccine by examining the interaction between the antigen (influenza) and its corresponding antibody, it is representative of what is actually going on in the body. While it does not give any information about whether an immune response can be elicited, it does show whether or not the antigen can be recognised by an antibody.

Titre determination

A second test for potency or titre determinations is the haemagglutination assay, an influenza-specific protein quantification assay[9]. HA is the primary antigen protein present on the surface of influenza viruses that causes red blood cells to clump together, or agglutinate. The influenza sample is incubated with a dilute solution containing any one of several species of red blood cells (eg, chicken, turkey, horse, guinea pig, rabbit or sheep), and the virus dilution at which agglutination starts is identified. Agglutination is the point where the solution is visually cloudy or hazy. However, this has to be determined by eye, and most often the agglutination point is not straightforward. There are intermediate phases between not- and fully-agglutinated. For example, a donut-shaped ring will form during partial agglutination, where the ring diameter can provide an estimate of potency by comparing ring sizes. In this case, the dilutions near the point of agglutination are typically further diluted to allow a better estimate of potency. Since the test is typically conducted visually, the accuracy is extremely dependent on the skill of the operator.

While these assays are variable and not particularly sophisticated, they are accepted by the regulators to demonstrate vaccine potency, despite a widespread recognition of their limitations. Alternative tests are being developed that will be more amenable to automation and thus have less likelihood of variability, as well as being faster to run because of the potential for high throughput techniques being applied to them.

SDS-PAGE

A third test relies on separation of proteins in a gel matrix to provide identity and purity characteristics[10]. In this case, SDS-PAGE (sodium dodecyl sulphate – polyacrylamide gel electrophoresis) is used to separate proteins based on molecular weight. By comparing the sample banding pattern to a reference standard, the identity and purity can be confirmed. For further confirmation, Western blotting is used where the proteins are transferred to a membrane, which is then immunostained, allowing another level of specificity. Although SDS-PAGE and Western blotting are well-established techniques, they are largely manual and semi-quantitative as best, leading to high variability.

Potential for automation and minimisation

BTEC is addressing these deficiencies by working on ways to automate the primary vaccine analyses. First, HPLC is being investigated for hemagglutinin content or potency[11-12]. In this technique, HA and its subunits are separated and quantitated with a high degree of precision and accuracy. Furthermore, HPLC is already automated from sample injection through data analysis, thus addressing the manual liabilities realised by SRID and the hemagglutination assay. The second technique is an intermediate solution – still carrying out the current style of haemagglutination assay but using a plate-based technology to supplant visual reading and interpretation. Instead, a spectrophotometer could be used to look at turbidity at a particular wavelength. Potency would be estimated by comparison to fully agglutinated and non-agglutinated samples. Removing the human subjectivity and replacing it with a data-driven system would minimise the potential for variability and improve accuracy.

Finally, microfluidic platforms are being investigated as an orthogonal approach to SDS-PAGE. Specifically, microfluidics platforms such as the Agilent 2100 Bioanalyzer or the BioRad Experion automated electrophoresis systems are well-suited for QC operations in that the sample separation, detection and data analysis are all conducted in a lab-on-a-chip environment which is both automated and enclosed. Furthermore, the data are captured as electropherograms, allowing peak migration times to be more accurately determined and the peaks areas are integrated, allowing improved quantitation in terms of accuracy and precision.

The ability to automate QC tests like these would not only speed up testing and improve accuracy and precision by reducing manual manipulation and interpretation, it would also ensure sample traceability. If an operator misplaces a sample in a particular well because they are working with hundreds or even thousands of samples, it can negatively impact the integrity of the QC process. By automating QC procedures sample mislabelling or misplacement in a rack are greatly reduced. Automation allows pipette tips to be tracked, for example, even for more manual-style assays such as haemagglutination. More modern assays based on HPLC or some form of plate reader will also have an indexing capability, with data available for download and computer analysis. The data are more robust, particularly in terms of sample identification, with every sample tracked and identified correctly throughout.

Automated liquid handling systems are another way in which indexing can be ensured. Where each sample is coming from is certain, as is where it is going. Samples of batches coming off the manufacturing floor have to be fully traceable, with multiple samples of the same batch destined for different assays. Critical decisions are made on the results of these assays, and if a sample does not meet the required standards the batch does not move forward to the next stage of the manufacturing process, and will likely have to be thrown away. Data quality is of the utmost importance. Clearly, patient safety is the overriding driver behind the whole QC process, so any substandard batches must be eliminated. However, false positives must also be avoided for economic reasons – bad data that incorrectly indicates that a batch does not meet the required standards could cost millions of dollars.

Although automated liquid handlers greatly increase the precision of assays, inaccuracy in liquid transfer processes can also be a major source of assay error[13]. While the trade-off between quality and productivity will never truly be resolved, advances in liquid handling quality assurance are easing that struggle. The Artel MVS® Multichannel Verification System is the only technology able to verify the accuracy and precision of each tip of an automated liquid handler in one rapid experiment. This allows laboratories to more frequently verify the performance of their instrumentation so that liquid handling errors can be identified and removed before they impact upon assay results. The MVS is based on a technique known as ratiometric photometry, which produces measurements that are traceable to international standards, allowing comparability of all liquid handling devices regardless of model, location or number of dispensing channels.

References

1 Jefferson, T et al. BMJ 2009, 339, b5106.

2 Osterholm, MT et al. Lancet Infect. Dis. 2012, 12, 36.

3 Hickling, J and D'Hondt, E. A review of production technologies for influenza virus vaccines, and their suitability for deployment in developing countries for influenza pandemic preparedness. World Health Organization Initiative for Vaccine Research, Geneva Switzerland, Dec. 20, 2006, 1-34.

4 Partridge, J and Kieny, MP. Global production capacity of seasonal influenza vaccine in 2011. Vaccine, 2012, article in press http://dx.doi.org/10.1016/j.vaccine.2012.10.111.

5 Potency Determination of Inactivated Influenza Virus Vaccines by the Single Radial Immunodiffusion (SRID) Method. U.S. Food and Drug Administration/CBER Document ID: 000333 7/21/2011.

6 Wood, JM et al. Journal of Biological Standardization, 5 (1977) 237-247.

7 Williams, MS et al. Journal of Biological Standardization, 8 (1980) 289-296.

8 Vaccines for Human Use – General Considerations. US Pharmacopeia 1235, 2011.

9 Killian, ML (2010). Chapter 7: Hemagglutination Assay for the Avian Influenza Virus. In E. Spackman, Methods in Molecular Biology, Vol. 436: Avian Influenza Virus (pp. 47-52). Totowa, NJ: Humana Press.

10 Shaw, ML et al. PLoS Pathog, 4(6), 2008, e1000085.

11 Kapteyn, JC et al. Vaccine 27, 2009, 1468–1477.

12 Lorbetskie, B et al. Vaccine 29, 2011, 3377–3389.

13 Hentz, N. SLAS tutorial 2012, 02,06.

June 22, 2011 – Trainee uses Artel PCS system to practise pipetting technique as part of the QC/analytical lab during the BARDA-sponsored Fundamentals of cGMP Influenza Vaccine Manufacturing course at BTEC

Ultimately, our best chance of preventing an influenza pandemic in future will involve the development of more effective vaccines as well as speedy manufacture, increase in number of doses and preventing spread at the origin. Universal vaccines are being investigated as these could give much broader protection against different influenza strains, and remove the need for annual vaccinations. However, these are still some way in the future, and until then the need for increased global capacity for influenza vaccine manufacture remains. Training operators from new facilities in modern quality control methods and current analytical techniques, and adoption of robust quality systems, will be vital if the goal of being able to manufacture sufficient vaccine for the world's entire population is to be achieved. DDW

Dr Nathaniel Hentz *is the Assistant Director of the BTEC Analytical Lab at North Carolina State University. Prior to this current role, Dr Hentz served as an independent consultant working with Artel offering guidance on their efforts toward automated liquid handling quality control within high throughput screening laboratories. Dr Hentz's tenure in the HTS industry includes nearly two years as Senior Research Investigator at Bristol-Myers Squibb in Wallingford, CT where his team supported the fully-automated screening systems within the Lead Discovery group. Prior to BMS, Dr Hentz enjoyed seven years at Eli Lilly RTP Laboratories in North Carolina. During his tenure at Lilly RTP, Dr Hentz was responsible for Tier 1 ADMET screening in 2004 and led the New Technologies group. He received his PhD in analytical chemistry from the University of Kentucky in 1996 and joined Lilly as a postdoctoral scientist the same year.*

Preventing pandemics

Over the years, influenza has taken the lives of millions. While vaccines are helping to reduce the damage caused by the virus, there is still a major capacity shortfall. Could access to US and European expertise and quality training improve capacity and prevent the next pandemic?

By Dr Nathaniel Hentz

When new and deadly strains of influenza appear, their effects can be devastating, since a lack of immunity among the population can lead to pandemic disease. There were three pandemics in the 20th century – in 1918, 1957 and 1968 – which killed millions of people around the world. While flu shots have been available since the 1940s, when a virulent new strain appears there is a real scramble to get sufficient doses of the vaccine ready to protect the population.

A significant problem derives from the fact that the vaccine required to give protection changes every year. Unlike the situation with viruses such as measles, the antigen required to give protection varies depending on the predominant circulating strains of the influenza virus in any given year, as it constantly mutates to form new strains. Vaccination remains the only realistic weapon against a flu pandemic. The antiviral drugs oseltamivir (Tamiflu) and zanamivir (Relenza) that were stockpiled by governments in response are not particularly effective[1], whereas vaccination can be efficacious two-thirds of the time, according to a large meta-analysis published in Lancet Infectious Diseases in 2012[2]. While it is clearly less than perfect – and the protection figures are even worse for the elderly – vaccination is still the best weapon we have against influenza.

H1N1 pandemic underlines capacity challenge

The H1N1 'swine flu' strain was first identified in April 2009, and by June its geographic spread was so vast that the World Health Organization (WHO) declared it to be the first pandemic of the 21st century. By this time, the seasonal flu vaccine for the year was already in production. (The annual make-up of the vaccination is determined by WHO's Influenza Surveillance Network, which monitors the circulating influenza strains. It usually contains three antigens, two of which are influenza A and one influenza B, and while it may be the same from one year to the next, more commonly one or two antigens are changed.) An add-on vaccine was hastily prepared to provide protection against the new strain – but the twin problems of time and capacity meant there were never going to be sufficient doses in time for everyone who wanted one, despite the pandemic preparedness activities that were already in place at the vaccine manufacturers (**Figure 1**).

Influenza vaccines have historically been egg-based, with the antigen for each dose requiring one egg to grow, and the whole process taking several months. Capacity is finite, and when the pandemic was announced most of this capacity was already committed to producing the normal seasonal vaccine[3]. Cell culture is now starting to be used to produce influenza vaccines, with the first cell culture-derived vaccine, from Novartis, having been approved by the FDA in November. This will significantly reduce the time it takes to make the vaccine and increase capacity. Cells can be banked and stored for long periods of time, ultimately allowing manufacturing capacity to surpass that for egg-based influenza vaccines. Furthermore, viruses do not have to be adapted as they currently are for egg-based vaccine product. Finally, vaccines produced in cells do not have the same immunogenicity profile as with egg-based, potentially allowing a wider patient population to be immunised.

If sufficient vaccine is ever going to be available for the entire global population in the case of an influenza pandemic, then the global manufacture of influenza vaccine will also have to increase. In the US alone, more than 130 million doses were

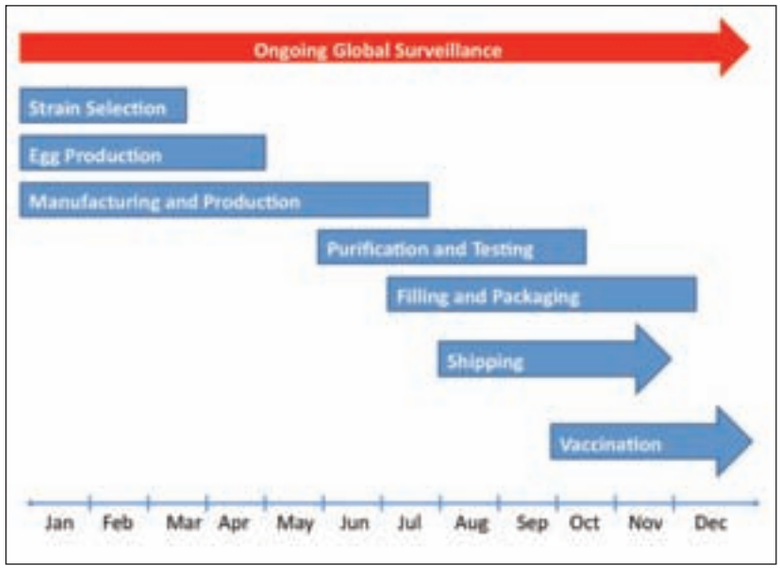

Figure 1
Annual influenza production timeline

distributed for the 2011-2012 influenza season. Globally, manufacturers produced 620 million doses of seasonal trivalent influenza vaccine in 2011[4]. At the time of the 2009 H1N1 pandemic, the best estimate was that maybe one billion doses of a pandemic vaccine could be made. More could be made if adjuvants that enable smaller amounts of antigen to be used in each dose were more widely accepted. With a global population now almost seven times the maximum non-adjuvanted capacity, manufacturing capacity was clearly insufficient.

Quality challenges put capacity at risk

While there may be sufficient capacity in a normal, non-pandemic year to meet demand for seasonal vaccine doses, if one of the manufacturers has a production problem, this would adversely affect availability. This was brought into sharp focus in October, when several countries suspended the distribution of Novartis's seasonal flu vaccine after protein aggregates of predominantly haemagglutinin protein were found in doses. Rapid evaluation showed that the doses were effective and the doses were released to patients. Dutch vaccine manufacturer Crucell – now part of Johnson & Johnson – has also been hit by manufacturing issues, withdrawing its Inflexal V vaccine after two of 32 batches failed quality control tests.

Issues like this highlight the potential problems that can result from a quality problem at a manufacturing site, and thus the importance of ensuring product quality throughout the manufacturing process. The majority of flu vaccines are currently manufactured in the US and Europe, where quality systems are embedded into the manufacturing culture – and yet quality problems still occur. There is a real drive to expand the global reach of influenza vaccine manufacture as this will increase both manufacturing capacity and responsiveness in the case of a pandemic. But what impact might this have on quality? And what is being done to ensure that quality standards are maintained, and that quality control processes ensure that any sub-standard batches are not released to patients?

What is being done about vaccine capacity?

To address these capacity issues, WHO instituted a global action plan for influenza vaccines in 2006. One of its main objectives is to increase production capacity for pandemic vaccines without altering the capacity for normal seasonal vaccines. The aim is that, by 2015, enough vaccine for two billion people should be available six months after the vaccine prototype strain is transferred to manufacturers. In the longer term, the hope is that sufficient vaccine for the entire world's population could be prepared. This is to be done by expanding vaccine capacity, particularly in those regions where capacity is lacking or absent, and the development of high yield technologies to enable surge capacity in the event of a pandemic.

The project has already had some success – since 2006, global seasonal influenza vaccine capacity had increased from less than half a billion doses to nearly a billion doses by 2010. In addition, more than a dozen developing countries have been awarded grants to establish their own influenza vaccine capacity, including Brazil, China, Egypt, India, Indonesia, Romania, Russia, South Korea and Thailand. Several of these now have their own vaccines on the market, with the rest in the late stages of development. WHO has also set up technology transfer hubs in the Netherlands for the production of inactivated influenza vaccine in eggs, and Switzerland for adjuvant production.

With much of the capacity and technology in the US and Europe, pharmaceutical manufacturers, academic groups and health authorities in those regions are working with WHO to help achieve increased production. For example, the US Department of Health and Human Services' Biomedical Advanced Research and Development Authority, or BARDA, has awarded several multi-million dollar grants to WHO to assist developing countries to foster pandemic influenza vaccine manufacturing infrastructure, train staff about influenza vaccine manufacturing and establish and

distribute technologies that will be useful in the event a pandemic vaccine is required.

As part of the BARDA-WHO partnership, North Carolina State University's Biomanufacturing Training and Education Center (BTEC) was awarded a significant grant to assist in the training of personnel from vaccine manufacturing or research organisations in WHO member countries. The goal is to provide key staff from these institutions with hands-on instruction in the latest FDA good manufacturing practices in vaccine production technology, so that these can be implemented back home to improve quality systems and quality control within the facility.

The unique role of BTEC

The BTEC training programme started in 2010, where three cohorts of 12 students each were trained in the first year. At first, managers from some of these vaccine-producing institutions attended a three-week long course on the fundamentals of vaccine manufacture. The course provides an A-Z on how to work with eggs and cells, right through the whole processing of the influenza vaccine from production to purification, analysis and the final aseptic fill. The idea was that they would be able to provide their own people with some training in vaccine manufacture. While this will certainly be helpful, it was clear from the outset that it would not be sufficient, particularly as some of these institutions were more based in research than manufacture, and thus did not have the background in working under GMP conditions.

Now, more advanced courses are being run, and instead of managers the attendees are people who are actively involved in the production of vaccines. In addition to the first course on the fundamentals of cGMP influenza vaccine manufacturing, two further courses are now being run on advanced processes for influenza vaccine manufacturing, one looking at upstream aspects and the second downstream. The objective is to get detailed knowledge of best practices from the US into the hands of operators in places without a history of vaccine manufacture. If the number of countries where good quality vaccines are made is increased, then WHO's goal of raising the global capacity and spread of influenza vaccine manufacture is more likely to become a reality.

In addition to training scientists from around the world the process of vaccine manufacturing under GMP conditions, BTEC also focuses on QC fundamentals such as basic statistics, data analysis, troubleshooting, instrument calibrations and even pipetting, while using state-of-the-art instrumentation. The trainees can alter their pipetting technique and measure the effect on accuracy and precision. The underlying importance of this programme is that emphasis on quality permeates through all levels of scientists within vaccine manufacturing process. Training normally occurs in the specific area that the scientist works. For example, QC training is typically reserved for QC scientists, but through this unique training programme at BTEC, scientists that normally work upstream (virus production) or downstream (virus processing and purification) have an opportunity to learn the QC side. By using this approach the importance of quality is instilled into all manufacturing areas, with the assumption that the quality of the product will ultimately improve.

Quality control in vaccine manufacture

One of the key issues that the BTEC courses cover is quality control. Fundamentally, QC ensures that a product is what it says it is, and meets all quality and purity standards. In terms of influenza vaccine manufacture, while the assays that are currently required by the regulators are fairly unsophisticated, they are labour-intensive and require care.

SRID Assay

Potency is measured using the single radial immunodiffusion, or SRID, assay[5-8]. The antigen is introduced on to the centre of a gel containing an antibody to that antigen. The antigen diffuses through the gel, creating a precipitin ring that continues to grow until it reaches equilibrium, a process that can take hours to overnight. The

May 2012 – Trainees learn SDS-PAGE for assessing influenza vaccine purity and identity in the QC/analytical lab of the BARDA-sponsored Fundamentals of cGMP Influenza Vaccine Manufacturing course at BTEC

May 10, 2011 – Trainees participate in a hands-on activity in the intermediate scale upstream processing lab during the BARDA-sponsored Fundamentals of cGMP Influenza Vaccine Manufacturing course at BTEC

diameter of the precipitin rings are proportional to the concentration of hemagglutinin (HA) on the surface of the antigen presenting vaccine product. To estimate potency, the samples rings are compared to those created by reference standards (provided by approved agencies such as CBER and NIBSC) and whether this meets required specifications. Although ring diameter measurement is automated through imaging software, the whole testing process is manual, and there is little opportunity for automation. The gels are prepared as needed at the time of the analysis. Furthermore, the gels are prepared with antibodies to the particular influenza strains announced each year. Finally, this assay requires an overnight incubation, followed by staining, destaining and drying steps. While it does give an indication of the potency of the antigen, the variability of the assay is quite high and could use improvement. However, because SRID measures the effectiveness of a vaccine by examining the interaction between the antigen (influenza) and its corresponding antibody, it is representative of what is actually going on in the body. While it does not give any information about whether an immune response can be elicited, it does show whether or not the antigen can be recognised by an antibody.

Titre determination

A second test for potency or titre determinations is the haemagglutination assay, an influenza-specific protein quantification assay[9]. HA is the primary antigen protein present on the surface of influenza viruses that causes red blood cells to clump together, or agglutinate. The influenza sample is incubated with a dilute solution containing any one of several species of red blood cells (eg, chicken, turkey, horse, guinea pig, rabbit or sheep), and the virus dilution at which agglutination starts is identified. Agglutination is the point where the solution is visually cloudy or hazy. However, this has to be determined by eye, and most often the agglutination point is not straightforward. There are intermediate phases between not- and fully-agglutinated. For example, a donut-shaped ring will form during partial agglutination, where the ring diameter can provide an estimate of potency by comparing ring sizes. In this case, the dilutions near the point of agglutination are typically further diluted to allow a better estimate of potency. Since the test is typically conducted visually, the accuracy is extremely dependent on the skill of the operator.

While these assays are variable and not particularly sophisticated, they are accepted by the regulators to demonstrate vaccine potency, despite a widespread recognition of their limitations. Alternative tests are being developed that will be more amenable to automation and thus have less likelihood of variability, as well as being faster to run because of the potential for high throughput techniques being applied to them.

SDS-PAGE

A third test relies on separation of proteins in a gel matrix to provide identity and purity characteristics[10]. In this case, SDS-PAGE (sodium dodecyl sulphate – polyacrylamide gel electrophoresis) is used to separate proteins based on molecular weight. By comparing the sample banding pattern to a reference standard, the identity and purity can be confirmed. For further confirmation, Western blotting is used where the proteins are transferred to a membrane, which is then immunostained, allowing another level of specificity. Although SDS-PAGE and Western blotting are well-established techniques, they are largely manual and semi-quantitative as best, leading to high variability.

Potential for automation and minimisation

BTEC is addressing these deficiencies by working on ways to automate the primary vaccine analyses. First, HPLC is being investigated for hemagglutinin content or potency[11-12]. In this technique, HA and its subunits are separated and quantitated with a high degree of precision and accuracy. Furthermore, HPLC is already automated from sample injection through data analysis, thus addressing the manual liabilities realised by SRID and the hemagglutination assay. The second technique is an intermediate solution – still carrying out the current style of haemagglutination assay but using a plate-based technology to supplant visual reading and interpretation. Instead, a spectrophotometer could be used to look at turbidity at a particular wavelength. Potency would be estimated by comparison to fully agglutinated and non-agglutinated samples. Removing the human subjectivity and replacing it with a data-driven system would minimise the potential for variability and improve accuracy.

Finally, microfluidic platforms are being investigated as an orthogonal approach to SDS-PAGE. Specifically, microfluidics platforms such as the Agilent 2100 Bioanalyzer or the BioRad Experion automated electrophoresis systems are well-suited for QC operations in that the sample separation, detection and data analysis are all conducted in a lab-on-a-chip environment which is both automated and enclosed. Furthermore, the data are captured as electropherograms, allowing peak migration times to be more accurately determined and the peaks areas are integrated, allowing improved quantitation in terms of accuracy and precision.

The ability to automate QC tests like these would not only speed up testing and improve accuracy and precision by reducing manual manipulation and interpretation, it would also ensure sample traceability. If an operator misplaces a sample in a particular well because they are working with hundreds or even thousands of samples, it can negatively impact the integrity of the QC process. By automating QC procedures sample mislabelling or misplacement in a rack are greatly reduced. Automation allows pipette tips to be tracked, for example, even for more manual-style assays such as haemagglutination. More modern assays based on HPLC or some form of plate reader will also have an indexing capability, with data available for download and computer analysis. The data are more robust, particularly in terms of sample identification, with every sample tracked and identified correctly throughout.

Automated liquid handling systems are another way in which indexing can be ensured. Where each sample is coming from is certain, as is where it is going. Samples of batches coming off the manufacturing floor have to be fully traceable, with multiple samples of the same batch destined for different assays. Critical decisions are made on the results of these assays, and if a sample does not meet the required standards the batch does not move forward to the next stage of the manufacturing process, and will likely have to be thrown away. Data quality is of the utmost importance. Clearly, patient safety is the overriding driver behind the whole QC process, so any substandard batches must be eliminated. However, false positives must also be avoided for economic reasons – bad data that incorrectly indicates that a batch does not meet the required standards could cost millions of dollars.

Although automated liquid handlers greatly increase the precision of assays, inaccuracy in liquid transfer processes can also be a major source of assay error[13]. While the trade-off between quality and productivity will never truly be resolved, advances in liquid handling quality assurance are easing that struggle. The Artel MVS® Multichannel Verification System is the only technology able to verify the accuracy and precision of each tip of an automated liquid handler in one rapid experiment. This allows laboratories to more frequently verify the performance of their instrumentation so that liquid handling errors can be identified and removed before they impact upon assay results. The MVS is based on a technique known as ratiometric photometry, which produces measurements that are traceable to international standards, allowing comparability of all liquid handling devices regardless of model, location or number of dispensing channels.

References

1 Jefferson, T et al. BMJ 2009, 339, b5106.

2 Osterholm, MT et al. Lancet Infect. Dis. 2012, 12, 36.

3 Hickling, J and D'Hondt, E. A review of production technologies for influenza virus vaccines, and their suitability for deployment in developing countries for influenza pandemic preparedness. World Health Organization Initiative for Vaccine Research, Geneva Switzerland, Dec. 20, 2006, 1-34.

4 Partridge, J and Kieny, MP. Global production capacity of seasonal influenza vaccine in 2011. Vaccine, 2012, article in press http://dx.doi.org/10.1016/j.vaccine.2012.10.111.

5 Potency Determination of Inactivated Influenza Virus Vaccines by the Single Radial Immunodiffusion (SRID) Method. U.S. Food and Drug Administration/CBER Document ID: 000333 7/21/2011.

6 Wood, JM et al. Journal of Biological Standardization, 5 (1977) 237-247.

7 Williams, MS et al. Journal of Biological Standardization, 8 (1980) 289-296.

8 Vaccines for Human Use – General Considerations. US Pharmacopeia 1235, 2011.

9 Killian, ML (2010). Chapter 7: Hemagglutination Assay for the Avian Influenza Virus. In E. Spackman, Methods in Molecular Biology, Vol. 436: Avian Influenza Virus (pp. 47-52). Totowa, NJ: Humana Press.

10 Shaw, ML et al. PLoS Pathog, 4(6), 2008, e1000085.

11 Kapteyn, JC et al. Vaccine 27, 2009, 1468–1477.

12 Lorbetskie, B et al. Vaccine 29, 2011, 3377–3389.

13 Hentz, N. SLAS tutorial 2012, 02,06.

June 22, 2011 – Trainee uses Artel PCS system to practise pipetting technique as part of the QC/analytical lab during the BARDA-sponsored Fundamentals of cGMP Influenza Vaccine Manufacturing course at BTEC

Ultimately, our best chance of preventing an influenza pandemic in future will involve the development of more effective vaccines as well as speedy manufacture, increase in number of doses and preventing spread at the origin. Universal vaccines are being investigated as these could give much broader protection against different influenza strains, and remove the need for annual vaccinations. However, these are still some way in the future, and until then the need for increased global capacity for influenza vaccine manufacture remains. Training operators from new facilities in modern quality control methods and current analytical techniques, and adoption of robust quality systems, will be vital if the goal of being able to manufacture sufficient vaccine for the world's entire population is to be achieved. DDW

Dr Nathaniel Hentz *is the Assistant Director of the BTEC Analytical Lab at North Carolina State University. Prior to this current role, Dr Hentz served as an independent consultant working with Artel offering guidance on their efforts toward automated liquid handling quality control within high throughput screening laboratories. Dr Hentz's tenure in the HTS industry includes nearly two years as Senior Research Investigator at Bristol-Myers Squibb in Wallingford, CT where his team supported the fully-automated screening systems within the Lead Discovery group. Prior to BMS, Dr Hentz enjoyed seven years at Eli Lilly RTP Laboratories in North Carolina. During his tenure at Lilly RTP, Dr Hentz was responsible for Tier 1 ADMET screening in 2004 and led the New Technologies group. He received his PhD in analytical chemistry from the University of Kentucky in 1996 and joined Lilly as a postdoctoral scientist the same year.*