WHO Report

Strengthening the influenza vaccine virus selection and development process
Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1–3 April 2014

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ABSTRACT

Despite long-recognized challenges and constraints associated with their updating and manufacture, influenza vaccines remain at the heart of public health preparedness and response efforts against both seasonal and potentially pandemic influenza viruses.

Globally coordinated virological and epidemiological surveillance is the foundation of the influenza vaccine virus selection and development process. Although national influenza surveillance and reporting capabilities are being strengthened and expanded, sustaining and building upon recent gains has become a major challenge.

Strengthening the vaccine virus selection process additionally requires the continuation of initiatives to improve the timeliness and representativeness of influenza viruses shared by countries for detailed analysis by the WHO Global Influenza Surveillance and Response System (GISRS).

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[1] For Declarations of Interest see Annex 1.

Efforts are also continuing at the national, regional, and global levels to better understand the dynamics of influenza transmission in both temperate and tropical regions. Improved understanding of the degree of influenza seasonality in tropical countries of the world should allow for the strengthening of national vaccination policies and use of the most appropriate available vaccines.

There remain a number of limitations and difficulties associated with the use of HAI assays for the antigenic characterization and selection of influenza vaccine viruses by WHOCCs. Current approaches to improving the situation include the more-optimal use of HAI and other assays; improved understanding of the data produced by neutralization assays; and increased standardization of serological testing methods.

A number of new technologies and associated tools have the potential to revolutionize influenza surveillance and response activities. These include the increasingly routine use of whole genome next-generation sequencing and other high-throughput approaches. Such approaches could not only become key elements in outbreak investigations but could drive a new surveillance paradigm. However, despite the advances made, significant challenges will need to be addressed before next-generation technologies become routine, particularly in low-resource settings.

Emerging approaches and techniques such as synthetic genomics, systems genetics, systems biology and mathematical modelling are capable of generating potentially huge volumes of highly complex and diverse datasets. Harnessing the currently theoretical benefits of such bioinformatics ("big data") concepts for the influenza vaccine virus selection and development process will depend upon further advances in data generation, integration, analysis and dissemination.

Over the last decade, growing awareness of influenza as an important global public health issue has been coupled to ever-increasing demands from the global community for more-equitable access to effective and affordable influenza vaccines. The current influenza vaccine landscape continues to be dominated by egg-based inactivated and live attenuated vaccines, with a small number of cell-based and recombinant vaccines. Successfully completing each step in the annual influenza vaccine manufacturing cycle will continue to rely upon timely and regular communication between the WHO GISRS, manufacturers and regulatory authorities.

While the pipeline of influenza vaccines appears to be moving towards a variety of niche products in the near term, it is apparent that the ultimate aim remains the development of effective "universal" influenza vaccines that offer longer-lasting immunity against a broad range of influenza A subtypes.

1. Introduction

For over 60 years the WHO Global Influenza Surveillance and Response System (GISRS)[1] has served as the foremost global coordination mechanism for monitoring and responding to the evolution and spread of influenza viruses, and ensuring the use of the most up-to-date vaccine formulations. The GISRS vaccine virus selection process involves the coordinated collection and laboratory analysis of hundreds of thousands of clinical specimens each year, with the goal of determining which vaccine compositions will best protect against disease during upcoming northern and southern hemisphere influenza seasons. Due to severe time and other production constraints inherent in current influenza vaccine manufacturing technologies, the vaccine virus selection process must be completed almost a year in advance of the predicted peak of influenza activity in the season in which the vaccine is to be used. The GISRS also continually monitors and assesses the risks posed by potential pandemic viruses and provides guidance on appropriate public health responses. In recent years, data similar to that used for seasonal influenza vaccine development have been used to select viruses for use in 2009 A(H1N1) pandemic vaccines, and in vaccines against other influenza virus subtypes, including A(H5), A(H7) and A(H9) for pandemic preparedness purposes.

In 2010, the convening of the first WHO Informal Consultation for Improving Influenza Vaccine Virus Selection provided a unique opportunity to review in detail this highly complex and collaborative process [1]. Building upon the outcome of this review, a second consultation was held in 2011 to discuss the key principles of influenza surveillance and representative virus sharing, the virological characterization of candidate vaccine viruses, vaccine manufacturing and regulatory requirements, and the potential application of new and emerging vaccine technologies [2]. In the intervening period between these two meetings, the Pandemic Influenza Preparedness (PIP) Framework for the sharing of influenza viruses and access to vaccines and other benefits was adopted. This important milestone event reflected growing recognition of the importance of the timely sharing and characterization of viruses, and of the equitable provision of effective vaccines against both seasonal and pandemic influenza. In response, WHO has continued working to improve knowledge of the global patterns of influenza activity; support the development of informed national policies, aided by the work of its Strategic Advisory Group of Experts (SAGE) on Immunization; increase global influenza production capacity and supply as part of its Global Action Plan for Influenza Vaccines (GAP); and promote expanded access to vaccines under the PIP Framework.

Since the re-emergence of human cases of H5N1 influenza ("bird flu") in 2003 and the 2009 H1N1 pandemic, growing awareness of influenza as an important threat to public health has driven an expansion of surveillance and response capacities in many countries. Nevertheless, many countries are now facing major challenges in sustaining and building upon the gains made. In light of recent national, regional and global initiatives to promote efficient surveillance and representative virus sharing, allied to ongoing advances in vaccine development and production technologies, it was felt timely to convene a third WHO informal consultation in order to:

- update participants on the progress made since the previous meeting;
- further discuss surveillance as the foundation of vaccine virus selection;

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[1] Formerly known as the Global Influenza Surveillance Network prior to the adoption of the World Health Assembly Resolution WHA 64.5 on 24 May 2011. As of May 2014, the GISRS consisted of 141 National Influenza Centres (NICs) in 111 countries, six WHO Collaborating Centres (WHOCCs), 12 WHO H5 Reference Laboratories and four WHO Essential Regulatory Laboratories (ERLs).

- discuss newly emerging insights into the circulation and virological characteristics of influenza in tropical regions with the potential to strengthen vaccine composition and deployment decisions;
- discuss new assays, new technologies and new approaches and their potential for bringing about improvements in both vaccine effectiveness and manufacturing efficiency;
- discuss the regulatory and other practical issues that must be considered in relation to both existing and emerging vaccine technologies; and
- continue to provide a forum for stakeholders to review and evaluate potential improvements to the influenza vaccine virus selection process.

Approximately 128 participants drawn from 51 countries covering all six WHO regions, and representing a wide range of WHO partner organizations and other stakeholders attended (Annex 2). Participants were drawn from WHO Collaborating Centres (WHOCCs), WHO Essential Regulatory Laboratories (ERLs), National Influenza Centres (NICs), WHO H5 Reference Laboratories, the academic research community, National Regulatory Authorities (NRAs), national public health agencies, veterinary institutions and organizations, vaccine manufacturers, donor agencies and other stakeholders.

2. Strengthening influenza surveillance and improving the representativeness, timeliness and availability of candidate influenza vaccine viruses

2.1. Efforts at national, regional and global levels

National influenza surveillance, reporting and response capabilities continue to be strengthened and expanded in many countries, particularly during and since the 2009 H1N1 pandemic. However, despite increasing awareness of the incidence, transmission and disease burden associated with both seasonal and year-round influenza activity, and the continuing pandemic threats posed by A(H5N1) and other zoonotic viruses, sustaining and building upon recent gains has become a major challenge. Key elements in strengthening and sustaining influenza surveillance and response systems include:

- National surveillance system building;
- National laboratory capacity building;
- improved reporting and virus-sharing procedures;
- enhanced capacity to rapidly detect and respond to zoonotic influenza outbreaks.

Recent national surveillance system building efforts in Viet Nam have included the establishment of hospital-based sentinel screening for severe acute respiratory infection (SARI). The prompt transfer of clinical samples to laboratories allows for feedback to the participating sentinel sites and simultaneous onward reporting of results to national authorities. However, these activities currently rely upon external funding from the United States Centers for Disease Control and Prevention (CDC) and the number of sentinel sites is decreasing. The viruses and data shared may therefore not be fully representative in terms of geography, climate, age groups and epidemic timing. Selecting representative viruses to send to a WHOCC is further complicated by the two peaks of influenza activity typically observed in tropical countries. As in many other settings, obtaining good-quality denominator data in hospitals to produce meaningful estimates of the real burden of disease caused by influenza is also problematic. Efforts to improve reporting and virus-sharing procedures have included the

production of a widely circulated weekly newsletter summarizing national influenza activity, weekly reporting to the WHO FluNet platform and the submission of representative circulating viruses to a WHOCC. As a result, the quality of data on the impact and seasonality of influenza in Viet Nam has improved, along with the ability to rapidly detect influenza outbreaks and monitor circulating viruses in the context of national prevention and control efforts.

In terms of national laboratory capacity building, the challenges experienced in Pakistan illustrate the issues NICs facing in many countries as influenza activities compete for funding with other public health priorities. Foremost among these is ensuring the sustainability of funding to meet the running costs of laboratories, and maintain the momentum of recent capacity-building activities. In addition, virus-isolation rates are low and more training in the required skills is needed for laboratory staff. Retaining suitably qualified, trained and motivated staff at all levels of any national system will be a key factor in ensuring the quality, completeness, relevance and timeliness of virus sharing and data reporting.

In Madagascar, the national influenza sentinel surveillance network monitors and reports upon both SARI and influenza-like illness (ILI) across five different bio-climates. The network is based upon both clinical and biological surveillance activities, with specimens submitted weekly to the NIC for analysis. Data-collection and reporting activities include the production of a weekly report on national influenza activity; daily and event-specific sharing of epidemiological data with the Ministry of Health; regular feedback of laboratory results to sentinel sites; and weekly reporting to the WHO FluNet platform and to the WHO Regional Office for Africa. In common with all NICs, the Madagascar NIC aims to ensure the representativeness of the virus types and subtypes submitted to WHOCCs, with all unsubtypable viruses promptly shipped to a WHOCC for further investigation. Future strengthening activities include the provision of support to regional (sub-national) collaborating laboratories, conducting cost-effectiveness analyses to enhance the sustainability of national influenza surveillance activities and research into topics with particular relevance to Madagascar. These include understanding the aetiology of viral ILI (and factors associated with its severity) through integrated genomic, immunological and other approaches; the spatiotemporal dynamics of influenza virus circulation; and estimating the risk of human infections caused by swine influenza viruses by identifying the genetic and antigenic characteristics of viruses that infect both humans and animals.

Experiences gained in China during the strengthening of national influenza surveillance capacities and capabilities – including for the detection of zoonotic influenza outbreaks – have provided valuable insights that may be highly applicable to other countries and regions. Following its rapid expansion since 2009 (Fig. 1), the Chinese National Influenza Surveillance Network (NISN) comprised 408 network laboratories and 554 sentinel hospital sites by 2013. In that same year, the NISN was able to rapidly detect and characterize A(H7N9) and A(H10N8) viruses causing human infections. In the case of H10N8, this rare infection was detected through the Pneumonia of Unknown Etiology system, highlighting the importance of having the necessary testing and reporting systems in place. For H7N9, confirmed human cases were reported to WHO on 31 March 2013 with candidate vaccine viruses published on the WHO website by 10 May followed on 31 May by formal WHO vaccine virus recommendations.

The WHOCC Beijing promotes a national strategy which places laboratory capacity-building at the centre of surveillance-strengthening efforts. Important principles identified in achieving the goals of national surveillance include the collection, reporting and consolidation of data, along with regular data analysis and interpretation. Continuous efforts are required to detect, evaluate and respond to any unusual patterns in the data. The quality of
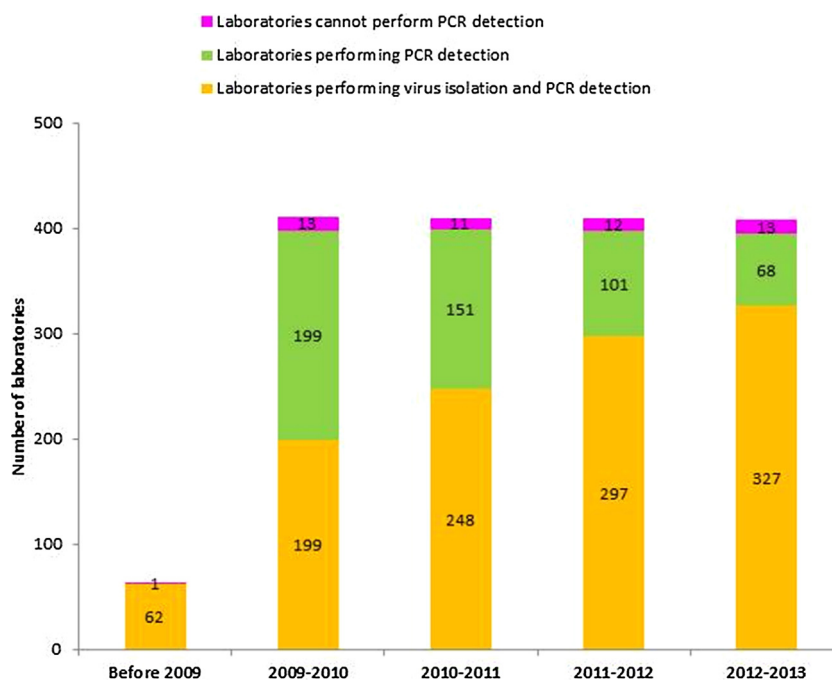
Fig. 1. Expanded and improved influenza surveillance laboratory capacity in China.

laboratory testing and related activities is assessed through participation in both national and international initiatives. These include a well-established national quality-evaluation system based upon core timeliness and performance indicators, and the WHO External Quality Assessment Project (EQAP) for the detection of influenza viruses by polymerase chain reaction (PCR).

Recent initiatives at the regional level have included a study of the patterns of influenza virus submission by countries in the WHO European Region. Despite being the cornerstone of GISRS vaccine virus selection activities, and a crucial element in meeting the obligations of the PIP Framework, no systematic analysis had previously been made of the temporal and epidemiological representativeness of the viruses shared with WHOCCs, or of the timeliness of such sharing in relation to the February WHO Vaccine Composition Meeting (VCM). An analysis was made of the influenza surveillance samples submitted by NICs in the region to the WHOCC London over two seasons (2010–11 and 2011–12). The degree of completeness of data provided in conjunction with each virus sample was also evaluated. Aggregated data for both seasons indicated that a total of 2954 viruses were shared, with 1741 (59%) collected prior to the February VCM deadline; however, only 946 viruses (32%) were shipped in time for consideration by the VCM. This overall figure also masked clear sub-regional variations, with a number of highly significant sub-regions being underrepresented. The average periods between specimen collection and shipment were 90 days and 54 days for the first and second seasons respectively, with both the numbers of viruses submitted and delays in their shipping varying significantly between different sub-regions. Missing data precluded further analysis of the level of demographic and epidemiological representatives achieved over the two seasons. Future study objectives include determining the causes of limited, late or lack of isolate sharing in some countries and sub-regions, identifying the sampling strategies and criteria used by countries to select positive specimens for virus isolation, and evaluating the impact of increasingly PCR-based surveillance on the availability and representativeness of viruses reaching NICs.

Such analyses of regional submission patterns could potentially aid in the revision and refining of current WHO guidance for NICs on which viruses to share with WHOCCs and by when in order to strengthen the VCM process and its outcomes [3]. At the same time, NICs need to be able to recognize both usual and unusual circulating viruses and to decide upon an optimum submission strategy in the context of their own situation, particularly as the timing of seasons varies. It also remains the case that virus submission patterns may be adversely affected by external factors in many countries, for example, customs requirements and other causes of shipping delays. Although delaying the February VCM deadline by up to three weeks could potentially bring significant gains, any such shift would have to be evaluated for feasibility within the very narrow overall timeframes currently available for vaccine development and manufacture. In the United States, the WHOCC Atlanta has provided guidance to individual States on the number of samples needed for different epidemiological situations. Such an approach could help to improve the representativeness of viruses, and may also be applicable to other regions of the world.

At the global level, following adoption of the PIP Framework and other international initiatives, the demands placed on the GISRS have expanded, with activities now including:

- comprehensive support for high-quality influenza surveillance and virus detection, sharing and characterization;
- maintaining and enhancing electronic reporting platforms such as WHO FluNet (http://www.who.int/influenza/gisrs_laboratory/flunet/en/) and FluID (http://www.who.int/influenza/surveillance_monitoring/fluid/en/) to facilitate global reporting;
- the development and revision of WHO guidance on epidemiological and virological surveillance;
- supporting national capacity-building and sustainability improvements (including through PIP Framework mechanisms and the WHO Shipping Fund);
- collaboration with vaccine manufacturers, associated laboratories and regulatory agencies to facilitate vaccine-production and licensing processes;
- supporting research into new surveillance and vaccine technologies;

- strengthening collaboration with veterinary and other animal-sector agencies working at the human–animal interface.

Ongoing efforts in these and other areas will be the key to meeting the twin demands of continually improving influenza vaccines while ensuring their availability to an increasingly larger proportion of the world's population.

2.2. Efforts to increase the availability of egg isolates

Influenza viruses isolated in eggs are still needed to meet current regulatory requirements for vaccine manufacture. There are three main challenges in obtaining such isolates, namely: low isolation rates in eggs; the occurrence of egg-adaptive changes in the haemagglutinin (HA) gene that can lead to changes in virus antigenic profile; and other changes associated with the development of high-growth reassortants (hgrs) for use in vaccine production. Such difficulties continue to be compounded by the reduced provision of egg isolates by NICs.

Potential solutions to the problem of low isolation rates identified by the WHOCC Atlanta include increasing the age at which embryonated eggs are inoculated from 9–10 days to 13–15 days; changing the inoculation route to the allantoic cavity; and changing the egg incubation temperature from 33 °C to 35 °C. The use of such approaches has progressively increased the percentage of A(H3N2) viruses isolated from 0.8% in 2011 to 11% in 2013, with the trend appearing to continue in 2014 (18% to date). Similar success was also reported by the WHOCC London following a switch to eggs obtained from a specific breed of hen. Modifying egg-isolation parameters has thus resulted in significantly improved egg-isolation rates for A(H3N2) viruses in recent years. In relation to the issue of egg-adaptive changes to the HA gene and further changes associated with the development of hgrs, the use of egg/cell paired viruses in routine virus characterization can reveal how differences in the substrate used for virus propagation can impact upon antigenic profiles, thus aiding selection of the best A(H3N2) candidate vaccine viruses.

Further studies of the egg-adaptation pathways of A(H3N2) viruses may eventually allow for the selection of viruses that are antigenically more similar to their mammalian cell propagated counterparts. In the future, such issues may be overcome altogether through the use of alternative or emerging technologies such as reverse genetics or the development of synthetic viruses. At present, however, significant adaptive mutations associated with egg-propagated A(H3N2) viruses continue to occur and are being monitored by WHOCCs (Fig. 2). Given the challenges inherent in the isolation and characterization of A(H3N2) egg isolates, consideration could be given in the short term to requesting that NICs increase the submission of matching clinical materials along with virus isolates to WHOCCs.

2.3. Efforts from vaccine manufacturers

The provision of human-vaccine serum panels by vaccine manufacturers remains an essential element in the broad range of well-established cooperative activities between the WHO GISRS and Industry [1,2]. Such panels enable assessments to be made of the reactivity of pre- and post-vaccination sera with influenza viruses of interest (both seasonal and pre-pandemic) and thus generate extremely valuable data for the WHO VCM. At the request of WHO, manufacturers continue to provide human serum panels from different regions of the world – either through contracts or in the case of Europe as part of annual manufacturer clinical trials. Following the decision to phase out clinical trials from European Union annual update licensing requirements (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/07/WC500170300.pdf), alternative arrangements will be needed in this region and discussions are now being held on the best way forward.

Through the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) Influenza Vaccine Supply (IVS) Task Force, Industry also supports research and development into hgrs, which have long been used as the basis of influenza type A components in seasonal influenza vaccines. As the difficulties in obtaining A(H3N2) egg isolates in recent years have at least partially been addressed, the number of such isolates has greatly increased thus permitting the improved selection of optimal high-yield strains for vaccine production. In addition to
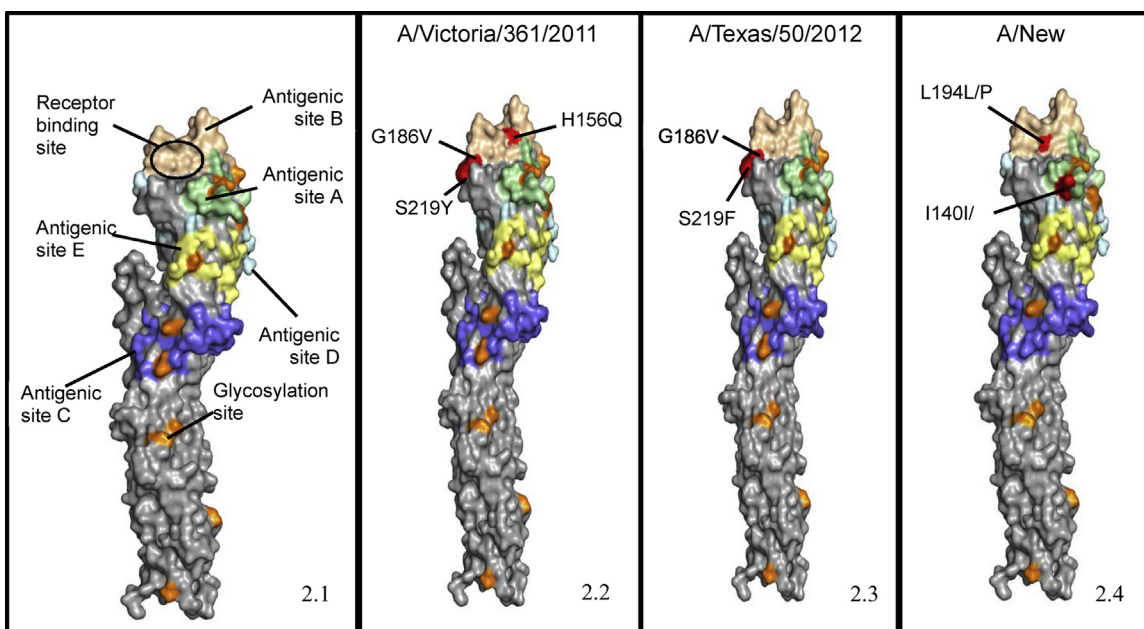

Fig. 2. Egg adaptive changes in the HA of A(H3N2) influenza viruses.

providing isolates of antigenic significance to Industry and reassortant laboratories, WHOCCs also perform the subsequent antigenic analysis and sequencing of the resulting strains, thus highlighting the interdependence of GISRS and Industry in producing hgr vaccine viruses that are antigenically closely related to wild-type viruses. Related advances such as the use of monoclonal rather than polyclonal antibodies as selection reagents has the potential both to significantly accelerate the speed of production of reassortants and increase the number of suitable candidate vaccine viruses. Recent advances in influenza B reassortant technologies could also soon be applied to overcome a currently rate-limiting step in influenza vaccine production, particularly as manufacturers move towards the production of quadrivalent vaccines in which type B viruses will require 50% of vaccine virus production capacity.

The IVS Task Force has also collaborated with several WHOCCs and reassortant producers in evaluating the feasibility of developing virus isolates in qualified cell lines that would meet with regulatory acceptance. Preliminary data indicate that MDCK qualified cell lines are suitable for influenza virus isolation, with the majority of viruses produced retaining the properties of the corresponding WHO reference viruses. In conjunction with the acceptable growth rates achieved using MDCK cell isolates in both cell-culture and egg-based manufacturing processes, and the outcome of risk assessments and supporting literature review, the position taken by Industry is that the approach appears to be suitable. It is envisaged that a regulatory framework could be developed for the use of cell isolates as candidate vaccine viruses, potentially involving the provision of approved cell lines to WHOCCs if this were feasible.

The use of "synthetic" technologies may also bring significant gains by allowing for the accelerated production of better-matched and high-yielding vaccine viruses. This technology was used during the 2013 H7N9 outbreak response, with the HA and neuraminidase (NA) genes being synthesized and vaccine viruses rescued within one week of the China CDC posting the genetic sequencing data. Clinical trials indicated that the resulting vaccine was both safe and immunogenic. The routine early and continuous sharing of sequencing data could lead to the full realization of the potential public health benefits to be gained using synthetic seeds, including the acceleration of pandemic response activities, the rapid availability of higher-yielding and better-matched strains and the ability to generate candidate vaccine viruses in multiple locations as soon as epidemiological and virological data emerge. Other Industry efforts in this area will include collaborative efforts to identify genetic markers of yield that can be used to select for high-yielding strains.

2.4. Collaborative animal influenza surveillance and response activities

The importance of sustained and coordinated inter-sectoral influenza surveillance at the animal–human interface, and of collaborative assessment of the risk of a human pandemic, continues to be reflected in the FAO-OIE-WHO Tripartite Concept (http://www.who.int/influenza/resources/documents/tripartite_concept_note_hanoi_042011_en.pdf?ua=1). Initiated in January 2011 and renewed in January 2014 for a further 5 years, this concept sets out the roles of both WHO and the OIE-FAO Network of Expertise on Animal Influenza (OFFLU) in coordinating global activities to address health risks at the animal–human–ecosystems interface.

Recent progress has included improved sharing of viruses and reagents for their characterization, and the increased availability of appropriate OFFLU information and data at the biannual WHO VCMs. Key information now routinely shared includes epidemiological overviews and phylogenetic data of highly pathogenic avian H5N1 in animals; the results of antigenic testing of specified isolates using ferret-derived antisera; and information on a broad range of animal viruses considered to be of public health concern, including H9, H7 and H5 (other than H5N1) subtypes.

Long-standing obstacles to sustainable influenza surveillance in animals include limited awareness of the need to manage pandemic risks; the lack of drivers for non-notifiable influenza surveillance; and associated absence of legislative frameworks. In many settings, under-resourced veterinary services face challenges in securing sustainable funding and obtaining industry involvement, particularly when balancing financial and other incentives against potential disincentives. As a result, advocacy efforts are still needed to further strengthen the commitment of national veterinary agencies to global public health objectives, and to make the case for the extra resources and increased awareness that are needed to support the strengthening and sustaining of veterinary services.

Improving influenza surveillance in farmed animal populations, and clearly establishing the roles and responsibilities of all agencies working in this area, will become increasingly necessary, especially as surveillance capacities and technologies evolve. For example, in 2004 the very limited capacity for H5 surveillance led to the establishment of GISRS H5 Reference Laboratories and the resulting close collaboration with OFFLU in a broad range of surveillance, pandemic risk assessment and vaccine development activities. Following a subsequent dramatic increase in H5 surveillance, including by WHOCCs, consideration is being given to the current role of H5 reference laboratories.

3. Improving the understanding of influenza activity and addressing the complexities related to vaccines in tropical regions

3.1. The concept of influenza transmission zones

WHO is working to identify epidemiological "transmission zones" in which countries with similar influenza activity and transmission trends are grouped together in order to better reveal global and regional patterns of influenza spread. Eighteen transmission zones were provisionally created, initially based upon existing geographical regions and adjusted according to knowledge of influenza transmission trends (Fig. 3). Following analysis of FluNet data submitted by countries that met specified criteria, it was concluded that the provisionally identified zones did appear to consist of countries exhibiting similarities in their patterns of influenza transmission. Further time-series analyses were carried out to better categorize tropical transmission patterns, again using countries with continuous FluNet data for a specified period. In line with recent published findings [4], statistical assessment of the number and timing of national influenza activity peaks indicated that although influenza patterns definitely exist in tropical regions of the world, such patterns were less evident than those observed in the northern and southern hemispheres. Quality surveillance data are now being generated to allow for more-detailed trend analyses and for periodic review of transmission zone divisions.

Improving the methodology used and supplementing data with literature reviews could lead to a greatly improved understanding of global influenza trends; strengthened seasonal influenza preparedness; and a more accurate assessment of burden of disease to guide the development and expansion of national vaccination policies. The continuous collection of both laboratory and epidemiological influenza surveillance data will be the key to achieving such goals. Current limitations include the reliance on national level data which may not be regionally representative, due for example to the widely differing sizes of different countries. In some cases, data may not even be nationally representative due to wide variations in

Fig. 3. Influenza transmission zones.

seasonal patterns across very large countries such as Brazil, China and India. There is thus a need to better understand the underlying factors that result in the creation of both broad transmission zones and of regional outliers. As understanding increases, it may even become feasible to reduce the number of transmission zones, while still bringing about significant practical refinements and other benefits not available using the current three "de facto" zones (southern hemisphere, northern hemisphere and the tropics).

3.2. Addressing the complexities related to influenza vaccines in tropical regions

WHO issues its recommendations for influenza vaccine formulations in February and September each year in preparation for the corresponding northern and southern hemisphere influenza seasons (November–April and May–October respectively). However, in tropical regions of the world, such well-defined seasonality does not always occur. For example, in Kenya and many other countries in tropical Africa, influenza viruses circulate year-round and despite limited epidemiological data, there is growing evidence of a significant burden of influenza disease in the region. Following improved surveillance and virus-sharing efforts in Kenya and other African countries, it appears that selected priority populations would stand to benefit from receiving either the northern or southern hemisphere vaccine formulation. Further work is needed to evaluate the optimal delivery mechanisms in a country with almost year-round virus circulation, and to determine the added benefits and costs of strategically using both vaccine formulations as opposed to a single formulation. As influenza vaccine use in tropical Africa grows, more data will be needed in countries to inform national vaccination policies.

In the American tropics, similar issues exist in determining the degree of influenza seasonality, predicting annual transmission patterns and selecting between southern or northern vaccine formulations. An analysis of data from countries in the region found that for many countries influenza epidemics typically occurred between May and September during the austral winter and lasted for 4–5 months, rather than year round. Although the specific formulation used in individual countries varies, an estimated 81% of the predominant strains in the American tropics were represented in the southern rather than northern hemisphere vaccine

formulation, with the result that this was the most up-to-date composition. The viruses that circulate in the American tropics tend to be similar throughout the region but those characterized as predominant one year in a particular sub-region tend to become dominant in other sub-regions in successive years in a clearly discernible geographical order.

In Americas, influenza activity in the tropics is often preceded by the release of the southern hemisphere vaccine and by Vaccination Week in April each year. Against this regional backdrop, national patterns of influenza transmission and seasonality are complex. Nevertheless, as quality surveillance data become available for more years, the optimal timing of vaccination is likely to become clearer. In Brazil, a large latitudinal range encompassing both temperate and tropical regions, with good epidemiological data available in certain of its sub-regions, provides an example of how the optimum timing of vaccination cannot be reduced to the current northern–southern hemisphere paradigm. One study comparing the historic use of southern hemisphere vaccine recommendations and schedule against a hypothetical northern hemisphere vaccine scenario concluded that a higher degree of matching between circulating and vaccine viruses would have been achieved had the northern hemisphere vaccine composition and vaccination schedules been followed [5]. Although based on relatively few virus isolates, this study highlights the complexities of influenza vaccination in a large tropical country.

Although most tropical countries in the Asian region technically lie in the northern hemisphere, they do not exhibit the influenza seasonality seen in temperate regions, with some level of influenza virus circulation typically occurring throughout the year. Asian countries lying between the equator and approximately 30° N in latitude experience a peak in influenza during the monsoon season (June–September). In countries closest to the equator, there is year-round circulation with no discrete peak season. In order to better understand patterns of influenza virus transmission in the region and inform national vaccination approaches, a study was conducted which aimed to characterize influenza seasonality in tropical and subtropical countries of southern and south-eastern Asia; identify latitude gradients associated with discrete seasonality; and determine the best time of the year for national influenza vaccination campaigns [6]. Weekly surveillance data from 10 Asian countries over the period 2006–2011 clearly indicated peak

periods of influenza activity in seven of the countries, with no distinct seasonality in the remaining three. In many cases, it was apparent that the current vaccination schedules used were suboptimal and that most tropical countries in Asia might beneficially consider conducting vaccination in April–June each year, i.e., prior to influenza peak circulation, using the most recent WHO-recommended vaccine formulation.

Compiling and sharing data on influenza from individual countries in a given region appears to potentially allow for a regional consensus to be reached on the circulation patterns and seasonality of influenza, which could also incorporate latitudinal differences. At present, the effects of climatic factors in particular are poorly understood especially in remote areas of large tropical countries and in countries where the required data remain sparse. Even in those tropical countries where there are likely to be at least two peaks of influenza activity each year, improved knowledge of the main peak has the potential to result in improved vaccination approaches. Across all the tropical regions of the world there now appears to be a paradigm shift occurring based upon improved understanding of the extent to which influenza is seasonal in order to strengthen national vaccination policies and select vaccines of optimal composition.

4. Improving the characterization and selection of influenza vaccine viruses

Maintaining good levels of influenza vaccine effectiveness [7] requires regular vaccine composition updating and annual administration. Until radically different approaches such as the use of universal influenza vaccines become feasible, the updating process is likely to remain based primarily upon the antigenic characterization and selection of egg-isolated (and potentially cell-isolated) candidate vaccine viruses for the production of both inactivated and live attenuated vaccines. WHOCCs combine the data obtained from the antigenic characterization of viruses using HAI and virus neutralization assays and the serological reactivity of pre- and post-vaccination human sera with extensive genetic sequencing data and epidemiological and clinical information. The resulting datasets form the scientific basis for expert consideration at the biannual WHO VCMs.

Despite being the traditional assay of choice since the 1940s, there remain a number of limitations and difficulties associated with the use of HAI assays. As a result, a range of corrective techniques and complementary assays are used by WHOCCs and other GISRS laboratories [2]. Efforts are also currently under way to address issues such as the differential reactivities of egg-derived and cell-derived viruses with ferret sera, and the complications arising from the binding of the virus NA surface protein to red blood cells. As a result, new approaches continue to be needed to improve assay sensitivity and accuracy, streamline the throughput of samples and improve the reproducibility of data between laboratories. Such approaches would also need to be sufficiently flexible to be used in the analysis of antibody responses to emerging viruses.

Until significant advances are made in the development of new laboratory assay platforms and/or vaccine technologies WHOCCs will continue to face acute time pressures, particularly around the time of the biannual VCMs. Any requirement for the introduction of further assays, or for the greatly expanded use of existing but selectively applied approaches such as microneutralization (MN) assays, are likely to prove problematic, particularly given the rate-limiting step of growing viruses and developing the reassortants required for some assays. Realistic aims at present would appear to include the more-optimal use of HAI and other assays; improved understanding of the data produced by neutralization assays; and strengthening of national and global initiatives such as CONSISE (Box 1) for the standardization of serological testing methods.

Attempts to overcome the inherent limitations of the traditional HAI assay through the development of assays based upon synthetic beads or solid matrices have had only limited success. Studies into the development of synthetic red blood cells consisting of beads coated in either purified natural glycans or synthetic sialyl-glycans have highlighted that, despite evidence of high reproducibility, a range of complex issues (including the prohibitive cost of synthetically produced glycans) would need to be addressed before the approach could become feasible. Efforts to harness a number of non-bead technologies based upon the use of sialyl-glycans or red blood cell membrane fragments is ongoing. Despite the adoption of multiple strategies for developing HAI replacement assays, no viable alternative has yet emerged. The development of such assays would be enhanced by improved understanding of the glycans recognized by different influenza types/subtypes.

As previously reported [2], the NA surface protein plays a key role in the life cycle of the influenza virus and its transmission. Despite ongoing high levels of interest in the potential role that antibodies to NA could play in the development of more-effective vaccines, there is presently no regulatory requirement for the precise determination and standardization of vaccine NA content. Nevertheless, different NA subtypes are known to be genetically distinct, exhibit discontinuous antigenic drift and give rise to antibodies associated with protection against homologous, and to a lesser extent heterologous, influenza viruses. Efforts are therefore continuing to improve understanding of the patterns of antigenic drift in the NA of seasonal influenza viruses.

The application of "antigenic landscape" modelling approaches may also provide enhanced understanding of the quality and breadth of human antibody responses elicited against HA (and potentially against NA) following infection or vaccination, and of the influence of prior immunity on vaccination responses. If corresponding advances in predicting the course of viral evolution prove to be feasible then such approaches could be used to inform and

evaluate vaccination strategies. Antigenic mapping (cartography) techniques have been studied for about ten years and despite variable red blood cell binding effects, and other practical issues, can help to visualize the antigenic evolution of predominant viruses [8]. Antigenic landscaping approaches have now been used to examine historic HAI datasets from a household cohort study in Viet Nam in order to recreate the recent course of influenza virus evolution and explore pre- and post-infection responses. Observations of increasing immunity over time clearly highlighted a persistent "back-boost" effect in which vaccination with antigenically advanced vaccines appeared to stimulate a recall of previous antibody responses. In terms of vaccination strategies such a phenomenon may imply that the optimum vaccine strain to use might be ahead of the centre of the current cluster of evolving strains, thus providing both recall benefits as well optimal de novo responses to new epitopes. By combining such insights with improved predictions of the course of future virus evolution it may be possible to improve the vaccine virus selection process and increase the effectiveness of influenza vaccines. At present, there are a number of practical and theoretical assumptions underlying the approach and further studies are required. These could include prospective studies of vaccination with an antigenically advanced virus; studies across different age groups; and studies based upon data from a range of alternative laboratory assays (such as NA assays, MN and assays that detect stalk-reactive antibodies).

5. New technologies and tools for improving influenza vaccine virus selection

A number of emerging new technologies and tools have the potential to revolutionize influenza virological surveillance and response activities. In the context of national surveillance activities, the increasingly routine use of whole genome sequencing and other high-throughput approaches in large and technologically capable NICs is providing significant insights in areas such as virulence assessment, phylogenetic and transmission studies and evaluation of antiviral susceptibility. As such high-throughput methodologies change the focus of surveillance away from single HA and NA characterization and towards whole genome sequence determinations they will become not only a key element in outbreak investigations but will also drive a shift towards a new surveillance paradigm (Fig. 4).

Further development and increased availability of next-generation sequencing and associated technologies and equipment, together with reduced operating costs and data-analysis requirements, could also allow for the examination of global gene expression at the level of pathogen and host. Such approaches have the potential not only to greatly improve understanding of circulating viruses and their evolution, but to reveal the nature and extent of intrahost viral diversity and degree of fitness; signal the potential emergence of drug resistance; aid in vaccine virus selection and allow for the accurate assessment of risk, including pandemic risk. Datasets and pipelines could also be rapidly generated for examining genetic variation in populations of viruses that may be associated with a particular phenotype. The use of synthetic genomics technologies based on the sequencing data obtained would then allow for the rapid synthesis for future use of "libraries" of numerous HA, NA and other gene segments. Efforts under way in this area include the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH)-supported Synfluenza project at the J. Craig Venter Institute (http://gsc.jcvi.org/projects/gsc/Synfluenza/index.php) and the United States Department of Health and Human Services (HHS)-supported synthesis of whole viruses for vaccine production (as occurred during the production of inactivated H7N9 vaccines in 2013) and for associated challenge, transmission and pathogenesis studies.

As evidenced in 2013, assessing and responding to the risk to human health posed by zoonotic influenza infections is currently highly problematic for national surveillance systems. Substantial viral diversity exists within animal populations, even within the same subtype, and only limited genetic sequencing and antigenic data are typically available. Responding to outbreaks of zoonotic influenza viruses is also compounded by pronounced variability in their growth properties. Efforts undertaken to address this situation include virus-challenge studies conducted at the WHOCC Memphis, which have highlighted a number of potentially attainable benefits of genomic and bioinformatics approaches to avian influenza surveillance, while providing insights into the methodological and other challenges that will need to be overcome. In addition, a case study conducted by the United States CDC, and based upon clinical samples taken during the H7N9 event in China, indicated that despite multiple challenges, properly applied next-generation sequencing technologies to detect and assess the properties of emerging intrahost genetic variants can lead to improved risk assessment. Studies have also been conducted on the potential of use of reconstructed ancestral A(H5N1) influenza viruses to develop cross-clade protective vaccines [10], and on the utility of reverse genetics techniques to improve H5N1 vaccine virus growth rates [11]. Long-term goals in efforts to detect and respond to zoonotic influenza outbreaks include the routine use of surveillance and risk-assignment activities based upon the use of genetic sequencing data, followed by the use of reverse genetics approaches to rapidly generate suitable candidate vaccine viruses.

Despite the advances made to date, significant challenges will need to be addressed before next-generation technologies become a routine part of national surveillance and response paradigms, particularly in low-resource settings. These include the need to fully understand the utility of more-comprehensive genomic datasets in vaccine virus selection and development. In addition, the use of current technology platforms requires considerable expertise, with advanced research and development efforts now under way to refine and harmonize the pipelines and bioinformatics tools required at different stages of the process. Bioinformatics demands in particular remain high given the ever-evolving nature of instrumentation and protocols, and the costs associated with the analysis of sequencing data.

Interest also remains high in the development and potential applicability of mathematical modelling approaches for predicting the course of influenza virus evolution, and thus potentially accelerating the selection of new vaccine viruses. Recent work on integrating data on the antigenic and genetic evolution of influenza viruses indicates that such a combined approach can provide potentially valuable new insights into the factors that determine observed patterns of antigenic drift [8]. By pinpointing the precise mechanisms by which changes in viral genes result in antigenic change it may soon be feasible to predict which of the viruses circulating at the start of the influenza season will come to predominate in terms of number of new infections. The related concept of viral "fitness" provides a further potential means of evaluating and predicting patterns of virus evolution. This approach is based upon the integration of publicly available HAI, sequence and biophysical data plus regional information to develop a joint epitope/non-epitope fitness model. Preliminary retrospective studies indicate that such an approach can successfully predict the evolution of HA sequence clades year on year. The predictive potential of such a model at the phenotypic level remains to be demonstrated. Potential further improvements and refinements include more-rigorous data-quality control, use of a wider range of input-data categories and improved integration of selected datasets.

Approaches based on systems genetics and systems biology concepts also have the potential to provide valuable new insights. Systems genetics approaches can capture human genetic diversity,
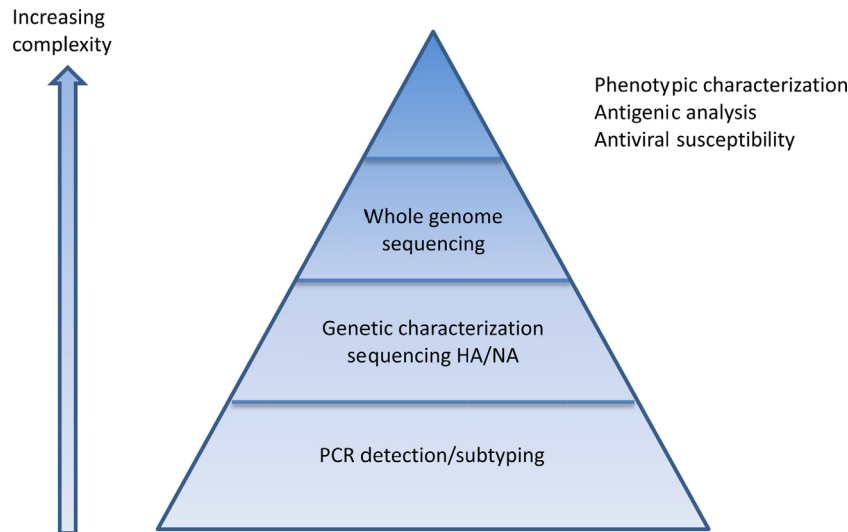
Fig. 4. Paradigm shift—the new surveillance pyramid.

and allow, for example, the identification of the specific host-susceptibility genes that regulate disease outcomes following viral infections. Systems biology approaches provide an opportunity to generate new types of datasets with the potential to identify both diagnostic and prognostic markers; understand pathogenic and virulence mechanisms; evaluate vaccine performance and responses; generate improved cell lines for virus cultivation; and identify correlates of protection. Despite being at an early stage, systems biology approaches allied to current statistical capabilities could feasibly be used to elucidate some of the molecular correlates of immune responsiveness and related immunogenicity issues. Such associative studies may soon be feasible in the context of clinical trials to determine their potential application. However, in terms of understanding causality much remains hypothetical at this stage and further work is required to identify the key pathways of interest and develop effective therapeutic approaches. As viruses rely upon host factors to replicate, and often hijack the cellular processes initiated in response to infection, such approaches could even be based upon the suppression of host responses.

Human influenza vaccine responses and course of infection have also been evaluated during a longitudinal study which combined genetic, transcriptional and immunological data [12]. Such an integrative genomics approach allows for the identification of host genetic factors that contribute to variations in vaccine responsiveness, and may uncover important mechanisms affecting vaccine efficacy. The results indicate that genetic variations between individuals are important determinants of vaccine immunogenicity. Understanding the complex mechanisms that underlie variations in vaccine responses may allow for the identification of individuals who do not develop a protective antibody response following influenza vaccination. Appropriate modifications to the dose or vaccine type given to such individuals could potentially lead to a reduction in the proportion of the population who would otherwise remain unprotected. The approach may also be useful in guiding the modification of factors, such as adjuvant use, intended to enhance the immune response to influenza vaccines in all recipients.

Taken together, the range of new technologies and tools now under development are capable of generating potentially huge volumes of highly complex and diverse datasets. Recent trends in the acceleration of data volumes, velocity and variety are driving a newly emerging concept of "big data" (Fig. 5). Efforts will be required to ensure the availability, accessibility and quality of the data generated, including through the validation of datasets; to enable the meaningful integration of often highly
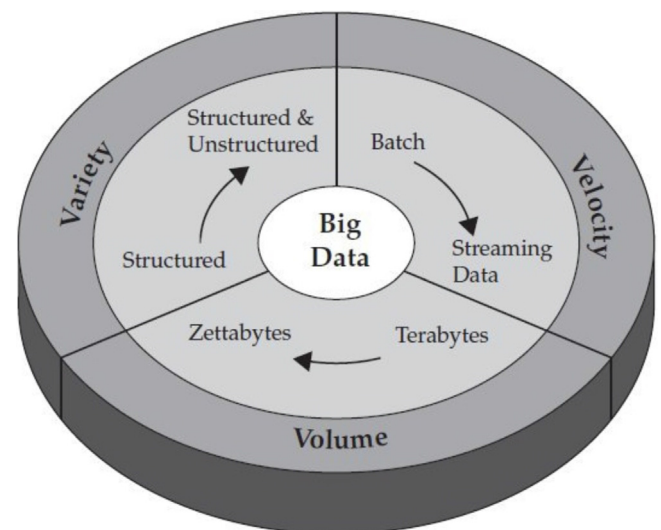

Fig. 5. The three "V"s of big data.[2]

disparate datasets; and to ensure the broadest possible relevance and application of the resulting findings. As part of its activities in these areas, the NIAID/DMID Genomics Program has invested in a number of Bioinformatics Research Centers (BRCs), including the Influenza Research Database (www.fludb.org). This free-to-use comprehensive collection of influenza-related data and analysis tools is intended to support a process of data standardization and integration encompassing a range of sequence, surveillance and immunological data categories.

In addition to expanded data volumes and the accelerated velocity of data generation, current efforts to map the organizing principles of big data and its application increasingly highlight the need for parallel advances in the underlying knowledge base and interpretive context in which to set the data generated. Discerning the key patterns in data relies crucially on the capture of data variety ("metadata"). A collaborative United States Genome Sequence Centers-BRC metadata working group has now been established in an attempt to support the capture of relevant, standardized and

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[2] Source: Journal of PayDeg. Issue 03. August 2012 (http://paydeg.com/CloudComputing3.html, accessed 20 April 2015).

consistently reproducible metadata by its member projects. Harnessing the currently theoretical benefits of concepts such as big data for the influenza vaccine virus selection and development process will depend upon consolidating and building upon these and further advances in the generation, integration, analysis and dissemination of potentially huge and complex datasets.

6. Manufacturing and regulatory aspects of improved vaccine virus selection and development

Over the last decade, growing awareness of influenza as an important global public health issue has been coupled to increasing demands for more-equitable access to effective and affordable influenza vaccines. However, efforts to increase global vaccine production by establishing manufacturing and regulatory capacity in some developing countries have met with a number of challenges. In Brazil, for example, influenza vaccine production at the Butantan Institute was initiated through a technology-transfer programme involving Sanofi Pasteur that started in 1999 and was completed in 2010 as part of the WHO Global Action Plan for Influenza Vaccines (GAP). The transferred technology enabled the manufacture of egg-based split virus vaccine with an annual production capacity of 20 million doses, based primarily upon the estimated size of the elderly population targeted in national policy for vaccination in 1999. This unprecedented initiative proved to be a long and involved process for all stakeholders, including government, Sanofi Pasteur and the federal Brazilian regulatory agency Agência Nacional de Vigilância Sanitária (ANVISA). However, despite the obstacles encountered, the technology-transfer process not only resulted in seasonal vaccine production in Brazil, but also allowed for the development and production of H5N1 vaccines, pandemic H1N1 vaccine lots for clinical studies and H7N9 vaccine lots for pre-clinical studies. Current challenges include the need to accelerate vaccine production and to conduct clinical trials as required by ANVISA, and overcoming the distribution logistics associated with a vast land area. It is intended that improvements in the acquisition of eggs, reassortants and reagents; strengthened communications with federal customs and ANVISA; improved collaboration with influenza reference laboratories; and enhanced production planning will result in an increased production capacity sufficient to meet the needs of a range of newly identified groups targeted for inclusion in expanded national influenza vaccination policies.

Successfully completing each step in the annual influenza vaccine manufacturing cycle relies upon timely and regular communication between the WHO GISRS, manufacturers and regulatory authorities. For northern hemisphere vaccines, production must begin almost a year in advance of their eventual deployment (Fig. 6). The seasonally shifted schedule for southern hemisphere vaccines involves a shorter lead time between the WHO VCM announcement in September and final formulation and distribution. Despite early access by manufacturers to epidemiological data, regular teleconferences prior to the VCM and prompt information on available reassortants and reagents, at least one component virus of seasonal influenza vaccines must be manufactured "at risk" and up to two working seeds prepared prior to the WHO VCM (Fig. 6). Subsequent steps in the manufacturing cycle are equally time critical and include the optimization and validation of the manufacturing process; the supply of calibrated reagents for the single radial immunodiffusion (SRID) assay by ERLs; and the need to annually update the product licences in an often complex regulatory and manufacturing environment. These timelines have now come under further pressure with the introduction of several quadrivalent vaccines. In view of such time constraints, there appears to be no obvious means of accommodating any proposal to improve the representativeness of viruses sent to WHOCCs by strategically delaying the timing of the WHO VCM.

Eliminating avoidable delays in the vaccine production cycle caused by the use of suboptimal PR8 master donor viruses remains a key aim, including during the time-critical development of candidate pandemic vaccine viruses. The United States Biomedical Advanced Research and Development Authority (BARDA) is working to develop panels of optimized viral "backbones" that can be selectively used in the accelerated production of inactivated influenza vaccines. Three representative PR8 donor viruses from GISRS partner laboratories were compared in terms of their impact on HA yield across a broad range of different influenza virus subtypes and lineages. In some subtypes (for example, the H5N1 strain A/Hubei/1/2010) optimizing the PR8 internal genes was associated with an up to eight-fold increase in HA yield compared to suboptimal donor use. Although the switch to a 5:3 genotype (containing wild type PB1 as well as HA and NA genes) generally had detrimental effects on the yields achieved with the classical 6:2 genotypes, this did not apply to certain low-yielding 6:2 viruses in which yield could be doubled.

In terms of vaccine potency, SRID tests remain the established gold standard for determining the antigen content of influenza vaccines, with studies indicating acceptably low inter-laboratory variability. However, the 2009 H1N1 pandemic placed increased pressures on vaccine production and release timelines, with clinical trials being either required or desirable. The availability and early use of other types of vaccine potency assays prior to the production of SRID reagents early in the pandemic resulted in renewed interest in the development of alternative approaches. The leading candidates are either physicochemical assays – such as high-performance liquid chromatography (HPLC); sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE); and mass spectrometry – or biological assays ("bioassays") such as ELISA/enzyme immunoassay (EIA); surface plasma resonance (SPR); and immunocapture isotope dilution mass spectrometry (IC-IDMS). Physicochemical assays potentially have the advantages of being immediately available, rapid, reproducible and well suited for use in automated high-throughput approaches. Disadvantages include the inability to take into account the conformation, antigenicity or immunogenicity of the measured HA; the potential need for reference reagents; and the expense and technical difficulty of some methods. Bioassays measure vaccine biological activity or reactivity and are usually specific for the antigen being assayed. Their main advantage is their potential for measuring biologically active HA and for evaluating its stability. Moreover, some assay formats, such as ELISA, are already well established and easily implementable. Disadvantages include the need for specific reagents and the expense and technical complexity of some assays. Despite this, bioassays are preferred over physicochemical assays with the combination IC-IDMS assay also offering some potential. Workshops have now been held and studies initiated to compare and evaluate a range of physicochemical and biological assays as potential alternatives to SRID.

In the United States, regulatory experience at the Food and Drug Administration (FDA) Center for Biologics Evaluation and Research suggests that the current influenza vaccine virus selection process generally works well, and does not in itself present significant regulatory issues for vaccine manufacturing. However, the required analysis of manufacturer's seed viruses and the preparation and calibration of potency reagents are highly resource intensive. This situation is now being exacerbated by an increase in the number of available reference viruses and the licensure of new vaccine manufacturers. In addition, recent licensure processes for quadrivalent, cell-based, recombinant-protein and adjuvanted vaccines have highlighted a number of regulatory challenges associated with new influenza vaccine types.

For quadrivalent vaccines, licensure is based upon safety and immunogenicity studies and the demonstration of non-inferiority
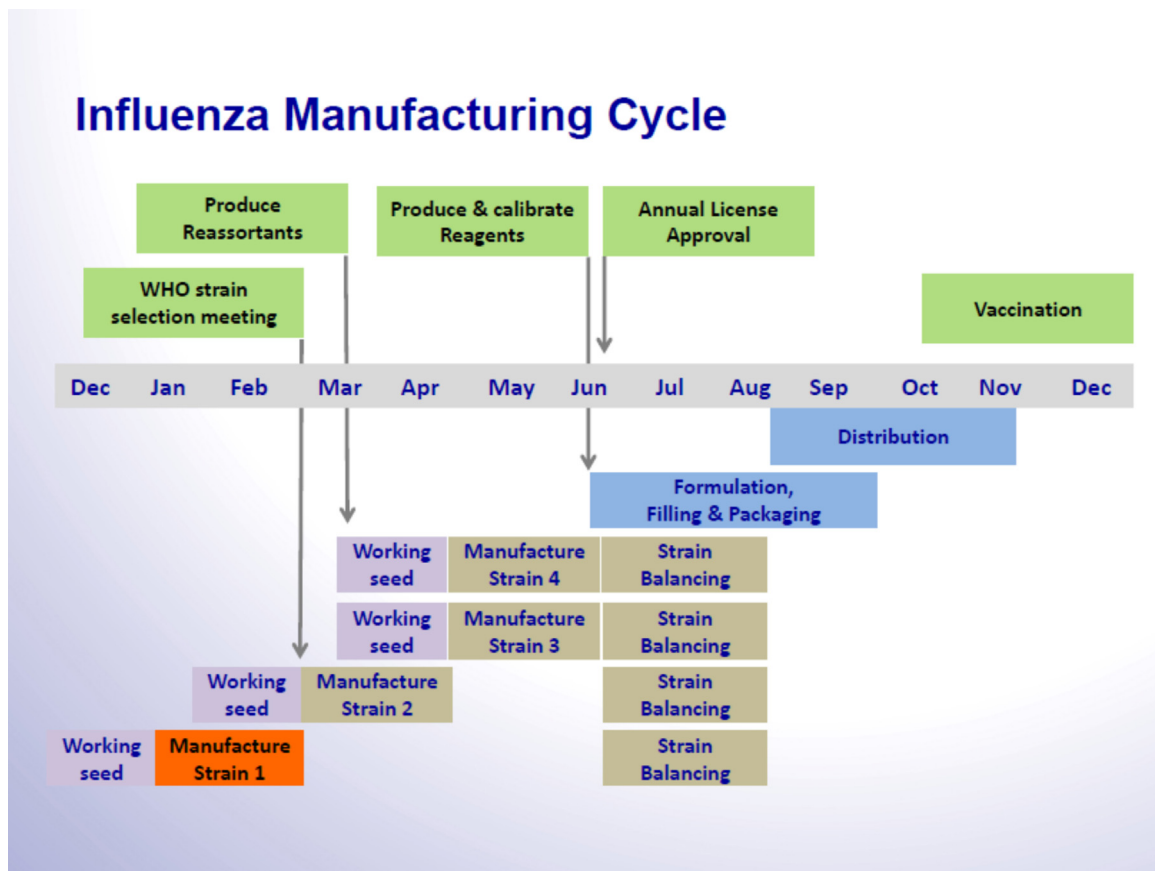
Fig. 6. The manufacturing cycle for influenza vaccines for use in the northern hemisphere winter season.[3]

to licenced trivalent vaccines. Related key issues include the need for four sets of specific reagents for potency testing; the potential for both wild-type and reassortant viruses to be used as vaccine viruses; and cross-reactivity and other difficulties in SRID and identity testing arising from the relatedness of the two B lineages. Influenza B strains are also typically the slowest growing strains and the lack of ideal high-growth reassortants may result in manufacturing and lot-release delays. Currently, there is only one mammalian cell based influenza vaccine licenced in the United States, with studies indicating that initial concerns around the potential tumorigenicity of MDCK cell lines were unfounded. A single recombinant protein influenza vaccine has also been licenced in the United States and is based upon an insect virus (baculovirus) expression system and recombinant DNA technology. Although candidate vaccine viruses are not needed to produce such a vaccine (only sequence information for the recommended HA is needed) there are likely to be issues in relation to the determination of appropriate potency assays and current lack of established pathways for product improvement.

Any development and licensure pathway for adjuvanted vaccines will require careful attention to preclinical testing, study design, dosing decisions and safety monitoring. The complex nature of adjuvants and adjuvanted vaccines necessitate the collation of extensive safety data, including clear indications of duration of follow-up, monitoring of adverse events of special interest and a focus on the potential for autoimmune/auto-inflammatory disease development. The only adjuvanted influenza vaccine currently licenced in the United States is an AS03-adjuvanted H5N1

monovalent vaccine produced under government contract as part of national pandemic preparedness. A detected association between narcolepsy and AS03 following the use of 2009 H1N1 pandemic vaccines in a small number of Scandinavian countries highlights the case-by-case approach required while emphasizing the need for appropriate safety data for all adjuvants.

The emergence of potentially pandemic influenza viruses creates specific regulatory and associated challenges in terms of clinical study design, timeline and interim data analysis. Overcoming such challenges requires collaboration and cooperation between multiple agencies and manufacturers. Since 2009, NIAID clinical vaccine trials have been conducted to determine the appropriate response to emerging influenza viruses in both pandemic and non-pandemic scenarios. The ongoing emergence of multiple potentially pandemic influenza viruses (such as A(H3N2)v, A(H5N1) and A(H7N9) viruses) will continue to create challenges for vaccine development in terms of clinical trial study design and resource requirements. During the 2009 H1N1 pandemic, 12 clinical trials were undertaken by the NIAID in the context of the United States national emergency preparedness framework. These studies were complementary to planned Industry trials, and were primarily intended to generate data on safety and vaccine use in selected populations.

Despite the challenges and constraints of current vaccine-production technologies, the influenza vaccine landscape continues to be dominated by egg-based inactivated and live attenuated vaccines, with a small number of cell-based and recombinant vaccines. During a pandemic all these platforms would potentially necessitate antigen-sparing strategies and possibly gains in manufacturing efficiencies to meet demand. While the field of influenza vaccines thus appears to be moving towards a variety of niche products in the near term, it is apparent that the ultimate goal

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[3] Source: International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) Influenza Vaccine Supply (IVS) Task Force.

remains the development of "universal" influenza vaccines offering longer-lasting immunity against a broad range of influenza A virus subtypes. Such vaccines would likely remove the need for annual vaccine virus selection, reduce production costs, eliminate potential vaccine mismatches and shortages, and greatly increase the global supply of both pandemic and seasonal vaccines.

7. Conclusions and future directions

Virological and epidemiological surveillance is the foundation of the influenza vaccine virus selection and development process, and national, regional and global efforts will continue to be needed to improve monitoring and reporting activities, and to ensure the timeliness and representativeness of virus sharing. In many countries, much remains to be done in establishing, expanding and sustaining ILI and SARI sentinel surveillance, motivating staff, improving laboratory capacity and providing training for sub-national and regional laboratory personnel. In some parts of the world, severe economic and/or logistical hurdles continue to restrict the timeliness and temporal and geographical representativeness of the viruses sent to WHOCCs for detailed analyses. To help countries make the best use of available resources while meeting their obligations under the PIP Framework, WHO and its partners will continue to work to develop regional and global guidance on optimal approaches for ensuring the timeliness and representativeness of influenza isolates and clinical specimens shared with the WHO GISRS.

Improved knowledge of influenza transmission "zones" could allow for refined and more-targeted surveillance activities, and greater understanding of virus circulation and transmission patterns, including in tropical regions. Further refinement of the currently provisional basis used to determine functional influenza transmission zones should be considered. Even without the development of specific recommendations for tropical areas, such an approach could bring about much-needed improvements in current guidance on where and when to use northern or southern hemisphere influenza vaccine formulations in tropical countries. As part of this, consideration could also be given to promoting the wider use and potential refinement of reporting platforms such as WHO FluNet and FluID, for example to accommodate multiple seasonality and/or wide geographical variety within a single country.

The need for sustained and coordinated inter-sectoral influenza surveillance at the animal–human interface, and collaborative assessment of the risk of a human pandemic, continues to be reflected in the FAO-OIE-WHO Tripartite Concept ("One Health"). Given the long-standing obstacles in implementing sustainable influenza surveillance in animals, and in building upon recent progress in integrating the outcome of this into the public health context, improved collaboration between the WHO GISRS and OFFLU is needed.

Opportunities to improve the antigenic characterization and selection of influenza vaccine viruses include the strategic use of neutralization assays to support HAI assay data, and the further strengthening of standardization and information-sharing initiatives. Efforts to develop alternative simplified and high-throughput methods and quantitative assays will also continue, along with further research into the utility of approaches based upon the use of synthetic red blood cells, characterization of virus NA and antigenic landscape technologies. Realistically, such aims must be weighed against the finite capacity of WHOCCs to antigenically characterize candidate viruses in typically limited timeframes.

A number of emerging new technologies and tools have the potential to revolutionize influenza virological surveillance and response activities. These include the increasingly routine use of whole genome sequencing and other high-throughput approaches, and the potential application of synthetic genomics, systems genetics, systems biology, mathematical modelling and bioinformatics approaches. However, despite the advances made to date, significant challenges will need to be addressed before such technologies become a routine part of national surveillance and response paradigms, particularly in low-resource settings.

Although vaccine technology is evolving, and despite long-recognized challenges and constraints associated with current vaccine-production technologies, the influenza vaccine landscape continues to be dominated by egg-based inactivated and live attenuated vaccines, with a small number of cell-based and recombinant vaccines. The research and development of hgrs will for the foreseeable future continue to be a major focus of Industry and government agency efforts as such viruses form the basis of the influenza type A components of current seasonal influenza vaccines.

While the pipeline of influenza vaccines appears to be moving towards a variety of niche products in the near term, it is apparent that the ultimate aim remains the development of effective "universal" influenza vaccines that offer longer-lasting immunity against a broad range of influenza A subtypes. In this and other related areas of research and knowledge development, WHO and its partners will work to ensure that the advances made are translated into improved influenza preparedness and response activities and into far more equitable global public health outcomes.

Appendix A. Declarations of interest

The 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection, 1–3 April 2014, was attended by experts from the WHO Global Influenza Surveillance and Response System (GISRS), national epidemiological institutions, national regulatory authorities, research and academic laboratories, institutions and organizations, veterinary institutions and organizations, human influenza vaccine manufacturers, and donor agencies and other stakeholders.

In accordance with WHO policy, the meeting Chair, co-Chairs and joint Rapporteurs were required to complete the WHO form for the Declaration of Interests for WHO Experts prior to the consultation. During the opening session, the interests declared by these experts were disclosed to all participants.

No current or recent (within the last 4 years) personal financial or other interests relevant to the subject of the consultation were declared by the Chair (Dr. N Cox), co-Chairs (Dr. M Siqueira, Dr. M Giovanni, Dr. G Grohmann, Dr. J Katz and Professor M Rahman) or joint Rapporteur Dr. A Waddell. A single financial interest declared by the joint Rapporteur Dr. J Wood was reviewed by the WHO Secretariat. As the joint Rapporteurs were to work collaboratively with the formal writing group shown above, and under the direct guidance of the Chair and co-Chairs, it was decided that the interest disclosed did not present a conflict of interest in relation to full meeting participation or the preparation of this report.

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