Improving influenza vaccine virus selection

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Executive summary

Starting in 2010 the World Health Organization (WHO) has held a series of regular informal consultations on improving the selection of influenza vaccine viruses. The aim of these consultations is to explore ways of improving the highly complex and time-pressured virus selection process by providing a platform for expert discussion across a broad range of key areas.

In November 2015 the fourth informal consultation brought together experts from around the world to review the current status of efforts in the field and to explore ways of improving the extent, timeliness and quality of influenza surveillance data, evaluate the development and application of new laboratory assays and related initiatives, and assess the progress made in the application of next generation sequencing, synthetic genomics technologies and potentially predictive modelling approaches. Discussions were also held on the production of pandemic and broadly protective vaccines and on strengthening understanding of regulatory and manufacturing perspectives and constraints.

In light of the widely reported late emergence of an antigenic variant of influenza A(H3N2) viruses and the resulting influenza vaccine virus mismatch which occurred in the 2014–15 northern hemisphere season, specific attention was also given to the issue of late-emerging variants and to potential strategies for responding to them. There was broad acknowledgement that under the current and long-established influenza vaccine virus selection and development paradigm that such a mismatch was inevitable on this occasion given the timing of the emergence of the A(H3N2) variant in question.

Against a backdrop of increasing awareness of the health and economic burdens caused by seasonal influenza, the ever-present threat posed by zoonotic influenza viruses and the 2014–15 vaccine virus mismatch this consultation provided a highly timely opportunity to share recent developments in the field, exchange views via both panel-based and plenary discussions and to propose a coherent and feasible set of core action points. Efforts will now be accelerated to progress the near- and mid-term actions identified.

Introduction

Efforts to establish a global network to detect and identify new and potentially dangerous influenza viruses predate the adoption of the WHO Constitution in 1948. Today, the WHO Global Influenza Surveillance and Response System (GISRS)\(^1\) serves as the global coordination mechanism for monitoring and responding to the threat posed by influenza viruses, and for ensuring the use of the most up-to-date and geographically appropriate vaccine formulations. Since 1998, separate and appropriately timed recommendations for the northern and southern hemispheres have been issued each year following the WHO vaccine composition meetings (VCMs) held in February and September, respectively.

Retrospective studies have shown that with only few exceptions these WHO recommendations have resulted in good antigenic correspondence between the viruses in the

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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 September 2015 the GISRS consisted of 143 National Influenza Centres (NICs) in 113 countries, six WHO Collaborating Centres (WHOCCs), 13 WHO H5 Reference Laboratories and four WHO Essential Regulatory Laboratories (ERLs).
vaccines and influenza viruses circulating during the following influenza season. Nevertheless, various levels of antigenic divergence between vaccine and circulating viruses have occurred as a result of the ongoing antigenic variation of viruses, issues related to egg adaptation and, occasionally, the unpredictable emergence of an antigenically variant virus following the biannual WHO VCMs – as occurred during the 2014–15 northern hemisphere season. These and other factors lead to a range of complex challenges and unavoidable constraints in implementing the current vaccine virus development and production process.

Despite significant scientific and technical advances and improved understanding of influenza evolution, the approaches used to select and develop vaccine viruses and to produce vaccines have remained fundamentally unchanged for decades. In order to overcome the severe time constraints and other challenges inherent in the current system a number of current knowledge gaps will need to be addressed. These include improved understanding of the gradual changes in A(H3N2) viruses that are now making virus detection and antigenic analysis problematic. The in vivo implications of changes in both haemagglutinin (HA) cell receptor properties and neuraminidase (NA) binding of red blood cells (RBCs) and their interrelationships are not well understood. In addition, the precise contribution made by NA antibodies to protective immunity is not fully known, and efforts to potentially standardize the NA content of vaccines are at an early stage. Although the evolution of the HA gene can be retrospectively tracked, and sequence changes followed, it is not yet possible to recognize significant changes at the early stages or to apply such data with any certainty to predicting future virus evolution. In addition, the occurrence of drug-resistance mutations in the apparent absence of drug pressure is currently not well understood. Despite a reasonable degree of understanding of the role of HA receptor binding in the adaptation of avian viruses to humans, the much less understood polymerase mechanics means that the risk of a pandemic caused by any particular avian virus cannot be accurately estimated. Despite associated progress in the preparation of therapeutic and potentially broadly reactive antibodies to conserved HA stem epitopes the extent to which these can form the basis of a so-called universal vaccine remains unclear. Improved understanding is also required of the practical applications of other emerging technologies, such as the use of synthetic genomics, next generation sequencing, and the analysis of virus and host factors responsible for the emergence of seasonal and potentially pandemic viruses.

Following on from the first three meetings in this series (1–3) the specific objectives of this fourth informal consultation were to review and discuss:

- strengthening of influenza surveillance activities to improve vaccine composition;
- virological characterization and evaluation of candidate vaccine viruses;
- challenges posed by late-emerging variants;
- application of next generation sequencing technology;
- potential role of enhanced evolutionary analysis and predictive modelling;
- development of pandemic and broadly protective vaccines;
- regulatory issues for influenza vaccines.

In each of these areas, a set of proposed action points was then identified and discussed. The resulting matrix of proposed near- and mid-term actions for implementation by suggested parties was viewed as a key meeting outcome and is presented in Annex 1.

Growing awareness of the public health importance of seasonal influenza epidemics and of the ever-present threat of influenza pandemics continues to drive increasing global demand
for effective and timely influenza vaccines. Such increasing awareness brings with it a unique opportunity to leverage technical and financial support from a broad range of international initiatives, including the forthcoming 3–5 year workplan of the Pandemic Influenza Preparedness (PIP) Framework for the sharing of influenza viruses and access to vaccines and other benefits. The time is now right to review the broad and complex landscape of vaccine virus selection and development activities and to make progress in all the key areas outlined in this report.
1. Strengthening influenza surveillance to improve vaccine composition

1.1 Overview and current issues

WHO global influenza surveillance was formally initiated in 1952 and centred on the activities of 26 laboratories, principally in Europe and the United States of America (USA). As of November 2015, the WHO GISRS comprised six WHO Collaborating Centres (WHOCCs), 143 National Influenza Centres (NICs) in 113 countries, four Essential Regulatory Laboratories (ERLs) and 13 WHO H5 Reference Laboratories. National and international quality-assessment programmes have consistently indicated that GISRS laboratory virus-testing capacity and accuracy have both increased dramatically over the last 20 years. In addition, since 2007, there has been a significant increase in the number of laboratories sharing viruses with WHOCCs, and a corresponding increase in the number of countries sharing virological and epidemiological surveillance data through the WHO FluNet and other reporting platforms. Recent examples of WHO efforts to further improve the quality and comparability of global influenza data have included the publication of guidance on epidemiological influenza surveillance (4).

In an effort to better define the exact amount of virological surveillance data required to meet surveillance objectives, and in light of economic considerations, the United States Centers for Disease Control and Prevention (CDC) initiated a “right-size” project in the USA. This initiative is driven by a statistical, systematic approach based upon the required degree of accuracy of surveillance rather than upon local and national surveillance capacity. The defined surveillance objectives were to provide situational awareness, detect novel viruses, monitor antiviral resistance and ensure the forwarding of sufficient influenza-positive samples of each subtype/lineage by contributing laboratories to detect antigenic drift and inform seasonal vaccine virus selection. In the context of this latter objective, sampling parameters were based upon detection of at least one drift variant at a prevalence of $\geq 3\%$ with 95% confidence at a national level and a monthly time frame. For the purposes of seasonal vaccine development it was assumed that influenza A subtypes and B lineages were both of equal concern. Retrospective analysis of the effect of applying this right-size principle to the 2011–12 and 2013–14 influenza seasons indicated that a significantly more even workflow throughout the year at CDC would have resulted.

Despite these and other strides made in the expansion, strengthening and refining of global influenza surveillance a number of significant gaps and discontinuities remain in influenza reporting and virus-sharing activities in some areas of the world. For example, in tropical and subtropical zones an estimated 74 out of 138 countries – representing around 60% of the world population – do not have a national policy for influenza vaccination. In addition, there is a lack of clarity and guidance regarding the basis upon which countries select either the northern or southern hemisphere influenza vaccine for use in their national programme. Following antigenic analysis of A(H3N2) virus evolution in Thailand, and associated analysis of limited data for influenza H3 and B-Victoria and B-Yamagata lineages in 17 tropical and subtropical countries, it was concluded that there was no evidence to support the need for a separate influenza vaccine composition consultation for the tropics and subtropics. On this basis, WHO undertook to develop guidance for tropical and subtropical countries on how to determine which of the two annual influenza vaccine compositions (northern or southern hemisphere) to use and to establish the optimal timing of vaccination. As discussed during a series of WHO Expert Group meetings, influenza seasonality in the tropics and subtropics
was independently assessed by CDC, the Netherlands Institute of Health Services Research (NIVEL), PATH and WHO using different data sources and analytic approaches and the resulting data analyzed as part of an ongoing process of developing and implementing WHO guidance and recommendations in this area (5–8).

1.2 Challenges and opportunities

Current challenges for GISRS include ensuring the timeliness, representativeness and optimal scale of surveillance activities. In the context of seasonal influenza vaccine virus selection, timeliness specifically refers to the routine monitoring of circulating influenza virus variants and their epidemiological significance against a backdrop of other circulating viruses, prompt reporting to WHO FluNet and other platforms, and the timely forwarding of emerging antigenic variant specimens and/or virus samples to WHOCCs. Factors affecting the timely sharing of viruses include the sampling strategies used by NICs, the frequency and timing of virus shipping in the context of the biannual WHO VCMs and potential financial considerations. Other potential factors include the impact of international agreements on the sharing of seasonal influenza viruses, such as the recently implemented Nagoya Protocol. The related challenge of ensuring the representativeness of sentinel surveillance sites, and of NIC sampling, selection and shipping strategies, is compounded by the crucial importance of the timing of influenza epidemics in relation to the timing of the VCMs.

Despite the historical expansion of GISRS coverage there remains a need for improved reporting of influenza surveillance data and for the more timely and routine sharing of influenza viruses, especially by countries in currently under-represented regions of the world. The absence of national influenza vaccination policies in many tropical and subtropical countries also means that a large percentage of the world’s population does not have access to the benefits of such vaccination. Even where vaccines are used in such regions of the world there is often a lack of clarity regarding the basis upon which vaccines are selected for national programmes and a lack of surveillance and other data to support vaccination timing decisions.

It has been calculated that simplistically applying the principles of the right-size approach outlined above to international seasonal vaccine virus selection, but using a 5% threshold for classifying drift variants, would require 59 viruses per virus subtype/lineage per month per WHO region. This equates to 16 992 viruses forwarded each year for the purpose of developing the WHO recommendations on vaccine composition. Recent WHO data indicate that this would result in an increased workload for WHOCCs, potentially requiring an increased frequency of NIC shipments, and increased complexity necessitating the revision of current WHO guidance on sample submission, participation reminders, and the monitoring of submissions and surveillance data to allow for samples of lower prevalence to be actively solicited. Although challenging and requiring an increased frequency of specimen and/or sample shipping, such an approach might mean that relatively minor changes in the current system could result in meaningful improvement in both data strength and interpretation without any reduction in current surveillance goals, coverage and effectiveness. Potential specific gains include improved geographic representativeness and better understanding of the statistical strength of data prior to the biannual VCMs, a more-even annual workflow for laboratories and the more-timely follow-up of seasonal viruses of high interest.

Although WHO GISRS capacity has been strengthened in recent years much of this has focused on ensuring the detection of viruses with pandemic potential. There is thus a need for
A systematic review of seasonal influenza surveillance capacities and capabilities, which could potentially be facilitated by the forthcoming 3–5 year workplan of the Pandemic Influenza Preparedness (PIP) Framework for the sharing of influenza viruses and access to vaccines and other benefits. Such a systematic review could then inform a process of national capacity-building for influenza surveillance within WHO GISRS. This could include the enhancement of NIC technical capacities for analysing influenza A viruses beyond simple subtype identification, and the acceleration of new laboratory assay development by WHOCCs. In the medium to longer term such a process of review and capacity-building could also be applied to the integration of emerging technologies into routine surveillance and virus-characterization, in particular the use of next generation sequencing techniques (see section 4).

1.3 Proposed action points

- a. Continue to promote global NIC capacity building by communicating to national authorities the vital public health contributions made by NICs, and the need for continued and enhanced support for their activities.
  
  [Near-term action – WHO]

- b. Review the current global influenza surveillance landscape and approaches in the context of the vaccine virus selection process in order to promote the optimal and strategic deployment of available resources, and to identify opportunities for strengthening WHO GISRS epidemiological and laboratory capacities, including in under-represented world regions.

  [Near-term action – WHO]

- c. Provide support to national and regional efforts to improve the collection, analysis and use of influenza surveillance and burden data in tropical and subtropical countries.

  [Near- to mid-term action – WHO]

- d. Provide revised detailed operational and other guidance to NICs in key areas such as virus sampling and selection strategies, the reporting of virus activity to the WHO FluNet platform at both national and subnational levels, the timely provision of genetic data to publicly available databases, and the optimal frequency of virus sharing – including original clinical specimens – to facilitate the timely shipment of representative viruses to WHOCCs in time for characterization prior to the biannual VCMs.

  [Near-term action – WHO and WHOCCs]

- e. Further strengthen WHO technical, logistical and other assistance to NICs across the broad range of sampling, reporting, shipping and related activities required to ensure the timely sharing of information and representative viruses.

  [Near-term action – WHO]

- f. Improve communications between WHOCCs and NICs, and between NICs and national laboratories, with a greater emphasis on tailoring guidance to specific seasonal and national circumstances, including for example the provision of timely and up-to-date information from WHOCCs to NICs on difficulties in virus isolation as well as on genetic markers observed in low-reacting samples.
[Near-term action – WHOCCs and NICs]

g. Further develop and evaluate the concept of “right-sizing” of global seasonal influenza surveillance and assess relevant WHOCC capacities as part of evaluating its feasibility.

[Mid-term action – WHO and WHOCCs]
2. Virological characterization and evaluation of candidate influenza vaccine viruses

2.1 Overview and current issues

For over 60 years the antigenic characterization of influenza viruses has traditionally relied upon the use of the haemagglutination inhibition (HI) assay to evaluate the ability of specific antibodies to inhibit the binding of virus HA to RBC receptors. As a surrogate for virus neutralization, the HI assay is routinely used to inform the biannual WHO recommendations on influenza vaccine composition. However, since its emergence in 1968, the current A(H3N2) sub-type has progressively displayed reduced avidity in terms of HA binding to chicken, turkey and guinea-pig RBC receptors, with the resulting loss of binding to all but the latter type of RBC. In addition, a mutation in the NA of A(H3N2) viruses at residue 151, which occurs in recent MDCK cell isolates, results in RBC binding by the NA component, necessitating the addition of the antiviral oseltamivir to the HI assay to exclude this effect. During 2013–14 further evolution in the HA resulted in some MDCK-cell propagated viruses failing to agglutinate guinea-pig RBCs, impacting upon both the detection and antigenic analysis of the seasonal A(H3N2) component. Even with the addition of oseltamivir the majority of viruses in the H3 clade 3C.2a that emerged during 2014–15 could not be analysed by the HI assay.

In combination with the above HA assay issues, it has long been recognized that the traditional propagation of influenza viruses in embryonated chicken eggs can select for HA sequence changes that influence antigenicity. This complicates vaccine composition decisions, particularly in relation to recent A(H3N2) viruses in the 3C clade. Contributing to difficulties in interpreting HI data for vaccine virus selection, ferret sera raised against high-yield reassortant viruses shows a greater differential reactivity with recent isolates than sera raised against the parental egg isolate.

Increasingly, virus neutralization (VN) assays that directly detect antibodies that prevent cell infection have been used to complement HI antigenic analysis using the same reference sera, and to more sensitively measure human antibody titres, particularly at the threshold of detection. VN assays developed to date include a WHO two-day assay originally developed in 1988 (9) and modified in 1999 (10) using an ELISA readout – a variation of this assay using a three-day incubation with CPE or HA readout has been shown to be equivalent (11). In addition, pseudotype neutralization assays using retroviral vectors expressing HA and NA (12–14) have been used primarily for human serology for highly pathogenic avian influenza viruses to overcome biocontainment issues. In recent years a number of plaque/focus reduction VN assays based on micro-plaque reduction assays have also been developed and optimized using MDCK cells for A(H1N1) and B viruses, and MDCK-SIAT cells (expressing increased levels of α2,6-linked sialic acid) for A(H3N2) viruses (15–17). Such assays are completed in 1 day rather than 3 and demonstrate 10- to 100-fold higher virus titres compared to other microneutralization assays and are more suitable for early-passage isolates.

Substantial experimental evidence is accumulating which indicates that antibodies directed against influenza virus NA contribute to protection from illness. For example, individuals with previously acquired NA antibodies that were cross reactive with the 1968 A(H3N2) or 2009 A(H1N1) pandemic viruses were found to be less likely to be infected or to suffer
infection-related illness. An enzyme-linked lectin assay (ELLA) for quantitating antibodies against NA has now been evaluated and shown, through the CONSISE consortium,\(^1\) to exhibit good consistency when used in conjunction with an antibody reference preparation (18). Published ELISA assays are also available for quantitating NA in vaccines (19, 20) and the results of NA-capture ELISA assays have been demonstrated to be indicative of the amount of active native NA present and hence of NA immunogenicity.

Vaccine effectiveness (VE) studies also represent another potential approach to the strengthening of vaccine virus selection. The recently developed “test-negative” design has now been widely adopted for VE estimations in which a sample of patients presenting with influenza-like illness (ILI) are tested for influenza and a comparison made between the odds of vaccination among those with laboratory-confirmed influenza infection and the odds of vaccination among those who test negative. Such an approach could, at a cost, be built into existing ILI surveillance programmes in a number of countries. However, as VE estimates are influenced by the accuracy of patient vaccination data, and subject to limitations based on sampling size and vaccination rate in the sampled population, subgroup analysis for age or virus type/subtype are not currently possible in many settings. The Global Influenza Vaccine Effectiveness (GIVE) initiative currently provides end-of-season VE estimates to the biannual VCMs. This initiative coordinates and reports on findings from both the northern and southern hemispheres based on a combination of general practitioner and hospital surveillance.

### 2.2 Challenges and opportunities

VN assays using reference ferret antisera perform comparably to HI assays in differentiating between viruses. In addition, plaque-reduction VN assays display two- to four-fold higher antibody titres, can be used to characterize recent A(H3N2) viruses that lack HA binding and appear not to be significantly affected by factors such as NA mutations that contribute to RBC binding. VN assays are also generally less likely to be affected by the passage history of viruses. The disadvantages of VN assays relate to throughput limitations compared to HI (particularly automated HI) and longer completion times (1–3 days compared with ~8 hours for HI). In addition, the sensitivity and consistency of VN results are more dependent upon reagent (cell and virus) quality.

In VN assays, antisera against cell culture propagated viruses also show the greatest specificity for isolates in their particular clade (for example, 3C.2a, 3C.3a or 3C.3b, with those for the latter clade being highly specific) whereas antisera raised against egg-propagated viruses are less specific, show high homologous titres and significantly lowered titres against recent isolates even in their own clade. Each of these clades also displays different and distinct patterns of egg-adaptive changes, including frequent receptor binding substitutions or polymorphisms for the aspartic acid residue at HA1 residue 225.

Although NA antigen content is indicative of vaccine immunogenicity and generally provides a broadly reactive immune response within a subtype, virus-to-virus variability in the HA:NA ratio has been observed. While vaccine manufacturers test for NA activity in bulk vaccines there is currently no requirement to quantify the antigen in the final product and content

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\(^1\) Following its establishment in 2011, the Consortium for the Standardization of Influenza Seroepidemiology (CONSISE) has worked to standardize the seroepidemiology of influenza and other respiratory pathogens, and to develop comprehensive investigation protocols for use in responding to both seasonal and potentially pandemic influenza viruses, and other respiratory pathogens. See: https://consise.tghn.org/
standardization is thus not possible. Genetic and antigenic mapping of HA and NA indicate that NA antigenic drift is discordant with that of the HA for both N1 and N2 NA subtypes. The selection of a virus with the appropriate NA may thus be important in vaccine production, particularly where there is an HA mismatch. If future vaccine developments were to offer the opportunity to standardize NA content, the amount of NA needed to generate the required level of antibody would need to be determined.

End-of-season VE studies have recently been used to influence vaccine virus selection for the subsequent season. This required observational studies based on real-world vaccine performance rather than experimental studies that demonstrate efficacy in an idealized environment. There is also a need to compare data from different representative populations, and to consider how late in a season useful data on antigenically drifted viruses could be obtained. Related efforts already under way have included evaluation of the feasibility of measuring product-specific VE in the EU/EEC against the backdrop of the newly implemented 2015 requirement by the European Medicines Agency (EMA) that immunogenicity criteria be replaced by the monitoring of vaccine performance. Initial simulation of the requirements for such monitoring by the GIVE partner Epiconcept indicated that for the most commonly used influenza vaccine product (achieving 6.2% vaccination coverage in the population) and a case–control ratio of 1:3 the calculated sample size required to detect a VE of 32.5% with a confidence interval spanning 20% would be 5648. However, assessing VE for all vaccine brands in the elderly, at current uptake levels, would require almost 30 000 ILI patients and approximately 6000 participating general practitioners which would not be achievable.

In light of the central importance of pre- and post-vaccination human serum in all the above and other key areas, efforts could also be usefully undertaken to improve the sourcing and availability of human serum panels to facilitate the use of such serum in a broad range of both routine and advanced antigenic characterization and related activities.

2.3 Proposed action points

a. Identify additional sources of human pre-/post-vaccination human serum panels to better characterize human antibody responses to newly circulating viruses for enhanced identification of significant antigenic variants.

[**Near-term action** – WHOCCs, WHO ERLs; and WHO]

b. Create a VN working group to further optimize and harmonize protocols and VN testing strategies to improve robustness and simplicity, and thus allow for higher throughput antigenic characterization of seasonal influenza viruses based upon recent advances in this technology.

[**Near-term action** – WHOCCs and WHO ERLs]

c. Establish a broadly based NA interest group to address issues related to the feasibility of NA as a potentially quantified vaccine antigen, particularly as next-generation recombinant vaccines are developed.

[**Mid-term action** – WHO, WHOCCs and manufacturers]

d. Harmonize VE protocols among different studies/sites so that data can be combined for more robust analyses.

[**Mid-term action** – GIVE, I-MOVE and other VE networks and initiatives]
3. The challenge of late-emerging variants

3.1 Overview and current issues

During the 2014–15 northern hemisphere winter, a number of variant A(H3N2) viruses emerged (Fig. 1) which were not well neutralized by antisera to the selected vaccine virus (A/Texas/50/2012). The subsequent predomination of these viruses then resulted in low VE (21–23).

Fig. 1. Phylogenetic comparison of A(H3N2) genes – November 2013 to March 2015
In the USA this precipitated a Congressional hearing on the reasons for the mismatch and on the decision not to produce a supplementary monovalent A(H3N2) vaccine. A corresponding CDC review of the relevant surveillance data, presented at the meeting, showed that:

- During a predominantly A(H1N1) season from October 2013 to 1 February 2014, CDC characterized 86 A(H3N2) viruses, all of which were vaccine (A/Texas)-like.
- In February, 80% of the 161 A(H3N2) viruses characterized belonged to the 3C.3 clade and 20% to 3C.2, with only 2 viruses (<1%) exhibiting a reduced titre to the A/Texas/50/2012 vaccine virus sera.
- By March the corresponding figure for reduced titres was 4%, increasing to 11% in April, 31% in May and 36% during June–August.
- Candidate viruses A/Palau/6759/2014 and A/Switzerland/9715293/13 were sent for reassorting on 23 June and 2 July respectively. A/Palau was unsuitable but A/Switzerland was successful and selected at the WHO September VCM as the A(H3N2) component for the upcoming 2015 southern hemisphere vaccine. By this time, 49% of the A(H3N2) viruses analysed exhibited reduced titres to the northern hemisphere vaccine virus sera but the United States vaccination campaign had already commenced.
- On 10 October, testing of the A/Switzerland reassortant was complete and it was shipped to vaccine manufacturers.
- During October–November 2014, 67.5% of tested A(H3N2) viruses exhibited reduced titres to the A/Texas sera, and in November the level of ILI visits in the USA exceeded the national baseline thus marking the beginning of the influenza season. During February–May hospitalization rates among those aged ≥65 years was the highest since record keeping commenced in 2005–6.

Following the antigenic drift and consequent antigenic divergence between vaccine and circulating viruses, the United States Biomedical Advanced Research and Development Authority (BARDA) assessed the potential for improving seasonal influenza vaccine development and production, and revising vaccination timelines, as possible strategies for increasing the likelihood of achieving a timely and antigenically well matched influenza vaccine for use in the USA. Following the establishing of an agreed baseline “process map” for current vaccines (including the vaccine administration campaign) a table-top exercise was undertaken which simulated: (a) a scenario involving a delayed vaccine virus decision, designed to stress but not change the current process in which the WHO decision on one vaccine virus is delayed until mid-April; and (b) a scenario involving a revised vaccine virus selection in which a changed recommendation is made for one vaccine virus as late as mid-June. The outcome of the exercise indicated that a recommendation for a single subtype that was changing could be made in March or possibly later, facilitated by a staggered process in which the recommendations for the more stable subtypes were made in January or February. It was also apparent however that delaying the vaccine composition recommendations beyond mid- to late March would adversely affect vaccine administration and consequent uptake and was not considered feasible.

In Europe, influenza vaccination policies and programmes are decided upon by individual European Union member states, with non-binding European Council seasonal influenza vaccination recommendations made for vaccination in older age groups (ranging from >50 to >65 years across various countries), for those with chronic medical conditions and for health care workers. The European Centre for Disease Prevention and Control (ECDC) is working
to improve influenza surveillance and to explore ways of shortening the vaccine production timeline. Conversely, the extent to which a delayed vaccination programme could be accommodated varies widely by country with least tolerance observed in countries in Western Europe which have the greatest vaccination coverage and where the influenza season starts early. The examples of England and Wales in the United Kingdom and Finland illustrate the variability of influenza epidemiology and vaccination scheduling within Europe. In England and Wales vaccination in recent years has typically commenced in early October and approaches peak uptake by early December for both adult and paediatric vaccination programmes. Based on ILI consultation figures for 1988–2012, the influenza season normally commences prior to the end of the year with only limited opportunities to delay vaccination. In Finland, although vaccine also becomes available in October, vaccination commences in early November with peak uptake preceding Christmas and the peak of the influenza season in January–February. The current lead-in time between vaccine receipt and administration may thus provide some degree of flexibility. Nevertheless, the mid-season updating of a multivalent vaccine is not regarded as a viable option for European countries and production of an off-cycle monovalent vaccine, essentially equivalent to a pandemic vaccine, would need to be a global decision presumably led by WHO and based on a global threat assessment.

The International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) Influenza Vaccine Supply (IVS) Task Force was established in 2002 to serve as a single point of contact between manufacturers and public health stakeholders and to strengthen collaborative approaches with regard to seasonal and pandemic influenza preparedness. The Task Force also provides significant funding for research and development activities in areas such as virus isolation in eggs and the preparation of high growth reassortants which has long been the basis of influenza type A seasonal influenza vaccine production. IVS has now initiated a cross-functional group to review potential areas for improvement in the seasonal vaccine production process, focusing on the selection of candidate vaccine viruses for production, timely preparation and supply of calibrated reagents, and the ability to respond to the delayed supply of candidate vaccine viruses. In 2015, IVS participated in two mismatch-mitigation discussions in the USA in relation to late-emerging antigenic variants.

3.2 Challenges and opportunities

Under current system constraints, the recognition of a vaccine mismatch in April–May – as occurred in the 2014–15 northern hemisphere mismatch – does not allow sufficient time to prepare a well-matched vaccine for the upcoming influenza season. To compound the complexity of the situation, the late emergence of a variant does not in itself equate to its subsequent predominance, and time is therefore needed to evaluate its spread and likely epidemiological significance. As a result, there is currently no scope for bringing forward by a month or more the dates of the biannual VCMs. Conversely, in the longer term, significantly delaying the VCMs would only be feasible if significant programmatic delivery time savings and other mitigating gains could be made to alleviate the degree of at-risk manufacture and ensure no adverse public health impact caused by delayed vaccine delivery.

The industry preference for working to the current recommendations timeline was emphasized and the potential adverse impacts of any delays or late vaccine virus changes highlighted. Concerns were specifically expressed regarding potential product write-offs, impacts on contractual obligations to ensure vaccine availability, decreased vaccination rates and the need for public information programmes in the event of delays or a follow-on monovalent vaccine. Further detailed studies and analysis would be required of the costs and
benefits of delayed vaccine decisions, the use of follow-on monovalent vaccine, the influence of market forces and cost-liability issues, and of effective public communication strategies.

It was acknowledged however that if absolutely required then delaying the decision on a single vaccine virus until mid-March was theoretically feasible, especially in the context of a staggered vaccine production process supported by open information sharing on an ongoing basis to allow manufacturers to prepare for delays. To facilitate this, and given the apparent greater flexibility in the vaccine development and production timeline compared to the vaccine administration timeline, broad agreement was reached and support expressed, including by industry, for efforts to strengthen and refine early-stage activities. These include surveillance and related laboratory testing for the early detection and notification of variants, timely shipping of virus samples and clinical specimens, preparation of high-yield candidate vaccine viruses (including influenza B viruses) and ensuring the availability of alternative potency assays and reagents. It was recognized that in some of these areas further careful consideration would need to be given to regulatory, cost and other issues, particularly in relation to the use of technologies such as reverse genetics and the use of heterologous reagents for potency testing. At the same time, it was felt that should the public health threat appear to be sufficiently high that a favourable regulatory environment may result.

3.3 Proposed action points

a. Communicate on a more regular basis with industry on the characterization of circulating viruses, and on the availability of potential wild-type vaccine viruses and reassortant candidate vaccine viruses.

[Near-term action – WHOCCs, WHO ERLs and WHO]

b. Develop a framework process for a scenario in which the WHO recommendation for a single vaccine virus is delayed, incorporating the prompt initiating of ad hoc teleconferences and other strategies and guidance for communicating with IFPMA in the event of the late emergence of significant drift variants.

[Near-term action – WHOCCs, WHO ERLs and WHO]

c. Improve the development and timing of vaccine potency reagents and assays by further evaluating the use of heterologous reagents for potency testing and of potency reference antigen reagents using viruses other than candidate vaccine viruses (such as reverse genetics viruses or wild-type viruses, and recombinant-derived proteins).

[Near-term action – WHO ERLs]

d. Develop alternative potency assays using both antibody-based and antibody-independent technologies.

[Near-term action – WHO ERLs]

e. Improve the current characterization and suitability evaluations of cell-propagated viruses to support the work of manufacturers towards the licensure of cell-based influenza vaccines that use cell-propagated vaccine seed viruses.

[Mid-term action – WHOCCs]

f. Develop candidate vaccine viruses with improved yield by expanding current laboratory capacity for classical reassortment, developing improved influenza B high-growth donor viruses, and initiating a systematic evaluation of the potential
application of synthetic biology and reverse genetics techniques to the production of egg-based A(H3N2) candidate vaccine viruses with high-growth properties.

[Mid-term action – WHOCCs and WHO ERLs]
4. Application of next generation sequencing to influenza surveillance and vaccine virus selection

4.1 Overview and current issues

Activities are continuing in a range of countries, agencies and GISRS laboratories in relation to the anticipated paradigm shift in influenza surveillance and related processes associated with the introduction of next generation sequencing (NGS) and whole genome sequencing (WGS) of influenza viruses. In England, influenza surveillance through the public health laboratory network is geared towards providing early warning of winter pressures on the public health system, triggering antiviral prescribing, monitoring vaccine uptake and effectiveness, and identifying new variants and disease syndromes. Influenza virological surveillance is largely focused on community cases through sentinel practice reporting – with a subset of ILI patients being swabbed – plus hospitalized cases. However, in recent years, the increasing use of PCR diagnosis in hospitals has resulted in a decline in the submission of influenza isolates from hospital laboratories, with none being received in 2014–15. This development has prompted a shift towards the submission of original clinical material to the NIC. The criteria established for submission include a Ct< 32 (PCR cycle threshold); representative (early, middle and late season) and unusual samples; samples from vaccine failures and fatalities; samples from suspected drug resistance; and (since the 2009 H1N1 pandemic) increased sampling from intensive care unit cases. In 2007–09, a Sanger WGS methodology was developed and initially applied to fatal cases, and then expanded from 2009 to A(H1N1) pandemic samples, fatal cases, household transmission studies and antiviral resistance cluster transmission. In light of the perceived benefits of WGS in surveillance, and following a successful comparison of Sanger and NGS (Illumina) methodologies, a partial switch to the latter occurred in 2014–15. ISO 15189 validation and the development of work systems for data analysis and direct uplift of sequences to GISAID are in progress.

The purchasing and successful trialling of a Personal Genome Machine (Ion Torrent) by the WHOCC Melbourne in late 2014 was quickly followed by collaboration with the Duke-NUS Medical School in Singapore in the development of a data-analysis pipeline, based largely on publically available software programmes. Once optimized, and its broader utility on other NGS platforms evaluated, the WHOCC Melbourne will make this pipeline freely available. Following confirmation of the complete comparability of results using the NGS and Sanger platforms, a series of applications are now planned, including the conducting of clade-specific VE studies by direct sequencing from original clinical samples. Since 2005, the NIAID Collaborative Influenza Genome Sequencing Project has generated publically available sequence data for 18 000 influenza genomes of viruses collected worldwide.¹ Examples of the use of the generated data include the conducting of sequence alignments, constructing phylogenetic trees, tracking the evolution of seasonal influenza viruses, including the 2012–13 A(H3N2) viruses (24), and tracking the global movement of human and animal influenza viruses. In addition, a method of combining antigenic mapping with sequence data – Distancing of Antigenicity by Sequence-based Hierarchical Clustering (DASH) – in order to determine the suitability of candidate vaccine viruses is currently under assessment.

¹ Sequences are publically available on GenBank and the Influenza Research Database – http://www.fludb.org – which provides the facility to search the database, analyse data online and submit sequences.
A CDC programme to transform the virological surveillance paradigm from phenotyping first to genotyping first is dependent upon high-throughput NGS and a strong informatics infrastructure. The goal is to genotype all viruses received in order to propagate a selected subset based on bioinformatic genome analysis. Genotypic determination of viruses obtained from original clinical samples is based upon multi-segment RT–PCR and MiSeq platform NGS and has resulted in greatly increased genome and isolate analysis over the previous two seasons. Genome assembly and curation has now been migrated to a “virtual private cloud” to allow for the transfer of the NGS pipeline to United States Public Health Laboratories. A 2015 pilot study at the Wisconsin State Laboratory of Hygiene, involving the sequencing of viruses received from a catchment area of submitting laboratories, successfully validated the genomic NGS sequencing approach to be used and will be extended to two additional catchment areas in 2016 and 2017 to cover all CDC National Reference Laboratories.

In relation to potential pandemic vaccine virus selection, an A(H5N8) virus (A/gyrfalcon/Washington/41088-6/2015) was identified in the USA in December 2014 and was subsequently recommended by the following VCM for use as a candidate vaccine virus. Despite the successful application of a synthetic genomics approach based on the use of a PR8 backbone, this virus exhibited poor immunogenicity against subsequent H5 isolates found to have several HA mutations. H5 variants with these specific point mutations are now being synthesised using a DNA printer (https://www.sgidna.com/bxp3200.html)⁴ to determine the impact of such mutations on the key vaccine-development characteristics of antigenicity and growth. Such synthetic genome approaches are likely to find increasing application in combating emerging viruses and strengthening seasonal influenza surveillance.

4.2 Challenges and opportunities

It is clear that NGS is a powerful tool with a broad range of current and potential future applications in influenza surveillance and vaccine development activities. Key benefits of using sequence data include the ease with which direct comparisons can be made, as opposed to HI data comparisons which require algorithms and expertise. Furthermore, sequence data can be provided by NICs at the time of sample shipping to facilitate the selection viruses for egg or cell culture isolation and to permit clade-specific VE estimations. Such early provision of sequence information by NICs can also potentially shorten the time frame for deriving suitable egg isolates and reassortants for use as candidate vaccine viruses, which currently takes several months.

At present, the use of NGS can allow WHOCCs, and in some cases NICs, to sequence influenza virus isolates and clinical specimens without prior knowledge of type or subtype. In addition to being potentially faster and cheaper than Sanger sequencing for whole genome analysis it can also provide high throughput for routine HA, NA and M gene sequencing. NGS technologies are also capable of identifying mixed infections and single nucleotide polymorphisms and are likely to become increasingly important in ensuring the timely selection and virological characterization of candidate vaccine viruses.

A number of robust NGS instruments and platforms are now available that are suitable for use with influenza viruses, each of which has specific advantages and disadvantages. For

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¹ The mention of specific companies or of certain manufacturers’ products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned.
individual laboratories, the optimal selection is likely to be determined by issues of budget, required throughput and related factors (Fig. 2). The adoption of multiple platforms across different settings is likely to highlight, and thus help to reduce, any platform-specific effects, with both multiplex real-time PCR and single primer amplification providing a suitable basis for use. Cloud computing approaches in which data can be uploaded and analysed using a series of free or commercial applications are currently under development, and are likely to become more accessible in the near future thus improving the feasibility of broader NGS uptake.

Fig. 2. Comparative features of different NGS platforms

Achieving the economy-of-scale and related benefits of NGS is, however, also accompanied by the demands of initial sample preparation, and of “big data” storage and analysis which are currently rate-limiting steps. Associated challenges include the need for adequate viral loads in original specimens, preventing host and other pathogen gene interference, and avoiding biases in coverage and sampling. Improving capability will thus require addressing issues such as sampling and operating protocols, data quality, process efficiency, workforce planning, automation, and the handling, analysis, storage and sharing of data and metadata. Corresponding efforts will also be required to preserve laboratory virus-culture capabilities at the national level and to right-size and improve culture systems. In order to fully incorporate NGS and WGS technologies into the vaccine virus selection process new approaches will also be needed for better integrating genetic and phenotypic data. The use of sequence data to prioritize the phenotypic characterization of viruses also has the potential to improve data timeliness and availability during the biannual WHO VCMs. Current constraints include the need to conduct VCMs before the end of the season (with inadequate opportunity for HI or VN analysis of samples received in the preceding 3 weeks) and delays in virus shipment.

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1 The mention of specific companies or of certain manufacturers’ products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned.
As with other influenza surveillance activities (see section 1 above) the concept of capacity building in NICs and other GISRS laboratories provides an overarching approach for systematically addressing the broad range of capabilities needed to realize the possible gains of a paradigm shift towards WGS and NGS. Potentially crucial opportunities in this area could include: (a) the putting in place of broad integrated national approaches to disease surveillance involving other public health needs; (b) strengthened engagement with the global donor community; and (c) harnessing of the capacity-building components of major international health agreements such as the forthcoming 3–5 year workplan of the Pandemic Influenza Preparedness (PIP) Framework for the sharing of influenza viruses and access to vaccines and other benefits.

4.3 Proposed action points

a. Conduct a survey of current Sanger and NGS sequencing capabilities and activities undertaken by NICs, which also incorporates evaluation of the needs of laboratories in moving towards a sequencing-driven paradigm of influenza surveillance and vaccine virus development.

[Near-term action – WHO and WHOCCs]

b. Document the range of current capacity-building opportunities and potential synergies for expanding the use of NGS within the WHO GISRS, including training provision by WHOCCs and guidance development for NICs on the steps required in areas such as platform selection, large-scale computerized data storage, and data analysis and reporting.

[Near-term action – WHO]

c. Develop revised and strengthened guidance for NICs on the required characteristics and optimal extent of sequence and antigenic data, and on the key need to ensure the timely submission of sequence data and virus samples to WHOCCs, along with prompt uploading of data and other information to publicly available reporting platforms and databases, in support of vaccine virus selection.

[Near-term action – WHOCCs]

d. Identify approaches to facilitate the capture of genetic polymorphisms in NGS databases, and the routine retrieval of such data.

[Near-to mid-term action – WHOCCs]

e. Work with the animal health sector on cross-cutting issues such as quality assurance standards, data storage, analysis and management, sharing of bioinformatics pipelines and capacity building.

[Near-term action – WHOCCs]
5. Potential role of enhanced evolutionary analysis in informing influenza vaccine virus selection

5.1 Overview and current issues

A computer package (BEAST)\(^1\) has been developed to integrate sequence data with other virus characteristics such as geographical location. This approach has now been used to analyse the factors that influence the rates of transition of influenza viruses between locations. In line with earlier findings, the highest correlation was found between A(H3N2) virus transition and aircraft passenger numbers flying from one location to another. The geographical location of the trunk of the phylogenetic tree reveals those locations where the virus has persisted for longer periods – in the case of the A(H3N2) viruses studied these tend to be high-population areas with less-marked influenza seasonality such as Southern China, South-East Asia and India. In highly seasonal locations, genetic variants appear to persist for less time, in most cases less than a year for A(H3N2) viruses, highlighting the fact that tracking evolution in a single country is unlikely to be informative.

A publically available online database (nextflu – http://nextflu.org/H3N2/3y/) has been designed to provide an almost real-time sense of what is happening with A(H3N2) influenza and to support vaccine virus selection by determining the predictors of successful virus clades. This database collects genetic and epidemiological information from the GISAID epiflu database which is then processed and displayed as annotated phylogenetic trees; the data for which can be explored for a range of variables including sampling date, geographical region, epitope and non-epitope mutations, specific mutations and their frequency, and specific virus strains (25). Further model development based upon the mapping of A(H3N2) HI data onto the phylogenetic tree has been undertaken which allows for prediction of antigenicity from HA sequence data, and a browser application has been developed (26) that visualizes antigenic data on a continuously updated phylogeny (http://HI.nextflu.org). This model has an accuracy of 81\% in predicting the growth and decline of clades, indicating that unknown factors are also contributing in some cases. When used to predict the A(H3N2) clade that would become prominent in 2014–15 using samples collected in May 2014, the 3C.3a clade appeared to be the most fit and it was not until collection dates reached early 2015 that clade 3C.2a emerged as the most fit.

Viral fitness, defined as the expected growth rate of genetically or antigenically related strains which translates into higher frequencies of clades at later time points is a potential predictor of virus evolution. Following the proposal of one such model based purely on genetic data and mutations outside and within epitopes (27) a two-phase fitness model has now been developed based on the use of integrated sequencing, phylogenetic, HI and epidemiological data (28). In the first phase, sequence data alone are used to identify potential variants and to select viruses for neutralization tests (27) followed in the second phase by the prediction of vaccine efficacy using HI data.

Investigations have also been carried out into the utility of generating potentially genetically advanced viruses by inducing the random mutation of the gene coding for the globular head of the HA protein. Following the compiling of a mutant virus library using reverse genetics, viruses with the potential to escape existing immunity can then be detected using ferret or

\(^1\) Bayesian Evolutionary Analysis Sampling Trees (BEAST) – http://beast.bio.ed.ac.uk/
human antibodies directed towards the parental virus. Application of this technique to pandemic A(H1N1), A(H3N2) and A(H5N1) strains demonstrated that: (a) the A(H1N1) variants detected were able to infect ferrets that had previously been infected by, and become immune to, the parental virus; (b) A(H3N2) (A/Texas/50/2012) virus mutants were created that clustered phylogenetically with clades 3C.2a and 3C.3a and contained the F159Y and F159S substitutions; and (c) a series of H5 virus mutants filled an antigenic space around a hypothetical ancestral node of clade 2, and were thus potential candidate vaccine viruses.

5.2 Challenges and opportunities

Complex modelling based on modified antigenic cartography can reduce the “noise” caused by serum quality and potency variability, and has the potential to combine antigenic and phylogenetic data into a single evolutionary analysis that quantifies both the antigenic and evolutionary distances between strains (29). Such approaches have shown that: (a) the antigenic drift of influenza A(H3N2) is more rapid than that of seasonal A(H1N1), followed by the type B Victoria lineage, with the B/Yamagata lineage being the slowest, with all drift approximately occurring in proportion to the rate of genetic change; and (b) the rate of antigenic drift correlates well with the relative incidence of the various types/subtypes based on data from the USA.

Using this approach to antigenically compare vaccine viruses with circulating strains for the H3 subtype 1987–2009 has also demonstrated that at the time of selection vaccine viruses were on average 0.46 antigenic units ahead of the mean (where one antigenic unit translates to a two-fold drop in HI titre between virus and sera). However, by the time of vaccine use their antigenic distance from the mean was 3.25 antigenic units. As antigenic evolution is a discrete and punctuated process determined by a limited set of amino acid residues, further work is under way to identify the amino acids in the HA of A(H3N2) viruses involved in (though not necessarily responsible for) the jump from one antigenic cluster to the next. The amino acid residue 159 is of particular interest in view of its involvement in the evolution of clades 3C2.a (phenylalanine to tyrosine) and 3C3.a (phenylalanine to serine).

A study based on the A(H3N2) antibody landscape of individuals before and after infection, or following vaccination, highlighted the potential benefits of using antigenically advanced A(H3N2) viruses as vaccine viruses (30). Such viruses were either selected from isolates that lay beyond the current clade or were generated by random mutation. The study found that antibody levels following vaccination increased broadly to both contemporary and previously encountered viruses back to those circulating at the time of initial exposure, with immunization with the more advanced virus providing as good or better responses against an earlier strain compared with immunization with the earlier strain, possibly due to a lessened effect of pre-existing antibodies. Such an approach may also provide protection against potential pandemic viruses such as current H5 clade 2 viruses, where the creation of a hypothetical HA around the hypothetical ancestral node of this diversified clade might potentially allow for a second vaccine dose capable of diversifying the immune response.

5.3 Proposed action points

a. Organize a meeting to broaden and strengthen understanding of different modelling systems between the modellers themselves and WHOCCs, and to assess how best to evaluate and harness the potential season-by-season contributions of each system to
the vaccine virus selection and development process; including through retrospective evaluation of modelling performance.

[Near-term action – WHOCCs, with support from WHO and BARDA]

b. Develop a route for the more formalized incorporation of modelling data and findings into the considerations of the biannual WHO VCMs.

[Near-term action – WHOCCs]
6. Development of pandemic and broadly protective vaccines

6.1 Overview and current issues

The selection, development and evaluation of candidate pandemic vaccine viruses are limited to viruses that have caused human infections during the preceding period. This process involves both antigenic and genetic characterization by the same laboratories involved in seasonal vaccine decisions plus the H5 influenza reference laboratories and representatives of the OIE–FAO Network of Expertise on Animal Influenza (OFFLU). There are significant constraints on this process arising from the wide diversity of viruses under consideration, the small numbers characterized (particularly antigenically), the generation of a substantial proportion of the total data outside the WHO GISRS and a lack of epidemiological data. In addition, the majority of candidate pandemic vaccine viruses are likely to never be used in vaccine manufacture.

In the 7 months preceding the September 2015 southern hemisphere VCM high levels of influenza H5 activity had been detected in birds, involving multiple HA clades and multiple NA subtypes. Despite the occurrence of human cases, no human isolates were received by the WHOCC Memphis during this period and sequence information was available for only two viruses. This situation reflected recent trends of reduced availability and receipt of viruses from human cases in a number of affected countries, and inconsistent receipt of representative animal viruses. In the absence of human viruses, animal viruses can be a good surrogate and may provide an early indication of the potential need for a new vaccine candidate. However timeliness is vital, as evidenced by the selection of one previously widely spreading H5 pandemic vaccine virus in the February VCM which by the September VCM of the same year was assessed as being a poor candidate against circulating strains.

In June 2015, a WHO consultation was held to develop a draft operational framework to guide a pandemic vaccine response, which included the recommending of mechanisms for switching from seasonal to pandemic vaccine production. A further objective of the consultation was to help finalize the incorporation of a specific section on pandemic vaccine response into the 2013 WHO Interim Pandemic Influenza Risk Management (PIRM) framework for pandemic preparedness that had been developed in light of the 2009 H1N1 pandemic.1 A number of complexities and time-related challenges were highlighted in relation to pandemic vaccine production, with a detailed schematic process for a WHO pandemic vaccine response to emerging or potential influenza pandemics incorporated into the final consultation report (31). Other key consultation outcomes included: (a) improved understanding of the roles and responsibilities of stakeholders in terms of communication and interaction; (b) recognition of the need for WHO leadership through advisory bodies such as the IHR Emergency Committee in recommending a switch to pandemic vaccine production; (c) greater awareness of the importance of risk assessments in making the decision to stop seasonal vaccine production and switch to pandemic vaccine production; and (d) recognition of the need to review and improve current procedures for assessing the biocontainment requirements of candidate vaccine viruses.

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1 Other relevant WHO pandemic response initiatives and resources include: (a) the International Health Regulations (2005) under which a public health emergency of international concern can be declared and the WHO Director-General advised to declare a pandemic; (b) the Pandemic Influenza Preparedness Framework for the sharing of influenza vaccines and access to vaccines and other benefits; and (c) the Global Action Plan to increase supply of pandemic influenza vaccines.
In relation to the development of broadly protective (“universal”) influenza vaccines a number of studies have focused on the potential role of either cross-reactive antibodies or T-cell immunity. Potential viral targets for cross-reactive antibodies include the M2 and NA proteins, and the stalk region of the HA protein, while targets for cross-reactive T-cell immunity include the structural proteins, particularly NP and M1, non-structural proteins and polymerase proteins. Recent animal-model and human studies have therefore involved the investigation of a range of potential approaches, including cytotoxic T-cell (CTL) immunity, M2 protein ectodomain (M2e)-specific antibodies and HA stalk region antibodies.

The ability of CTLs to confer hetero-subtypic protection has been demonstrated for a number of influenza A subtypes in mouse-challenge systems. This has also been inferred from recent human studies in which a high frequency of pre-existing T-cells to conserved epitopes correlated with less severe A(H1N1) pandemic infections. CTLs acquired by exposure to seasonal influenza A viruses have also been shown to cross react with viruses such as A(H5N1), A(H3N2)v and A(H7N9). Such immunity is however dependent upon successful delivery to antigen processing cells and requires the use of specialized delivery systems. The use of Modified Vaccinia virus Ankara (32) as a delivery system for nucleoprotein and M1 protein epitopes has been demonstrated to be effective in both mice and humans, including older humans. Challenge studies in humans have indicated that although such vaccination reduces disease severity it does not protect against infection and numerous issues remain to be addressed.

The protective effect of M2e-specific antibodies, via antibody-dependent cell cytotoxicity and antibody dependent phagocytosis of infected cells mediated through immunoglobulin Fc receptors, has long been demonstrated in animal models. However, it remains to be confirmed that human Fc receptors will contribute to this process. Although, early-stage human trials with a variety of M2e carrier protein constructs indicated that such vaccines were immunogenic and well tolerated, protection in humans has not been confirmed.

Antibodies directed against the relatively conserved HA stalk region have been shown to be protective in mice and have been the subject of recent interest. Monoclonal antibodies of both mouse and human origin have been derived that neutralize viruses of either group 1 or group 2 of the influenza A HA subtypes, or the entire group in the range H1–H17. A number of strategies have been explored for preparing vaccines which produce anti-stalk antibodies, including the use of free mini-HA particles (33) and a ferritin conjugate (34). When injected with adjuvant in mouse challenge studies both vaccines conferred protection against homologous A(H1N1) and selective heterologous influenza A viruses. Immunization with free mini-HA particles also reduced fever against sub-lethal H5 challenge in non-human primates, while the ferritin conjugate was partially protective against hetero-subtypic A(H5N1) challenge in ferrets.

6.2 Challenges and opportunities

A number of significant challenges and fundamental gaps remain to be addressed in determining optimal approaches to pandemic vaccine preparedness. Despite some indication that the degree of cross reactivity of antibodies generated by the current list of candidate pandemic vaccine viruses may be broader than that indicated by ferret data there is a lack of data on the precise nature of responses in humans. In light of substantial development and other costs associated with candidate pandemic vaccine viruses, and an ever-increasing list of
H5 candidate viruses being selected based on reactions with ferret sera, there is a need for more human serology data to more accurately assess the number of such viruses required.

There is also a need for continuing and strengthened collaboration between the human and animal influenza surveillance sectors, particularly with respect to those animal subtypes not currently responsible for human infection, and a systematic approach adopted towards reagent development. Currently only subtypes responsible for human infections are subject to review at the biannual WHO VCMs.

Serious constraints also persist on the distribution of reagents for candidate pandemic viruses, with a requirement for high-containment laboratories and a variety of different national shipping laws and restrictions involving both departments of health and agriculture. Reagent preparation itself can also prove problematic as many of the animal influenza viruses are poor immunogens and often require boosting by the injection of adjuvanted virus without clear evidence regarding the influence this may have on the specificity of the antibodies produced. Unlike seasonal influenza candidate vaccine viruses, most zoonotic candidate viruses are produced by reverse genetics as this can be achieved relatively quickly. However, a significant bottleneck exists in the form of regulatory requirements to test the safety of such viruses in ferrets and chickens. Given the satisfactory experience to date of H5 vaccine safety evaluations there may now be sufficient data to allow for a review of these and related requirements. In support of this, a review of the current evidence could be conducted to evaluate the case for de-risking the use of reverse genetics approaches in this context in order to better inform current national and other mandated risk–benefit analysis requirements.

Although strategies for streamlining a switch from seasonal to pandemic vaccine production and expediting licensing have been explored and further developed, the speed of switching will be dependent upon a clear signal to switch production. This switch is likely to be required before all relevant information is available thus introducing an element of risk and the possibility of unnecessarily compromising seasonal vaccine supply. In addition, vaccine manufacturers will face a number of contractual and supply issues following a decision to switch, particularly those committed to year-round seasonal vaccine production. Given these and other implications of switching from seasonal to pandemic vaccine manufacture, which cannot proceed concurrently, coordinated international leadership and risk assessment from WHO will be crucial. Immediate next steps in this area include finalization of the WHO risk-assessment tool, draft operational framework for pandemic vaccine response, and PIRM framework.

Recent zoonotic infections and the ever-present threat of a pandemic have led to an exponential increase in the volume of scientific papers relating to the development of broadly protective influenza vaccines over the last decade, illustrating the high level of interest in this area. In addition, as highlighted by the 2014–15 northern hemisphere influenza season, there is a risk of unavoidable antigenic mismatch under the current seasonal vaccine production paradigm. The further development of anti-HA stalk vaccines will require human immunogenicity and protection studies, determination of correlates of protection, establishment of safety (including absence of vaccine-associated enhanced respiratory disease), and confirmation that the targeted portion of the HA molecule remains conserved under immune pressure.

6.3 Proposed action points
a. Reiterate to NICs, as outlined in the PIP Framework, the need to promptly share with WHOCCs all viruses, clinical samples and information relating to all cases of human zoonotic infections.

   [Near-term action – WHO and WHOCCs]

b. Review the evidence base to support the case for reduced animal testing of candidate pandemic vaccine viruses derived using reverse genetics and intended for use against highly pathogenic avian influenza viruses.

   [Near-term action – WHO, WHOCCs and WHO ERLs]
7. Regulatory issues for seasonal and pandemic influenza vaccines

7.1 Overview and current issues

Only three laboratories within the WHO GISRS produce reassortant candidate vaccine viruses using the classical procedure. Although a number of laboratories produce candidate vaccine viruses for inactivated virus vaccines using reverse genetics, the use of this technology is currently restricted to zoonotic and pre-pandemic candidate viruses. Candidate vaccine viruses for producing inactivated vaccines have on occasion been made outside the GISRS and offered for use but have not to date been taken up by manufacturers. Manufacturers are also working on their own systems to generate high-growth reassortants including those optimized for improved yield in cell substrates. In some cases this involves the use of reverse genetics from synthetic DNA to produce “synthetic viruses”.

Conventionally developed candidate vaccine viruses are subjected to a broad range of tests prior to use, including identity of the HA and NA genes, full genotyping, HA gene sequence, absence of parental high-yield PR8 HA, antigenicity in a two-way HI test, sterility, HA titre and infectivity. Estimates of yield are also considered desirable by manufacturers. The Quality Module of the recent EMA Guideline on Influenza Vaccines contains separate chapters on candidate vaccine viruses and vaccine seed lots for seasonal vaccines, pre-pandemic vaccines and pandemic vaccines, plus a guideline on the quality of candidate influenza vaccine viruses isolated in cell culture. The Guideline is now open to the use of demonstrably suitable new or modified technologies and permits the use of candidate viruses not provided by a WHO or otherwise-approved laboratory, including those prepared by a vaccine manufacturer, provided they represent the vaccine viruses recommended by WHO and/or the EMA Committee for Medicinal Products for Human Use. The Guideline also specifies that it is the responsibility of the manufacturer to demonstrate the suitability of a candidate virus, and establish a seed lot in line with European Union recommendations for seasonal vaccine composition, with confirmation of antigenic similarity to the WHO-recommended virus by a WHOCC using two-way HI testing.

7.2 Challenges and opportunities

The preparation and standardization of currently accepted potency reference reagents for inactivated influenza vaccines can represent a timeline bottleneck for both seasonal and pandemic influenza vaccines. This was recognized in the BARDA table-top exercise regarding the possibility of delayed vaccine formulation decisions (see section 3). In addition, the shipping and sharing of reagents for candidate pandemic vaccine viruses are often subject to high-containment laboratory requirements, thus accentuating the problem. In addition, because vaccine manufacturer seed laboratories are typically limited to BSL-2 enhanced containment, and global vaccine manufacturing capacity meets BSL-2 enhanced containment, manufacturers are highly dependent upon rapid safety testing and clear communication of the outcome when seeking to commence work on pandemic or potential pandemic candidate vaccine viruses.

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1 New York Medical College, USA; National Institute for Biological Standards and Control, United Kingdom; and bioCSL, Australia (now Seqirus). The Chinese Centre for Disease Control and Prevention, China is currently gearing up to produce classically reassorted viruses.
In light of the current constraints in both seasonal and pandemic vaccine production there is industry support for: (a) the development of alternative potency assays; (b) convergence of national regulatory authority evaluations needed to ensure the quality, safety and efficacy of vaccines; (c) establishment of a mutual recognition system for pandemic vaccine registration; and (d) use of international WHO package labelling to ensure robust safety standards and avoid delays in pandemic vaccine deployment.

Candidate vaccine viruses prepared by reverse genetics are considered to constitute genetically modified organisms (GMOs) in some countries and as a result are subject by law to various national regulations and restrictions. There is an opportunity to review these and related issues as part of a broader exploration of the current landscape of candidate vaccine virus development, including evaluation of prevailing global regulatory opinions, resource-prioritization strategies and associated aspects. Such an exploration might now be beneficially informed by a systematic comparative review of the potential advantages and safety of viruses generated by reverse genetics compared to conventionally generated viruses. The related issue of intellectual property barriers was highlighted but was considered to be outside the scope of the present consultation.

7.3 Proposed action points

a. Review and compare existing data on the time required to generate candidate vaccine viruses (optimized for growth in eggs and/or cells, and with acceptable antigenicity) by reverse genetics or by classical reassortment.
   [Near-term action – WHOCC Atlanta with support from other GISRS entities]

b. Make the initial scientific case for the equivalence of candidate vaccine viruses generated by reverse genetics and those generated by classical reassortment with respect to GMO status, as part of a process of early case-building and advocacy.
   [Near-term action – WHOCC Atlanta with support from other GISRS entities]
References


28. Lukša M, Lassig M; to be published.


## Annex 1
### Summary matrix of proposed action points

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<tr>
<th>Proposed action point</th>
<th>Timeframe</th>
<th>Actor</th>
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<tr>
<td>1. Strengthening influenza surveillance to improve vaccine composition</td>
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<td>1.a Continue to promote global NIC capacity building by communicating to</td>
<td>Near term</td>
<td>WHO</td>
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<td>national authorities the vital public health contributions made by NICs, and the</td>
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<td>need for continued and enhanced support for their activities.</td>
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<td>1.b Review the current global influenza surveillance landscape and</td>
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<td>approaches in the context of the vaccine virus selection process in order to</td>
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<td>promote the optimal and strategic deployment of available resources, and to</td>
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<td>identify opportunities for strengthening WHO GISRS epidemiological and laboratory</td>
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<td>capacities, including in under-represented world regions.</td>
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<td>1.c Provide support to national and regional efforts to improve the collection,</td>
<td>Near to mid-term</td>
<td>WHO</td>
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<td>analysis and use of influenza surveillance and burden data in tropical and</td>
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<td>subtropical countries.</td>
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<td>1.d Provide revised detailed operational and other guidance to NICs in key areas</td>
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<td>WHO and WHOCCs</td>
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<td>such as virus sampling and selection strategies, the reporting of virus activity to</td>
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<td>the WHO FluNet platform at both national and subnational levels, the timely</td>
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<td>provision of genetic data to publically available databases, and the optimal</td>
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<td>frequency of virus sharing – including original clinical specimens – to facilitate</td>
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<td>the timely shipment of representative viruses to WHOCCs in time for</td>
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<td>characterization prior to the biannual VCMs.</td>
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<td>1.e Further strengthen WHO technical, logistical and other assistance to NICs across</td>
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<td>the broad range of sampling, reporting, shipping and related activities required</td>
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<td>to ensure the timely sharing of information and representative viruses.</td>
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<td>1.f Improve communications between WHOCCs and NICs, and between NICs and</td>
<td>Near term</td>
<td>WHOCCs and NICs</td>
</tr>
<tr>
<td>national laboratories, with a greater emphasis on tailoring guidance to specific</td>
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<tr>
<td>seasonal and national circumstances, including for example the provision of timely</td>
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<tr>
<td>and up-to-date information from WHOCCs to NICs on difficulties in virus isolation</td>
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<tr>
<td>as well as on genetic markers observed in low-reacting samples.</td>
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<tr>
<td>1.g Further develop and evaluate the concept of “right-sizing” of global seasonal</td>
<td>Mid-term</td>
<td>WHO and WHOCCs</td>
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<tr>
<td>influenza surveillance and assess relevant WHOCC capacities as part of evaluating</td>
<td></td>
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<tr>
<td>its feasibility.</td>
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<tr>
<td>2. Virological characterization and evaluation of candidate influenza vaccine viruses</td>
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<tr>
<td>2.a Identify additional sources of human pre-/post-vaccination human serum panels</td>
<td>Near term</td>
<td>WHOCCs, WHO ERLs; and WHO</td>
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<tr>
<td>to better characterize human antibody responses to newly circulating viruses for</td>
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<tr>
<td>enhanced identification of significant antigenic variants.</td>
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<tr>
<td>2.b Create a VN working group to further optimize and harmonize protocols and VN</td>
<td>Near term</td>
<td>WHOCCs and WHO ERLs</td>
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<tr>
<td>testing strategies to improve robustness and simplicity, and thus allow for higher</td>
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<tr>
<td>throughput antigenic characterization of seasonal influenza viruses based upon</td>
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<td>recent advances in this technology.</td>
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<tr>
<td>2.c Establish a broadly based NA interest group to address issues related to the</td>
<td>Mid-term</td>
<td>WHO, WHOCCs and manufacturers</td>
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<tr>
<td>feasibility of NA as a potentially quantified vaccine antigen, particularly as next-</td>
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<td>generation recombinant vaccines are developed.</td>
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<tr>
<td>2.d Harmonize VE protocols among different studies/sites so that data can be</td>
<td>Mid-term</td>
<td>GIVE, I-MOVE and other VE networks and</td>
</tr>
<tr>
<td>combined for more robust analyses.</td>
<td></td>
<td>initiatives</td>
</tr>
</tbody>
</table>
3. The challenge of late-emerging variants

<table>
<thead>
<tr>
<th>3.a</th>
<th>Communicate on a more regular basis with industry on the characterization of circulating viruses, and on the availability of potential wild-type vaccine viruses and reassortant candidate vaccine viruses.</th>
<th>Near term</th>
<th>WHOCCs, WHO ERLs and WHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.b</td>
<td>Develop a framework process for a scenario in which the WHO recommendation for a single vaccine virus is delayed, incorporating the prompt initiating of ad hoc teleconferences and other strategies and guidance for communicating with IFPMA in the event of the late emergence of significant drift variants.</td>
<td>Near term</td>
<td>WHOCCs, WHO ERLs and WHO</td>
</tr>
<tr>
<td>3.c</td>
<td>Improve the development and timing of vaccine potency reagents and assays by further evaluating the use of heterologous reagents for potency testing and of potency reference antigen reagents using viruses other than candidate vaccine viruses (such as reverse genetics viruses or wild-type viruses, and recombinant-derived proteins).</td>
<td>Near term</td>
<td>WHO ERLs</td>
</tr>
<tr>
<td>3.d</td>
<td>Develop alternative potency assays using both antibody-based and antibody-independent technologies.</td>
<td>Near term</td>
<td>WHO ERLs</td>
</tr>
<tr>
<td>3.e</td>
<td>Improve the current characterization and suitability evaluations of cell-propagated viruses to support the work of manufacturers towards the licensure of cell-based influenza vaccines that use cell-propagated vaccine seed viruses.</td>
<td>Mid-term</td>
<td>WHOCCs</td>
</tr>
<tr>
<td>3.f</td>
<td>Develop candidate vaccine viruses with improved yield by expanding current laboratory capacity for classical reassortment, developing improved influenza B high-growth donor viruses, and initiating a systematic evaluation of the potential application of synthetic biology and reverse genetics techniques to the production of egg-based A(H3N2) candidate vaccine viruses with high-growth properties.</td>
<td>Mid-term</td>
<td>WHOCCs and WHO ERLs</td>
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</tbody>
</table>

4. Application of next generation sequencing to influenza surveillance and vaccine virus selection

<table>
<thead>
<tr>
<th>4.a</th>
<th>Conduct a survey of current Sanger and NGS sequencing capabilities and activities undertaken by NICs, which also incorporates evaluation of the needs of laboratories in moving towards a sequencing-driven paradigm of influenza surveillance and vaccine virus development.</th>
<th>Near term</th>
<th>WHO and WHOCCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.b</td>
<td>Document the range of current capacity-building opportunities and potential synergies for expanding the use of NGS within the WHO GISRS, including training provision by WHOCCs and guidance development for NICs on the steps required in areas such as platform selection, large-scale computerized data storage, and data analysis and reporting.</td>
<td>Near term</td>
<td>WHO</td>
</tr>
<tr>
<td>4.c</td>
<td>Develop revised and strengthened guidance for NICs on the required characteristics and optimal extent of sequence and antigenic data, and on the key need to ensure the timely submission of sequence data and virus samples to WHOCCs, along with prompt uploading of data and other information to publically available reporting platforms and databases, in support of vaccine virus selection.</td>
<td>Near term</td>
<td>WHOCCs</td>
</tr>
<tr>
<td>4.d</td>
<td>Identify approaches to facilitate the capture of genetic polymorphisms in NGS databases, and the routine retrieval of such data.</td>
<td>Near to mid-term</td>
<td>WHOCCs</td>
</tr>
<tr>
<td>4.e</td>
<td>Work with the animal health sector on cross-cutting issues such as quality assurance standards, data storage, analysis and management, sharing of bioinformatics pipelines and capacity building.</td>
<td>Near term</td>
<td>WHOCCs</td>
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</tbody>
</table>

5. Potential role of enhanced evolutionary analysis in informing influenza vaccine virus selection

<table>
<thead>
<tr>
<th>5.a</th>
<th>Organize a meeting to broaden and strengthen understanding of different modelling systems between the modellers themselves and WHOCCs, and to assess how best to evaluate and harness the potential season-by-season contributions of each system to the vaccine virus selection and development process; including through retrospective evaluation of modelling performance.</th>
<th>Near term</th>
<th>WHOCCs, with support from WHO and BARDA</th>
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</thead>
<tbody>
<tr>
<td>5.b</td>
<td>Develop a route for the more formalized incorporation of modelling data and findings into the considerations of the biannual WHO VCMs.</td>
<td>Near term</td>
<td>WHOCCs</td>
</tr>
</tbody>
</table>
### 6. Development of pandemic and broadly protective vaccines

| 6.a | Reiterate to NICs, as outlined in the PIP Framework, the need to promptly share with WHOCCs all viruses, clinical samples and information relating to all cases of human zoonotic infections. | Near term | WHO and WHOCCs |
| 6.b | Review the evidence base to support the case for reduced animal testing of candidate pandemic vaccine viruses derived using reverse genetics and intended for use against highly pathogenic avian influenza viruses. | Near term | WHO, WHOCCs and WHO ERLs |

### 7. Regulatory issues for seasonal and pandemic influenza vaccines

| 7.a | Review and compare existing data on the time required to generate candidate vaccine viruses (optimized for growth in eggs and/or cells, and with acceptable antigenicity) by reverse genetics or by classical reassortment. | Near term | WHOCC Atlanta with support from other GISRS entities |
| 7.b | Make the initial scientific case for the equivalence of candidate vaccine viruses generated by reverse genetics and those generated by classical reassortment with respect to GMO status, as part of a process of early case-building and advocacy. | Near term | WHOCC Atlanta with support from other GISRS entities |
## Annex 2

### Meeting agenda

#### Day 1: Wednesday 18 November 2015

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Topics</th>
<th>Speakers</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00–9:45</td>
<td>Opening and welcome</td>
<td>C Chan, W Wong</td>
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<tr>
<td></td>
<td>Welcome and introduction to meeting objectives</td>
<td>W Zhang</td>
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<td></td>
<td>Selection of chair and rapporteurs</td>
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<td></td>
<td>Administrative announcements</td>
<td>J Tam</td>
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<td></td>
<td>Adoption of the agenda and chair’s introduction</td>
<td>Chair</td>
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</table>

#### Session 1: Influenza surveillance and special studies  
**Co-chair:** F Motta

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Topics</th>
<th>Speakers</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:45–10:05</td>
<td>Overview of WHO influenza surveillance – status and challenges from the vaccine virus selection perspective</td>
<td>W Zhang</td>
<td></td>
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<tr>
<td>10:05–10:25</td>
<td>Vaccine composition in tropical and subtropical regions</td>
<td>S Hirve</td>
<td></td>
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<tr>
<td>10:25–10:45</td>
<td>Application of the right-size approach to vaccine virus</td>
<td>L Brammer</td>
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<tr>
<td>11:15–11:40</td>
<td>Emerging issues with influenza A(H3N2) viruses</td>
<td>J McCauley</td>
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<tr>
<td>11:40–12:05</td>
<td>Overview of virus neutralization assays and application to virus characterization</td>
<td>J Katz</td>
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<tr>
<td>12:05–12:30</td>
<td>Update on neuraminidase-related developments since 2014 from the perspective of vaccine virus selection</td>
<td>M Eichelberger</td>
<td></td>
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<tr>
<td>12:30–12:50</td>
<td>Vaccine effectiveness and its potential contribution to vaccine virus selection</td>
<td>S Sullivan</td>
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</tbody>
</table>

#### Session 2: Response to a late-emerging antigenic variant  
**Co-chair:** T Odagiri

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Topics</th>
<th>Speakers</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:25–14:45</td>
<td>An introduction to the situation in 2014</td>
<td>J Katz/D Jernigan</td>
<td></td>
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<tr>
<td>14:45–15:15</td>
<td>Perspectives from the United States and feedback from the table-top exercise in November</td>
<td>A Donabedian</td>
<td></td>
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<tr>
<td>15:40–16:00</td>
<td>Perspectives from Europe</td>
<td>P Penttinen</td>
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<tr>
<td>16:00–16:25</td>
<td>Perspectives from vaccine manufacturers</td>
<td>B Taylor</td>
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</tbody>
</table>
Day 2: Thursday 19 November 2015

9:00–9:10 Recap of Day 1 and introduction to Day 2
Chair

Session 3: Vaccines, regulatory issues and pandemic preparedness
Co-chair: J Tam

9:10–9:35 Selection of zoonotic influenza vaccine viruses
– challenges and actions
R Webby

9:35–10:05 Pandemic vaccine response – report from a WHO
consultation in June
J Wood

10.05–10:25 Update on universal influenza vaccine development
G Rimmelzwaan

10:55–11:15 Timeline and bottlenecks in pandemic vaccine response
G Grohmann

11:15–11:40 Regulatory perspectives on developer-generated
viruses/seeds in vaccine production
O Engelhardt

11:40–12:00 Vaccine viruses for pandemic preparedness
– an industry perspective update
S Lee

12:00–12:45 Panel discussion: speakers and A Donabedian,
M Eichelberger, O Kistner, E Mol, E Sparrow and Z Ye

Session 4: Next generation sequencing and its application to influenza vaccine
virus selection
Co-chair: R Donis

13:45–14:15 Integration of whole genome sequencing/next generation
sequencing into national surveillance programmes
M Zambon

14:15–14:45 Public health use of influenza virus genetic sequence
information
I Barr

14:45–15:15 Next generation sequencing platforms, virus diversity and
population dynamics – implications for influenza vaccine
virus selection
M Adams

15:40–16:10 Combating influenza through evolutionary analysis and
synthetic genomics – reality and the ideal
D Wentworth

16:10–16:55 Panel discussion: speakers and P Daniels, J McCauley,
T Mokhtari-Azad, R Njouom and G Yi

Day 3: Friday 20 November 2015
09:00–09:10 Recap of Day 2 and introduction to Day 3 Chair

Session 5: Predictive modelling and beyond Co-chair: J McCauley

09:10–09:40 Evolution and epidemiology – genomic sequencing and surveillance A Rambaut

09:40–10:10 Quantitative approaches to virus evolution T Bedford

10:10–10:40 Modelling and influenza fitness M Lässig

11:10–11:50 A new strategy for vaccine targets and moving forward Y Kawaoka/ D Smith

11:50–12:35 Panel discussion: speakers and I Barr, B Cowling, A Hay, M Luksza and R Neher

Session 6: Way forward Co-chair: W Zhang

13:25–13:55 Knowledge gaps in the hi-tech era on influenza vaccine virus selection A Hay

13:55–15:45 Review of sessions 1–5 Session co-chairs

15:45–16:05 Priority actions to be taken Chair

16:05–16:15 Meeting closure W Zhang
Annex 3

Meeting participants

Mark Adams, J Craig Venter Institute, Rockville, USA
Malti R. Adhin, ADEK Universiteit van Suriname, Paramaribo, Suriname
Meral Akçay, Sanofi Pasteur, Istanbul, Turkey
William Ampofo, University of Ghana, Accra, Ghana
Hana Apsari Pawestri, Jakarta, Indonesia
Albert Au, Department of Health China, Hong Kong SAR
Juliana Barbosa Ramírez, National Influenza Centre, Bogota, Colombia
Ian Barr, WHO Collaborating Centre for Reference and Research on Influenza (VIDRL), Melbourne, Australia
Trevor Bedford, Fred Hutchinson Cancer Research Center, Seattle, USA
Mohamed Ali Ben Hadj Kacem, Charles Nicolle Hospital, Tunis, Tunisia
Peter Bogner, Global Initiative on Sharing All Influenza Data, Munich, Germany
Karoline Bragstad, Norwegian Institute of Public Health, Oslo, Norway
Lynnette Brammer, CDC, Atlanta, USA
Amparo Larrauri Camara, Instituto de Salud Carlos III, Madrid, Spain
Jorge Augusto Camara, Universidad Nacional de Córdoba, Ciudad de Córdoba, Argentina
Mandeep Chadha, National Institute of Virology, Pune, India
Constance Chan, Department of Health, China Hong Kong SAR
Desmond Chan, Department of Health, China Hong Kong SAR
Sheng Chen, The Hong Kong Polytechnic University, China Hong Kong SAR
Li Mei Chen, National Center for Immunization and Other Respiratory Diseases, Atlanta, US
Amber Jia-chi Chiou, The Hong Kong Polytechnic University, China Hong Kong SAR
Malinee Chittaganpitch, National Institute of Health (NIH), Nonthaburi, Thailand
Shuk-kwan Chuang, Department of Health, China, Hong Kong SAR
Ben Cowling, The University of Hong Kong, China, Hong Kong SAR
Nancy Cox, CDC Atlanta, USA
Peter Daniels, OFFLU, Australia
Badarch Darmaa, National Center of Communicable Diseases (NCCD), Ulaanbaatar, Mongolia
Armen Donabedian, Biomedical Advanced Research and Development Authority (BARDA), Washington DC, USA
Ruben Donis, National Center for Immunization and other Respiratory Diseases, Atlanta, USA
Vladimir Drazenovic, Croatian National Institute of Public Health, Zagreb, Croatia
Nkwembe Ngabana Edith, Institut National de Recherche Biomédicale (INRB), Kinshasa/Gombe, Democratic Republic of the Congo
Maryna Eichelberger, Food and Drug Administration (FDA), Center for Biologics and Evaluation and Research, Silver Spring, USA
Amany Elghohary Sheta, Central Public Health Laboratories, Ministry of Health and Population, Cairo, Egypt
Othmar Engelhardt, Hertfordshire, UK
Vasily Aleksander Evseenko, FORT Biopharmaceutical Company, Moscow, Russian Federation
Rodrigo Fasce, Instituto de Salud Publica de Chile, Santiago, Chile
Rebecca Garten, CDC, Atlanta, USA
Gary Grohmann, Campbell, Australia
Yi Guan, The University of Hong Kong, China, Hong Kong SAR
Julia Guillebaud, Institut Pasteur de Madagascar, Antananarivo, Madagascar
Alan Hampson, Interflu Pty Ltd, Victoria, Australia
Alan Hay, The Francis Crick Institute, London, UK
Zhu Huachen, The University of Hong Kong, China, Hong Kong SAR
Jude Jayamaha, National Influenza Centre, Colombo, Sri Lanka
Daniel Jernigan, CDC, Atlanta, USA
Jackie Katz, CDC, Atlanta, USA
Yoshihiro Kawaoka, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, USA
Balkrishna Khakurel, Department of Drug Administration of Nepal, Kathmandu, Nepal
Kisoon Kim, National Institute of Health, Chungcheongbuk-do, Republic of Korea
Otfried Kistner, Wien, Austria
Igor Krasilnikov, Krasnoe Selo, Russian Federation
Gordana Kuzmanovska, Institute of Public Health, Skopje, The former Yugoslav Republic of Macedonia
Yonnie Lam, Department of Health, China, Hong Kong SAR
Michael Lässig, University of Colgne, Köln, Germany
Terence Lok-ting Lau, The Hong Kong Polytechnic University, China, Hong Kong SAR
Sam Lee, Pandemic & New Influenza Products, Swiftwater, USA
Min-Shi Lee, Taiwan, China
Alex Leung, Department of Health, China, Hong Kong SAR
Min Levine, National Center for Immunization and Other Respiratory Diseases, Atlanta, USA
Yan Li, Public Health Agency of Canada, Canadian Science Centre for Human and Animal Health, Winnipeg, Canada
Jih-Hui Lin, Taipei, Taiwan, China
Samuel Chun-lap Lo, The Hong Kong Polytechnic University, China, Hong Kong SAR
Janice Lo, Department of Health, China, Hong Kong SAR
Irma Lopez Martínez, Instituto de diagnóstico y Referencia Epidemiologicos, Mexico
Marta Luksza, Institute for Advanced Study, Princeton, USA
Ann Machablishvili, National Centre for Disease Control and Medical Statistics (NCDC), Tbilisi, Georgia
Ignacio Martin-Loeches, St James's University Hospital, Dublin, EIRE Ireland
John McCauley, The Francis Crick Institute, London, UK
Janet Mghamba, Ministry of Health and Social Welfare, Dar es Salaam, United Republic of Tanzania
Kenji Minari, Takeda Pharmaceutical Company Limited, Yamaguchi, Japan
Donan Mmbando, Ministry of Health and Social Welfare, Dar es Salaam, United Republic of Tanzania
Talat Mokhtari-Azad, Iranian National Influenza Center, Tehran, Iran (Islamic Republic of)
Els Mol, Abbott Biologicals BV, Weesp, Netherlands
Fausta Mosha, National Health Laboratory, Ministry of Health and Social Welfare, Dar-es-salaam, United Republic of Tanzania
Fernando Motta, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil
Richard Neher, Max Planck Institute for Developmental Biology, Tübingen, Germany
Mbayame Niang, Institut Pasteur de Dakar, Dakar, Senegal
Richard Njouom, Centre Pasteur du Cameroun, Yaounde, Cameroon
Obaidullah, Drug Regulatory Authority of Pakistan, Islamabad, Pakistan
Takato Odagiri, National Institute of Infectious Diseases, Tokyo, Japan
Pasi Penttinen, European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
Mahmudur Rahman, Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh
Andrew Rambaut, Ashworth Laboratories, University of Edinburgh, Edinburgh, UK
Samir Abdelaziz Refaey, Ministry of Health and Population, Cairo, Egypt
Guus Rimmelzwaan, Erasmus MC, University Medical Center, Rotterdam, Netherlands
Olga Sadikova, Centre for Disease Prevention and Control, Tallinn, Estonia
Pretty Multihartina Sasono, National Institute of Health Research and Development, Jakarta, Indonesia
Derek Smith, University of Cambridge, Cambridge, UK
Kellie So, Department of Health Hong Kong, China, Hong Kong SAR
Sheena Sullivan, WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia
John Siu-lun Tam, The Hong Kong Polytechnic University, China, Hong Kong SAR
Beverley Taylor, NVS Influenza Vaccines, Liverpool, UK
Florette Treurnicht, National Institute for Communicable Diseases, Johannesburg, South Africa
Theodore F Tsai, Takeda Vaccines, Cambridge, USA
Phengta Vongprachanh, National Center for Laboratory and Epidemiology(NCLE), Ministry of Health, Vientiane, Lao People's Democratic Republic
Tony Waddel, Durham, UK
Ralf Wagner, Paul-Ehrlich-Institut, Langen, Germany
Alexander Ping-kong Wai, The Hong Kong Polytechnic University, China, Hong Kong SAR
Niteen Wairagkar, Bill & Melinda Gates Foundation, Seattle, USA
Shinji Watanabe, National Institute of Infectious Diseases, Tokyo, Japan
Richard Webby, St Jude Childrens Research Hospital, Memphis, USA
Jerry Weir, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, USA
David Wentworth, CDC, Atlanta, USA
Wing-tak Wong, The Hong Kong Polytechnic University, China, Hong Kong SAR
Man-kin Wong, The Hong Kong Polytechnic University, China, Hong Kong SAR
John Wood, Bushey Herts, UK
Xiyan Xu, National Center for Immunization and Other Respiratory Diseases, Atlanta, USA
Maria Zambon, Public Health England, London, UK
Ye Zhiping, Food and Drug Administration (FDA), Center for Biologics and Evaluation and Research, Silver Spring, USA

WHO Secretariat
Claudia Alfonso WHO/HQ/HIS/EMP
Humayun Asghar EM/RGO/DCD
Sylvie Briand WHO/HQ/HSE/PED

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Nancy Cox was selected as chair of the meeting. Fernando Motta, Takato Odagiri, John Tam, Ruben Donis, John McCauley and Wenqing Zhang were appointed as co-chairs for sessions 1–6 respectively. Alan Hampson and Anthony Waddell were appointed as co-rapporteurs.
Annex 4

Declaration of Interests

The 4th WHO Informal Consultation on Improving Influenza Vaccine Virus Selection, 18–20 November 2015, 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, all participants were required to complete the WHO form for Declaration of Interests for WHO Experts. With the exceptions of Alan Hampson, Daniel Jernigan, David Wentworth, Guus Rimmelzwaan, Ian Barr, Jacqueline Katz, John McCauley, John Tam, John Wood, Maria Zambon, Mark Adams, Michael Lässig, Nancy Cox, Othmar Engelhardt, Pasi Penttinen, Sam Lee, Trevor Bedford and Yoshi Kawaoka, no personal current or recent (within the last 4 years) financial or other interests relevant to the subject of the meeting were declared.

All declarations made were evaluated by the WHO Secretariat prior to the meeting. It was concluded that the interests declared did not conflict with the objectives of the meeting and that the above individuals could participate in full. At the start of the meeting the interests that had been declared were disclosed to all participants.