

Improving influenza vaccine virus selection Report of a WHO informal consultation held at WHO headquarters, Geneva, Switzerland, 14–16 June 2010

WHO Writing Group

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Executive summary

- For almost 60 years, the WHO Global Influenza Surveillance and Response System (GISRS) has been the key player in monitoring the evolution and spread of influenza viruses and recommending the strains to be used in human influenza vaccines. The GISRS has also worked to continually monitor and assess the risk posed by potential pandemic viruses and to guide appropriate public health responses.
- The expanded and enhanced role of the GISRS following the adoption of the International Health Regulations (2005), recognition of the continuing threat posed by avian H5N1 and the aftermath of the 2009 H1N1 pandemic provide an opportune time to critically review the process by which influenza vaccine viruses are selected. In addition to identifying potential areas for improvement, such a review will also help to promote greater appreciation by the wider influenza and policy-making community of the complexity of influenza vaccine virus selection.
- The selection process is highly coordinated and involves continual year-round integration of virological data and epidemiological information by National Influenza Centres (NICs), thorough antigenic and genetic characterization of viruses by WHO Collaborating Centres (WHOCs) as part of selecting suitable candidate vaccine viruses, and the preparation of suitable reassortants and corresponding

reagents for vaccine standardization by WHO Essential Regulatory Laboratories (ERLs).

- Ensuring the optimal effectiveness of vaccines has been assisted in recent years by advances in molecular diagnosis and the availability of more extensive genetic sequence data. However, there remain a number of challenging constraints including variations in the assays used, the possibility of complications resulting from non-antigenic changes, the limited availability of suitable vaccine viruses and the requirement for recommendations to be made up to a year in advance of the peak of influenza season because of production constraints.
- Effective collaboration and coordination between human and animal influenza networks is increasingly recognized as an essential requirement for the improved integration of data on animal and human viruses, the identification of unusual influenza A viruses infecting human, the evaluation of pandemic risk and the selection of candidate viruses for pandemic vaccines.
- Training workshops, assessments and donations have led to significant increases in trained laboratory personnel and equipment with resulting expansion in both geographical surveillance coverage and in the capacities of NICs and other laboratories. This has resulted in a significant increase in the volume of information reported to WHO on the spread, intensity and impact of influenza. In addition, initiatives such as the WHO Shipment

Fund Project have facilitated the timely sharing of clinical specimens and virus isolates and contributed to a more comprehensive understanding of the global distribution and temporal circulation of different viruses. It will be important to sustain and build upon the gains made in these and other areas.

- Although the haemagglutination inhibition (HAI) assay is likely to remain the assay of choice for the antigenic characterization of viruses in the foreseeable future, alternative assays – for example based upon advanced recombinant DNA and protein technologies – may be more adaptable to automation. Other technologies such as microtitre neuraminidase inhibition assays may also have significant implications for both vaccine virus selection and vaccine development.
- Microneutralization assays provide an important adjunct to the HAI assay in virus antigenic characterization. Improvements in the use and potential automation of such assays should facilitate large-scale serological studies, while other advanced techniques such as epitope mapping should allow for a more accurate assessment of the quality of a protective immune response and aid the development of additional criteria for measuring immunity.
- Standardized seroepidemiological surveys to assess the impact of influenza in a population could help to establish well-characterized banks of age-stratified representative sera as a national, regional and global resource, while providing direct evidence of the specific benefits of vaccination.
- Advances in high-throughput genetic sequencing coupled with advanced bioinformatics tools, together with more X-ray crystallographic data, should accelerate understanding of the genetic and phenotypic changes that

underlie virus evolution and more specifically help to predict the influence of amino acid changes on virus antigenicity.

- Complex mathematical modelling techniques are increasingly being used to gain insights into the evolution and epidemiology of influenza viruses. However, their value in predicting the timing and nature of future antigenic and genetic changes is likely to be limited at present. The application of simpler non-mechanistic statistical algorithms, such as those already used as the basis of antigenic cartography, and phylogenetic modelling are more likely to be useful in facilitating vaccine virus selection and in aiding assessment of the pandemic potential of avian and other animal influenza viruses.
- The adoption of alternative vaccine technologies – such as live-attenuated, quadrivalent or non-HA-based vaccines – has significant implications for vaccine virus selection, as well as for vaccine regulatory and manufacturing processes. Recent collaboration between the GISRS and vaccine manufacturers has resulted in the increased availability of egg isolates and high-growth reassortants for vaccine production, the development of qualified cell cultures and the investigation of alternative methods of vaccine potency testing. WHO will continue to support these and other efforts to increase the reliability and timeliness of the global influenza vaccine supply.
- The WHO GISRS and its partners are continually working to identify improvements, harness new technologies and strengthen and sustain collaboration. WHO will continue in its central role of coordinating worldwide expertise to meet the increasing public health need for influenza vaccines and will support efforts to improve the vaccine virus selection process, including through the convening of periodic international consultations.

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Introduction

The historic initiative to establish a global network to detect and identify new and potentially dangerous influenza viruses predates the adoption of the WHO Constitution in 1948. With memories of the 1918–1919 influenza pandemic still vivid, and the ever-evolving threat posed by influenza recognized, the WHO Global Influenza Surveillance and Response System (GISRS)[†] was formally established in 1952. Influenza thus became one of the first diseases to

highlight the importance of international monitoring and collaboration in protecting human health.

Following the re-emergence of human cases of highly pathogenic avian H5N1 influenza in 2003 and the adoption of the International Health Regulations (2005), the GISRS was strengthened and its role in protecting public health enhanced. In addition to tracking the course and impact of annual influenza epidemics and monitoring the evolution of seasonal influenza viruses, the GISRS also acts as a global alert mechanism for the emergence of influenza viruses with the potential to cause a human pandemic. The Network provides support to both seasonal and pandemic influenza preparedness and response activities in areas such as diagnostics, vaccine development, virological surveillance and risk assessment. It also acts as the focus of WHO efforts to

[†] Former WHO Global Influenza Surveillance Network (GISN), which has been renamed as WHO Global Influenza Surveillance and Response System (GISRS) since 24 May 2011, when the World Health Assembly Resolution WHA 64.5 was adopted.

assist Member States in strengthening their national capacity for the surveillance, diagnosis, characterization and sharing of influenza viruses.

As a key player in global influenza risk assessment and response, the GISRS continues to evolve and expand, and as of December 2010 consisted of 135 National Influenza Centres (NICs) in 105 countries, six WHO Collaborating Centres (WHOCCs), 11 WHO H5 Reference Laboratories and four WHO Essential Regulatory Laboratories (ERLs). The GISRS also works to ensure the successful coordination of WHO activities with those of external agencies such as the Global Outbreak Alert and Response Network (GOARN), national regulatory authorities, academic and veterinary institutes, and the pharmaceutical industry.

The first formal WHO recommendations on influenza vaccine composition were issued in 1971. Since 1998, separate and appropriately timed recommendations for the Northern and Southern Hemispheres have been issued each year in February and September, respectively. These biannual recommendations are based upon the virological and epidemiological information generated by the GISRS and play a crucial role in the development, production and availability of effective influenza vaccines.

The continuing threat posed by avian H5N1, the aftermath of the 2009 H1N1 pandemic, the increased knowledge of influenza, and the development and availability of new technologies provide a timely opportunity to review the complex processes and issues involved in influenza vaccine virus selection and to identify potential areas for improvement. This WHO informal consultation represents the latest step in an ongoing process of GISRS strengthening and was convened with the following objectives:

- to review the current vaccine virus selection process, including its constraints and limitations;
- to identify opportunities for improving influenza surveillance and representative virus sharing;
- to assess the potential for improving the assays and technologies used for vaccine virus selection; and
- to assess the potential impact of new vaccine technologies on the vaccine virus selection process.

Participants were drawn from a broad and highly diverse range of institutes and sectors including the following: WHOCCs, NICs, WHO ERLs, WHO H5 Reference Laboratories, national regulatory authorities, public health agencies, academia, influenza vaccine manufacturers, and veterinary laboratories and organizations.

The GISRS vaccine virus selection process

The primary goal of the GISRS vaccine virus selection process (Annex 1) is to generate and analyse the data needed to recommend the influenza vaccine viruses that will most closely match the influenza viruses likely to be circulating

during forthcoming influenza seasons. Current vaccine technologies and production schedules mean that decisions on vaccine composition have to be made almost a full year in advance of the peak of seasonal influenza activity. As a result, the process relies upon the earliest possible detection of emerging antigenic variants and the most up-to-date information on their potential future epidemiological significance. Information must therefore be collected year round on the continuous evolution and global circulation of human influenza viruses to provide a sound basis for the biannual WHO recommendations on the composition of influenza vaccines for use in the Northern and Southern Hemispheres. For countries in equatorial regions, epidemiological considerations influence which recommendation (February or September) individual national and regional authorities consider more appropriate.

Role of National Influenza Centres

National Influenza Centres (NICs) play a vital role in this complex process. Their core activities include collating epidemiological information, diagnosing cases of influenza A and B infection, and identifying the subtype or lineage of the viruses responsible. The primarily molecular diagnosis of infection using RT-PCR techniques is based upon standardized primers and probes provided by the GISRS. Viruses must also be isolated to allow their antigenic identification using the type- and subtype-specific reference reagents provided in annually distributed WHO kits. Further detailed characterization may include sequence analyses to monitor genetic changes and assessment of virological traits such as resistance to antiviral drugs. Sequence data are shared within the GISRS using public databases such as GenBank and GISAID EpiFlu. NICs in some settings then attempt to relate potentially important virological changes observed with clinical and epidemiological information and trends and may even conduct serological studies to evaluate the immune status of the population.

Weekly reports on the virological characteristics and epidemiology of circulating viruses are submitted to the WHO FluNet – an internet-based data-query and reporting tool. Information on the virus subtypes and lineages is collated, together with observations of potential clinical or epidemiological importance, and regular summaries of the geographical spread, intensity and impact of influenza are produced by WHO.

If human infection with an avian or other animal influenza virus is suspected, a suitably equipped NIC or other national influenza reference laboratory can conduct preliminary diagnostic testing using RT-PCR protocols and/or reagents for H5, H7 and H9 subtypes provided by WHO. Such RT-PCR testing does not require high-level biocontainment facilities. However, it is expected that the detection of any unusual influenza A virus distinct from known

circulating viruses, especially one suspected to be of animal origin or unsubtypeable using current WHO reagents, will immediately be reported to WHO and collaboration urgently initiated with a WHO Collaborating Centre (WHOCC). If the required laboratory biosafety facilities and procedures are not available, then virus isolation should not be attempted in the national laboratory and the sample should be promptly sent to a WHOCC.

Role of WHO Collaborating Centres

The routine and timely sharing of representative circulating influenza viruses and unusual viruses with a WHOCC is an essential step in the vaccine virus selection process. The criteria for forwarding viruses include their temporal, geographical and age-group distribution, severity of cases and virological characteristics such as unidentified subtype and antiviral drug resistance. WHOCCs are then responsible for the systematic antigenic characterization of the thousands of viruses forwarded each year by NICs and other laboratories, and for the detailed genetic characterization of a selected subset. Such detailed antigenic and genetic characterization is a necessary step in monitoring virus evolution and detecting any distinct antigenic variants that may necessitate updating the seasonal vaccine composition. The process also allows for the identification and characterization of animal viruses causing sporadic human infections, assessment of the risk they pose and the potential development of candidate vaccine viruses as part of pandemic preparedness.

Antigenic characterization

Of prime importance in immunity to influenza is the production of antibodies to the virus haemagglutinin (HA) protein. Such antibodies can neutralize the infectivity of viruses, and their level in the blood has been shown to correlate with the level of protection against infection with a homologous virus. As a result, influenza vaccine virus selection has primarily been based upon the antigenic characterization of virus HA using the haemagglutination inhibition (HAI) assay. HAI tests provide a visual readout of the ability of specific antibodies to prevent the attachment of HA to red blood cells (RBCs) and thus prevent their agglutination. Antigenic drift in the HA of circulating viruses in response to host immunity reduces the effectiveness of vaccines and is therefore the major consideration when recommendations are made on the composition of influenza vaccines.

The HAI test is likely to remain the assay of choice for the antigenic characterization of virus HA for the foreseeable future. Strain-specific antisera are produced by infecting previously unexposed ('naive') ferrets with either vaccine viruses, reference viruses representative of circulating viruses or viruses that appear in HAI tests to be potential antigenic

variants. The resulting sets of reference viruses and antisera are then used to evaluate the antigenic characteristics of the HAs of recent isolates. Where antigenic differences are detected, these are likely to affect human immunity against the new variants. The HAI test is a surrogate for the more complicated and time-consuming virus neutralization assay used to clarify antigenic relationships when observed variations in HAI titre reflect, for example, changes in receptor binding rather than differences in antigenicity.

Genetic characterization

A subset of between 10% and 20% of all viruses received is selected for genetic sequencing and more detailed analysis – principally of their HA and NA components. This subset is selected to include representative circulating viruses, as well as apparent antigenic variants and viruses from severe or fatal cases. Phylogenetic analyses are carried out to better understand the evolution of circulating viruses, their degree of genetic heterogeneity and the emergence of new genetic clades. Antigenic or other phenotypic variants may thus be defined in terms of separate genetic clades with distinct amino acid signatures. Relating the locations of amino acid substitutions to antigenic, receptor-binding or glycosylation sites on the 3D structure of the HA molecule then helps to identify the individual substitutions associated with phenotypic (antigenic) changes. Identifying such amino acid signatures also facilitates global monitoring of the emergence, distribution and impact of different genetic variants. This is particularly helpful when data on emergent variants are limited at the time of a WHO vaccine consultation. Comparisons of the sequences found in clinical specimens and virus isolates are also useful in revealing amino acid substitutions which result from passage in different substrates, mainly MDCK cells and eggs. Up-to-date sequence data are shared within GISRS and made publically available via the GISAID EpiFlu database.

Complete genome sequencing is necessary to identify animal (including avian) viruses causing human infection and is important in detecting the emergence of reassortant viruses among co-circulating human viruses or between human and animal viruses. WHOCCs maintain panels of reference reagents for all influenza A subtypes. These include H5 (especially H5N1), H9 and H7 avian viruses and various H1N1 and H3N2 swine viruses, as well as viruses present in other animals such as horses and dogs.

Studies using human sera

WHOCCs also collaborate with the WHO ERLs in serological studies of representative human sera from previously vaccinated individuals. Sera are provided by vaccine manufacturers and are used in HAI tests to assess whether or not the antibodies induced by current vaccines are likely to be effective against currently circulating viruses. The results

provide important supplementary evidence for vaccine composition decisions.

WHO recommendations on influenza vaccine composition

The principal criteria used to decide whether or not to recommend changes to influenza vaccine components include:

- the emergence of an antigenically and genetically distinct variant among circulating viruses (including a novel influenza A virus with the potential to cause a pandemic);
- evidence of the geographical spread of such a distinct variant and its association with outbreaks of disease, indicating its future epidemiological significance;
- the reduced ability of existing vaccine-induced antibodies to neutralize the emergent variant; and
- the availability of suitable candidate vaccine viruses.

To facilitate collaborative studies by the WHOCCs and WHO ERLs and ensure that appropriate potential candidate vaccine viruses are identified in advance of the WHO vaccine composition consultation, the most recent virological and epidemiological data are shared and discussed via teleconferences held 6 and 2 weeks before the WHO consultation. A summary of each teleconference is promptly distributed to keep all NICs and vaccine manufacturers informed of the developing situation. In addition, potential candidate vaccine viruses are provided to manufacturers.

During the formal biannual consultations, the technical advisory group considers the cumulative antigenic and genetic data on the viruses characterized by WHOCCs. The data are set against the broader epidemiological context collated by WHO and are supported by serological data from WHOCCs and WHO ERLs, as well as by additional information provided by NICs. HAI data obtained in the different centres using a wide variety of reference viruses and ferret antisera are correlated using common reference reagents. In recent years, antigenic cartography has been used to collate and statistically visualize the degree of antigenic variation. The interpretation of HAI data may, however, be complicated by the influence of changes in the receptor-binding properties of natural viruses or by the selection of variants during isolation and passaging in different cell or egg substrates. Comparisons with sequence data are made to relate any differences in antigenicity with specific HA genetic clades and to more precisely define the identity of antigenic variants. The results of virus neutralization tests, which usually correspond to those of HAI tests, are used to clarify the true antigenic relationships between different viruses.

If the antigenic data, supported by genetic and serological data, indicate that a new antigenic variant is spreading globally, then a change in that component of the seasonal vaccine is considered to be warranted. The implementation of a rec-

ommendation to update a vaccine component is, however, contingent upon the availability of suitable vaccine viruses. Only after all the factors have been taken into account is a decision taken on whether or not to recommend a change in influenza vaccine virus composition. The decision is announced at an Information Meeting immediately following each WHO consultation and published on the WHO web site and in the WHO Weekly Epidemiological Record.

Since the re-emergence of human cases of highly pathogenic H5N1 avian influenza in 2003, WHO has also regularly reviewed the available antigenic and genetic data on human and avian viruses in relation to the epidemiology of H5N1 influenza among birds. To support the development of safe and effective human H5N1 vaccines, WHO has coordinated the development of a number of candidate attenuated vaccine viruses (Annex 1) and made them available to vaccine producers. Clinical trials have been conducted to evaluate the immunogenicity of different H5N1 vaccine formulations and the breadth of antibody responses elicited. In addition, as part of pandemic preparedness, WHO has coordinated the ongoing development and updating of an inventory of H2, H7 and H9 candidate vaccine viruses.

Vaccine development considerations

Important constraints on the vaccine virus selection process include the tight timelines involved (Annex 1), particularly in the Northern Hemisphere, where since recent years seasonal influenza activity tends to start increasing in middle or late January in general. As a consequence, decisions often have to be made relatively early in the influenza season. In addition, post-infection ferret antisera against potential antigenic variants are urgently required to define their antigenic relationships to previously circulating viruses. Panels of recent isolates must also be prepared to assess the degree to which they are neutralized by antibodies in the sera of previously vaccinated individuals. Finally, potential new candidate vaccine viruses must be prepared and evaluated for their suitability in vaccine production. Ensuring the timely availability of viruses with suitable growth properties is a crucial step in ensuring that sufficient quantities of vaccine can be produced in time for administration prior to the next influenza season. Although cell culture has steadily replaced the use of embryonated eggs for the primary isolation of viruses, candidate vaccine viruses must still be isolated directly in eggs according to current regulatory requirements. The limited availability of egg isolates, particularly of recent H3N2 viruses which generally grow poorly in eggs, has led to the establishment of Cooperative Research and Development Agreements (CRADAs) and similar agreements between the vaccine industry and a number of WHOCCs to increase the availability of egg isolates for vaccine use.

The GISRS vaccine virus selection process necessarily involves a series of collaborative steps, including the selection of prototype antigenic variants and suitable vaccine viruses, and the provision of standardizing reagents by the WHO ERLs. The process thus impacts directly upon the subsequent authorizing of vaccine composition by national and regional regulatory authorities and upon the large-scale production of vaccine by manufacturers. Mismatches have occasionally occurred as a result of the emergence of variant strains shortly after the recommendations have been made, highlighting one of the unavoidable consequences of current vaccine development and production constraints. Nevertheless, retrospective studies have shown that with very few exceptions WHO vaccine virus recommendations have closely matched the influenza viruses that have circulated during the following influenza season. In addition, following the out-of-season emergence of the pandemic A(H1N1) 2009 virus, this closely integrated system demonstrated its unique ability to very rapidly orchestrate the development and provision of appropriate (suitably attenuated) candidate vaccine viruses for pandemic vaccine production.

Improving influenza surveillance and representative virus sharing

Global influenza surveillance has always presented a major challenge as it is a highly demanding public health need with a significantly uneven distribution of surveillance capacity worldwide. Since the outbreak of severe acute respiratory syndrome (SARS) in 2003, the re-emergence of H5N1 infection in humans and the 2009 H1N1 pandemic, it has become ever clearer that surveillance and the prompt sharing of viruses and information are central to the broad range of influenza preparedness and response activities.

Enhancing NIC surveillance capacity

Although the known impact and the awareness of seasonal influenza vary in different parts of the world, the threat posed by avian H5N1 viruses has galvanized influenza surveillance efforts in all countries. Improving surveillance and acquiring the capacity to detect and report unusual cases of influenza are essential components of global pandemic planning and are enshrined in the International Health Regulations (2005). Successful efforts to increase the capacity of NICs and other laboratories have been made, and in a number of settings the development, revision and adoption of guidelines on strengthened national, regional and global surveillance and collaboration is under way.

Global influenza surveillance has also been strengthened through expanded geographical coverage and the collection of more data of better quality. For example, in Africa there are now 25 influenza laboratories in 21 countries, including 12 recognized NICs, almost all of which have the capacity

to conduct RT-PCR diagnosis of influenza infection. In less than two years, the percentage of African countries with an NIC increased from 17% to 26% with the number of countries with no influenza laboratory markedly decreasing.

Global, regional and national training workshops, assessments and donations have all led to significant increases in trained personnel, equipment procurement and laboratory capacity, resulting in the increasingly widespread use of molecular techniques such as real-time RT-PCR and genetic sequencing. Recent WHO capacity-building activities have included BSL-3 training courses for NICs to promote safe practices when working with highly pathogenic influenza viruses, and courses on virus isolation, gene sequencing and antiviral resistance detection. Increased participation in both internal and external quality assurance programmes such as the WHO external quality assessment project (EQAP) has contributed to marked improvements in laboratory proficiency.

These and other efforts enabled a more effective response to the emergence of the 2009 H1N1 pandemic in many countries. However, the pandemic also revealed significant limitations in the analysis and integration of epidemiological and virological surveillance data. In addition, few early seroprevalence surveys were conducted to allow for the timely assessment of the extent and impact of the pandemic. The pandemic also revealed significant gaps in laboratory infrastructure and personnel, equipment procurement and funding, particularly in developing countries. Improvements and training in areas such as web-based integration and analyses of clinical, epidemiological and virological data are being implemented but care must be taken to ensure that such activities are not conducted at the expense of detection, characterization and virus-sharing activities in less well-resourced settings.

Identified research priorities in influenza surveillance and response include evaluation of the temporal and geographical circulation of influenza viruses and of the burden of influenza. In all settings, establishing a sound evidence base will support the development or updating of national, regional and global policies, plans and guidelines. This in turn could lead to greater acceptance of the use of influenza vaccines, particularly seasonal vaccines, and assist in the development of vaccination policies.

Virus and information sharing

The primary requirement of NICs will remain the prompt diagnosis of influenza infection and the timely sharing of clinical specimens and virus isolates – especially those obtained from unusual, severe or fatal cases – backed up by appropriate epidemiological and clinical information. Procedures should be in place to ensure that the increasingly predominant use of molecular diagnostic techniques, particularly real-time RT-PCR, does not adversely affect the timely isolation and for-

warding of viruses. Improved communication between NICs and WHOCCs on how best to facilitate prompt virus sharing, including discussion of the constraints faced, could improve coordination and avoid potential delays.

A more systematic approach to engaging NIC information and expertise would also lead to significant benefits. Such an approach is likely to be facilitated by a number of developments in the use of WHO web-based tools. For example, NICs with enhanced capabilities currently strengthen the collaborative characterization of viruses and aid early assessment of the significance of genetic and antigenic changes by sharing detailed virological information (especially HA sequences) on selected viruses, either directly or via public databases. As technologies advance, national patterns of seropositivity to circulating influenza viruses may also become available on a more timely basis and could thus guide vaccine use. This is particularly important given the increasing emphasis now placed on assessing vaccine effectiveness. Comprehensive NIC summary reports forwarded just prior to each WHO consultation also provide highly beneficial additional data to inform WHO recommendations on vaccine composition.

To overcome logistical and other obstacles to the safe and efficient shipping of clinical specimens and virus isolates to WHOCCs, a WHO Shipment Fund Project was established. The project provides support to NICs and other influenza laboratories in all countries by arranging the transport of specimens and isolates along a guaranteed cold chain, especially in settings where there are severe financial and infrastructural constraints. As a direct result of the project, and associated 'infectious substances shipping' workshops conducted in all WHO regions, there has been a significant increase in the number of countries sharing specimens and isolates, especially following the outbreak of the 2009 H1N1 pandemic. Furthermore, the expansion and harmonization of the information currently provided in the accompanying standard shipping form to include information such as clinical outcome, patient vaccination status or recent travel history would greatly enhance understanding of the epidemiological context associated with the spread of viruses.

Animal viruses

A better understanding of the diversity and evolution of animal influenza viruses is essential for evaluating the pandemic risk posed by subtypes currently causing sporadic human infections (such as H5N1 and H9N2) and informing the selection of candidate vaccine viruses. The emergence of H5N1 in particular led to the establishment in 2005 of the OIE–FAO Network of Expertise on Animal Influenza (OFFLU) – a worldwide network of approximately 20 laboratories and institutions that coordinates the global surveillance of animal influenza. A number of joint WHO–OFFLU tech-

nical initiatives on influenza at the human–animal interface have been conducted (including successful collaboration during the 2009 H1N1 pandemic) and reciprocal participation in annual meetings has taken place. There remains, however, considerable scope for improved coordination and collaboration with the animal influenza surveillance sector, especially in the collection and analysis of antigenic and genetic data, the timely exchange of representative viruses and reference reagents, and the conducting of serological studies of human exposure to zoonotic infection.

Influenza is an important disease of many avian and mammalian species with serious economic consequences for livestock industries and has potential adverse impacts on human food supplies. Despite this, animal influenza surveillance coverage is limited with a shortage of epidemiological data on the circulation of various viruses in different countries. Efforts are now under way to establish triggers for initiating enhanced surveillance that go beyond animal disease notification and sporadic human infections. Although there is increasing understanding of the interrelationships between animal and human influenza and the need for 'integrated' surveillance, full collaboration at both national and global levels is currently constrained by a number of practical, funding, regulatory and policy issues. Maintaining a regular dialogue based upon the mutual interests of the different networks will be an important public health activity and may also help to enhance the sustainability of animal influenza surveillance in particular settings. A more formal collaborative mechanism might allow for the improved integration of animal virus data into the WHO candidate vaccine virus selection process. Increased awareness of the content and extent of use of animal influenza vaccines would also aid understanding of their impact on virus evolution.

Improving the process of vaccine virus selection

A range of laboratory assays and other techniques provide the complementary information on changes in the antigenic and genetic characteristics of influenza viruses needed to select the most appropriate influenza vaccine viruses. However, inherent limitations in the biological assays used and significant variations in the results obtained by different laboratories complicate the collation and definitive interpretation of data.

Assays for characterization of antigenic properties and antibody responses

Because the HAI test outlined previously is a simple, rapid and reproducible surrogate assay for virus neutralization, it is widely used to measure the antigenic relationships between different viruses as well as antibody responses to

infection or vaccination. In addition, the test provides the basis of the only current quantitative correlate of protection against infection (serum HAI antibody titre ≥ 40) used to standardize inactivated vaccines. However, variations in the physical characteristics of RBCs obtained from different species and differences in the receptor-binding properties of different viruses influence both the sensitivity and the utility of the assay. Furthermore, changes in receptor-binding affinity or specificity associated with adaptation, antigenic drift or the isolation and passage of viruses in eggs and cell culture may also affect HAI titres. Standardization between laboratories has also proved difficult, and the assay is currently not suitable for use in a fully automated system.

A range of practical refinements such as attempts to develop 'synthetic' RBCs (for example using glycan-coated beads) have been unsuccessful. Given the currently limited knowledge of the principal natural receptors for influenza viruses, such approaches are unlikely to circumvent the virus-dependent shortcomings of assays based upon natural RBCs which are therefore likely to remain the primary approach to antigenic characterization for the foreseeable future. Recent developments based on the use of panels of recombinant HA do offer alternative or supplementary microtitre or microarray binding-assay formats for assessing antibody specificity and antibody inhibition of the HA-glycan receptor interaction. Although such approaches are relatively expensive and require a high degree of skill to implement, they are potentially highly suited to automation and in time may reduce the need for virus isolates. In addition, such formats can readily be adapted to incorporate biosensor technologies to provide more quantitative analyses of binding characteristics. A number of such assays are currently being validated using ferret and human antisera.

The contribution of antibodies against virus NA in conferring protection following natural infection or vaccination is still not well understood. Studies of NA antigenic variation have been limited, and the NA content of influenza vaccines is not currently standardized. Although neuraminidase inhibition (NAI) assays were conducted more routinely in the past, these were cumbersome to perform and were complicated by the relatively low levels of antibodies against NA in post-infection ferret sera and by interference from antibodies against HA. A number of different NAI microtitre assay formats have recently been developed. These have been used to correlate antigenic changes with sequence variations in the NA component, provide more precise information on the evolution of NA and assess NA antibody responses following vaccination. Improved understanding of antigenic drift in NA and of the role of anti-NA antibodies in conferring immunity might have significant implications for both vaccine virus selection and vaccine development.

Microneutralization (MN) assays – based on measuring virus replication, cell viability or NA activity – provide an important adjunct to HAI tests in antigenic characterization. MN assays are generally more sensitive and measure a broader repertoire of functional antibodies that neutralize viral replication, with potential advantages in the evaluation of human serological responses. In addition, comparisons of MN and HAI tests for measuring antibody responses in vaccinated individuals have shown a consistent degree of correlation and have confirmed the utility of MN assays in analyses of human antibody responses to H3 vaccine components. Techniques for simplifying assay formats and making them more readily applicable to the routine testing of low-titre viruses are under investigation, and efforts are under way to use MN assays for H1 and B viruses. This should facilitate the use of MN assays to overcome the variable nature of interactions between viruses and RBCs, and hence in interpreting 'anomalous' HAI results which complicate vaccine virus selection. Pseudo-type virus neutralization assays may also offer some advantages in scale and standardization over conventional MN assays for measuring serological responses to particular viruses, especially highly pathogenic viruses. Furthermore, ongoing improvements in automation will potentially enable the more labour-intensive MN assay to be applied to large-scale serological analysis. Epitope mapping using genome fragment phage display libraries provides another powerful technique for further dissecting the fine specificity of antibody responses to vaccination and infection and should allow for a better assessment of the quality of a 'protective' immune response and aid the development of additional correlates of immunity.

Serological studies

To encourage the performance of seroepidemiological surveys to assess the impact of influenza in a population, countries should be supported in establishing well-characterized serum banks of age-stratified representative sera as a national, regional and global resource. Current advantages of the GISRS serological activities undertaken in support of vaccine virus selection include the use of shared serum panels and common antigens, with frequent consensus obtained from participating WHOCCs and WHO ERLs. Limitations include the large variability of HAI data, a requirement for antibody standards and a need for MN or other assays to resolve inconsistencies. The availability of antibody standards would not only enhance the comparability of serological data generated in different laboratories and countries but also facilitate the comparison of antibody responses to different vaccines.

Increasing attention to influenza vaccine effectiveness studies will lead to the availability of more real-time data for comparing clinical benefit with the degree of antigenic

relatedness of vaccine and circulating viruses. Such studies, especially those based upon laboratory-confirmed outcomes, should provide evidence of the specific benefits of vaccination. Consistent studies providing estimates of vaccine efficacy over successive influenza seasons should improve understanding of the effects of small rather than major antigenic differences between vaccine and circulating viruses on clinical outcomes and should help to allay concerns arising from a perceived vaccine mismatch caused by the emergence of virus clades exhibiting little or no antigenic drift.

Technological developments

Recent advances in high-throughput genetic sequencing could potentially lead to a greatly enhanced understanding of the genetic changes occurring in influenza viruses and the evolutionary interactions that occur between co-circulating viruses. In-depth analyses of the precise mechanisms involved in the evolution and epidemiology of influenza would require advanced bioinformatics tools to comprehensively mine the data produced. Such an approach should reveal, for example, the broader genetic changes that underlie antigenic variation in HA and thus allow for a better understanding of the relationship between genetic evolution and antigenic drift. Increased information from X-ray crystallography on the structural features of the HAs of recent viruses and specific mutants, together with developments in computer modelling, should assist in attempts to predict the likely influence of amino acid substitutions on the antigenic and receptor-binding properties of new variants. Further development of high-throughput laboratory systems for integrated and automated genetic and phenotypic analyses – from initial sample accession to data management – offers the intriguing prospect of a futuristic standardized virtual network for virus characterization in an epidemiological context. As such systems will have broad implications, not only for vaccine virus selection, but also for the organization and conduct of global influenza surveillance, it is extremely important that their development and deployment are integrated with the activities of the WHO GISRS.

Mathematical modelling

Numerous mathematical modelling techniques have now been used to gain insights into the mechanisms that underlie both the evolution and the epidemiology of influenza viruses. For example, exploratory models have been developed to generate and test various hypotheses to explain the relatively restricted diversity of influenza viruses in terms of constrained antigenic repertoire, and to explore the underlying nature of immunity. They have also been used to improve understanding of the extent of between-subtype and between-type competition and of the potential conse-

quences of such interactions for trends in the incidence of seasonal influenza viruses.

Phylogenetic models have also been used to identify changes in selective constraints in relation to antigenic drift and inter-species transmission. When based upon the amino acid substitutions associated with mammalian host adaptation, such models may aid assessment of the pandemic potential of avian and other animal viruses. Phylodynamic modelling based upon available sequence data, supplemented with antigenic data, has already been successfully used to trace the emergence of new antigenic and genetic variants and track their geographical spread.

However, in the absence of greatly improved understanding of the underlying evolutionary and biological mechanisms and other processes involved, the capacity of current mathematical modelling techniques to predict the timing and nature of future antigenic and genetic changes is limited. The intrinsically stochastic nature of influenza evolution may make such predictive modelling extremely challenging. Where changes occur over short time scales, the application of simpler non-mechanistic statistical algorithms, such as those used as the basis of antigenic cartography, is likely to be more useful in facilitating vaccine virus selection than attempts to develop predictive models from the existing complex dynamical models of influenza evolution and transmission. Such predictive models might presently be better suited for use in understanding the possible long-term effects of vaccination, optimizing the timing and location of focused surveillance efforts and predicting the possible consequences of the emergence of a novel virus. Eventually, these models should be able to take advantage of integrated immunological and antigenic surveillance data to develop predictions of short-term dynamics in specific locations.

Impact of new vaccine technologies

All new influenza vaccine technologies have implications for vaccine virus selection and for regulatory and manufacturing processes. However, any potential requirement to tailor the virus selection process to specific types of vaccine is unlikely to be a crucial issue, especially if advances in vaccine technology and speed of production lead to greater flexibility in the timing of recommendations. Although live-attenuated vaccines are not yet universally licensed, the current vaccine composition recommendation process is used. However, antibody response is not a good correlate of protection for such vaccines and the identification of a true correlate might affect the requirement for annual updating. Several quadrivalent vaccines are also now under development that contain representative strains of the two influenza B virus lineages (B/Victoria and B/Yamagata) together with influenza A(H1N1) and A(H3N2) viruses.

This raises a number of issues that could affect vaccine supply, including the possibility of two poorly growing vaccine viruses; the likely variable impact of a fourth component on vaccine yields and timing of manufacture; the prioritization of influenza B lineage viruses in the context of both trivalent and quadrivalent vaccine production; and the need for a fourth set of reagents. Adjuvanted vaccines have been licensed with the primary aims of inducing better immune responses in certain age groups and allowing 'antigen sparing'. Although there has been no specific intention to provide a broader spectrum of immunity to circumvent the need for annual vaccine updates, different products are likely to show a different breadth of response. Providing recommendations in relation to product-specific cross-reactivity over successive influenza seasons is unlikely to be a feasible option for the WHO GISRS. In addition, various types of recombinant vaccines are now under development, including protein subunit, DNA, vector and VLP vaccines – none of which are presently licensed.

In the case of non-HA-based vaccines, different guidelines will apply and all such vaccines are likely to impact the current vaccine virus selection process in various ways depending upon their precise type and mechanism of protection. The level of protection afforded by immunity to NA is receiving continued interest. Currently, this component is included as part of the candidate vaccine virus and is selected on the basis of its sequence but not antigenicity. Standardization of the NA component would require antigenic characterization during the virus selection process, while antigenic changes in NA in the absence of a corresponding change in HA antigenicity may on its own necessitate the updating of vaccine composition. For all such vaccines, HA variant selection may become less crucial than it is for current vaccines.

Although high-growth reassortants have been used to manufacture influenza A vaccine components for many years, their yields have been variable and there is continued need to identify the molecular determinants of high yield to engineer a more reliable and reproducible production process. Reverse genetics, now used in the United States to produce virus reassortants for live-attenuated vaccines, has also been used to produce attenuated candidate H5N1 vaccine viruses suitable for inactivated vaccine manufacture. This approach was, however, less successful than classical reassortment in obtaining a suitable 2009 H1N1 pandemic vaccine virus, emphasizing the need for further investigation of the applicability of reverse genetics in the routine provision of suitable vaccine viruses.

Following the licensing of cell culture vaccines, the feasibility of isolating seasonal vaccine viruses in qualified¹ cell

lines is being evaluated in a collaboration involving a number of WHOCCs and WHO ERLs under CRADAs with vaccine manufacturers. These studies should provide the basis for the introduction of a universal qualified cell culture system for providing mammalian cell-derived seasonal influenza candidate vaccine viruses. This would result in a greater choice of candidates, especially for recent H3N2 viruses, and may provide greater flexibility in responding to the 'late' emergence of a variant necessitating a vaccine composition change. Such virus isolates would not be subject to undesirable egg-selected changes and would potentially provide a better match to the natural virus. However, the relative merits of egg and cell culture candidate vaccine viruses have still to be rigorously evaluated. Guidance on quality assurance aspects has already been published by the European Medicines Agency (EMA). The finalization of new EMA regulatory guidelines may be accompanied by a WHO technical document on harmonizing regulatory approaches worldwide and the engagement of other regulatory authorities in vaccine-manufacturing nations.

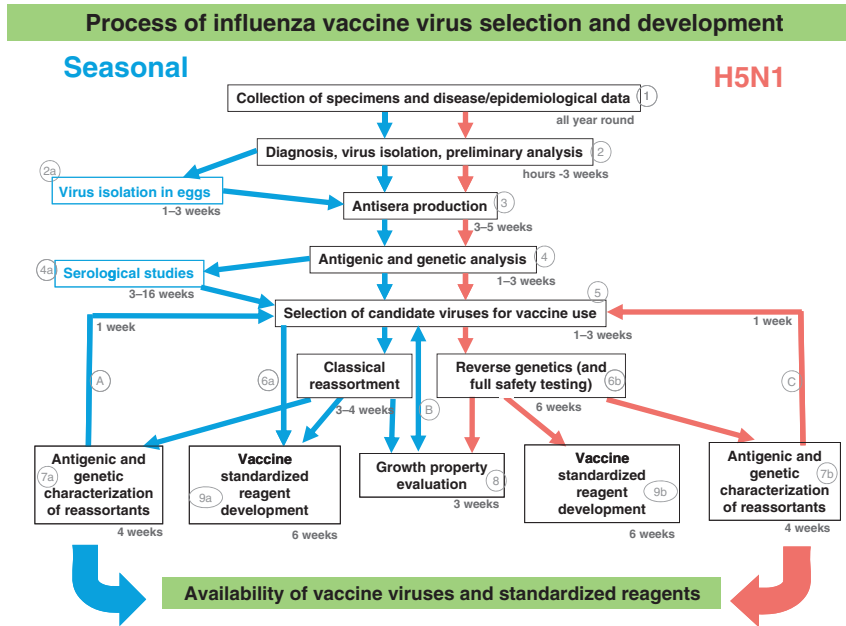
Vaccine manufacturers and the WHO ERLs are also collaborating in an evaluation of cell culture-based reagents for use in single radial immunodiffusion (SRID) potency testing, due for completion in early 2011. In addition, despite international consensus on the key quality specifications for 2009 H1N1 pandemic influenza vaccines, reagents to calibrate the majority of candidate vaccines using conventional potency tests only became available immediately prior to the initiation of clinical trials. In some cases, candidate vaccines were available ahead of the reagents. Although national authorities proved flexible in accepting the use of validated alternative potency tests to allow clinical trials to proceed, newer methods such as high-performance liquid chromatography (HPLC) and mass spectrometry are now being evaluated.

Conclusions and future perspective

The GISRS has a long history of success in recommending influenza vaccine compositions that have closely matched the combination of viruses circulating during subsequent influenza seasons. Based upon the voluntary participation of its many constituent partners, the GISRS enjoys strong institutional and governmental support.

Global influenza surveillance is the foundation of the vaccine virus selection process. Efforts to enhance and strengthen national, regional and global laboratory capacity for virological surveillance and representative virus sharing must continue. As part of this, improved integration of virological and disease surveillance data will be a key aim and will help to build the foundations for future studies of the impact and burden of influenza worldwide.

¹ Defined as cell lines accepted by regulatory authorities as suitable substrates for vaccine manufacture.



To strengthen the pandemic influenza preparedness, collaboration between the GISRS and veterinary laboratories and organizations such as OFFLU in relation to zoonotic influenza infection has been greatly enhanced and has included the development of appropriate candidate human vaccine viruses from animal viruses. However, there remains considerable scope for improvement in this area, including the more timely exchange of information, viruses and reagents, and strengthened technical collaboration at all levels.

Although antigenic characterization of promptly forwarded virus isolates will remain the central criterion for selecting influenza vaccine viruses in the foreseeable future, technological developments (such as advanced recombinant DNA and protein technologies, and high-throughput sequencing and advanced bio-informatics tools) will inevitably impact current GISRS surveillance and virus selection activities. In the interests of global public health, it will be important to integrate into the GISRS system appropriate information and data generated by various networks using emerging technologies.

Antigenic cartography has been adopted by the GISRS in recent years as a means of integrating HAI data from different laboratories to allow for statistical comparison and visual display. The development of new statistical algorithms to complement the use of antigenic cartography may further facilitate vaccine virus selection.

Greater emphasis should be placed on conducting human serological studies which incorporate the use of antibody standards to improve the comparability of results. Such studies would improve current understanding of the prevalence and spread of influenza, and complement the

development of improved epidemiological models. Greater collaborative effort is needed to generate randomly sampled, representative and integrated serological, epidemiological and evolutionary data that provide snapshots of host and viral populations suitable for modelling hypotheses on virus evolution and host immunity. The application of advanced techniques for dissecting the fine specificity of antibody responses to vaccination and infection should also lead to improvements in understanding the quality of a 'protective' immune response and aid in the development of additional correlates of immunity.

Recent collaboration between the GISRS and external partners including academic institutions and vaccine manufacturers has resulted in the increased availability of egg isolates and high-growth reassortants. New approaches to the generation of high-growth vaccine viruses involving the use of reverse genetics and qualified cell cultures will continue to be evaluated and developed, as will alternative methods of vaccine potency testing. WHO will continue to support these and other efforts to increase the reliability and timeliness of global influenza vaccine supply.

New vaccine types currently under development may allow more flexibility in the timing of recommendations on vaccine virus composition. Conversely, alterations to the virus selection process and additional information may be needed in relation to new-generation vaccine types with different compositions and mechanisms of protection.

The WHO GISRS vaccine virus selection process lies at the heart of global efforts to address the constantly evolving threat posed by influenza. For decades, this highly collaborative and complex process has ensured a continued supply

of effective seasonal vaccines and was able to respond very rapidly to the emergence of the 2009 H1N1 pandemic. If the current limitations and constraints inherent in the process are to be overcome, ongoing efforts by the WHO GISRS and its partners must continue to identify improvements, harness new technologies and strengthen collaboration. WHO will continue in its central role of developing and coordinating worldwide expertise to meet the increasing public health need for influenza vaccines and will support this process through the convening of periodic international consultations on improving influenza vaccine virus selection.

Annex 1: Process of influenza vaccine virus selection and development²

The diagram shows that the individual steps in the selection of candidate vaccine viruses and development of standardizing reagents for seasonal influenza and for a potential H5N1 influenza pandemic are essentially equivalent. For seasonal vaccines *the timelines are:*

- Steps 1–4: the collection, isolation and thorough antigenic and genetic characterization of recent virus isolates continues *throughout the year*;
- Step 4a: comparisons of the recognition of representative recent viruses by vaccine-induced antibodies in human sera are conducted *2–3 weeks before the biannual WHO vaccine consultation meetings*;
- Steps 5, 6a and 7a: candidate viruses for vaccine use are reviewed and selected, and high-growth reassortants prepared and characterized *following identification of (potential) antigenic variants* – these steps are not solely dictated by the recommendations of the WHO biannual vaccine virus consultations.
- Step 8: Evaluation of their growth properties is conducted in a timely manner around the time of the WHO vaccine virus consultations and *prior to authorization of vaccine composition by national authorities*.
- Step 9a: Preparation of the standardizing reagents for new vaccine components is initiated *once the particular vaccine virus has been selected following the WHO recommendation*.

Annex 2: Declaration of interests

The WHO Informal Consultation for Improving Influenza Vaccine Virus Selection, 14–16 June 2010, was organized by the Virus Monitoring and Vaccine Support (VMV) Unit of WHO, with participation from WHO Collaborating Centres on influenza, ERLs, National Influenza Centres,

² For further information see: http://apps.who.int/gb/pip/pdf_files/Fluvaccirusselection.pdf

national control laboratories, national regulatory authorities, academic and veterinary institutions, influenza vaccine manufacturers and other collaborating organizations.

In accordance with WHO policy, all members of the writing group that assisted WHO in the development of this meeting report had completed the WHO Declaration of Interests for WHO experts. These declarations were then evaluated by the WHO Secretariat prior to the consultation.

The members of the writing group declared the following personal current or recent (within the last 4 years) financial or other interests relevant to the subject area:

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The interests declared by Drs Levandowski, Monto and Zambon were reviewed by the WHO Secretariat and were considered not to present a conflict of interest with their role in the writing group.

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