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Annex 5

# WHO biosafety risk assessment and guidelines for the production and quality control of human influenza pandemic vaccines

This document provides guidance to national regulatory authorities and vaccine manufacturers on the safe production and quality control of human influenza vaccines produced in response to a threatened pandemic. The document details international biosafety expectations for both pilot-scale and large-scale vaccine production and control and is thus relevant to both development and production activities. It should be read in conjunction with the WHO *Laboratory Biosafety Manual* (*1*) and replaces the earlier WHO guidance *Production of pilot lots of inactivated influenza vaccines from reassortants derived from avian influenza viruses: Interim biosafety risk assessment* (*2*). Tests required to evaluate the safety of candidate influenza vaccine reference viruses by WHO Reference Laboratories prior to release to vaccine manufacturers are also specified in this document.

The following text is written in the form of guidelines rather than recommendations. Guidelines allow greater flexibility than recommendations with respect to expected future developments in the field. These guidelines specify steps to minimize the risk of introducing influenza virus strains with pandemic potential from a vaccine manufacturing facility into the community. If a national regulatory authority so desires, these guidelines may be adopted as definitive national requirements, or modifications may be justified and made by a national regulatory authority. It is recommended that modifications to the principles and technical specifications of these guidelines be made only on condition that the modifications ensure that the risks of introducing influenza virus to the community are no greater than as outlined in the guidelines set out below.

## Summary

International biosafety expectations for both the pilot-scale and large-scale production of human vaccines for a response to a pandemic influenza strain, and the quality control of these vaccines, are described in detail in these WHO Guidelines. Tests required to evaluate the safety of candidate influenza vaccine reference viruses prior to release to vaccine manufacturers are also specified in this document which is thus relevant to both development and production activities, and also to vaccine and biosafety regulators. A detailed risk assessment is presented that concludes that the likelihood of direct harm to human health would be high if non-reassortant H5 or H7 viruses with multiple basic amino acids at the haemagglutinin (HA) cleavage site and high *in vivo* pathogenicity are used for vaccine production. Such viruses could also pose a significant risk to animal health. Stringent vaccine biosafety control measures, defined as Biosafety Level (BSL)3 enhanced (pandemic influenza vaccine) are defined to manage the risk from vaccine production and quality control using such viruses in the pre-pandemic period. For all other vaccine strains, for example reassortants derived from H5 or H7 strains in which the multiple basic amino acid HA0 cleavage site has been removed, the direct risk to human health is very remote. Nevertheless, there is an indirect risk to human health due to a theoretical risk of secondary reassortment with normal human influenza viruses, resulting in a virus with avian-like coat proteins capable of replicating in humans. Although very unlikely, the secondary reassortant could become adapted to human infection and transmission which, if vaccine production was taking place in the pre-pandemic period, would have serious public health consequences. The biosafety control measures that are proposed, defined as BSL2 enhanced (pandemic influenza vaccine), take this and also potential risks to animal health into account. Facility and personal protection specifications are provided for both BSL2 enhanced and BSL3 enhanced bioafety levels and guidance is provided on biosafety management and implementation within a vaccine production facility. Tests to be performed on candidate vaccine reference strains prior to release to vaccine developers depend on the type of virus but include, at a minimum, in vivo tests on ferrets or other susceptible mammals, and, where appropriate, chickens and egg embryos, plaque assays and sequencing.

## Glossary

The definitions given below apply to the terms used in these guidelines. They may have different meanings in other contexts.

*Aerosol*

A dispersion of solid or liquid particles of microscopic size in a gaseous medium.

*Air balance*

The necessity to keep air supply and exhaust systems in balance by means of measurements of static pressure, fan and motor performance, and air volumes.

*Airlock*: Areas found at entrances or exits of rooms that prevent air in one space from entering another space. These generally have two doors and a separate exhaust ventilation system. In some cases a multiple-chamber airlock consisting of two or more airlocks joined together is used for additional control.

*Biosafety committee*

An institutional committee of individuals versed in the subject of containment and handling of infectious materials.

*Biosafety level 2 (or 3) (enhanced pandemic influenza)*

A specification for the containment of pandemic influenza during vaccine manufacture and quality control testing with specialized air handling systems, waste effluent treatment, immunization of staff, specialized training, and validation and documentation of physical and operational requirements.

*Biosafety manual*

A comprehensive document describing the physical and operational practices of the laboratory facility with particular reference to infectious materials.

*Biosafety officer*

A staff member of an institution who has expertise in microbiology and infectious materials, and has the responsibility for ensuring the physical and operational practices of various biosafety levels are carried out in accordance with the standard procedures of the institution.

*Biological indicators*

The use of organisms to test the efficacy of sterilization processes.

*Biological safety cabinet*

Primary and partial containment work enclosure used for manipulation of materials that may cause infections or sensitization to workers. They are equipped with high-efficiency particulate air (HEPA) filters and may or may not be open-fronted.

*Certification*

Documentation that a system qualification, calibration, validation, or revalidation has been performed appropriately and the results are acceptable.

*Decontamination*

A process by which an object or material is freed of contaminating agents.

*Floor dams*

Purpose-built elevations to enclose liquid spills.

*Fumigation*

The process whereby gaseous chemical is applied to an enclosed space for the purpose of sterilizing the area.

*Good manufacturing practices*

That part of quality assurance which ensures that products are consistently produced as controlled to the quality standards appropriate to their intended use and as required by the marketing authorization.

*HEPA filter*

A filter capable of removing at least 99.97% of all particles with a mean aerodynamic diameter of 0.3 micrometres.

*Inactivation*

To render an organism incapable of replication by application of heat, or other means.

*Seed lot*

A culture of microorganism distributed from a single bulk container in a single operation, in such a manner as to ensure uniformity and stability and to prevent contamination.

*Positive pressure laminar flow hood*

An enclosure with unidirectional outflowing air, generally used for product protection.

*Primary containment*

A system of containment, usually a biological safety cabinet or closed container, which prevents the escape of a biological agent into the immediate working environment.

*Respirator*

A respiratory protective device with an integral perimeter seal, valves and specialized filtration, used to protect the wearer from toxic fumes or particulates.

*Risk analysis*

A formalized documented process for analysing risks.

*Secondary containment*

A system of containment, usually involving specialized air-handling, airlocks and secure operating procedures, which prevents the escape of a biological agent into the external environment or into other working areas.

*Sterilization*

Sterility is the absence of viable microorganisms. In general, an item is assumed to be sterile if the validation of the sterilization process applied to it indicates that only one item in one million items subjected to the process will contain a viable microorganism.

*Validation*

The documented act of proving that any procedure, process, equipment, material, activity, or system actually leads to the expected results.

## Introduction

The earlier WHO guidance *Production of pilot lots of inactivated influenza vaccines from reassortants derived from avian influenza viruses. An interim biosafety risk assessment* (*2*) was prepared in response to the threat of a pandemic posed by the highly pathogenic H5N1 avian influenza viruses and the need to begin development of experimental vaccines. This threat persists and several countries are now planning large-scale production of H5N1 vaccine. The risk assessment that informed the WHO biosafety guidance for pilot-lot vaccine production (*2*) has therefore been reassessed in light of the intended greater scale of vaccine production and because production facilities are likely to be different from those used in developing small pilot lots, and also taking into account the experience gained from developing and testing vaccine reference viruses derived by reverse genetics from highly pathogenic avian influenza viruses.

This document follows the risk-assessment scheme used in the WHO biosafety guidance for pilot-lot vaccine production, but is extended to include considerations relating to the greater production-scale needed to supply large quantities of vaccines. The risks associated with large-scale production are likely to be different from pilot lots, e.g. the "open" aspect of some production processes and quantity of virus-containing waste. It also takes into account the considerable experience gained from highly pathogenic avian influenza viruses, and the hazards associated with such strains.

Furthermore, the range of options for vaccine development is broader than originally considered in the WHO risk assessment for pilot lot production and the present document has been expanded to encompass current vaccine development pathways.

## 1. Scope of the risk assessment

Much effort has recently gone into the development of H5N1 vaccine and manufacture and the guidance presented is strongly influenced by the experience gained with this strain and our greater knowledge of H5

strains in general. It is, nevertheless, intended that the guidance will also be applicable to future threats from other potential pandemic strains, such as H2 or highly pathogenic H7.

There is a range of possible pathogenicities in the viruses used in candidate vaccine production not only for humans but also for other mammals and avian species. On the one hand, H5 viruses that can be highly pathogenic to both humans and chickens have been used to produce reassortant viruses genetically modified to be of low pathogenicity for chickens and mammals. On the other hand, for strains inherently less pathogenic for humans, wild-type virus might be used directly for vaccine production. Thus reassortants derived by reverse genetics, empirically-derived reassortants, which may or may not be genetically modified, and native wild strains are within the scope of these guidelines.

Eggs have traditionally been used for the production of influenza vaccines, but cell culture techniques have been recently introduced and international expectations for production and quality control specifications defined (3). For the development of pandemic vaccine, either method may be used; thus both egg and cell culture production methodologies are within the scope of this document.

Most effort to date with candidate pandemic vaccine development has been targeted towards inactivated vaccines. In one country however two live attenuated virus vaccines for potential pandemic strains are under development. This may raise important issues beyond the risks to humans, namely the potential for excreted viruses or their derivatives to infect and replicate in non-human species particularly in those raised for commercial purposes. As the detection of H5 and H7 influenza strains are notifiable strains to the Office International des Epizooties (OIE), widespread dissemination of such vaccine strains could have a significant economic impact as well as ramifications for international trade. Developers and regulators will need to assess both the human and the agricultural risk of live pandemic strain vaccines under development should shedding and replication be possible. Both vaccine types (inactivated and live) are therefore covered in the scope of these guidelines.

Furthermore it is intended that the risk assessment and the guidelines on containment measures should apply to all facilities and laboratories that have a need to handle live vaccine virus. This includes not only the vaccine manufacturing facility but also to the quality control laboratories of the manufacturer and, if appropriate, to National Control Laboratories. The transport of live virus materials within and between sites should comply with international specifications (*4*).

Finally it should be noted that the risk assessment for vaccine manufacture will vary according to whether production is occurring in an interpandemic

period, in a pandemic alert period (as for example early in 2004 when H5N1 was threatening to circulate extensively in South East Asia) or in a pandemic period. These guidelines are intended to describe steps to minimize the risks associated with the production and testing of vaccines with emphasis on the interpandemic period, while indicating modifications that may be found appropriate during other periods.

## 2. Hazard identification

Hazards associated with pandemic vaccine manufacturing and laboratory testing are dependent on the type of pandemic vaccine strain (reassortant or wild type), method of production (egg-based or cell-based) and whether it is an inactivated or live attenuated virus vaccine. The type of vaccine strain, the proposed testing schedule and containment level are illustrated in Table 1.

### 2.1 Hazards associated with the type of pandemic vaccine virus

#### 2.1.1 *Hazards associated with the recipient virus in a reassortant strain*

Pandemic vaccine reassortants have been produced on the human strain A/PR/8/34 (PR8) as recipient virus. PR8 has had over 100 passages in each of mice, ferrets and embryonated chicken eggs. The result of such a passage history is complete attenuation of the virus and its inability to replicate in humans (*5*).

PR8 reaches a high titre in embryonated chicken eggs and since the late 1960s, it has been used to produce "high growth reassortants" in combination with the prevailing influenza A vaccine strain. The use of such reassortants as vaccine strains has increased vaccine yield many-fold. The reassortants are produced by a mixed infection of eggs with PR8 and the nominated vaccine strain, combined with a selection system based on anti-PR8 antibody and growth at high dilution.

Live attenuated influenza vaccines are licensed in some countries. The parental strains used in such vaccines, e.g. A/Ann Arbor/6/60, are also potential recipient strains for the development of pandemic reassortant vaccines. These parental strains possess phenotypic markers of vaccine safety, such as temperature sensitivity, cold-adaptation and attenuation in ferrets or rodents and moreover have a demonstrated attenuated phenotype in humans.

#### 2.1.2 *Hazards arising from the inserted gene product in a reassortant vaccine strain*

The products of the inserted genes will be, at a minimum, the haemagglutinin (HA) and neuraminidase (NA) of the pandemic strain virus. For reassortants derived from highly pathogenic H5 or H7 strains by reverse genetics, the

HA will have been modified so that the multiple basic amino acids at the HA cleavage site, which are associated with high pathogenicity, will be reduced to a single basic amino acid. Any protein derived from the wild-type strain on its own will be neither inherently infectious nor harmful.

### 2.1.3 *Hazards arising from reassortant viruses*

#### 2.1.3.1 *Direct hazards*

Without treatment, reassortant viruses may be expected to survive for at least a short time (hours) on surfaces or in a laboratory environment and thus provide a potential means of infection for laboratory workers. Although the surface antigens of reassortants, particularly the HA, can contribute to pathogenicity (*5*, *6*) published information indicates that a reassortant between PR8 and a wild-type human influenza virus is likely to be avirulent in humans (5, *7–9*). Although such information is difficult to interpret because the genetic composition of the reassortants was not clear, it is known that the degree of attenuation increases as reassortants include more PR8 genes (*10*, M Tashiro, unpublished data). The reassortants created by reverse genetics as H5N1 pandemic reference strains contain six out of eight viral genes from PR8 and the NA and modified HA genes of the H5N1 virus. Furthermore, the H5 HA retains a preference for $\alpha$2,3 linked residues (see below), so the ability of the H5N1 reassortants to bind to and replicate in human cells should be minimal. It is therefore envisaged that an H5N1 reassortant derived by reverse genetics according to WHO guidance (*11*) would be attenuated for humans compared to the H5 wild type. Furthermore, it is clear that such reassortants are expected to be of low pathogenicity in chickens and other animals compared to the highly pathogenic parental wild strains, and this expectation has been borne out by experience to date. Nevertheless, as the factors affecting pathogenicity are not fully understood (see below), genetic manipulation to remove the polybasic sites could theoretically have unpredicted effects on both transmissibility and pathogenicity.

For reassortants derived by traditional co-cultivation methods, the gene constellation is less predictable. There is a theoretical possibility of developing reassortants with more than two wild-type parental genes or even of selection of a mutant (non-reassortant) wild-type virus with improved growth characteristics. If vaccine production takes place in the interpandemic phase there would be a need to determine the gene constellation of reassortants derived by traditional co-cultivation methods in order to conduct a full risk assessment.

Reassortants with a 6:2 gene constellation based on live attenuated recipient strains such as A/Ann Arbor/6/60, or other strains used as live attenuated vaccines, may also be used for the production of pandemic influenza vaccine. The attenuated A/Ann Arbor/6/60 strain has been used

as a backbone in 6:2 reassortant live attenuated vaccines in clinical studies for more than 30 years using approximately 30 different vaccine strains, and the data demonstrate that the Ann Arbor/6/60 virus produces reassortant vaccine strains that are attenuated for humans (*12*). Live vaccines derived from the Ann Arbor strain have been licensed in one country. An adequate level of attenuation should be expected for modified H5 reassortant strains. For each candidate pandemic strain, this should be verified by testing as described below (section 3.6.1).

Reassortants may be also be derived from non-H5 or non-H7 viruses (e.g. H9N2, H2N2) and may use either PR8 or an attenuated vaccine strain. The hazards associated with such reassortants depend on HA receptor specificity. If a reassortant has a preference for avian cell receptors ($\alpha$2,3 linked sialic acid e.g. avian H2N2 viruses), the hazards are considered to be no different from those associated with the above-mentioned 6:2 reassortants derived from attenuated H5 or H7 viruses (see section 3.3). However, if a reassortant has a preference for mammalian cell receptors ($\alpha$2,6 linkages, e.g. human H2N2 pandemic virus from 1957), or possesses both avian and mammalian receptor specificities (e.g. H9N2), there is a greater risk of human infection (see Table 1).

#### 2.1.3.2 *Indirect hazards*

Although it is considered that, for example, an H5N1/PR8 reassortant will be either attenuated or possibly non-infectious to humans, an indirect hazard may exist through secondary reassortment with a human or animal influenza virus as influenza viruses are known to exchange genes by the process of reassortment. For secondary reassortants to be generated, several events need to occur; firstly infection of the production staff with the reassortant strain; secondly, for the infected worker to have a mixed infection with a wild type influenza virus, and thirdly for a reassortment event to take place. In practice, manufacturers have 30 years of experience with large scale production of vaccines based on PR8 reassortants and there have been no reported cases of human illness. However, it should be noted that this does not rule out the possibility of infections having occurred. Additionally, at the point when seasonal influenza vaccines become available, production staff can be vaccinated to reduce the chances of an infection with a circulating wild-type virus.

In practice, the lack of success in producing H5N1 reassortant vaccine strains in 1997 (UK: avian and swine viruses; Australia and USA: avian and PR8 viruses) suggests that the probability of producing H5 reassortants between mammalian and avian viruses in human cells is slight. It should also be considered that poultry and pig farmers are continually exposed to animal influenza viruses and there have been few documented cases of human infection in this population with a reassortant between an avian or

porcine and a human influenza virus. Based on these considerations the probability that a PR8 reassortant strain will replicate and combine with another influenza virus(es) in human cells is considered to be minimal. The risk of such secondary reassortments for animal species will be considered in the environmental risk assessment section (see section 3.2).

#### 2.1.4 *Hazards arising from the use of wild type viruses for pandemic strain vaccine production*

Wild-type strains may be considered for production purposes and different potential vaccine candidates could be:

— an avian strain with no record of human infection (surrogate virus);
— an avian strain with documented human infection (potential pandemic virus);
— an actual human pandemic virus, or a past H2N2 pandemic virus.

The hazards from wild type vaccine strains will differ according to the category of wild-type virus used but in all cases are compounded during vaccine manufacturing and associated vaccine product testing, due to the high volumes and high titres encountered. With the exception of surrogate viruses, the use of wild type pandemic-like influenza viruses to develop pandemic vaccine strains presents considerable biosafety risks to personnel in vaccine manufacturing facilities and testing laboratories, and also to the general community if manufacture is taking place for clinical studies or stockpiling of vaccines during the interpandemic period.

### 2.2 Hazards arising from the production process

Vaccine manufacture follows *Good manufacturing practices for biologicals* (*13*). Good manufacturing practices (GMP) require protection of the product from the operator and the environment and thus amelioration of certain hazards associated with production will require the establishment of a suitable balance between GMP and biosafety requirements.

#### 2.2.1 *Production in eggs*

Influenza vaccine has been produced in embryonated hens' eggs on a large scale since the early 1950s. Much experience has been gained and some facilities are capable of handling large numbers of eggs on a daily basis with the aid of mechaniyed egg handling, inoculation and harvesting machines.

Hazards occur only during the production stages and quality control laboratory activities prior to virus inactivation. The most hazardous production stage is egg harvesting when the eggs have to be opened to harvest the allantoic fluid. The volume and titre of virus is higher at this

stage than at any other. The open nature of the operations leads to a greater exposure to aerosols and spills. In contrast during egg inoculation, the virus used is dilute and of a relatively small volume. The allantoic fluid that is harvested from the eggs is invariably manipulated thereafter in closed vessels and hazards arising from live virus during downstream processing and during the virus inactivation process, if used, are therefore less than during virus harvest. Collection and disposal of egg waste is potentially a major environmental hazard. Safe disposal of the waste from egg-grown vaccines, both within the plant and outside, is therefore critical.

#### 2.2.2 *Production in cell cultures*

For pandemic influenza vaccines produced on cell cultures, the biosafety risks associated with manufacturing will depend primarily on the nature of the cell culture system employed. Closed systems, such as bioreactors, normally present little to no opportunity for exposure to live virus during normal operation, but additional safety measures must be taken during procedures where samples are introduced into or removed from the bioreactor, and during procedures to deal with accidental spills. Roller bottles and cell culture flasks used for virus production may allow exposure to live virus through aerosols, spills, and other operations during virus production and, thereafter, additional risks are associated with the inactivation and disposal of the large quantities of contaminated solid waste generated by this method.

The possibility exists that genetic mutations may be selected in pandemic vaccine viruses during passage in mammalian cells that render them more adapted to humans. Sequence analysis of the region of the HA gene encoding the receptor binding site may be useful. However, it should be noted that little is known about the relation between cell substrate and virus reversion or adaption. Beare et al. (*5*) tried to de-attenuate PR8 by multiple passage in organ cultures of human tissue, but failed, whereas studies with MDCK cells (*14*) demonstrated that human viruses that retained their $\alpha$2,6 receptor specificity (human-like) were likely to mutate to an $\alpha$2,3 specificity (avian-like) as this provided a replicative advantage on MDCK cells, rather than the reverse. Overall, hazards arising from the inherent properties of a reassortant or wild type virus are likely to be far greater than the probability of adaptation of the virus to a more human-like phenotype.

## 2.3 Factors affecting pathogenicity for humans

#### 2.3.1 *HA receptor specificity*

The influenza HA is responsible for attachment of virus to the target cell and has specificity for sialic acid receptors on cell surface molecules. The HAs present on human influenza A viruses preferentially bind to receptors

containing α2,6-linked sialic acid residues, whereas avian influenza viruses preferentially bind to α2,3-linked sialic acid (*15*). Human tracheal cells have mainly α2,6 linked residues (*16*), so the acquisition of an avian HA by PR8 virus is expected to minimize potential binding to human respiratory epithelial cells. Although the α2,3 receptor specificity of avian viruses will reduce the efficacy of such binding, it may not completely prevent infection in humans. Moreover, the presence of avian-like receptors has been demonstrated in human respiratory tract epithelium (*17*). Beare and Webster (*18*) found that over 100 fold higher quantities of avian viruses (between 106.8 and 109.2 egg infectious doses) were needed for replication in humans and, because replication was poor, that it was not possible to induce person-to-person transmission.

There have been many reports of human infections with avian H5N1 viruses since 1997 in south-east Asia. It is possible that exposure to high-titre H5N1 virus in contaminated chicken or duck carcasses or animal products may have overcome the avian specificity of HA receptor binding. Virus replication in such human cases was much better than in the earlier experimental studies of avian influenza viruses in humans (*18*); however, the extensive replication of H5N1 viruses in these people is inexplicable on the basis of current knowledge of receptor specificity because the viruses isolated from them retained the α2,3 avian specificity.

### 2.3.2 *HA cleavability*

The HA of influenza virus must be cleaved into HA1 and HA2 by host cell proteases as a prerequisite for infectivity, and this cleavage has been correlated with virulence. The pathogenicity of H5 and H7 influenza A viruses in chickens is largely determined by the nature of the amino acids at the HA cleavage site. H5 and H7 viruses with multiple basic amino acid sequences are highly pathogenic and their HA can be effectively cleaved by the ubiquitous furin-like proteases, which are expressed in most organs of birds and humans. In contrast, the HA of H5 and H7 viruses of low pathogenicity for birds and certain laboratory animals contain a single basic residue at the cleavage site, a feature common to all other subtypes of influenza HA, and which can only be cleaved by trypsin-like proteases, which are restricted to certain cell types, e.g. epithelial cells lining the respiratory tract of humans and the gut of birds. Thus, HA cleavability influences tissue specificity and is a major determinant of pathogenicity for H5 and H7 viruses in chickens and certain laboratory animals. Multiple basic amino acids at the cleavage site have not been observed for any other HA subtype.

Direct evidence has been obtained that both HA cleavage and HA receptor specificity have an effect on tissue tropism of an avian H7N1 virus, A/

Fowl Plague/Rostock/34 in chicken embryos (*19*). Similarly, the available evidence from the H5N1 infections in 1997 demonstrates that the high degree of pathogenicity in chickens, mice and ferrets is directly influenced by the presence of the multiple basic amino acids. Webby et al. (*20*) demonstrated that removal of the basic amino acids changed H5N1 infections from a fatal systemic infection to a localized non-pathogenic infection in chickens (i.e low pathogenicity for chickens), mice and ferrets. Hatta et al. (*21*) and Lipatov et al. (*22*) have also shown by reverse genetics that high cleavability of H5N1 HA due to the presence of multiple basic amino acids was an essential requirement for a lethal mouse infection. It is not ethical to examine the pathogenicity of influenza virus infection in humans, but an examination of H5N1 viruses by Gao et al (*23*) provided evidence that pathogenicity in mice can resemble that in humans. The occurrence of multiple organ failure after human H5N1 infections is suggestive of an unusual tissue tropism. Although evidence for viral replication outside the lung has been described for at least one human case (*24*), such evidence remains difficult to document (*25*).

The available evidence suggests that virulence of the 1997 and later H5N1 viruses for humans is related to the presence of the HA multiple basic amino acids. It is therefore considered imperative to remove them, if present in the HA of any H5N1 virus being developed as a vaccine strain, to reduce the potential for harm to humans. This procedure will also increase the safety of the reassortants for avian species (see below under environmental risk assessment) as cleavage site modifications have resulted in a reduction of their pathogenicity in avian embryos (*26*). It should be noted that during production of reassortants by reverse genetics, base substitutions are introduced to stabilize the removal of multiple basic amino acids during passage of reassortants.

### 2.3.3 *Other factors affecting pathogenicity*

Although it is clear from experience in south-east Asia from 1997 to the present that H5N1 influenza viruses that display $\alpha$2,3 sialic acid specificity could replicate in humans, it must be noted that influenza virus pathogenicity does not depend solely on HA, but is a polygenic trait. The 1997 H5N1 virus had unusual PB2 and NS1 genes that influenced pathogenicity whereas the 2004 H5N1 viruses possess complex combinations of changes in different gene segments that affect pathogenicity in ferrets (*27*). Changes in the PB2 gene of the 1997 H5N1 viruses were sufficient to attenuate them for mice (*21*) and changes in the NS1 protein rendered these viruses resistant to the effects of interferons and other cytokines produced as part of the innate immune response (*28*). The changes to NS1 conferred a highly virulent phenotype which allowed replication to proceed unchecked in vivo. In this case even a virus with a poor affinity for its receptor was able to replicate

(although not to transmit). In contrast, viruses with a gene constellation producing PR8 internal proteins were clearly sensitive to the innate immune mechanisms which prevent the establishment of infection by an avian virus in humans. This may well explain why in the outbreaks of H5 avian influenza before 1997, no evidence of transmission from birds to humans was noted. Further, prior to the 2003 outbreak in the Netherlands, only two cases of transmission of H7 viruses from birds to humans were documented (*29*, *30*). Also during the many years of laboratory handling of high-titre avian viruses (of which one H7 strain (A/FPV/Dobson) is known to contain a gene which adapts it for replication in mammalian cells (*31*)), there has only been one report in the literature of a worker being affected by these viruses. This was a laboratory worker in Australia who developed conjunctivitis after accidentally being exposed to a H7N7 virus directly in the eye (*32*). The PR8/H5N1 6:2 reassortants and the A/Ann Arbor/6/60 live attenuated 6:2 reassortants created by reverse genetics for the production of H5N1 vaccine do not contain the gene constellation considered necessary for pathogenicity in chickens, mice and ferrets and in contrast have internal genes that confer sensitivity to the innate immune response.

## 2.4 Hazards arising from the vaccine

Inactivated pandemic influenza vaccines present no biosafety risks provided that the results of the inactivation steps show complete virus inactivation, as the viral vaccine is rendered incapable of replication.

In an interpandemic or pandemic alert period, pilot-scale live attenuated pandemic influenza vaccines may be developed for clinical evaluation. As there is some uncertainty concerning the biosafety risks associated with shedding or other unintentional release into the environment following vaccination, subjects participating in clinical trials in the interpandemic or pandemic alert phase should be kept under appropriate clinical isolation conditions. If this is not done, indirect hazards for humans could arise as considered in section 3.1. Furthermore, for pandemic human influenza vaccine strains that express H5 or H7 avian influenza genes, there will be potential consequences for agricultural systems (section 3.2.2). If viruses of the H5 or H7 subtype become transmissible in livestock, this would be notifiable to OIE and could result in sanctions with serious economic and trade implications to prevent the spread of disease.

If a human pandemic has already started, the hazards from live attenuated vaccines elaborated above will not be relevant.

## 2.5 Previous large-scale experience with reassortants

Reassortants derived from PR8 have been used routinely for the production of inactivated influenza vaccines for the past 30 years. This work involves

the production of many thousands of litres of infectious egg allantoic fluids, which create substantial aerosols of reassortant virus within manufacturing plants. Most of the reassortants were made from wild type human strains that had not yet been in widespread circulation. Thus, although the manufacturing staff would have some susceptibility to infection with the wild type virus, there have been no anecdotal or documented cases of work-related human illness resulting from occupational exposure to the reassortants.

Similarly, reassortants derived from the A/Ann Arbor/6/60 strain have been used for the production of live attenuated vaccine for at least 3 years and no anecdotal or documented cases of work-related human illness have been reported. While to date no conclusive study has been conducted to detect silent infections for either the PR8 or live attenuated strains, and thus infectivity in humans cannot be fully assessed, the attenuation status of these vaccine strains continues to be supported by their excellent safety record to date

However, unlike the situation with the human influenza strains selected for the annual vaccine formulation, staff manufacturing an H5N1 vaccine would have no previous immunological experience of the avian virus, and would therefore be expected to be susceptible, although the risk of work-related human illness and of transmission outside the facility is expected to be slight and lower than for non-reassortant strains.

## 2.6 Testing of reference viruses being considered for vaccine production

Vaccine reference viruses will be developed by a WHO laboratory or by a laboratory approved by a national regulatory authority (hereafter, for ease of reference, referred to as a WHO laboratory). The following tests and specifications have been developed based on experience gained in the evaluation of 6:2 reassortant H5N1 viruses produced on the PR8 and A/Ann Arbor/6/60 backbones. The principles outlined should be applicable during the interpandemic period to other reassortant strains, but exceptions may be made if appropriately justified. Tests on wild-type viruses being considered for vaccine production will need to be selected on a case-by-case basis. The tests described below are usually conducted by the WHO laboratory developing the reference strain.

In a pandemic alert period or a pandemic period, the requirement for the conduct or the completion of some or all of these tests prior to the distribution of a candidate reference strain may be relaxed based on the risk assessment. For example, in a pandemic alert period, a candidate reference strain which on the basis of molecular analyses, is expected to have a low risk of human infection and transmission could be distributed to vaccine manufacturers to enable them to begin preparation of their seed stocks prior

to the completion of time-consuming tests such as the chicken and the ferret pathogenicity tests. If a pandemic has already begun, and the pandemic virus has become adapted to human infection, there may be no need to perform all the pathogenicity tests indicated below. A risk assessment should be performed for each candidate reference strain and the outcome will depend on the nature of the strain and the pandemic period declared by WHO.

### 2.6.1 *In vivo tests to evaluate pathogenicity of H5 and H7 viruses*

For optimal interpretation of tests, the pathogenic properties of the candidate reference virus, should be compared with those of the parental backbone strain and the wild-type strain.

These tests should be performed under appropriate high laboratory containment conditions (see section 4.3). Tests to be performed on the candidate vaccine reference strain (see Table 1) by the WHO reference laboratory that develops the reassortant strain include:

- *The ability to plaque in the presence or absence of added trypsin*. Viruses with high pathogenicity can replicate in mammalian cell culture in the absence of added trypsin, whereas those with low pathogenicity generally do not.
- *The ability to cause chicken embryo death.* Highly pathogenic viruses cause rapid chicken embryo death upon inoculation into eggs whereas removal of the multiple basic amino acids from a highly pathogenic strain results in embryo survival (*26*).
- *Pathogenicity in chickens.* The chicken intravenous pathogenicity (IVP) test is an important statutory test required by veterinary authorities, and a reassortant virus must have an index of 1.2 or less before it can be removed from high-level containment (*33*). Development of specifications to indicate that the test articles have been correctly administered in the IVP test would be beneficial.
- *Attenuation in ferrets.* The viruses should be shown to be attenuated in ferrets or in other suitable animal models, provided they have virus sensitivity equivalent to that of ferrets and a similar ability to discriminate between highly pathogenic and non-pathogenic influenza viruses. These tests compare the candidate reference virus with the wild type virus. Detailed test procedures are described in Appendix 1. In the case of H5N1 reassortants, the criteria used to evaluate this test are that virus replication and clinical symptoms should be comparable to those induced by the attenuated PR8 parent virus and should be milder than the wild-type human H5N1 virus infection.

Ferrets were chosen because they have been used extensively as a good indicator of influenza virus virulence for humans (reviewed by Smith and Sweet, *34*). Typically, human influenza viruses cause lethargy, nasal discharge and occasionally fever in ferrets, and virus replication is usually

limited to the respiratory system. PR8 virus has been assessed in ferrets and found to cause few or no clinical signs, and virus replication is limited to the upper respiratory tract. However, the 1997 and 2004 wild-type human H5N1 viruses replicated in ferrets throughout the body, caused fever, weight loss and occasionally death (*27*, *35*). Thus, in the absence of human data, the ferret is the best model to predict whether a virus will be pathogenic or attenuated in humans.

It would be useful to be able to measure transmissibility as well as pathogenicity of virus strains, but currently a well-characterized methodology to do so is lacking. Intranasal administration of virus to chickens may be one such method, and has been shown to be possible, but to date the test is not standardized. Uninoculated birds in close contact with infected birds in the intravenous pathogenicity test may provide some information on transmissibility. Transmission studies in ferrets after oral and ocular inoculation are also potentially useful, but need to be standardized.

Tests for safety in mice may provide useful information if the parent strain is virulent in mice. Detailed test procedures are described in Appendix 1.

A reassortant virus should be used for vaccine manufacture only after appropriate results have been obtained in the above tests. For H5 and H7 strains, the nucleotide sequence corresponding to the HA cleavage site should be determined by the WHO laboratory to demonstrate the absence of multiple basic amino acids in the vaccine candidate. After WHO has declared a pandemic manufacturers may receive candidate reference strains that have not been assessed fully for pathogenicity. In this case they should handle the viruses appropriately depending on the nature of the virus and the pandemic situation.

### 2.6.2 *Genetic stability of H5 and H7 viruses*

Genetic stability is an important issue as it is known that in poultry, wild-type low-pathogenicity H5 and H7 avian viruses can become highly pathogenic by mutation (insertion of basic amino acids at the HA cleavage site) and this is the origin of the highly pathogenic H5 and H7 strains. Although the derivation of low-pathogenicity candidate reference viruses by reverse genetics involves the introduction of silent mutations in the region of the HA cleavage site that should minimize the re-insertion of multiple basic amino acids, during vaccine production, such viruses may be passaged several times and it is therefore important to evaluate their genetic stability at the cleavage site. Several attenuated reassortants have now been produced between PR8 virus and highly pathogenic H5N1, H5N3 and H7N1 viruses by reverse genetics (*20*, *26*, *36*, *37*, FLUPAN (http://www.nibsc.ac.ukflupan/)) and following extended passage in eggs (up to 10), they have each retained their attenuated phenotype.

Nevertheless, manufacturers should assess any H5 and H7 seed viruses and vaccine virus harvests by sequence analysis of the HA cleavage site. The need for studies of genetic stability for seed viruses prepared from candidate reference strains derived by other methods should be assessed on a case-by-case basis. At least one in vivo test (section 3.6.1) should be applied, for example the egg embryo lethality test.

#### 2.6.3 *Evaluation of wild-type non-pathogenic H5 or H7 viruses or reassortants derived from them*

In view of the propensity for non-pathogenic H5 and H7 viruses to acquire mutations leading to increased pathogenicity, it is advisable to conduct the full spectrum of pathogenicity tests (in ferrets, chickens and chicken embryos), as indicated in section 3.6.1.

#### 2.6.4 *In vivo evaluation of non-H5, H7 viruses or reassortants derived from them*

Ferret tests are required for non-H5, non-H7 candidate vaccine strains prior to manufacture. The tests should be conducted under biocontainment levels equivalent to that required for the production of the reference strain. The other tests (specified in sections 3.6.1 and 3.6.2) are not required because they are specific for reassortants derived from highly pathogenic H5 and H7 viruses.

# 3. Risk assessment

## 3.1 Health protection

### 3.1.1 *Likelihood of harm to human health*

By virtue of PR8 attenuation, avian receptor specificity, loss of multiple basic amino acids at the HA cleavage site and the absence of other H5N1 genes associated with pathogenicity in humans (i.e. NS1 or PB2 genes), it is envisaged that an PR8 x H5N1 6:2 reassortant, although possibly infectious to humans and ferrets, will have only a low probability of causing harm to human health. On the basis of these arguments, reassortants derived from H5 or H7 strains in which the multiple basic amino acid HA0 cleavage site has been removed, using either PR8 or strains attenuated for humans e.g. the A/Ann Arbor/6/60 as the recipient virus, would be likely to be similarly attenuated. Reassortants derived from all other subtypes or from low pathogenicity H5 and H7 subtypes, in which the multiple basic amino acids were not present, should also be attenuated by virtue of the receptor specificity of the avian HA and the attenuating effect of the 6 PR8 genome segments (absence of any other avian genes). The same arguments are also valid for reassortants prepared from live attenuated virus strains such as A/Ann Arbor/6/60.

If staff at a vaccine production plant are exposed to aerosols containing high-titre reassortant virus, sub-clinical infections could result. If this happened, it is very unlikely that a reassortant virus would transmit to human contacts as it is likely that replication will be attenuated and virus shedding, if it occurs, it would be well below the titres considered to be needed for human infection.

However, although there is no precedent, as described above there is a theoretical possibility of secondary reassortment with normal human influenza viruses and that such reassortant viruses may be replication-competent in humans, while having avian-virus like coat proteins. Although it is very unlikely that the secondary reassortant could become adapted to human infection and transmission, were this to happen the public health consequences would be serious. The likelihood of such occurences can be reduced through biosafety measures designed to limit exposure of personnel to high-titre materials during vaccine production and testing.

If non-reassortant wild-type viruses with multiple basic amino acids at the HA cleavage site and high in vivo pathogenicity are used for vaccine production they would potentially be highly pathogenic and transmissible in humans. Stringent vaccine biosafety control measures are required to manage the risk from vaccine production using such viruses. Non-reassortant wild-type viruses, without multiple basic amino acids at the HA cleavage site with low in vivo pathogenicity and avian receptor specificity are likely to be less pathogenic and less transmissible in humans (*18*) than the wild type viruses described above. However the risks of secondary reassortment with normal human viruses remain and the risk that such reassortant viruses may be able to replicate in humans. Appropriate vaccine biosafety control measures are required to manage the risk from vaccine production using such viruses. Non-reassortant wild-type viruses, without multiple basic amino acids at the HA cleavage site, with low in vivo pathogenicity and mammalian receptor specificity (e.g. human H2N2 and H9N2) are also likely to be less pathogenic than the wild-type viruses described above, but their ability to transmit to humans is unknown. Consequently, because of the risks of secondary reassortments, appropriate biosafety control measures should be considered.

## 3.2 Environmental protection

### 3.2.1 *Nature of the work*

Egg-based vaccine production represents a relatively open system with several operations likely to generate virus aerosols: namely, seed virus preparation, egg inoculation, harvest of infected egg fluids, use of laminar outward air flows, segregation of contaminated eggs, cleaning (that may include high powered spraying) and decontamination of contaminated egg trays, and disposal of waste products.

Prior to the virus inactivation step, cell culture production requires handling large volumes of high-titre preparations of live influenza virus. As mentioned above, even in closed systems such as bioreactors leaks can occur, and spillage or other operator contact with high-titre viral solutions during the introduction of materials into the bioreactor, taking of samples, or clean-up procedures is possible. If roller bottles or cell culture flasks are used in place of bioreactors there will be a higher risk of generating aerosols and spills due to the increased manipulations required, and the volume of materials to be properly decontaminated for disposal will be proportionally greater.

### 3.2.2 *Environmental considerations*

Influenza A viruses are endemic throughout the world in some farm animals (pigs and horses) and some populations of wild birds, specifically birds of the families *Anseriformes* (ducks, geese and swans) and *Charadriiformes* (shorebirds) (*38*). Of the influenza A viruses, a number can cause disease in domestic poultry, such as H5, H7 and H9. H5 and H7 are thought to be highly pathogenic in poultry, whereas H9 is typically less so. In addition, sporadic infections by influenza A viruses have been reported in farmed mink, wild whales and seals, dogs and captive populations of big cats (tigers and leopards) (*38*, *39*). In dogs, the influenza A infections were caused by H3N8 viruses closely related to endemic equine viruses, and in the big cats, the infections followed consumption of dead chickens infected with H5N1 viruses.

In the case of an H5N1 reassortant, the virus will have avian receptor specificity, and thus birds would theoretically be the species most susceptible. The contribution of the six PR8 internal genes to replication and virulence in birds is unknown.

However, Hatta et al. (*40*) have recently demonstrated, by the use of reverse genetics, that acquisition of only one PR8 gene by an avian influenza virus can abolish virus replication in ducks. Experimental evidence has demonstrated that PR8 virus is attenuated not only in humans (see above), but also chickens (*37*). Furthermore, a reassortant between PR8 (internal protein genes) and the 1997 Hong Kong H5N1 virus (NA and HA with a single basic amino acid) replicated poorly in chickens and was not lethal. Similar studies have been performed with the 2003 Hong Kong H5N1 virus at the WHO Collaborating Centre, Memphis, USA (R Webster, unpublished data), where the 6:2 PR8 reassortant did not replicate or cause signs of disease in chickens. The removal of the multiple basic amino acids from the H5 x PR8 reassortants in both studies probably played a major role in reducing the risk for chickens.

Although replication occurs in chicken embryos, for reasons that are unknown, the risk of environmental transmission via such replication in nature is remote.

Pigs are uniquely susceptible to infection by all strains of influenza A virus because they have both alpha 2,3 and alpha 2,6 receptors in abundance. Although pigs are not susceptible to infection with PR8, a reassortant containing a single gene (HA) from an A/New/Jersey/76 (H1N1) isolate, infected pigs and the animals excreted virus (*6*). It is thus conceivable that pigs are susceptible to infection by an H5N1 reassortant, as viruses with avian receptor specificity are known to replicate in this species. It is also possible that these species would be susceptible to secondary reassortments between the H5N1 reassortant and a pig virus. There is in fact evidence that triple reassortants between avian, pig and human influenza viruses have circulated in pigs (*41*).

## 3.3 Assignment of containment level

The production of influenza vaccine reassortant reference viruses, by WHO Collaborating Centres, from highly pathogenic H5 or H7 wild type viruses should take place at a high level of biocontainment (BSL-3 enhanced or BSL-4, as advised by WHO and national authorities) (*11*). The collaborating centres provide characterized reassortant reference viruses to vaccine manufacturers who may develop vaccine seeds and vaccines from these materials.

In consideration of the hazards associated with egg and cell culture H5 and H7 vaccine production and quality control with reassortant viruses of demonstrated low pathogenicity in chickens and/or in ferrets (and mice if applicable), as specified in sections 3.6.1 and 3.6.2, the assigned containment level is BSL-2 enhanced (pandemic influenza vaccine), as defined below (see Table 1). This applies to both pilot-scale and large-scale production during the interpandemic phase and pandemic alert period (*42*) when the site of vaccine production is geographically remote from the site of the emerging pandemic. Any subsequent relaxation of the levels of containment during the developing pandemic, should be decided on a case-by-case basis after careful evaluation of the risks.

In consideration of hazards associated with egg and cell culture vaccine production and quality control with wild-type viruses (non-H5 and non-H7) of demonstrated low pathogenicity in ferrets, as specified in section 3.6.3, the assigned containment level is BSL-2 enhanced (pandemic influenza vaccine), as defined below. This applies to both pilot-scale and large-scale production during the interpandemic phase and pandemic alert period (*42*) when the site of vaccine production is geographically remote from the site of the emerging pandemic. Any subsequent relaxation of the levels of containment during the developing pandemic, should be decided on a case-by-case basis after careful evaluation of the risks.

In consideration of hazards associated with cell culture vaccine production and quality control with highly pathogenic H5 or H7 wild-type viruses, the

assigned containment level is BSL-3 enhanced (pandemic influenza vaccine), as defined below. This applies to both pilot-scale and large-scale production during the interpandemic phase and pandemic alert period (*42*) when the site of vaccine production is geographically remote from the site of the emerging pandemic. Any subsequent relaxation of the levels of containment during the developing pandemic, should be decided on a case-by-case basis after careful evaluation of the risks. In addition, the parts of the facility where such work is done (both production and quality control) should meet the OIE requirements for containment, which include not only biosafety, but also requirements for biosecurity. (*33*). In view of the open nature of large scale egg-based vaccine production, it is not possible to operate at BSL-3 enhanced (pandemic influenza vaccine). Therefore egg-based vaccine production from high pathogenicity H5 or H7 wild-type strains is not recommended.

For vaccine production and quality control using other types of vaccine virus (e.g. reassortants derived from non-H5 or H7 viruses; wild-type low-pathogenic H5 or H7 viruses), the assigned containment level is BSL2 enhanced (pandemic influenza vaccine), as defined below. This applies to both pilot-scale and large-scale production during the interpandemic phase and pandemic alert period (*42*) when the site of vaccine production is geographically remote from the site of the emerging pandemic. Any subsequent relaxation of the levels of containment during the developing pandemic, should be decided on a case-by-case basis after careful evaluation of the risks.

It should be noted that implementation of the containment conditions described in this section within a production and quality control testing facility must take into account the large quantities and high titres of live virus that are produced, the industrial scale of facilities, as well as the rules and regulations governing the manufacture and testing of medicinal products known as good manufacturing practices (GMP) (*13*). The facility requirements for a specific biosafety level within a manufacturing plant will differ from the facility requirements within a laboratory handling smaller quantities of infectious material such as a laboratory producing reassortant reference viruses or in a pilot-scale facility. It should also be noted that these biosafety requirements apply to the production and quality control operations involving live viruses; virus lots shown to be inactivated by a validated process need not be handled under these conditions.

## 3.4 Environmental control measures

Each vaccine manufacturer must review their own control measures in light of the intended work, the nature of laboratory and production facilities and the need to maintain GMP. Influenza specific enhanced containment measures (defined in 4.4.1 and 4.4.2) should be in place for open manipulations with live

virus, especially virus harvesting in egg production facilities. Quality control facilities need to meet production containment requirements, and in some regions, a second approval will be needed to meet other requirements such as those regulating products containing materials derived from a genetically modified organism (GMO).

Local safety regulations provide guidance on the disposal of potentially infectious waste. Contaminated waste from current production facilities may reach high virus titres. Decontamination methods should be validated. If possible, decontamination of waste should take place on site. Where this is not possible, there should be procedures in place to ensure that material is safely contained and transported prior to decontamination off site. Guidance on regulations for the transport of infectious substances is available from WHO (*4*). In all cases the procedures should be validated to ensure that they function at the scale of manufacturing.

In view of the possible exposure to high titre pandemic strain virus and the need to reduce the chance of simultaneous infection with human influenza viruses, staff should be prophylactically vaccinated with seasonal influenza vaccines. It is anticipated that before large scale vaccine production is attempted, pilot lots of pandemic strain vaccine will have already been produced. Experimental vaccines inducing protective antibody levels are recommended for use by staff before large scale vaccine production commences if possible. Antiviral treatment must be available in case the situation warrants it.

Each manufacturer should also assess the risk of contamination of birds or pigs based on the likelihood of their being in the vicinity of the manufacturing plant, and the manufacturing controls in use. Staff or other personnel entering the area potentially exposed to live virus should avoid visiting pig, horse or bird facilities (e.g. farms, equestrian events, bird sanctuaries) for at least 14 days following occupational exposure. This period should be extended to 14 days after the symptoms resolve if conjunctivitis or respiratory signs indicating the potential development of influenza infection or disease develop during this 14 day period.

It is also known that mice can be experimentally infected with some influenza viruses and the PR8 strain is known to be lethal for mice. It is not known whether a reassortant based on PR8 will be able to replicate in mice, but steps should be taken to prevent exposure of wild mice and the escape of laboratory mice, and rodent control measures should be in place.

### 3.4.1 *Specifications for "BSL2 enhanced (pandemic influenza vaccine)"*

Specifications for BSL2 enhanced (pandemic influenza vaccine) facilities include the following in addition to the principles for BLS2 facilities as specified in the WHO *Laboratory biosafety manual* (*1*).

#### 3.4.1.1 *Facility*

The facility should be designed and operated according to the stage of the manufacturing process to meet the demands of protection of the recipient of the vaccine, the staff producing and testing the vaccine and of the environment. It is noted that different solutions may be needed depending on the risks inherent in the operation(s) conducted in an area. Specialized engineering solutions will be required that may include:

— use of relative negative pressure biosafety cabinets when possible;
— use of high-efficiency particulate air (HEPA) filtration of air prior to exhaust into public areas or the environment; and
— use of positive pressure with negative pressure in-line sinks prior to exhausting to the non-viral zone.

In addition the following decontamination procedures should take place:

— decontamination of all waste from BSL-2 enhanced (pandemic influenza vaccine) areas; and
— decontamination of manufacturing and quality control areas at the end of a production campaign through cleaning and validated decontamination for example gaseous fumigation.

#### 3.4.1.2 *Personal protection*

- Full-body protective laboratory clothing (for example Tyvek® disposable overalls) is to be worn in the controlled BSL-2 enhanced (pandemic influenza vaccine) area.
- If activities cannot be contained by primary containment and open activities are being conducted, the use of respiratory protective equipment, such as N95, FFP3 (*43*) or equivalent respirators is strongly recommended. Minimal specifications for the filtering/absorbing capacity of such equipment should be met, and masks, if used, must be fitted properly and the correctness of fit tested.
- Personnel should be instructed, in a written document to which they sign their agreement, not to have any contact with birds or pigs, in particular farm animals for 14 days after departure from the facility where vaccine has been produced. Currently the risks involved in contact with household dogs and cats are not considered to be significant, but the available scientific evidence is sparse.
- Staff should be prophylactically vaccinated with seasonal inactivated influenza vaccines.
- It is anticipated that before large scale vaccine production is attempted, pilot lots of pandemic strain vaccine will have already been produced. Experimental vaccines inducing protective antibody levels are recommended for use by staff before large scale vaccine production commences if possible.
- Antiviral treatment must be available in case the situation warrants it.

#### 3.4.1.3 *Monitoring of decontamination*

- Cleaning and decontamination methods need to be validated periodically as part of a master validation plan to demonstrate that the protocols, reagents and equipment used are effective in the inactivation of pandemic influenza virus on facility and equipment surfaces, garments of personnel and waste materials, and within cell growth and storage containers. Once decontamination procedures for influenza virus have been fully described and validated, there is no need to repeat them for each new strain. Validation studies using influenza viruses may be supplemented by studies with biological (for example bacterial) markers selected to be more difficult to inactivate than influenza.

### 3.4.2 *Specifications for "BSL3 enhanced (pandemic influenza vaccine)"*

Specifications for BSL3 enhanced (pandemic influenza vaccine) facilities include the following requirements in addition to the principles for BLS3 facilities as specified in the WHO Laboratory biosafety manual (*1*), and are additional to the specifications given above in section 3.4.1.

#### 3.4.2.1 *Facility*

The facility should be designed and operated to meet the demands of protection of the recipient of the vaccine, the staff producing and testing the vaccine and of the environment. This will require specialized engineering solutions that may include:

— negative pressure secondary containment areas
— HEPA filtration on supply and exhaust air
— on-site decontamination of liquid effluent
— floor dams should be erected around bioreactors or other large scale equipment including storage tanks to contain spillage of virus from large virus-containing vessels

#### 3.4.2.2 *Personal protection*

- All clothing worn outside the facility should be replaced by manufacturing facility garments upon entry into the facility.
- Upon entry into the containment zone personnel are to gown in full body protective single-use laboratory clothing (for example Tyvek® disposable overalls).
- When open activities are being conducted, eye protection and the use of respiratory protective equipment, such as N95, FFP3 (*43*) or equivalent respirators such as positive pressure air purifying respirators is required. Minimal specifications for the filtering/absorbing capacity of such equipment should be met, and masks, if used, must be fitted properly and the correctness of fit tested.

- Taking a full body shower upon exit from the BSL-3 enhanced (pandemic influenza vaccine) containment facility is recommended. It is mandatory following situations when staff may have been exposed to vaccine virus.
- Personnel should be instructed, in a written document to which they sign their agreement, not to have contact with animals, in particular farm animals 14 days following departure from the facility where vaccine has been produced. Currently the risks involved in contact with household dogs and cats are not considered to be significant, but the available scientific evidence is sparse.
- Staff should be prophylactically vaccinated with seasonal influenza vaccines.
- It is anticipated that before large-scale vaccine production is attempted, pilot lots of pandemic strain vaccine will have already been produced. Experimental vaccines inducing protective antibody levels are recommended for use by staff before large-scale vaccine production commences, if possible.
- Antiviral treatment must be available as necessary.

## 3.5 Biosafety management and implementation within a vaccine production facility

### 3.5.1 *Management structure*

The implementation of the biosafety levels described in these guidelines requires that the institution employ a biosafety officer who is knowledgeable in large-scale viral production and containment, but is independent of production in his or her reporting structure. The biosafety officer is responsible for the independent oversight of the implementation of the biosafety practices, policies and emergency procedures in place within the company or organization and should report directly to the highest management levels within the company. A biosafety officer is needed in addition to a qualified person who, in some countries, has overall responsibility for a medicinal product.

There should also be a Biosafety Committee comprising representatives of viral production and quality control that is responsible for reviewing the biosafety status within the company and for coordinating preventive and corrective measures. The institutional biosafety officer must be a member of the Committee. The chairperson should be independent of both the production and quality control functions. The management and governing board of the manufacturing company should ensure that adequate priority and resources are made available to the Committee to implement the required measures.

### 3.5.2 *Medical surveillance*

Occupational health departments at vaccine manufacturers of pandemic strain influenza vaccines should provide training in recognizing the clinical signs of influenza infection to company physicians, nurses and vaccine

manufacturing supervisors, who must make decisions on the health of personnel associated with the manufacture and testing of pandemic strain influenza vaccine. Local medical practitioners caring for personnel from the manufacturing site should receive special training in the diagnosis and management of pandemic influenza infection. Any manufacturer embarking on large-scale production should have documented procedures for dealing with influenza-like illness in the staff involved, or their family members, including diagnostic procedures and prescribed treatment protocols. Manufacturers should ensure that staff understand that they have an obligation to seek medical attention and to report any influenza-like illness to the occupational health department or equivalent. Manufacturers should hold supplies of one or more effective antiviral agent(s) and have defined means of quarantining staff if necessary.

### 3.5.3 *Implementation*

A detailed and comprehensive risk analysis should be conducted to define possible sources of contamination of personnel or the environment that may arise from the production or testing of live influenza virus within the establishment. For each procedure or system, this analysis should take into account the concentration and stability of the virus at the site, the potential for inhalation or injection that could result from accidents, and the potential consequences of a major or minor system failure. The procedural and technical measures to be taken to reduce the risk to workers and the environment should be considered as part of this analysis. The results of this risk analysis should be documented.

A comprehensive Biosafety Manual must be created and implemented that fully describes the biosafety aspects of the production process and of the quality control activities. It should define such items as emergency procedures, waste disposal, and the requirements for safety practices and procedures as identified in the risk analysis. The manual should be made available to all staff of the production and quality control units, with at least one copy present in the containment area(s). The manual should be reviewed and updated when changes occur and at least annually.

Comprehensive guidelines outlining the response to biosafety emergencies, spills and accidents should be prepared and made available to key personnel for information and for coordination with emergency response units. Rehersals of emergency response procedures are helpful. These guidelines should be reviewed and updated annually.

The implementation of the appropriate biosafety level status in the production and testing facilities should be verified through an independent assessment. National requirements concerning verification mechanisms should be in place and complied with.

Table 1

**Comparison of properties and proposed containment for pandemic vaccine production using different vaccine reference viruses**

| Vaccine virus | Haemagglutinin receptor specificity | Tests needed on reference virus[a] | Proposed containment for vaccine production |
|---|---|---|---|
| H5, H7 reassortants, from HP viruses[b] | α2,3 residues | Ferret, chicken, sequence, plaquing, egg embryo | BSL-2 Enhanced (pandemic influenza vaccine) |
| H5, H7 reassortants, from NP viruses[b] | α2,3 residues | Ferret, chicken, sequence, plaquing, egg embryo | BSL-2 Enhanced (pandemic influenza vaccine) |
| Non-H5, H7 reassortant | α2,3 residues | Ferret | BSL-2 Enhanced (pandemic influenza vaccine) |
| Non-H5, H7 reassortant | α2,6 residues | Ferret | BSL-2 Enhanced (pandemic influenza vaccine) |
| H5, H7 HP viruses | α2,3 residues | Not applicable | BSL-3 Enhanced (pandemic influenza vaccine) |
| H5, H7 NP viruses | α2,3 residues | Ferret, chicken, sequence, plaquing, egg embryo | BSL-2 Enhanced (pandemic influenza vaccine) |
| Non-H5, H7 viruses | α2,3 residues | Ferret | BSL-2 Enhanced (pandemic influenza vaccine) |
| Non-H5, H7 viruses | α2,6 residues | Ferret | BSL-2 Enhanced (pandemic influenza vaccine) |

[a] Test performed by WHO reference laboratory.
[b] Highly pathogenic and nonpathogenic viruses.

# Authors

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[1] The following series of background papers, commissioned by the WHO Secretariat, were prepared in the period April–July 2005.

[a] *A review of WHO biosafety guidelines for Manufacturing Avian Influenza Vaccines* (Frey, Richmond, Robinson).

[b] *A risk assessment for large scale manufacture of inactivated influenza vaccines from reassortants derived from avian influenza viruses* (Wood, Robertson, Logan).

[c] *Industry pandemic biosafety position paper* (IFPMA influenza vaccine supply international task force).

[d] *Conceptual risks of reassortants for the environment* (Swayne).

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1. *Laboratory biosafety manual,* 3rd ed. Geneva, World Health Organization, 2004 (WHO/CDS/CSR/LYO/2004.11).
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Appendix 1

# Testing for attenuation of influenza vaccine strains in mammals

## Titration of test virus

The dose of vaccine virus or parental strain virus that produces infection in 50% of cases should be determined by titration in eggs ($EID_{50}$) or cell culture ($TCID_{50}$), as appropriate. Titration of vaccine virus stock and parental virus stocks should be determined within the same laboratory and titres should be sufficiently high that these viruses can be compared using equivalent high doses in mice or ferrets ($10^7$ to $10^6$ $EID_{50}$ or $TCID_{50}$).

## Ferrets

### *Experimental procedure*

Outbred ferrets 4–8 months of age are sedated either by intramuscular inoculation of a mixture of anaesthetics (e.g. ketamine (25 mg/kg), xyalazine (2 mg/kg) and atropine (0.05 mg/kg)) or by a suitable inhalant. A standard dose of $10^7$ EID50/TCID50 (as appropriate) ($10^6$, if the higher dose is not possible) in 1 ml phosphate-buffered saline is slowly administered into the nares of the sedated animal, making sure that the virus is inhaled and not swallowed or expelled. A group of 4–6 ferrets should be infected. One group of ferrets (2–3 animals) should be killed on day 3 or 4 post-infection and the following tissues should be collected for estimation of virus replication: nasal turbinates and/or swabs, lung (tissue samples from each of four lobes and pooled), brain (tissues from anterior and posterior sections sampled and pooled), spleen and intestine. Additional lung tissue may be collected and processed for haematoxylin and eosin staining for microscopic evaluation of histopathology. The remaining animals are observed for 14 days for signs of weight loss, lethargy (based on a previously published index (*1*)), and respiratory and neurological symptoms. Neurological involvement may be confirmed by collection of brain tissue on day 14 post-infection at the termination of the experiment and processing as above for histopathology.

### *Expected outcome*

Viral titres of the vaccine strain in respiratory tissues should be no greater than in either parental strain; a substantial decrease in lung virus replication is anticipated. Replication of the vaccine candidate should also be restricted to the respiratory tract and replication in the spleen or intestine is not expected. Although isolation of the vaccine strain from the brain is not desirable, if high viral titres are found in the nasal turbinates, there may be some detection of virus in the brain based on previous results with non-

virulent human H3N2 viruses (*2*). The significance of such a finding may be confirmed by performing a histopathological analysis of brain tissue on day 14 post-infection. Neurological lesions detected in heamatoxylin and eosin-stained tissue sections confirm virus replication in the brain. Neurological symptoms and histopathology would indicate a lack of suitable attenuation of the vaccine candidate. Likewise clinical signs of disease such as weight loss and lethargy would indicate lack of attenuation in the vaccine strain, assuming that the wild-type avian virus also causes these symptoms.

### Mice

#### *Experimental procedure*

The 50% lethal dose ($LD_{50}$) of the vaccine strain and parental virus strains is determined in 6–8 week old female BALB/c mice. Mice are lightly anaesthetized with an inhalant and groups of mice (4–8 per group) are infected intranasally with 0.05 ml of serial 10-fold dilutions of virus (expected dose range $10^7$ to $10^1$ $EID_{50}$). Mice are observed daily for disease signs and the numbers of deaths at each virus dilution are recorded. The $LD_{50}$ values are calculated by the method of Reed and Muench (*3*). An additional three mice infected with a high dose of virus (e.g. $10^6$) are killed on day 3 or 4 post-infection and organs, including the lungs and brain, are harvested for estimation of virus replication.

#### *Expected outcome*

If the wild-type avian strain replicates in the brain and is highly lethal for mice, the vaccine candidate should exhibit at least a 1000-fold reduction in $LD_{50}$ values. Titres of the vaccine strain in lung and brain should be lower than those of either parental strain, consistent with an attenuation of replication in mouse tissues.

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