

Update of WHO biosafety risk assessment and guidelines for the production and quality control of human influenza vaccines against avian influenza A(H7N9) virus

As of 10 May 2013

Introduction

This document updates WHO guidance to national regulatory authorities and vaccine manufacturers on the safe production and quality control of human influenza vaccines produced in response to a pandemic threat¹. It details international biosafety expectations for both pilot-scale and large-scale production, and quality control of vaccines against avian influenza A(H7N9) virus now causing human infections in China. The updated guidance applies to both candidate vaccine development and to production activities for inactivated and live attenuated influenza vaccines. The general guidance in this document should be supplemented by specific risk assessments that should be carried out by laboratories or manufacturers intending to work with H7N9 virus-derived candidate vaccine viruses (CVV) and are to be updated as new characteristics of the viruses become available.

Development of the document

A small expert group convened by WHO held "virtual" consultations over the period 12 April – 6 May 2013. The group included biosafety experts, influenza virologists, veterinarians, representatives from laboratories involved in developing the vaccine virus strains and experts from the animal–human interface field². The group was asked to address questions about (1) testing of the CVVs being considered for human

¹ WHO biosafety risk assessment and guidelines for the production and quality control of human influenza pandemic vaccines, TRS No. 941, Annex 5 (2007), <http://www.who.int/biologicals/publications/trs/areas/vaccines/influenza/Annex%205%20human%20pandemic%20influenza.pdf>, accessed 03 May 2013

² The experts are listed in the Acknowledgements section. Each expert had completed a Declaration of Interest form and none had conflicts of interest concerning development of this guidance.

vaccine production; and (2) risk assessment for vaccine production of human vaccines to protect against H7N9 virus. The conclusions reached by the expert group form the basis of this updated guidance from WHO.

Background information

On 31 March 2013, WHO was informed by China of cases of human infections with avian influenza A(H7N9) virus. Since then, 128 human cases have been confirmed in China³. Related viruses have been identified in avian species, including pigeons, chickens and ducks.

The updated recommendations are predicated on the demonstration that the avian influenza A(H7N9) viruses are of low pathogenicity in chickens. The latter conclusion is based on the following evidence: (i) notifications that the virus currently fits the OIE definition of low pathogenicity avian influenza for poultry according to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals⁴; (ii) sequence analysis of the H7N9 viruses demonstrated that the haemagglutinin (HA) gene is consistent with phenotype of low pathogenicity in poultry⁵ (no accumulation of basic or other amino acids at the HA cleavage site); (iii) H7N9 viruses have been detected in live-bird markets and samples from poultry showing no overt disease signs.

At the time of this writing, preliminary results from multiple laboratories using the standard pathogenicity test (outlined in Appendix 1) indicate that H7N9 wild-type virus replicates efficiently in the upper and lower respiratory tract of infected ferrets, and may cause signs of disease, including weight loss, fever and transient lethargy, but does not cause severe or fatal disease.

³ For up-to-date information, see: http://www.who.int/influenza/human_animal_interface/influenza_h7n9/en/index.html, accessed 03 May 2013

⁴ 'On 4 April 2013, the Chinese Veterinary Authorities notified the occurrence of infection of pigeons and chickens with low pathogenic avian influenza virus H7N9 to the OIE.', <http://www.oie.int/for-the-media/press-releases/detail/article/questions-and-answers-on-influenza-ah7n9/>, accessed 03 May 2013; and subsequent follow-up reports http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=13391, accessed 7 May 2013

⁵ <http://www.oie.int/for-the-media/press-releases/detail/article/questions-and-answers-on-influenza-ah7n9/>, accessed 03 May 2013; and WHO RISK ASSESSMENT Human infections with influenza A(H7N9) virus) http://www.who.int/entity/influenza/human_animal_interface/influenza_h7n9/RiskAssessment_H7N9_13Apr13.pdf accessed 06 May 2013

Limited information is available on the pathogenicity of H7N9 viruses in humans. A substantial number of human cases have been severely ill, and many deaths have occurred, while a few milder cases have also been identified.

Testing of candidate vaccine viruses (CVV) considered for vaccine production

Taking into account the currently limited information about the pathogenicity of H7N9 viruses as well as the experience gained over decades with reassortant CVV incorporating HA and neuraminidase (NA) genes derived from both highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) viruses, the experts consulted by WHO concluded that the following tests should be conducted on reassortant CVV:

- Sequence analysis of the HA gene to confirm the presence of a cleavage site consistent with low pathogenic influenza viruses ;
- An assay to detect the ability of the virus to cause chicken embryo death;
- Pathogenicity test in ferrets; and
- An assay to determine the genetic stability of the HA gene of the virus upon multiple passaging.

A CVV is considered attenuated if the above tests show that (i) the sequence of the HA cleavage site is consistent with phenotype of low pathogenicity; (ii) the CVV does not cause embryo lethality; (iii) the pathogenicity of the CVV in ferrets is lower than that of the wild-type H7N9 virus from which it is derived⁶; and (iv) the CVV has been shown to maintain an HA cleavage site consistent with low pathogenic phenotype upon multiple passage in embryonated chicken eggs.

⁶ A genetically related wild-type virus may be used as comparator if its use is appropriately justified.

Such attenuated CVV should be handled in accordance with the recommendations described for 'CVV' in this document (see section Risk assessment for work with CVV derived from H7N9 viruses) and are distinguished from 'potential CVVs'⁷, for which the results of these tests have not been obtained or are unavailable.

Notes:

The expert group recommended that these tests be carried out on newly developed reassortant viruses, generated either by conventional reassortment or by reverse genetics, including, in the latter case, those viruses derived from synthetic nucleic acid. However, reassortant viruses that have HA and NA genes with sequences that are identical or nearly identical to those of a reassortant virus that has already been assessed using these tests may be exempted from safety testing. A review of safety data obtained from the first few reassortant CVV will be performed by the expert group to determine whether or not the suggested testing regimen should be changed.

The schedule of testing is based on the finding that the parental wild-type H7N9 viruses are of low pathogenic phenotype in chickens, as assessed according to the Manual for Diagnostic Tests and Vaccines for Terrestrial Animals 2012⁸. Should highly pathogenic H7N9 viruses emerge and be used for the derivation of reassortant CVV, the testing regimen described here would need to be re-evaluated; it is expected that in this case, testing as prescribed in TRS No 941, Annex 5 (2007) (sections 2.6.1 and 2.6.2) for reassortant viruses derived from highly pathogenic avian viruses of subtypes H5 or H7 would be appropriate, including pathogenicity testing in chickens¹.

In addition to the determination of the amino acid sequence of the HA cleavage site by sequencing of the HA gene, particular attention should be given to amino acid substitutions in genes derived from the wild-type H7N9 parent virus that indicate potential adaptation to mammalian hosts. Sequence information currently available for H7N9 viruses is indicative of some level of mammalian adaptation of these viruses; if any further adaptive changes are identified in CVV, a review of all safety data should be conducted.

⁷ Vaccine response to the avian influenza A(H7N9) outbreak – step 1: development and distribution of candidate vaccine viruses' (2 May 2013), http://www.who.int/influenza/vaccines/virus/CandidateVaccineVirusesH7N9_02May13.pdf, accessed 03 May 2013

⁸ Manual for Diagnostic Tests and Vaccines for Terrestrial Animals 2012; OIE. <http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/>, accessed 03 May 2013

It is recognized that the test for genetic stability takes a considerable amount of time and could delay the distribution of newly developed CVV. Work to date with CVV derived from highly pathogenic avian A(H5N1) viruses with modified HA genes (removal of polybasic cleavage site) has shown that these have, in all cases where this was assessed, retained their attenuated phenotype. It is therefore recommended that this test be carried out in parallel with other safety testing and in parallel with distribution of CVV. If loss of the attenuated phenotype, i.e. insertion of additional amino acids at the HA cleavage site, were identified for any newly developed H7N9 CVV during testing for genetic stability, an urgent signal would be sent to all recipients of the respective virus, through existing channels of communication used by WHO, WHO Collaborating Centres and WHO Essential Regulatory Laboratories.

Virus transmissibility is an additional aspect in the risk assessment of work conducted with influenza viruses. However, standard tests for evaluating this characteristic have not been validated and these tests have not been prescribed in previous guidance^{1,9}. The expert group concluded that, while it would be desirable to obtain data on the transmissibility of H7N9 wild-type viruses and CVV, formal testing of transmissibility would not be required for CVV derived from H7N9 viruses.

Risk assessment for work with candidate vaccine viruses (CVV) derived from avian A(H7N9) viruses

The expert group recommends the following assignments of containment levels for work with H7N9 viruses:

- For small-scale laboratory work:

- containment requirements for work with wild-type H7N9 virus should be BSL3, as specified elsewhere¹⁰

⁹ WHO biosafety risk assessment and guidelines for the production and quality control of human influenza pandemic vaccines: Update, 23 July 2009, http://www.who.int/biologicals/areas/vaccines/influenza/CP116_2009-2107_Biosafety_pandemicA_H1N1_flu_vaccines-Addendum-DRAFTFINAL.pdf, accessed 03 May 2013

¹⁰ Biosafety guidelines for handling highly pathogenic avian influenza viruses in veterinary diagnostic laboratories, in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2012, Chapter 2.3.4. adopted May 2012, Appendix 2.3.4.1, <http://www.oie.int/en/international-standard-setting/terrestrial-manual/access-online/>

- containment requirements for potential CVV, intended for use in the production of inactivated or live attenuated vaccines, should be as for wild-type viruses
- For large-scale work (pilot-scale vaccine production and commercial-scale vaccine production):
 - containment level for work with wild-type H7N9 virus, or potential CVV intended for use in the production of inactivated or live attenuated influenza vaccines, should be BSL-3 enhanced^{1,10}
 - containment level for work with characterized CVV (i.e. the attenuation of which has been demonstrated), intended for use in the production of either inactivated or live attenuated influenza vaccines, should be BSL-2 enhanced¹

The risk assessment underlying the present update took account of a number of characteristics of H7N9 virus that were known or imputed from preliminary data at the time of writing.

The general guidance in this document should be supplemented by specific risk assessments that should be carried out by laboratories or manufacturers intending to work with H7N9 virus-derived CVV and are to be updated as new characteristics of the agents become available. The elements of risk assessments to be incorporated can be found in the Laboratory Biosafety Manual, Third Edition,¹¹ and in the Laboratory Biorisk Management Standard¹². Risk assessments need to take into account, in addition to factors specific for the type of work and facility, the following non-exclusive list of virus characteristics and parameters in relation to H7N9 virus:

¹¹ 'WHO laboratory biosafety guidelines for handling specimens suspected of containing avian influenza A virus'
http://www.who.int/influenza/resources/documents/guidelines_handling_specimens/en/, accessed 02 May 2013

¹² COMITÉ EUROPÉEN DE NORMALISATION Laboratory biorisk management - Guidelines for the implementation of CWA 15793:2008

- Receptor binding properties of parental H7N9 virus: currently available sequence information suggests that these viruses can bind to mammalian-type receptors (α 2,6-linked sialic acid residues); this suggests that infectivity for and transmissibility to and between humans and other mammals may be higher than for avian influenza viruses considered in the past, such as avian H5N1 viruses.
- Amino acid residues at various positions in a number of genes of H7N9 virus suggest adaptation to mammalian hosts; if these genes are present in a reassortant CVV, their potential impact on infectivity and transmissibility should be considered.
- Passaging of viruses in various substrates can lead to the acquisition of sequence changes; while the HA gene of H7N9 virus is of the low pathogenicity type (no accumulation of basic or other amino acids at the HA cleavage site), it cannot be assumed that this genotype is stable.

Appendix 1 Safety Testing of Avian Influenza A(H7N9) Vaccine Viruses in Ferrets

Test virus

The 50% embryo or tissue culture infectious dose (e.g. EID₅₀, TCID₅₀) or plaque-forming units (PFU) of the reassortant candidate vaccine virus (CVV) and parental virus stock, or genetically similar wild-type virus will be determined. The infectivity titres of viruses should be sufficiently high such that these viruses can be compared using equivalent high doses in ferrets (10⁷ to 10⁶ EID₅₀, TCID₅₀ or PFU) and diluted not less than 1:10. Where possible, the pathogenic properties of the donor PR8 should be characterized thoroughly in each laboratory.

Laboratory facility

Animal studies with the vaccine strain and the parental wild-type strain should be conducted in animal biosafety level (BSL)-3 containment facilities with enhanced practices¹. An appropriate occupational health policy should be in place².

Experimental procedure

Outbred ferrets 4-12 months of age that are serologically negative for currently circulating influenza A and B viruses and the test virus strain are anesthetized by either intramuscular administration of a mixture of sedatives (e.g. ketamine (25 mg/kg) and xylazine (2 mg/kg)) and atropine (0.05 mg/kg) or by suitable inhalant anesthetics. A standard virus dose of 10⁷ to 10⁶ EID₅₀ (or TCID₅₀ or PFU) in 0.5 to 1 ml of phosphate-buffered saline is used to inoculate animals. The dose should be the same as that used for pathogenesis studies with the wild-type parental virus. The virus is slowly administered into the nares of the sedated animals, reducing the risk of virus being swallowed or expelled. A group of four to six ferrets should be inoculated. One group of two to three animals should be euthanized on day 3 or 4 after inoculation and samples should be collected for estimation of virus replication from the following organs in order: spleen, intestine, lungs (samples from each lobe and pooled), brain (anterior and posterior sections sampled and pooled), olfactory bulb of the brain, and nasal turbinates. If gross pathology demonstrates lung lesions similar to those observed in wild-type viruses, additional lung samples may be collected and processed with haematoxylin and eosin (H&E) staining for histopathological evaluation. The remaining animals are observed for clinical signs, which may include weight loss, lethargy [based on a previously published index³], respiratory and neurologic signs, and increased body temperature.

Collection of nasal washes from animals anesthetized as indicated above should be performed to determine the level of virus replication in the upper airways on alternate days after inoculation for up to nine days. At the termination of the experiment on day 14 after inoculation, a necropsy should be performed on at least two animals and organs collected. If signs of substantial gross pathology are observed (e.g. lung lesions), the organ samples should be processed as described above for histopathology.

Expected outcome

Clinical signs of disease such as lethargy and/or weight loss should be attenuated in the vaccine virus strain compared with the parental virus strains, assuming that the parental H7N9 donor virus also consistently causes these signs. Viral titres of the vaccine strain in respiratory samples should be no greater than either parental strain; a substantial decrease in lung virus replication is anticipated. Gross lung lesions seen at necropsy should be minimal. Replication of the candidate vaccine virus should be restricted to the respiratory tract. Virus isolation from the brain is not expected. However, detection of virus in the brain has been reported for seasonal H3N2 viruses⁴. Therefore, should virus be detected in the anterior or posterior regions of the brain (excluding the olfactory bulb) the significance of such finding may be confirmed by performing histopathological analysis of brain on day 14 after inoculation. . Neurological lesions detected in H&E-stained sections should confirm virus replication in the brain and observation of neurological signs. Presence of neurological signs and histopathology would indicate a lack of suitable attenuation of the vaccine candidate virus.

1. 'Biosafety guidelines for handling highly pathogenic avian influenza viruses in veterinary diagnostic laboratories' in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2012, Chapter 2.3.4. adopted May 2012, Appendix 2.3.4.1, <http://www.oie.int/en/international-standard-setting/terrestrial-manual/access-online/>, accessed 02 May 2013
2. 'WHO laboratory biosafety guidelines for handling specimens suspected of containing avian influenza A virus' http://www.who.int/influenza/resources/documents/guidelines_handling_specimens/en/, accessed 02 May 2013

3. Reuman PD, Keely S, and Schiff GM. (1989). Assessment of signs of influenza illness in the ferret model. *Laboratory Animal Science* 42:222-232.
4. Zitzow LA, et al., (2002) Pathogenesis of influenza A (H5N1) viruses in ferrets. *Journal of Virology* 76:4420-4429.

Table 1: Summary of results in ferrets infected intranasally with XXXX influenza A(H7N9) candidate vaccine virus

Virus	Dose (EID ₅₀) ^b	No. Animals	No. animals with clinical signs to day 14 p.i.				Mean max. % weight loss	Respiratory tract viral titers (log ₁₀ EID ₅₀ /ml or g) ^c		Lung lesions (day 3/4) ^{d,e}	Lung lesions (day 14) ^{d,e}	Detection of virus in other organ ^f
			Lethargy	Respiratory	Weight loss	Other (e.g. fever)		Mean peak nasal wash or nasal turbinate	Lung			
XXXX A/Shanghai/2/2013 ^a												
wt A/Anhui/1/2013												
hg or ca parental donor (e.g. A/PR/8/34)												

^a A/Anhui/1/2013 is antigenically and genetically similar to the parental wild-type donor virus A/Shanghai/2/2013.

^b Indicate whether dose is expressed as EID₅₀, TCID₅₀ or pfu.

^c Indicate whether respiratory viral titers are expressed as EID₅₀, TCID₅₀ or pfu per ml or g. Give lower limit of detection.

^d Score gross pathologic lung lesions as -, + ($\leq 20\%$), ++ (>20 and $< 70\%$), +++ ($>70\%$)

^e Indicate outcome of any histopathology evaluation

^f Indicate organ or not detected

p.i., post inoculation

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