

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

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Introduction

This document updates WHO guidance¹ 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. It details international biosafety expectations for both pilot-scale and large-scale production and control of vaccines against the influenza A (H1N1) virus causing the current international epidemics, and is thus relevant to both vaccine development and production activities.

Development of the document

A small expert group that was convened by WHO held "virtual" consultations over the period 14-22 May 2009. The group included biosafety experts, influenza virologists, representatives from laboratories involved in developing the vaccine virus strains and experts from the animal-human interface. The group was asked to address questions about (1) the testing of the reference viruses being considered for vaccine production; and (2) the risk assessment for influenza A (H1N1) vaccine production. A summary of the expert group's responses is given below and forms the basis of the updated guidance from WHO.

Issue 1. Testing of reference viruses being considered for vaccine production

Question 1

In the pandemic alert period (i.e. up to and including WHO phase 5) will it be necessary to conduct, and, if so, complete the ferret test on influenza A (H1N1) reassortants prior to the distribution of a candidate reference strain?

Response

Influenza A (H1N1) vaccine reassortants will need to be tested in ferrets. This is because it appears that some features of the infection (weight loss and lung pathology) may be more extensive than is seen with seasonal influenza viruses. Furthermore, it is not known to what degree the surface glycoprotein genes or internal protein genes contribute to the pathogenicity of these viruses.

¹ WHO Expert Committee on Biological Standardization: *fifty-sixth report*. Geneva, World Health Organization, 2007. (WHO Technical Report Series No. 941). Annex 5: WHO biosafety risk assessment and guidelines for the production and quality control of human influenza pandemic vaccines, pp. 265-299. Available at: http://whqlibdoc.who.int/trs/WHO_TRS_941.pdf

Until more is known, testing will be needed for each reassortant. If initial testing of candidate reassortants, obtained by either reverse genetics or classic reassortment, with a 6:2 gene constellation, and with the expected sequences, indicates that they are attenuated in ferrets, similar 6:2 reassortants with other related HAs need not undergo such testing. Decisions on reassortants with other gene constellations will need to be made on a case-by-case basis.

A standard protocol for testing in ferrets is given as Appendix 1.

Demonstration of attenuation should be sufficient to reduce the level of containment from the current WHO recommendation of biosafety level 3 (BSL-3) for culture of influenza A (H1N1) viruses². In order not to introduce delays into the vaccine production process, the prototype vaccine viruses may be distributed in parallel with initiation of ferret testing. Manufacturers who can work with the virus under BSL-2 conditions but with BSL-3 precautions² may initiate their adaptation process. Those manufacturers who do not have the facilities and systems in place should not initiate laboratory work until attenuation has been demonstrated by completion of the tests in ferrets.

Manufacturers receiving prototype vaccine viruses from WHO Collaborating Centres for Influenza or Essential Reference Laboratories will be provided further guidance after the ferret safety tests have been completed.

Question 2

If the ferret test is found to be unsuitable as a safety test for the influenza A (H1N1) reassortants are there other analyses that can be conducted to assess if the viruses are likely to have a low risk of human infection and transmission?

Response

At this time, there are no known genetic determinants in the HA and NA of the influenza A (H1N1) viruses that could be modified and act as markers of reduced virulence. Thus sequence analysis would not be useful as a surrogate to the ferret test. Under these circumstances, it will be necessary to follow a risk-assessment approach, based on prior experience of PR8/wild-type reassortants.

² *Laboratory biorisk management for laboratories handling human specimens suspected or confirmed to contain influenza A (H1N1) causing the current international epidemics.* World Health Organization, 6 May 2009. Available at: http://www.who.int/csr/resources/publications/swineflu/vaccine_production_biosafety/en/index.html

Issue 2. Risk assessment for influenza A (H1N1) vaccine production

Question 1

What containment level should be assigned for vaccine production from and quality control of influenza A (H1N1) reassortants?

Response

This should be BSL-2 enhanced (pandemic influenza vaccines) as described in WHO Technical Report Series No. 941¹.

Laboratory managers and workers should consult the biorisk management checklist published by WHO².

If there is widespread influenza A (H1N1) virus infection locally, yet pandemic phase 6 has not yet been declared, local relaxation of the level of containment may be considered in consultation with WHO.

For live attenuated influenza vaccines, the level of containment should be BSL-2 enhanced (pandemic influenza vaccines) and caution should be observed with clinical use of such vaccines in the absence of widespread spread of influenza A (H1N1) virus in the community and a pandemic being imminent (see WHO Technical Report Series No. 941, Annex 5, section 2.4, p. 279¹).

Question 2

What containment level should be assigned for vaccine production from and quality control of wild-type influenza A (H1N1) viruses?

Response

Containment level for vaccine production of wild-type influenza A (H1N1) should be BSL-3 enhanced (pandemic influenza vaccines) as described in WHO Technical Report Series No. 941¹.

Laboratory managers and workers should consult the biorisk management checklist published by WHO².

If there is widespread influenza A (H1N1) virus infection locally, yet pandemic phase 6 has not yet been declared, local relaxation of the level of containment could be considered in consultation with WHO.

Appendix 1

Safety testing of novel influenza A (H1N1) viruses in ferrets

Test virus

The 50% infectious dose (e.g. EID₅₀, TCID₅₀) or PFU of the reassortant vaccine virus and parental virus stock, or genetically similar wildtype virus, will be determined. The infectivity titres of viruses should be high enough for these viruses to be compared using equivalent high doses in ferrets (10⁷ to 10⁶ EID₅₀, TCID₅₀ or PFU) and diluted no 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 biosafety level 3 (BSL-3) containment facilities using BSL-3 practices in accordance with WHO guidance³. An appropriate occupational health policy should be in place.

Experimental procedure

Outbred ferrets aged 4-12 months that are serologically negative for currently circulating influenza A and B viruses and the test strain are sedated either by intramuscular inoculation of a mixture of anaesthetics (e.g. ketamine (25 mg/kg), xylazine (2 mg/kg) and atropine (0.05 mg/kg) or by a suitable inhalant. A standard dose of 10⁷ EID₅₀ (or TCID₅₀ or PFU) in 1 ml of phosphate-buffered saline is used to infect animals; if this dose cannot be achieved, a lower dose of 10⁶ EID₅₀ (or TCID₅₀ or PFU) may be used. The virus is slowly administered into the nares of the sedated animals, making sure that the virus is inhaled and not swallowed or expelled. A group of 4-6 ferrets should be infected. One group of animals (2-3 animals) should be euthanized on day 3 or day 4 post-infection and the following tissues be collected for estimation of virus replication in the order shown: intestines; spleen; lungs (tissues samples from each lobe and pooled); brain (tissues from anterior and posterior sections sampled and pooled); olfactory bulb of the brain; and nasal turbinates. If gross pathology demonstrates lung lesions, additional lung tissue may be collected and processed for haematoxylin and eosin staining for microscopic evaluation of histopathology. The remaining animals are observed for signs of weight loss, lethargy (based on a previously published index⁴), respiratory and neurologic signs. Collection of nasal washes on animals anaesthetized as indicated above should be performed to determine the level of virus replication in the upper airways on alternate days post-infection for up to 9 days. At the termination of the experiment on day 14 post-infection, a necropsy should be performed on at least 2 animals and if any signs of gross pathology are observed (e.g. lung lesions), the organs should be collected and processed as above for histopathology.

³ Laboratory biorisk management for laboratories handling human specimens suspected or confirmed to contain influenza A (H1N1) causing the current international epidemics. World Health Organization, 6 May 2009. <http://www.who.int/csr/resources/publications/swineflu/LaboratoryHumanspecimensinfluenza/en/index.html>

⁴ Reuman PD, Keely S, and Schiff GM. (1989). Assessment of signs of influenza illness in the ferret model. *Laboratory Animal Science* 42:222-232. [Journal of Virological Methods Volume 24, Issues 1-2](#), April-May 1989, Pages 27-34

Expected outcome

Clinical signs of disease such as lethargy and/or weight loss should be attenuated in the vaccine strain compared with the parental strains, assuming that the parental H1N1 donor virus also causes these symptoms. Viral titres of the vaccine strain in respiratory tissues should be no greater than those for either parental strain; a substantial decrease in lung virus replication is anticipated. Lung lesions seen at necropsy should be minimal. Replication of the vaccine candidate should be restricted to the respiratory tract; however, detection of the low levels of the vaccine strain in the intestine, may be acceptable. Isolation of the virus from the brain is not expected. However, detection of virus in the brain has been reported for seasonal H3N2 viruses⁵. Therefore, should virus be detected in any part of the brain, 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 H&E stained tissue sections should confirm virus replication in the brain and observation of neurological symptoms. Neurological symptoms and histopathology would indicate a lack of suitable attenuation of the vaccine candidate.

⁵ Zitzow LA, Rowe T, Morken T, Shieh WJ, Zaki S, Katz JM Pathogenesis of influenza A (H5N1) viruses in ferrets. *Journal of Virology*, 76:4420-29. Available at: <http://jvi.asm.org/cgi/content/full/76/9/4420?view=long&pmid=11932409>