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

23 July 2009

Introduction

This document updates guidance[1] from the World Health Organization (WHO) to national regulatory authorities and vaccine manufacturers on the safe production and quality control of human influenza vaccines produced in response to a pandemic. It details international biosafety expectations for both pilot- and large-scale production and control of pandemic influenza vaccines, including the current pandemic (H1N1) 2009 virus and is relevant to both vaccine development and production activities.

Development of the document

A small expert group convened by WHO has held virtual technical consultations in response to needs as vaccine development for pandemic (H1N1) 2009 has proceeded. The group includes biosafety experts, influenza virologists, representatives from laboratories involved in developing the vaccine virus strains and experts from the animal-human interface. It was asked to address questions about the testing of the reference viruses being considered for vaccine production and the risk assessment for pandemic (H1N1) 2009 vaccine production. A summary of the group's initial responses was published as WHO guidance on 28 May 2009.[2] This document updates that guidance and provides WHO's current position.

Testing of reference viruses being considered for vaccine production

On 28 May 2009, WHO issued guidance that all influenza A (H1N1)/09 reassortant viruses developed as candidate vaccine strains needed to be tested in ferrets. This was because some features of the infection with wild-type pandemic (H1N1) 2009 in animal models (virus replication in the lower respiratory tract and lung pathology) were 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.

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[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://www.who.int/biologicals/publications/trs/areas/vaccines/influenza/Annex%205%20human%20pandemic%20influenza.pdf, accessed 20 July 2009.

[2] Update of WHO biosafety risk assessment and guidelines for the production and quality control of human influenza pandemic vaccines. Available at http://www.who.int/biologicals/publications/trs/areas/vaccines/influenza/H1N1_vaccine_production_biosafety_SHOC.27May2009.pdf, accessed 20 July 2009.

A standard protocol for testing in ferrets is given as Appendix 1. Although other animal models, such as mice, are being investigated for evaluation of attenuation of H1N1 viruses[3], so far there is insufficient information to recommend alternatives to ferrets for safety testing of H1N1 viruses.

The 28 May 2009 guidance stated that, 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 would need to be made on a case-by-case basis.

Current guidance

As of 20 July 2009, four candidate reassortants have been safety tested in ferrets[4]. These are:

(a) X-179A virus, a 5:3 reassortant vaccine candidate that possesses the HA, NA and PB1 genes of A/California/07/2009 (H1N1v) and PA, PB2, NP, M and NS genes of A/PR/8/34 donated from the high-growth reassortant virus NYMC X-157 (A/New York/55/2004 x A/PR/8/34 (H3N2)). This virus was generated by traditional reassortment methods.

(b) A/Texas/05/2009(H1N1)-PR8-IDCDC-RG15 virus (RG15), which is a 6:2 modified reassortant vaccine candidate generated by reverse genetics and possesses the HA and NA from A/Texas/05/09 (H1N1) with mutation (Q226R) introduced into the HA and six internal genes from A/PR/8/34.

(c) NIBRG-121 virus, which is a 6:2 reassortant vaccine candidate generated by reverse genetics that possesses the HA and NA genes of A/California/07/2009 (H1N1)v and PA, PB1. PB2, NP, M and NS genes of A/PR/8/34.

(d) IVR-153 virus, which is a 6:2 reassortant vaccine candidate generated by traditional reassortment methods using A/California/7/2009 (H1N1)v and IVR-6. IVR-6 is a 5:3 reassortant of A/Texas/1/77 and A/Puerto Rico/8/34. IVR-153 possesses HA and NA genes from A/California/07/2009 (H1N1v), the PB1 gene from A/Texas/1/77 and PA, PB2, NP, M and NS genes of A/PR/8/34[5].

As a result of testing in ferrets, according to the protocol in Appendix 1, all of the above viruses are considered attenuated.

Consequently, the experts consulted by WHO agree that:

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[3] Transmission and pathogenesis of swine-origin 2009 A(H1N1) influenza viruses in ferrets and mice, Maines et. al., Sciencexpress, 2 July 2009, Page 1. Available at http://www.sciencemag.org/cgi/rapidpdf/1177238.pdf, accessed 20 July 2009

[4] Biocontainment for vaccine production and quality control of the reassortant vaccine candidate viruses IDCDC-RG15, NIBRG-121, and X-179A. Available at http://www.who.int/csr/resources/publications/swineflu/biocontainment_reassortant.pdf, accessed 20 July 2009.

[5] Biocontainment for vaccine production and quality control of the reassortant vaccine candidate virus IVR-153. Available at http://www.who.int/csr/resources/publications/swineflu/safety_results_reassortant_cw_ivr_153.pdf, accessed 20 July 2009.

- Any other vaccine reassortants with 6:2 gene constellation where donor strains are similar to those already safety tested, and with the expected sequences, do not need to undergo safety testing in ferrets.

- Vaccine reassortants with other gene constellations or with specific mutations introduced to enhance production characteristics or prepared from other donor strains should be evaluated for the need to safety test on a case-by-case basis.

Manufacturers receiving new vaccine reassortants from WHO Collaborating Centres for Reference and Research on Influenza (WHOCC) or WHO Essential Reference Laboratories (ERL) will be provided with information on whether ferret safety testing has been or should be further evaluated.

Risk assessment for pandemic (H1N1) 2009 vaccine production

Question 1

What containment level should be assigned for vaccine production from and quality control of attenuated pandemic (H1N1) 2009 reassortants?

Answer for inactivated virus vaccines

This should be biosafety level 2 (BSL-2) enhanced (pandemic influenza vaccines) as described in WHO Technical Report Series No. 941, Annex 5.[1]

Laboratory managers and workers should consult the biorisk management checklist published by WHO.[6]

If there is widespread pandemic (H1N1) 2009 virus infection locally, defined for example as when the relevant Health Authority stops laboratory-based diagnosis, local relaxation of the level of containment to BSL-2 may be permitted after a comprehensive risk assessment has been made and documented of each facility in question and if agreed upon by local regulatory authorities.

Answer 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 a widespread increase of pandemic (H1N1) 2009 virus in the community and a pandemic being imminent (see WHO Technical Report Series No. 941, Annex 5, section 2.4, p. 279[1]).

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[6] 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/Laboratorybiorisk management.pdf, accessed 20 July 2009.

As above, if there is widespread pandemic (H1N1) 2009 virus infection locally, defined, for example, as when the relevant Health Authority stops laboratory-based diagnosis, local relaxation of the level of containment to BSL-2 may be permitted after a comprehensive risk assessment has been made and documented of each facility in question and if agreed upon by local regulatory authorities.

Question 2

What containment level should be assigned for vaccine production from and quality control of wild-type pandemic (H1N1) 2009 viruses?

Response

Containment level for vaccine production of wild-type pandemic (H1N1) 2009 should be biosafety level 3 (BSL-3) enhanced (pandemic influenza vaccines) as described in WHO Technical Report Series No. 941[1].

Laboratory managers and workers should consult the biorisk management checklist published by WHO[6].

If there is widespread pandemic (H1N1) 2009 virus infection locally, local relaxation of the level of containment could be considered in consultation with WHO and if agreed upon by local regulatory authorities . However, it should be noted that the employer must always protect the worker from hazards at work, regardless of the hazards encountered outside the workplace. The potential exposure dose during work from wild-type pandemic (H1N1) 2009 virus may be much larger than what may be encountered in the community. This would have to be taken into account in any risk assessment.

Appendix 1:
Safety testing of novel influenza A (H1N1) viruses in ferrets

Test virus

The 50% infectious dose (e.g. EID_{50}, $TCID_{50}$) or PFU of the reassortant vaccine virus and parental virus stock, or genetically similar wild-type 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 (10^7 to 10^6 EID_{50}, $TCID_{50}$ 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 BSL-3 containment facilities using BSL-3 practices in accordance with WHO guidance[1]. 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 10^7 EID_{50} (or $TCID_{50}$ or PFU) in 1 ml of phosphate-buffered saline is used to infect animals; if this dose cannot be achieved, a lower dose of 10^6 EID_{50} (or $TCID_{50}$ 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 three or four post-infection and the following tissues should 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 should be observed for signs of weight loss, lethargy (based on a previously published index[7]), 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 nine days. At the termination of the experiment on day 14 post-infection, a necropsy should be performed on at least two animals and, if any signs

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[7] Reuman et. al.. (1989). Assessment of signs of influenza illness in the ferret model. Journal of Virological Methods, 24(1-2):27-34. Abstract available at: http://www.ncbi.nlm.nih.gov/pubmed/2760163, accessed 20 July 2009.

of gross pathology are observed (e.g. lung lesions), the organs should be collected and processed as above for histopathology.

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 pandemic (H1N1) 2009 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 the virus in the brain has been reported for seasonal H3N2 viruses.[8] Therefore, should the 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 hematoxylin and eosin (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.

[8] Zitzow et. al. (2002). Pathogenesis of avian influenza A (H5N1) viruses in ferrets. Journal of Virology, 76(9):4420-29. Full text available at: http://jvi.asm.org/cgi/reprint/76/9/4420, accessed 20 July 2009.