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4 Questions and answers on the Haemagglutination 5 Inhibition (HI) test for qualification of influenza vaccine 6 (inactivated) seed preparations

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30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

Questions and answers on the Haemagglutination Inhibition (HI) test for qualification of influenza vaccine (inactivated) seed preparations

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1. Questions and answers

1.1. Why has the EMA prepared a Q&A which considers Haemagglutination Inhibition (HI) testing for (inactivated) influenza vaccines?

Based on the experience from recent evaluations of Annual Update applications for influenza vaccines (inactivated), both regulators and industry have requested further guidance about the regulatory requirements of HI testing as applied for the qualification of influenza seed virus preparations.

Whilst some of the principles outlined below may be applicable to live attenuated influenza vaccines (LAIV), there are additional considerations towards the qualification of seed virus preparations using HI testing and hence LAIVs are outside the scope of this Q&A document.

1.2. Why is HI testing on influenza vaccine seed preparations requested?

As indicated in the CHMP Guideline on Influenza Vaccines – Quality Module [1] and the Ph.Eur. monographs on influenza vaccines [2,3], the haemagglutinin and neuraminidase antigens of each seed lot should be identified as originating from the correct strain of influenza virus by suitable methods.

The objective of HI testing of the manufacturers' seed preparations is to assure that these preparations are antigenically identical to the approved candidate vaccine virus (CVV), i.e. that no antigenic changes have been introduced when the CVV is propagated in the manufacturer's production system to prepare their seed material.

Therefore, the HI test result should not be justified as a Good Manufacturing Principle tool to confirm correct handling of a seed lot vial during vaccine manufacturing, i.e. to distinguish different strain preparations in the company's seed vial stock.

Although the technical challenges of the HI test are recognised, it remains the assay of choice for determining the antigenic characteristics of the majority of influenza vaccine virus preparations.

1.3. What details are expected in the MA dossier on the HI test?

As for any other analytical procedure, for which there is no published pharmacopoeial analytical method, the HI test shall be described in sufficient details. This would normally comprise the test principle, materials and equipment, procedures, validity/acceptance criteria and evaluation. This information should normally be part of the MA (core) dossier. However, virus specific aspects should be addressed in the Annual Update application, as appropriate. Full information about the viruses / anti-sera used should be provided. Any pre-treatment of the anti-sera to inactivate non-specific inhibitors of haemagglutination should be indicated. The type of red blood cells should be indicated and justified, i.e. the type of red blood cells should normally correspond to the type that is used by the WHO CC to certify the approved CVV using the 2-way HI assay.

Further suggested information about the HI testing can be found in the WHO Manual for the laboratory diagnosis and virological surveillance of influenza [4].

An example of how HI testing results could be presented is presented in Annex 2.

1.4. Which labs are responsible for testing at different stages of CVV/seed preparation, i.e. WHO c.c., national reference lab, company?

Whilst some manufacturers have contracted out the HI testing to external specialised laboratories, the responsibility of the HI testing remains with the vaccine company. The company has to choose a laboratory with adequate experience and access to the necessary reagents (antisera and viruses). The interpretation of the HI testing results may be complex and therefore adequate expertise should be available within the vaccine company and/or at the contract laboratories.

1.5. Should different reference viruses / antisera be included in HI test?

Normally, a one-way HI test is required to include, in addition to the seed preparation, the approved CVV and an antiserum against the approved CVV . In case there is a need to amplify the CVV in order to obtain sufficient material for analyses, this should ideally be restricted to a single passage to minimise the risk of introducing any antigenic changes.

It may also be useful to include the WHO recommended virus and/or the parent of the CVV and the antisera against these viruses. Other antisera can be used additionally to evaluate the pattern of reactivity, but this is not considered essential.

1.6. Are 'heterologous' antisera acceptable in HI test?

It is acknowledged that homologous antisera may not be available during the early phase of the production campaign and qualification of the seed preparations. In such cases, the use of 'heterologous' antisera may be useful to 'pre-qualify' the seed virus lots. However, at the time of formal seed virus lot / vaccine lot release and Annual Update MA procedure, the 'homologous' anti-sera (i.e. anti-CVV) should have been used in the crucial one-way HI test.

1.7. Are different approaches needed in case of wild type virus vs. reassortant virus, cell culture vs egg propagated influenza virus?

In principle, there should be no difference between the HI testing of egg-derived reassortant viruses and wild-type viruses, i.e. the seed virus of both virus types must antigenically match the approved CVV. Therefore, a cell culture-derived seed virus should be tested against the approved CVV from which it was derived.

1.8. What acceptance criteria should be used in HI test, difference titre reference virus: seed virus <4, <6, <8, …..?

The difference in titre between the approved CVV and seed virus should be less than 4-fold (< 4-fold) to conclude that the seed virus is antigenically identical to the approved CVV.

1.9. What is expected from MAHs in case of unexpected HI results (e.g. additional analysis by different lab, gene sequence data)?

In case the seed virus does not meet the < 4-fold difference acceptance criteria for identity, this should be followed up as soon as possible by careful investigation to exclude technical failures (e.g. suboptimal inactivation procedure of inhibitors). Additional HI testing should be considered, e.g. by an alternative (back-up) laboratory. Any difference in test results and subsequent conclusion about the antigenic profile of the seed virus will need to be discussed in detail, including number of analyses,

reagents used, etc. Furthermore, gene sequencing analysis might provide reassurance that the seed virus is genetically similar to the reference virus preparation and no amino acid(s) substitutions have been introduced into the virus that may have altered the antigenic profile of the seed virus. If further testing does not resolve the issue then a switch to another seed virus or CVV should be considered. The regulatory authorities could also be consulted for further guidance.

Glossary:

Approved CVV:	A Candidate Vaccine Virus (CVV) is a virus that has been certified by a WHO CC to be antigenically identical to the WHO-recommended virus. Hence, CVVs are antigenically representative of influenza viruses recommended by WHO/CHMP and which are suitable for establishment of seed virus lots for vaccine production. CVVs can be wild-type or reassortant viruses (the latter generated by classical reassortment or reverse genetics technology). Lists of egg- or cell culture-propagated CVVs suitable for use in human vaccine production are available on the WHO website[1]. CVVs acceptable in Europe are published by EMA in the 'EU recommendations for the seasonal influenza vaccine composition for the season, which are published annually on the EMA website [5].
WHO-recommended virus:	The influenza virus recommended by the WHO as the basis for an influenza vaccine composition. For example, an A/Michigan/45/2015 (H1N1)pdm09-like virus.
Seed virus preparation:	The seed virus prepared by the manufacturer to produce the influenza vaccine.
WHO CC:	WHO collaborating centres (WHO CC) are institutions designated as able to carry out activities in support of the WHO programmes for influenza vaccination campaigns.
parent virus:	Wild-type virus from which a CVV has been derived. The CVV typically contains two gene segments (segments 4 and 6, encoding the HA and NA proteins) from the parent virus, but may contain more.
Wild-type virus:	Wild-type influenza viruses or influenza virus isolates means naturally occurring influenza viruses that have been detected by any means including molecular methodology and/or cultured either in eggs or cells (i.e. isolated) directly from clinical specimens or subsequent culture passages and have not been purposefully modified [6].

[1] http://www.who.int/influenza/vaccines/virus/candidates_reagents/home

Annex 1. Attachments/ links

1. Guideline on Influenza Vaccines – Quality Module (EMA/CHMP/BWP/310834/2012). Committee for Medicinal Products for Human use (CHMP), 25 April 2014
2. European Pharmacopoeia monograph on influenza vaccine (split virion, inactivated); 01/2008:0158. European Pharmacopoeia Edition 2017 (9.2)
3. European Pharmacopoeia monograph on influenza vaccine (surface antigen, inactivated); 01/2008:0869. European Pharmacopoeia Edition 2017 (9.2)
4. Manual for the laboratory diagnosis and virological surveillance of influenza. WHO Global Influenza Surveillance Network. World Health Organization 2011.
5. Amended EU recommendations for the seasonal influenza vaccine composition for the season 2017/2018. EMA/CHMP/BWP/216216/2017. 06 April 2017
6. Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits. WHO. ISBN: 978 92 4 150308 2.

Annex 2. Example of presentation of Haemagglutination Inhibition (HI) testing results

Antigens		HI titres using reference sera[2][3]					
		A(H1N1) [*serum identification*]	A(H3N2) [*serum identification*]	B/Yam [*serum identification*]	B/Vic [*serum identification*]	Negative	Interpretation
Control antigens[4]							
1 [*CVV full name*]	A(H1N1)	$\geq$ 1280	< 10	< 10	< 10	< 10	
2 [*strain name*]	A(H3N2)	< 10	$\geq$ 1280	< 10	< 10	< 10	
3 [*strain name*]	B/Yam	< 10	< 10	320	40	< 10	
4 [*strain name*]	B/Vic	< 10	< 10	< 10	320	< 10	
Seed virus A(H1N1)							
5	Lot number	640	< 10	< 10	< 10	< 10	Identity seed virus complies, i.e. antigenically identical to the *approved CVV [full name]*(H1N1)

[2] Should be the homologous antisera raised against the CVV
[3] Titres are shown for illustrative purposes only.
[4] Should be the homologous antigen to which the antiserum was raised