Meeting Report

WHO Working Group Meeting on Revision of WHO TRS 941, Annex 5: WHO Biosafety Risk Assessment and Guidelines for the Production and Quality Control of Human Influenza Pandemic Vaccines

Domaine de Penthes, Geneva, Switzerland

9 – 10 May 2017

1 Disclaimer: This report contains the collective views of an international group of experts, and does not necessarily represent the decisions or the stated policy of the World Health Organization. The mention of specific companies or of certain manufacturers’ products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned.
Executive Summary

International biosafety expectations for pilot-scale and large-scale production of human vaccines in response to a pandemic influenza strain are crucially important to harmonize and facilitate the process of vaccine development and availability in a timely manner in the event of an influenza pandemic and in inter-pandemic work on pandemic preparedness. The World Health Organization (WHO) published the “biosafety risk assessment and guidelines for the production and quality control of human influenza pandemic vaccines” in 2007 in the Technical Report Series (TRS) No. 941, Annex 5. This guideline provides guidance to regulators, public health authorities, research laboratories and vaccine manufacturers on safe handling and testing of human pandemic influenza viruses during vaccine development, production and evaluation.

Experience with viruses of pandemic potential and with pandemic viruses has increased globally since the publication of TRS 941, Annex 5. Therefore, WHO organized a working group meeting, held in Geneva from 9 to 10 May 2017, to review up-to-date practice and knowledge on the safe production of influenza vaccines, identify gaps in the current TRS and discuss key issues that need to be addressed in a revision.

The expert group reviewed the existing document in light of new knowledge as well as taking into consideration input through a survey and an expert review of existing biosafety guidance. It concluded that the revised guideline should be expanded and should be more comprehensive, covering influenza viruses of all subtypes. The testing schedule for candidate vaccine viruses (CVVs) generated from viruses with pandemic potential was found to need adjustments: it was proposed that a test for genetic stability should be done for all CVVs, but the ferret pathogenicity and the chicken pathogenicity tests should be limited to certain CVVs (a summary of testing requirements will be shown in the revised Table 1 of the guideline). It was also proposed that the ferret pathogenicity test should not include comparison with the wild type parental virus, but that parameters of attenuation would be defined independently of parental viruses, using historic data and reference viruses of defined pathogenicity/attenuation. The sections on hazard identification and risk assessment will be updated to account for new knowledge and will be streamlined so as to avoid duplication between these sections. A clear distinction between hazards and mitigating factors/measures will be implemented in the revised guideline.
A first draft of the revised guideline will be ready for public consultation in the autumn of 2017. Further rounds of revision and public consultation will occur in 2018 with the aim to submit the final revised guidelines for consideration by the Expert Committee on Biological Standardization (ECBS) in October 2018.

I. Introduction

International biosafety expectations for pilot-scale and large-scale production of human vaccines in response to a pandemic influenza strain are crucially important to harmonize and facilitate the process of vaccine development and availability in a timely manner in the event of an influenza pandemic and in inter-pandemic work on pandemic preparedness. The World Health Organization (WHO) published the “biosafety risk assessment and guidelines for the production and quality control of human influenza pandemic vaccines” in 2007 in the Technical Report Series (TRS) No. 941, Annex 5. This guideline provides guidance to regulators, public health authorities, research laboratories and vaccine manufacturers on safe handling and testing of human influenza pandemic viruses during vaccine development, production and evaluation.

In the decade since the publication of this guideline, experience with viruses of pandemic potential and with pandemic viruses has increased globally. In response to the emergence of novel human influenza viruses, updates to the guideline have been published by WHO in 2009 in the early phase of the pandemic of H1N1 and in 2013 upon the emergence of human cases of infection with avian influenza viruses of subtype H7N9. During two informal consultations organised by WHO on the influenza vaccine response during the start of a pandemic in 2015 and 2016, the importance of updating TRS 941, Annex 5 was highlighted. Influenza vaccine manufacturers, through their trade association, the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA), submitted a White Paper to WHO in 2012, asking for a revision of the same guideline.

WHO organised a working group meeting, held in Geneva from 9 to 10 May 2017, to review up-to-date practice and knowledge on the safe production of influenza vaccines, identify gaps in the current TRS and discuss key issues that need to be addressed in a revision. The meeting was attended by experts and representatives of WHO Collaborating Centres (CC), Essential Regulatory Laboratories (ERL) for influenza, national regulatory authorities for vaccine regulation and for biosafety/biocontainment, World Organisation for Animal Health (OIE),
industry (IFPMA and Developing Country Vaccine Manufacturers Network-DCVMN), and WHO staff from relevant programmes (Norms and Standards, Global Influenza Programme, Global Action Plan for influenza, GMP inspection of prequalification).

The meeting was chaired by Dr Jerry Weir. Dr Othmar Engelhardt was the Rapporteur.

II. Background and objectives

Dr Ivana Knezevic welcomed all participants to the working group meeting; this meeting brought together a range of experts, including representatives from industry. Financial support for the meeting was provided by the Centre for Biologics Evaluation and Research (CBER)/FDA.

Dr Knezevic provided an update on WHO biological standardisation. Setting norms and standards and promoting and monitoring their implementation is one of WHO core functions. Although WHO is not a regulatory authority, it has, as part of its mandate, a unique role to support regulatory authorities in its 194 member states. WHO standardization programme includes two types of standards: written standards (currently 91 documents published as Recommendations or Guidelines on quality, safety and efficacy of biological products) and measurement standards (currently more than 400). WHO Guidelines provide key principles for evaluation of biologicals as a basis for setting national requirements, but leave space for national regulatory authorities (NRAs) to formulate additional or more specific requirements when needed. Furthermore, these documents serve as a benchmark for global acceptability of these products and for prequalification of vaccines for United Nations (UN) supply. WHO Guidelines are meant to complement guidance issued by other bodies, not to create conflicts between guidelines. These written standards are updated as required through revisions. Revisions can involve modifications of text of existing guidelines or the generation of new text. Dr Knezevic presented the WHO written standards on the work programme for WHO’s Expert Committee on Biological Standardisation (ECBS) in the period 2016 – 2018, which cover a wide range of topics. The revision of TRS 941, Annex 5 is due to be considered by ECBS in 2018.

Dr Wenqing Zhang presented on relevant issues raised from the WHO Global Influenza Programme (GIP). The Global Influenza Surveillance and Response System (GISRS) performs virus detection, surveillance and monitoring, risk assessment, vaccine virus selection/development/availability for seasonal and zoonotic/pandemic influenza viruses.
Biosafety recommendations apply for laboratory diagnostics, virus sharing (infectious substance transport) and the safety testing of candidate vaccine virus (CVVs). One of the key words in influenza is ‘timeliness’; this involves a balance between risk and benefit. For laboratory diagnostics, PCR detection and culture of seasonal influenza viruses are conducted at biosafety level (BSL) 2, culture of zoonotic or pandemic viruses at BSL3. Containment for certain viruses is based on a case-by-case risk assessment; for instance, pandemic H1N1 viruses were handled at BSL2 once the virus was widespread and these viruses are now considered seasonal viruses. Virus transport is conducted according to ICAO guidance; however, the available guidance does not cover all viruses, and a mechanism to make timely determinations regarding virus shipping would be useful. Testing of CVVs for seasonal influenza viruses does not raise any biosafety issues, whereas CVVs derived from zoonotic or pandemic influenza viruses need to be safety tested. In the pandemic of 2009, CVVs were shipped before the completion of the ferret pathogenicity test. In this case, a WHO expert working group defined safety testing requirements; it recommended that CVVs could be shipped before the ferret test but should be handled in BSL-2 laboratories with BSL-3 precautions. Dr Zhang recommended that a few points be considered: standardisation of terminology and associated definitions; a better understanding of the underlying science; a matrix of understanding from known viruses of different subtypes, risk assessment criteria and corresponding decisions with associated conditions; and the establishment of an expert group and corresponding functional mechanism that would be active now and during a situation requiring their input.

Dr Tiequn Zhou introduced the process of developing and revising WHO written standards. These are based on wide scientific consultation and international consensus and are living documents that may be revised in response to scientific advances. Consultations involve all interested parties and draft guidelines are circulated extensively for comments. The final draft must be reviewed by the WHO ECBS and, if adopted by ECBS, the document will be published in the WHO Technical Report Series (TRS) (available on: http://www.who.int/biologicals/technical_report_series/en/index.html). Influenza vaccine related standards can be found on the WHO website: http://www.who.int/biologicals/vaccines/influenza/en/. Dr Zhou explained why a revision of TRS 941, Annex 5 was considered now: the Guidelines are more than 10 years old (developed in 2005, published in 2007); since then, technologies have evolved in influenza virus testing and vaccine production; knowledge and experience have increased. There have
been calls for a review and revision of the Guidelines, by the WHO informal consultations on influenza vaccine response during the start of a pandemic in 2015 and 2016 and by IFPMA in 2012. There were also other developments since 2005 that may have implications on the TRS 941 Annex 5 revision e.g.: new guidance published under GIP and Pandemic Influenza Preparedness (PIP) framework; update on WHO GMP guidelines for biologicals in 2015; ongoing revision of WHO Laboratory Biosafety Manual (3rd edition, 2004). All these highlighted the need for a review and revision of the TRS 941, Annex 5. There are also challenges that need to be taken into account in the revision. The potential for a new influenza pandemic virus to emerge poses a constant threat to human health, but its nature is unpredictable (timing, type of virus, impact, etc.). Sufficient characterization and safe handling/containment of influenza viruses and associated hazardous biological materials are essential for the production of influenza vaccines for a pandemic. Meanwhile CVVs need to be made available to manufacturers as quickly as possible to enable earliest possible start of vaccine production and deployment in response to a pandemic. A risk-based approach is implemented in many aspects, such as risk assessment and management, biocontainment and emergency response. WHO guidance needs to be flexible to accommodate the evolving situation in a pandemic. The purpose of this working group meeting was to bring together experts representing interested parties including WHO CCs, ERLs, vaccine and biosafety regulators, manufacturers and other partners, to review up-to-date practice and knowledge on the safe production of influenza vaccines, identify gaps in the current TRS and discuss key issues that need to be addressed in a revision. This meeting should achieve consensus on the scope, outline and content of the TRS revision, such that they can serve as the basis for preparing the first draft of the revision for subsequent broader consultations and should agree on the work-plan for the revision towards submission to ECBS in 2018.

III. Current practice of safe production and control of human influenza vaccines in response to a pandemic, experiences and perspectives from stakeholders

Dr Richard Webby presented the perspective of a WHO Collaborating Centre generating and testing CVVs for zoonotic and pandemic influenza viruses. The bottleneck tests are the animal tests, specifically the tests in ferrets and in chickens. The ferret test takes 14 days with extra time before the test to acclimatise the animals. Four to six ferrets per virus (CVV or wild-type [wt] virus) are infected; three days later, two to three animals are euthanized for virus titration in organs. The remaining animals are observed until the end of the experiment and nasal washes are performed to determine virus shedding. The biggest issue with this test
is the wt virus: sometimes it is not available in a timely manner and sometimes comparison between wt virus and CVV is not straightforward. The question is whether demonstration of simple attenuation of a CVV is acceptable rather than “relative attenuation” to a wt virus? Dr Webby suggested that comparison with wt virus could be dropped and instead criteria for attenuation could be defined; these criteria could include loss of body weight and viral titres in nasal washes and lungs. The chicken test is defined by OIE and does not require comparison with a wt virus. Chickens are infected and monitored for 10 days; a score, the intravenous chicken pathogenicity index (IVPI), is calculated based on the observations. Experience from testing a large number of CVVs suggests that we can predict the outcome of the test with high accuracy and it is questionable whether the benefit of the assay is worth the additional time required. Dr Webby suggested that testing of CVVs in mice, which is mentioned in TRS 941, Annex 5, should be removed. The biggest lessons learned are that (1) the original document was built on emerging H5N1 viruses which are the most ferret lethal viruses, (2) the document was built on an abundance of caution due to lack of experience with zoonotic virus-based CVVs at that time and (3) the document needs to have some level of flexibility to respond to future emerging events.

Dr Othmar Engelhardt continued on the theme of presenting the perspective of CVV-generating laboratories. He showed examples of CVVs that have been tested in ferrets and that gave a range of results. Many CVVs derived from highly pathogenic avian influenza (HPAI) viruses of subtype H5N1 show a clear difference between the attenuated CVV and the parental wt virus, including in lethality, body weight and in virological parameters such as viral titres in organs. For a CVV (NIBRG-268) derived from a prototype H7N9 virus from 2013, the corresponding wt virus did not cause overt disease signs in ferrets; however, replication of the CVV was lower than that of the wt virus, as shown by reduced virus titres in lung, nasal turbinates and in nasal washes. By contrast, a CVV derived from the pandemic virus of 1957 [A/Singapore/1/57 (H2N2)] could not be distinguished by any of the measured parameters from its wt parent virus, which itself replicated to only low levels in infected ferrets, and could thus not be designated as ‘attenuated’ according to the current WHO Guidelines. Similarly, another CVV derived from a different H7N9 wt virus [a 1:7 reassortant containing 7 gene segments of the wt H7N9 virus and only one segment from the attenuated high-growth donor virus A/Puerto Rico/8/34 (PR8)] was not different from its wt parent virus in the ferret test; in this case, however, the wt virus showed robust replication in ferrets and
therefore the CVV had clearly failed the safety test, showing it was not sufficiently attenuated. These and similar results raise a number of questions:

- the meaning of attenuation and whether attenuation always has to be relative to wt virus;
- whether alternative read-outs in the ferret model or alternative animal models could solve the issue of wt viruses that do not themselves cause disease and robust replication in the ferret;
- whether all CVVs require safety testing (the concept of ‘similar’ viruses was introduced in the 2013 update of the TRS 941, Annex 5);
- whether the full complement of tests needs to be conducted for all CVVs; and
- who makes the decision on whether a CVV passed or failed the safety test(s).

Dr Engelhardt suggested the use of virus standards or references of pathogenicity as an alternative to the use of wt parental virus in ferret test.

The discussion following these two presentations covered a number of issues.

- Access to wt virus may become more challenging in the future, due to regulations on bioweapons. The question of whether ‘synthetic wt viruses’ (ie, viruses generated by reverse genetics from synthetic DNA, using the consensus sequences of all 8 gene segments of the wt virus) could be used instead was raised.
- It was pointed out that for live attenuated influenza vaccines (LAIV), safety tests were specified, at least in some cases, in the marketing authorisation. Also, criteria for attenuation in this case are explicit and not relative.
- Some participants thought that absolute attenuation criteria could be defined for CVVs. This may lead to a wt virus passing the test for attenuation.
- Standardisation of the ferret test between laboratories was seen as useful though not easy, but the guidelines need to include some flexibility. References/standard viruses for attenuation might be useful in this respect.
- New read-outs in ferrets, such as levels of cytokines, may be related to virus replication.
- The question of genetic stability of CVVs was raised. It was recommended that genetic sequence analysis and stability testing should be conducted on all CVVs, including wt viruses used as CVVs, in the revision.
**Dr Ponthip Wirachwong** presented GPO’s (The Government Pharmaceutical Organization, Thailand) programme of influenza vaccine development, which to date has led to two vaccines approved for pandemic use: an LAIV for H1N1pdm09 and an LAIV for H5N2. In both cases, attenuation of the CVV was demonstrated in ferrets; toxicity and immunogenicity were evaluated in mice; and human clinical trials were also conducted, with subjects in isolation for five days following administration of the vaccine. GPO has a pilot plant at BSL3. In reviewing TRS 941, Annex 5, Dr Wirachwong highlighted a few aspects: facility requirements, such as GMP, negative pressure biosafety cabinets, HEPA filtration of air prior to exhaust, proper decontamination of all waste, can be met by manufacturers. Most of the points listed in TRS 941, Annex 5 for personnel protection were followed except vaccination with experimental vaccine before the commencement of large scale production.

**Dr Beverly Taylor** presented on behalf of the IFPMA. She first acknowledged WHO for taking the TRS revision forward. During the pandemic of 2009, one of the key challenges was the delay in the ability of manufacturers to commence vaccine manufacturing prior to the establishment, through the completion of the safety testing, of the biocontainment level required to handle the virus. The IFPMA Influenza Vaccines Supply task force (IVS) group conducted a review of biocontainment standards in 2012. It concluded that influenza vaccine manufacturing facilities that meet cGMP guidelines also meet BSL3-like containment guidelines in general, accounting for biosecurity features inherent in both the design and operation of manufacturing facilities. Furthermore, the review found that the combination of facility design, room containment, individual operator protection and operational procedures provides a level of protection that is sufficient to allow manufacturing activities to be undertaken in the period prior to the completion of the vaccine virus pathogenicity testing. Manufacturers prefer a single ‘standard’ in describing the biosecurity compliance level required for manufacture. IFPMA proposes that manufacturing facilities should be certified by local authorities as compliant with the requirements of this standard and that this certification should be internationally accredited. Accredited manufacturing facilities should be able to receive and use CVVs of temporarily undefined pathogenicity in a pandemic alert or pandemic period. The review conducted by IVS identified nine gaps between GMP and BSL3 requirements; manufacturers believe these gaps can be addressed by enhanced work practices to provide secondary containment and operator protection.

The discussion after these two presentations covered the following issues:
• Rules and regulations related to biosafety and biosecurity are different in different countries and are administered and audited by different authorities. In some countries, a single facility is audited and inspected by a number of organisations, each dealing with different aspects (for example, protection of the environment, protection of staff, genetically modified organisms/micro-organisms).

• It was pointed out that in many countries vaccination of staff cannot be mandated; equally, availability of antiviral drugs may be limited, depending on prescription rules.

• WHO biosafety standards are being reviewed at the moment.

• An approach to biosafety that is focused on end results, and not so much on how these are achieved, was found to be useful.

• In some countries, the use of genetically modified viruses (CVVs generated by reverse genetics) complicates matters for manufacturers, requiring for instance special licences for transport of such viruses.

• It was pointed out that if a CVV is classified as a ‘select agent’ in the United States, it cannot be shipped to anybody who is not registered to handle select agents.

Dr Glen Gifford presented a perspective from the World Organisation for Animal Health (OIE). He explained that OIE’s goals are transparency, solidarity, standards and expertise and that the OIE recognises the importance of the One Health approach for global control of pandemic influenza. There are two technical standards for influenza vaccine manufacturing and testing facilities: the OIE Terrestrial Animal Health Code and the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. These standards contain chapters for avian, porcine and equine influenza, as well as a chapter on biosafety and biosecurity and chapters covering standards for manufacturing and testing of veterinary vaccines. The OIE also maintains networks of scientific expertise, including Collaborating Centres, reference laboratories and the joint OIE-FAO OFFLU animal influenza network. The latter comprises a network of global experts in animal influenza that, inter alia, collaborates with the WHO influenza network on animal-human interface, including human pre-pandemic vaccines. The OIE World Animal Health Information System (WAHIS) is a system for global reporting and tracking of animal diseases. Dr Gifford suggested some changes to text in the current TRS 941, Annex 5 that were duly noted by the working group.
Dr Jennifer Mihowich presented an overview of pathogen regulation in Canada. The Public Health Agency of Canada (PHAC) is the national authority on biosafety and biosecurity for human pathogens, but shares responsibility over the biosafety and biosecurity for animal pathogens with the Canadian Food Inspection Agency (CFIA). Pathogens are categorised into risk groups (RG) according to risk assessments conducted by PHAC’s Centre for Biosecurity; containment levels (CL) define the minimum physical and operational requirements for working with a biological agent in a laboratory setting. Influenza viruses are generally in RG2 and RG3 (with the exception of two viruses that are categorised as RG4) and work conducted with influenza viruses requires CL2 or CL3, depending on the activities. Dr Mihowich stated that the current WHO biosafety guidelines (TRS 941, Annex 5) facilitate the risk assessment process for CVVs and changes to the document could impact this process.

Dr Mihowich’s presentation led to a discussion on the definition of containment levels. There is no global standard for CL, biosafety level (BSL), RG, hazard groups (HG), etc. There could be value in having a global consensus on risk assessment and containment levels, but WHO can only recommend; biosafety regulations are under the authority of national authorities.

Discussion on key issues that will be considered in the revision of the document

In preparation for this session, a list of questions was included in the agenda of the working group meeting:

- What tests need to be done to determine safety of CVVs before release to manufacturers for shipment in response to a pandemic?
- What/how will manufacturers be guided to handle viruses?
- How to decide which tests should be used in the case of a newly emerging virus? How quickly can this be done?
- What to do in cases where the wt virus is non/low pathogenic in the animal model(s) used? How to show attenuation? Is it necessary to show attenuation?
- Does every CVV require testing? Concept of similar CVVs – when is a CVV similar to one already tested?
- Standardization/harmonization of safety testing?
- Is an “interim process” needed before the TRS revision is completed (i.e. Oct 2018) to provide guidance to manufacturers on CVVs handling?
• Interface with OIE and national veterinary authorities

The discussion was wide-ranging, and the main points are summarised below:

• Standardisation of CVV testing, especially the ferret test, was thought to be beneficial.
• Standards that could be used in ferret testing could be fully tested CVVs known to be attenuated. Additionally, a high-pathogenicity standard to ensure that a laboratory’s ferret test can detect high pathogenicity could be used. These standards would probably not have to be subtype-specific.
• Use of wt virus as comparator was not seen as essential. One could benchmark the ferret test instead.
• For H5N1 viruses, there has never been a CVV (with modified HA cleavage site) that has failed the ferret safety test. Not all tests may be necessary for new CVVs derived from HPAI H5N1.
• There is a good body of data from ferret testing; this could be reviewed and serve as a basis for formulation of pathogenicity/attenuation criteria.
• Many participants thought that for new subtypes, the ferret test would have to be conducted. It is unlikely that any new CVV (for zoonotic/pandemic influenza) will be released based on sequence data alone.
• Genetic stability of CVVs with modified cleavage site (H5, H7) was discussed. It is not known whether extensive passaging in cell culture exerts selective pressure for the CVV to acquire extra amino acids at the cleavage site, leading to a cleavage site characteristic of HPAI. In eggs, this would be easily noted due to increased embryo lethality.
• Removing the requirement for the IVPI for CVVs derived from H5 and H7 viruses was discussed. There are efforts underway to remove this requirement in individual countries, but a general move away from the chicken test was seen as difficult to achieve.
• The concept of similar CVVs that would not require animal testing was discussed. It was seen as a reasonable concept, but there is a question how specific the definition of ‘similar’ would have to be.
• It was agreed that a CVV with a new backbone (i.e. one that is not PR8 or one of the two approved LAIV backbones) would require animal testing in any case, regardless of whether or not the HA and NA genes are similar to those in already tested CVVs.

IV. Report of expert review of key relevant guidance documents on biosafety of influenza viruses

Dr Gary Grohmann opened his presentation by stating that the TRS 941, Annex 5 document was critical guidance to CVV and GISRS laboratories, national regulators and all manufacturers, as well as other international organizations such as the OIE and national agencies. He then presented a chronology of events leading to the present working group meeting: in 2009, manufacturers were delayed in starting vaccine manufacturing for H1N1pdm09 vaccine until the biocontainment level was determined by WHO. This led to concerns that if a pandemic happened again soon, the situation would not be different. IFPMA produced a White Paper in 2012 that suggested alterations to TRS 941, Annex 5 (for more details, see presentation by Dr Beverly Taylor above). The two WHO informal consultations on the influenza vaccine response during the start of a pandemic (2015 and 2016) identified bottlenecks in the manufacturing process which can cause a domino effect and affect both seasonal and pandemic vaccine production and availability; CVVs and determination of biocontainment level were among these. One of the proposed solutions in this area is for WHO to lead and coordinate biosafety assessment and to speed up the assessment. Dr Grohmann also reported on the outcome of a survey that was sent to 25 persons or institutions soliciting answers to the following questions: Can you identify (1) any gaps in the current document (TRS 941, Annex 5); (2) any changes that need to be made; (3) any challenges for the next pandemic (or threat) in the area of biocontainment; (4) lessons learnt from the 2009 pandemic regarding CVV production and from safety testing of zoonotic CVVs. From the responses to the survey the following recommendations were extracted:

1) The TRS 941 document should be expanded and become more comprehensive by covering all influenza viruses with pandemic potential, not just H5 and H7 viruses. A change in the title of the document is also recommended.

2) Separate specific documents on H1N1 and H7N9 should be replaced by the new revised TRS 941 document.

3) WHO should liaise with key agencies, laboratories and manufacturers on a regular basis to keep the TRS 941 guidance up-to-date.
4) Safety testing of CVVs should be standardized and periodically reviewed.
5) WHO should seek regulatory harmonisation and agreement on BSL levels according to risk assessment criteria so that any delay in manufacturing is avoided.
6) The TRS 941 document should clearly define biocontainment levels (BSL1 through BSL4) for the handling of novel influenza virus, CVVs and the production of pandemic influenza vaccines.
7) Biocontainment levels should be determined on the basis of risk assessment, but they should be flexible enough to accommodate new information as it becomes available.
8) Any change in the BSL for any part of influenza vaccine virus production must be accomplished quickly.
9) Considerations presented in the IFPMA White Paper should be largely adopted.
   Manufacturers should be allowed to proceed with pandemic vaccine production prior to completing safety testing during a pandemic alert period, provided agreed-upon BSL safety conditions can be met.
10) The new guideline should consider the use of other potential backbones, backbones with altered genes and synthetic viruses.

In the ensuing discussion, it was pointed out that definition of biosafety or biocontainment levels was a national responsibility. However, a WHO laboratory biosafety manual (available on: http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf?ua=1) exists and could be used in this context.

V. Initiation of revision of WHO TRS 941, Annex 5

Taking into consideration comments received in the survey prior to the meeting and discussion among working group participants, it was agreed to change the title of the revised document; the exact wording of the title will be defined later.

The working group also agreed that the scope of the guidelines should be expanded. The guidelines should cover aspects of safety testing and biosafety of CVVs for all virus subtypes, vaccine production and quality control (QC), different manufacturing platforms and potentially other work with live viruses for vaccine-related purposes (e.g. clinical serology), and provide minimum biosafety requirements, risk assessment and risk management and mitigation. It was agreed that safety testing of CVVs should stand as a separate section in the document, i.e. following hazard identification and followed by risk assessment sections.
The working group then split into three sub-groups focusing on respective sections: one group for hazard identification, one for safety testing of CVVs and one for risk assessment and control measures. The method of work varied between the three groups; some groups worked on text immediately (i.e. suggested modifications to relevant text in TRS 941, Annex 5), others laid down principles and concepts for and structure of the revised guidelines.

Following the work in subgroups, each group presented the outcomes of their discussions including proposed revision of the structure and/or text of respective sections.

Group 1 (hazard identification) presented an overall structure of the proposed revision of the section that includes: vaccine viruses (also covering future options, such as vectored vaccines using other viruses (VSV, MVA, adenovirus, etc.)); virulence factors associated with donor and wt viruses, such as gene segments, receptor specificity, transmissibility, cleavability, stability, drug resistance; manufacture in different substrates (eggs, cells) and at different scales; hazards from the vaccine (LAIV); and potentially genetic stability.

Group 2 (safety testing of CVVs) suggested a revision of Table 1 of TRS 941, Annex 5, which captures the essence of the safety tests required:

- the column detailing receptor specificity should be removed;
- the number of categories of vaccine viruses should be reduced from 8 to 6;
- the tests needed for vaccine viruses should be modified:
  - genetic stability and sequence identity should be included for all categories of CVVs
  - plaque assay (+/- trypsin) should not be required for CVVs derived from LPAI viruses of subtype H5 and H7
  - the IVPI should be removed for CVVs derived from LPAI viruses of subtype H5 and H7
- the proposed BSL levels were left open until definitions of BSL levels were clarified in the document

Group 2 also recommended that a WHO expert group be formed that reviews safety testing results and advises on biocontainment.

Other recommendations on the safety testing included:

- All mention of testing in mice should be removed.
• Two types of reference viruses/standards should be made available to testing laboratories: (1) a marker of pathogenicity and (2) a marker of attenuation. The latter could be an existing CVV.
• Criteria for definition of attenuation in ferrets should be determined. This will be done through internal review in the laboratories of those participants of the working group that perform ferret testing. The WHO CCs/ERLs (Drs Jacqueline Katz, Othmar Engelhardt, and Richard Webby) will review historical data on ferret testing in their labs in order to propose a revision of the protocol including attenuation criteria.
• Comparison with wt virus in the ferret test should be abandoned.
• The chicken test (IVPI) should only be conducted for CVVs derived from HPAI viruses of subtype H5 and H7.
• Genetic stability testing should be performed on all CVVs, including on wt viruses, if the latter are proposed for use in manufacturing; genetic stability testing should consist of 10 passages in a relevant substrate (eggs or cell culture).

Group 3 (risk assessment and control measures) reported that the structure of the original text on risk assessment and control measures should remain intact. Other comments and recommendations of the group were:

• Receptor specificity is not a reliable predictor of safety and should be qualified.
• The group recommended not making a distinction between scales of work; high concentrations of virus can occur at any scale.
• Activity-based risk assessments should be added. Consider small-pilot-large scale manufacturing as part of risk assessment.
• The amount of information on experimental infection should be reduced.
• Pigs are considered susceptible to all viruses, including CVVs with PR8 backbone and CVVs for LAIV.
• Text on relaxation of levels of containment should be changed to refer to authorisation by the competent authority.
• Mention of the geography of the manufacturing site should be deleted.
• Redundant text should be removed e.g. there are considerable redundancies between this section and the section on hazard identification.
• Clear distinction between hazards and mitigating factors/measures should be made.
• Regarding mitigation of possible exposure, vaccination should be recommended, as should antivirals, appropriate procedures and medical surveillance of staff. Use of experimental vaccines should be deleted in the text.
• Decontamination methods should be validated.
• The time frame for avoidance of contact with animals following possible exposure to virus could possibly be shortened, from 14 days as recommended in the current version of TRS 941, Annex 5, to 5 days, as required in Canada and the US.

Discussion continued on the description of biosafety levels. The updated WHO manual on biosafety (revision on-going) may not provide enough detail for the purposes of revision of TRS 941, Annex 5. An alternative approach to the exact definition of biosafety levels is to provide the definition of minimum requirements.

The working group meeting ended with an outlook on the work ahead and timelines. Dr Zhou presented the proposed workplan and timeline of the revision after this meeting and asked the group to provide continuous input. In the coming months, a draft of the TRS revision will be prepared taking into consideration discussions and recommendations by this working group and shared with the meeting participants prior to public consultation. A summary of the outcomes of the working group meeting will be presented at the 24th Meeting between WHO ERLs, CCs and influenza vaccine manufacturers at NIBSC, UK, 13 – 14 July 2017. The progress of this project will be reported to the ECBS in October 2017. The first round of public consultation on the WHO website is planned for November – December or even earlier if possible, followed by further revision of the draft during the first two months of 2018. A face-to-face consultation, to be convened in March or April 2018, will ask stakeholders to review the draft and propose further improvements. In May 2018, a second public consultation will take place to invite comments; the comments received will be taken into account in preparing the BS document. The final draft (BS document) will be submitted to ECBS by early July 2018; ECBS will consider the document at its meeting in October 2018. If approved, the document will then be published in the Technical Report Series (TRS).

Dr Knezevic and Dr Weir thanked all participants for their active participation and closed the working group meeting.

VI. Conclusion
During the 2-day meeting, participants reviewed lessons learnt and current practice on the safety testing and biocontainment aspects of CVVs and pandemic influenza vaccine production, discussed the critical issues that need to be updated and revised in the current TRS 941 Annex 5, proposed content and outline for the revision and agreed on the timeline and actions for finalizing the document towards submission to ECBS. These are summarized as below:

1. The title of the guideline should be revised to be more inclusive and appropriate.
2. The scope of the guideline should be expanded to cover all subtypes.
3. The structure of the guideline needs to be streamlined, e.g. safety testing of CVVs shall be a stand-alone section.
4. The entire document needs to be updated as needed, including all sections and references to reflect up-to-date development and knowledge. Detailed recommendations on the key issues to be addressed in respective sections are described in this report.
5. WHO CCs/ERLs (CVV-testing labs) will review historical data on ferret test and propose criteria to be included in the revised ferret test protocol.
6. Further need for standardization e.g. development of virus references or standards to be used in ferret test is identified. The proposal will be presented to ECBS in October 2017.
7. Following this meeting, a series of drafts of the revision will be prepared and consulted with stakeholders and public audience. Final revision will be submitted to ECBS for consideration in October 2018.

**Authors**

Othmar Engelhardt\(^a\) and Tiequn Zhou\(^b\), on behalf of the following participants\(^‡\) of the WHO Working Group Meeting on Revision of WHO TRS 941, Annex 5: WHO Biosafety Risk Assessment and Guidelines for the Production and Quality Control of Human Influenza Pandemic Vaccines.

\(^a\) National Institute for Biological Standards and Control, Medicines and Healthcare products Regulatory Agency, Potters Bar, United Kingdom.

\(^b\) World Health Organization, Geneva, Switzerland.
* Corresponding author: Tel: +41 22 791 4623. E-mail: zhout@who.int

‡ List of participants (alphabetically):

Othmar G Engelhardt, National Institute for Biological Standards and Control, Medicines and Healthcare products Regulatory Agency, Potters Bar, United Kingdom; Glen Gifford; World Organisation for Animal Health, Paris, France; Shigeyuki Itamura, Centre for Influenza Virus Research, National Institute of Infectious Diseases, Tokyo, Japan; Jacqueline M. Katz, WHO Collaborating Centre for the Surveillance, Epidemiology and Control of Influenza, Centers for Disease Control and Prevention, Atlanta, United States of America; Changgui Li, National Institutes for Food and Drug Control, Beijing, People's Republic of China; John McCauley, WHO Collaborating Centre for Reference and Research on Influenza, Crick Worldwide Influenza Centre, The Francis Crick Institute, London, United Kingdom; Jennifer Mihowich, Centre for Biosecurity, Health Security Infrastructure Branch, Public Health Agency of Canada, Ottawa, Canada; Ralf Wagner, Paul-Ehrlich-Institut, Langen, Germany; Richard Webby, WHO Collaborating Centre for Studies on the Ecology of Influenza in Animals, St. Jude Children's Research Hospital, University of Tennessee, Memphis, United States of America; Jerry Weir, Centre for Biologics Evaluation and Research, Food and Drug Administration, Maryland, United States of America; Gert Zimmer, Institute of Virology and Immunology, Mittelhäusern, Switzerland. Representatives of international federation of pharmaceutical manufacturers & associations (IFPMA): Matthew Downham, AstraZeneca/Medimmune; Lionel Gerentes, Sanofi Pasteur, France; Elisabeth Neumeier, GSK Vaccines, Germany; Beverly Taylor, Seqirus, UK. Representatives of developing countries vaccine manufacturers network (DCVMN): Pradip Patel, Cadila Healthcare Limited, Ahmedabad, India; Kittisak Poopipatpol and Ponthip Wirachwong, Government Pharmaceutical Organization, Bangkok, Thailand. WHO headquarters: Mustapha Chafai, Prequalification Team, Essential Medicines and Health Products (EMP) Department, Health Systems and Innovation (HIS) Cluster, World Health Organization (WHO), Geneva, Switzerland; Gary Grohmann, HIS/WHO, Switzerland; Ivana Knezevic, Technologies Standards and Norms (TSN) Team, EMP/HIS/WHO, Geneva, Switzerland; Wenqing Zhang, High Threat Pathogens (PAT), Infectious Hazard Management (IHM), Health Emergencies Programme (WHE), WHO, Geneva, Switzerland; Tiequn Zhou, TSN/EMP/HIS/WHO, Geneva, Switzerland.
Acknowledgement: Financial support from Center for Biologics Evaluation and Research (CBER), US FDA, provided through a Cooperative Agreement with WHO (Grant number 5U01FD005959-02) is very much appreciated.