

# Vaccines and biomedicines

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## Quality assurance and safety of biologicals

Many of the items debated at the 50th meeting of the WHO Expert Committee on Biological Standardization (ECBS)<sup>1</sup> reflect the increasing complexity of biomedicines. It seems possible that within the next few years the number of new biological medicines may supersede that of new chemical entities coming onto the market. The challenge now facing manufacturers and national regulatory authorities is how to continue to assure the quality and safety of new and existing biologicals. Biological standardization is set to play a key role in this respect.

## Recommendations for oral poliovirus vaccine

Revised recommendations (formerly requirements) for production and control of oral poliovirus vaccine (OPV) were agreed by the ECBS. New quality control procedures have now been introduced with the potential to increase the stringency of control. This is an important consideration given the considerable success of the polio eradication initiative and enhanced risk/benefit considerations of OPV use. The new quality control procedures will also decrease vaccine testing time. This will speed up OPV availability and respond to increased demands for supplies of vaccine to complete the eradication programme.

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<sup>1</sup>The WHO Expert Committee on Biological Standardization (ECBS) held its 50th meeting in October 1999. The ECBS is responsible for setting global standards for biological substances used in medicine, including vaccines, blood products, biological therapeutics and diagnostic procedures. Members were from Belgium, Canada, China, the Netherlands, the Russian Federation, the United Kingdom and the United States of America. Temporary Advisers were invited from Austria, Belgium, Canada, Finland, Germany, the Netherlands, Japan, South Africa, Switzerland, the United Kingdom and the United States of America. Representatives were present from the Council of Europe, European Association of the Plasma Products Industry, International Association for Biologicals, International Federation of Clinical Chemistry, International Federation of Pharmaceutical Manufacturers Associations, and the International Society of Thrombosis and Haemostasis.

For the first time, a test for molecular consistency of production for a live virus vaccine is now available. Mutant analysis by polymerase chain reaction (PCR) and restriction enzyme cleavage — MAPREC — quantifies reversion of a key base, 472C, that correlates in type 3 poliovirus vaccine with results of the WHO neurovirulence test (1). WHO-supported studies of the method have shown it to be a robust and reliable procedure. Results showed that MAPREC provided a very valuable additional test for consistency of production and the method was endorsed as the *in vitro* test of preference for control of poliovirus type 3. Excellent progress with MAPREC assays for poliovirus types 1 and 2 was also reported and the possibility of introducing MAPREC for these serotypes will be considered as soon as possible.

The discovery of the gene for the cellular receptor for poliovirus led to development of the TgPVR21 transgenic mouse susceptible to poliovirus infection (2, 3). A neurovirulence test for poliovirus vaccine has now been developed in the TgPVR21 transgenic mouse line and was shown in WHO-sponsored studies to be a suitable alternative to the test in monkeys for poliovirus type 3. It was therefore introduced in the new recommendations. Excellent progress with TgPVR21 neurovirulence tests for poliovirus types 1 and 2 was reported and the possibility of introducing a neurovirulence test in TgPVR21 mice for these serotypes will be considered as soon as possible.

The entire cycle from basic scientific research, through method development to standardization and application as control tests of MAPREC and the transgenic mouse model were all paradigms for regulatory research. This work clearly illustrated the need for long-term commitment of resources if significant advances in control and standardization of biologicals are to be made.

## Reference reagents and panels for diagnostic procedures for TSEs

A WHO Consultation on International Reference Materials for Diagnosis and Study of Transmissible Spongiform Encephalopathies (TSEs) held in May 1999 identified the need for international harmonization and the establishment of reference reagents and panels to compare diagnostic procedures.

Reference brain-derived materials and human and animal lymphoid tissue were considered necessary to compare assay systems. A working group established by WHO to develop appropriate standards was taking steps to obtain these materials. However, preparation of these reference materials will require dedicated facilities to comply with strict levels of laboratory containment. Candidate reference materials will be characterized in bioassays and immunoassays.

### International standards

The ECBS established 28 new or replacement International Standards and Reference Reagents covering a wide range of products (Table 1, page 234). Additionally, several International Standards, Reference Materials and Requirements that are no longer needed were discontinued following a public consultative process (Table 2, page 235) (5). The Committee also proposed to discontinue the first International Reference Preparation for protamine (salmon) at its next meeting, subject to public comment to this notice (Table 3, page 235).

### Discontinuation of requirements

As recommended by the 49th meeting of the ECBS, the intention to discontinue the WHO Requirements for Cholera Vaccine and Smallpox Vaccine was circulated for public comment. As a consequence, it was decided that the WHO Requirements for Cholera Vaccine should be discontinued in order to ensure correct testing of the new oral inactivated vaccines. However, in the case of smallpox virus, laboratory stocks had not been destroyed as expected and the Requirements for Smallpox Vaccine were retained.

### Database and electronic publication

Following a recommendation of the ECBS, the catalogue of WHO International Biological Standards and Reference Materials has been fully updated and made available on the Internet. The entire list of WHO biological standards and reference reagents is now available on the WHO home page at the following address:

<http://www.who.int/technology/biological.html>

A number of future developments concerning the new database include proposed linkages to the custodian laboratory holding the standards and to the corresponding WHO documents and publications and reports in the scientific literature.

### Other activities

The ECBS endorsed several other new projects including new or replacement reference materials for pertussis toxin and quality control of nucleic acid amplification tests (parvovirus B19 DNA, hepatitis A RNA, HTLV-1 RNA and HTLV-2 RNA). The Committee was also updated on many projects that are in progress. These included evaluation of mouse protection models for acellular pertussis vaccines; harmonization of antigen content and potency measurement of diphtheria and tetanus vaccines; standardization and control of oral cholera vaccines; standardization of antibody measurement; development of guidelines on preclinical and clinical testing of vaccines; abnormal toxicity test; consideration of the use of thiomersal as a preservative in vaccines, and evaluation of safety issues associated with the use of cell substrates for vaccine production.

Ongoing activities for blood and plasma derived products include a project on quality assurance of plasma-derived medicinal products and plasma fractionation activities; quality control of virus markers (HBsAg, anti-HCV and anti-HIV) in blood screening; standardization of unfractionated heparin and development of new international reference materials for blood grouping reagents.

Progress in standardization of biological therapeutics was also reported and the Committee endorsed proposals to establish new reference materials for vascular endothelial growth factor, ciliary neurotrophic factor, keratinocyte growth factor, neurotrophin-3 and relaxin. Given the rapidly expanding cytokines sector and limited resources available to carry out these activities, the Committee considered a policy to prioritize work in this area. A decision tree, developed during a WHO Consultation on Cytokine Standards, was modified for use in prioritizing work for all categories of biologicals.

### References

1. Chumakov, K.M. et al. Correlation of the amount of virus with altered nucleotide sequence and monkey test for acceptability of oral poliovirus vaccine. *Proceedings of the National Academy of Science*, **88**: 199–203 (1991).
2. Ren, R. Transgenic mice expressing a human poliovirus receptor: a new model for poliomyelitis. *Cell*, **63**: 353–362 (1990).
3. Koike, S. Transgenic mice susceptible to polioviruses. *Proceedings of the National Academy of Science*, **88**: 951–955 (1991).
4. *WHO Drug Information*, **13**: 2, 86–90 (1999).

**Table 1. International biological standards and reference reagents established by the 50th WHO Expert Committee on Biological Standardization**

<i>Preparation</i>	<i>Activity</i>	<i>Status</i>
<b>Antibodies</b>		
Islet cell antibodies	20 units/ampoule	First Reference Reagent 1999
	100 units/ampoule of anti-GAD65	First Reference Reagent 1999
	100 units/ampoule of anti-IA-2	First Reference Reagent 1999
Anti-pertussis serum, mouse	17 units per vial of anti-pertussis toxin	First Reference Reagent 1999
	143 units per vial of anti-filamentous haemagglutinin	First Reference Reagent 1999
	30 units per vial of anti-pertactin	First Reference Reagent 1999
	32 units per vial of anti-fimbriae types 2/3	First Reference Reagent 1999
<b>Antigens</b>		
Prostate specific antigen (PSA)	1 µg total PSA per vial	First International Standard 1999
Prostate specific antigen (90:10)	1 µg total PSA per vial	First International Standard 1999
Diphtheria toxoid, adsorbed	160 IU/ampoule	Third International Standard 1999
Hepatitis A vaccine, inactivated	100 IU/ml of immunogenic activity; 100 IU/ml of antigen content	First International Standard 1999
<b>Blood products and related substances</b>		
Blood coagulation factors II and X, concentrate, human	11.2 IU/ampoule of factor II 10.2 IU/ampoule of factor X	Third International Standard 1999 Third International Standard 1999
Blood coagulation factor IXa, concentrate, human, recombinant	11.0 IU/ampoule	First International Standard 1999
Fibrinogen, plasma, human	2.2 mg/ml	Second International Standard 1999
Tissue plasminogen activator, recombinant (alteplase)	10 000 IU/ampoule	First International Standard 1999
Human immunodeficiency virus type-1 RNA	100 000 IU/vial	First International Standard 1999
Hepatitis B virus DNA	500 000 IU/vial	First International Standard 1999
<b>Cytokines, growth factors and endocrinological substances</b>		
Insulin-like growth factor-II, human, recombinant	5000 units per ampoule	First Reference Reagent 1999
Hepatocyte growth factor/scatter factor	4000 IU/ampoule	First International Standard 1999
Hepatocyte growth factor/scatter factor (precursor)	2000 IU/ampoule	First International Standard 1999
Leptin, human	4000 IU/ampoule	First International Standard 1999
Leptin, mouse	4000 IU/ampoule	First International Standard 1999
Calcitonin, salmon	138 IU/ampoule	Third International Standard 1999
Interferon alpha, human leukocyte	11 000 IU/ampoule	First International Standard 1999
Interferon omega, human	20 000 IU/ampoule	First International Standard 1999
Interferon alpha 2c, human	40 000 IU/ampoule	First International Standard 1999
Interferon alpha 2b, human	70 000 IU/ampoule	Second International Standard 1999
Interferon alpha consensus, human	100 000 IU/ampoule	First International Standard 1999
Interferon alpha lymphoblastoid N1, human	38 000 IU/ampoule	Second International Standard 1999
Interferon alpha 2a, human	63 000 IU/ampoule	Second International Standard, 1999
Interferon alpha (leukocyte N3), human	60 000 IU/ampoule	First International Standard, 1999
Interferon alpha-1/8, human	27 000 IU/ampoule	First International Standard, 1999
Chorionic gonadotrophin	650 IU/ampoule	Fourth International Standard, 1999

**Table 2. Reference materials discontinued by the 50<sup>th</sup> WHO Expert Committee on Biological Standardization**

The First International Reference Reagent for adenovirus antisera, equine types 1, 2, 3, 5, 6, 7a, 8, 9, 10, 11, 13, 15, 17.  
 The First International Reference Reagent for adenovirus antisera, equine types 4, 19, 20, 22, 23, 24.  
 The First International Reference Reagent for adenovirus antisera, equine types 12, 18.  
 The First International Reference Reagent for adenovirus antisera, equine types 25, 26, 27, 28, 29, 30, 31, 32 and 33.  
 The First International Reference Reagent for adenovirus antisera, equine types 34, 35, 36.  
 The First International Standard anti-A,B blood typing serum, human.  
 The First International Reference Reagent for anti-HBs/ad serum, goat.  
 The First International Reference Reagent for anti-HBs/ad serum, guinea-pig.  
 The First International Reference Reagent for anti-HBs/ar serum, rabbit.  
 The First International Reference Reagent for anti-HBs/ay serum, goat.  
 The First International Reference Reagent for anti-HBs/ay serum, guinea-pig.  
 The First International Standard FITC-conjugated sheep anti-human immunoglobulins.  
 The First International Standard FITC-conjugated sheep anti-human IgG (anti-gamma chain).  
 The First International Standard FITC-conjugated sheep anti-human IgM (anti-mu chain).  
 The First International Standard for Thrombin, human.  
 The First International Reference Preparation for interferon, human, leukocyte (HuIFN-a (Le)).  
 The First International Reference Reagent for interferon, human, recombinant (rHuIFN-alpha2(alpha2b)).  
 The First International Standard for interferon, human, lymphoblastoid (Namalwa) (HuIFN-alpha (Ly)).  
 The First International Standard for interferon, human, rDNA (rHuIFN-alpha2 (alpha-A)).  
 The First International Working Standard for interferon, human, leukocyte (HuIFN-alpha (Le)).  
 The First International Standard for candicidin.  
 The First International Standard for rolitetracycline.  
 The First International Standard for desmopressin.  
 The First International Reference Preparation for gonadorelin.  
 The First International Reference Preparation for nisin.  
 The First International Reference Preparation for parathyroid hormone, bovine, for bioassay.

**Table 3 WHO International Reference Preparation proposed for discontinuation at the next meeting of the WHO Expert Committee on Biological Standardization**

**Blood products**

The First International Reference Preparation for protamine (salmon) (1954)

Comments on this proposal should be forwarded by 30 September 2000 to:  
 Dr E Griffiths, Quality Assurance and Safety of Biologicals, World Health Organization,  
 1211 Geneva 27, Switzerland, Fax +41 22 791 4210

### **Influenza preparedness plan: vaccine production and availability**

Influenza virus vaccines can take a minimum of 8 months to produce from the moment that a pandemic is identified to availability of a new vaccine. For this reason, WHO has devised an Influenza Pandemic Preparedness Plan (1) which outlines the separate but complementary roles of WHO and national authorities when an epidemic or pandemic is imminent or actually occurs. The following extract from the plan gives information on how any delay in influenza vaccine production can be minimized and also advises on how vaccine can be prepared for the following season.

Influenza virus vaccines are normally made by growing approved seed viruses in embryonated chicken eggs, purifying and chemically treating the harvest, including inactivating infectivity, and then adjusting the concentration against reference biological standards. In the case of a pandemic virus, however, special issues arise concerning vaccine composition that must be addressed before vaccine production can be completed. The lead-time from identifying a new strain to beginning vaccine production is usually 2–3 months, and vaccine lots first become available within about 4–5 months of inoculation of eggs.

Influenza epidemics usually peak between December and March in the northern hemisphere, and June and September in the southern hemisphere. To allow for the production of vaccines to be used before the winter season, a WHO meeting to select strains for vaccines takes place in February each year for the northern hemisphere, and in September for the southern hemisphere.

In some cases, newly recommended strains do not grow well in the embryonated chicken eggs used for vaccine production, so "high growth reassortant viruses" (hgr viruses) must be made. Once the WHO recommendation is known, vaccine production processes can begin immediately if the vaccine is multivalent and contains at least one previously used strain. Development of seeds suitable for production of new strains can be completed during this same time period. However, in a pandemic situation it is likely that monovalent vaccine would be made. In this case, efforts to reduce time for development of seed viruses should be actively pursued since all other activities are dependent on this phase.

Reagents for vaccine standardization are produced at the Center for Biologics Evaluation and Research (CBER), USA, the National Institute for Biological Standards and Control (NIBSC), United Kingdom, and the Therapeutic Goods Administration (TGA), Australia. These consist of sheep antiserum and calibrated antigen for use in the single-radial-diffusion (SRD) test. About 2–3 months are needed to prepare these reagents from the time each new strain is recommended, possibly adding a further important delay when only monovalent vaccines will be manufactured. Once SRD reagents have been received, manufacturers can standardize the potency of each monovalent vaccine batch and then blend the vaccines into a multivalent final product. At present three strains are used, type A(H3N2), type A(H1N1) and type B, at the required doses of 15 µg HA. Because each year's vaccines are made by the same process, many countries use a re-licensing procedure to approve the new strain(s). This may involve official batch release testing by multiple countries of either monovalent vaccine or final lots of multivalent product. Thus the whole process from identification of a new strain to first availability of vaccine cannot take less than about 8 months.

### **Reducing vaccine production time**

#### ***Early preparation of vaccine production seeds:***

If possible, production seeds ("reassortants") should be developed for killed and live attenuated vaccines (where licensed) as soon as pandemic viruses are detected and in advance of deciding whether they are needed. Under optimal circumstances this might be done in as little as 3–4 weeks. However, the H5N1 virus isolated from cases in Hong Kong in 1997 created several unanticipated difficulties because of its pathogenicity for chickens and embryonated chicken eggs, as well as the high case-fatality rate among infected people in Hong Kong. There was therefore a need for laboratories receiving the original isolates to have approved biological containment facilities and procedures which would protect laboratory workers and prevent any possible release of the virus into the environment. Contingency planning for future pandemic threats should include measures to ensure that the necessary laboratory facilities and procedures exist in numerous sites, including those involved in developing vaccine seeds or manufacturing processes.

#### ***Time-saving approaches***

Normally, 4–8 weeks are needed to produce SRD reagents for standardizing killed influenza virus

vaccines. It may be possible to reduce this time to one week if SRD reagents to potential pandemic strains are stockpiled, for example, reference strains for all haemagglutinin sub-types. An alternative would be to attain consensus that would allow different potency tests to be used. One possibility, in this regard, might be the determination of the amount of viral haemagglutinin by procedures, that do not use subtype-specific immunological reagents.

#### ***Reduce delays in licensing of vaccines***

More than one national control authority may be involved in approving the release of vaccine, since vaccines are used in many countries. Agreements on central licensing of vaccines for distribution in multiple countries could overcome this problem.

#### ***Develop alternative production procedures***

Orders for eggs to produce vaccines by current technology must be made at least 6 months in advance of production beginning. This may cause difficulties if a pandemic virus emerges outside the normal time when vaccine production is planned. Alternative methods of production based on fermentation technology, such as virus growth in tissue culture or antigen production by recombinant DNA technology, should be pursued.

#### ***Establish a research agenda***

It is possible that other approaches to vaccination may improve effectiveness. Live attenuated vaccines already offer the potential to immunize with a single dose those who have never experienced the antigens contained in the vaccine. This would appear to be a potentially important advantage in a pandemic, but needs further consideration and research, including addressing any special concerns arising from the introduction of a new haemagglutinin subtype in an infectious influenza vaccine during a pre-pandemic period. Mixing traditional vaccines with adjuvants may improve immunogenicity, and again could eliminate the need for two doses in unprimed populations, possibly also reducing the amount of antigen needed for each dose.

DNA vaccines represent another possibility of providing large numbers of doses in a short time. Since it is not known when the next pandemic will take place, intensive research could considerably improve the approaches available.

#### ***Vaccine valency***

##### ***Standardize pandemic virus vaccine to be monovalent***

Since 1977, WHO has recommended that influenza vaccines be trivalent, containing one type A(H3N2) virus, one type A(H1N1) virus and one type B virus. When responding to a pandemic threat, decisions must be taken whether the pandemic virus vaccine will be used alone or in combination with one or more other viruses. This will depend on surveillance results and best judgement at the time. If it is felt necessary for WHO or individual countries to recommend multivalent vaccines due to uncertainty about the disappearance of former strains, this could reduce the total supply of vaccines against the pandemic virus and complicate the international sharing of vaccines.

#### ***Purchasing and distribution***

##### ***Plan for emergencies when negotiating vaccine procurement contracts***

Many national governments and major pharmaceutical distributors have yearly contracts with manufacturers for influenza vaccines. In the event of a pandemic, those vaccines may prove unneeded, even though manufacturers may have begun or completed their production. Each vaccine manufacturer should discuss with the country(ies) where the influenza vaccine is usually produced or distributed how such contingencies can be addressed and in an emergency the expected rate of production. Production targets may depend on the type of packaging, single-dose or multidose vials, whether split vaccines or whole virus vaccines will be produced, and the potency of the vaccine. In preparing for a pandemic, it may be desirable to build flexibility into procurement procedures to allow for different vaccination strategies. Thus, a decision might be made in advance to have contracts permitting the emergency production of multidose vials of vaccine containing 7 µg per dose instead of the usual 15 µg to allow for stretching supplies or for a schedule of two 7 µg doses instead of one dose of 15 µg in order to maximize immune response in populations lacking prior exposure to an antigen related to that of the pandemic strain.

##### ***Explore possibilities for a "clearing house" to balance purchases and deliveries versus supply***

Each government and vaccine supplier will need to consider how much vaccine they will guarantee to purchase or sell in an emergency situation. The cost per dose may be different if vaccine is being

purchased by governments and made available to recipients without cost, or if vaccine is to be purchased at the user's expense. Without a clearing house to balance demand and supply, cost considerations rather than public health may drive vaccine distribution needs. The needs of non-industrialized countries without any resources to purchase vaccines may be completely overlooked. A mechanism such as a central clearing house operated and funded by a group of countries might allow for vaccine purchases to be pooled and distributed more equitably than otherwise. Such a system could also ensure that a portion of vaccines is purchased as a humanitarian donation for use by designated population sectors in non-industrialized countries, such as health care workers, pregnant women or others with high risk of exposure or severe disease who play essential roles in society.

***Design approaches to vaccine distribution that will be appropriate for an emergency situation***

Different countries have different systems of administration. These procedures may need modification in a pandemic situation. Plans will be needed on whether vaccine distribution will proceed only from facilities under direct government jurisdiction or through private distribution channels. Potential problems in ensuring vaccine security and accountability need to be considered. Timely and up-to-date statistics on vaccination supplies and use will be needed to guide the provision of a product expected to be in high demand and short supply. Control of stolen and counterfeit vaccines is likely to be a new problem.

***Establish international cooperation for assessing safety of influenza vaccines***

Considerable problems can develop if inappropriate reports are made of vaccine-related adverse reactions or failure to detect risks from a new vaccine that is to be widely used over a short period of time. As information travels rapidly, international cooperation is highly desirable to ensure immediate reporting of events.

**Reference:**

1. Influenza Pandemic Preparedness Plan. Communicable Disease Surveillance and Response, World Health Organization. <http://www.who.int/emc-documents/influenza/whocdscsredc991c.html>

## **Thiomersal: theoretical risk leads to phasing out**

Thiomersal, or mercuriothiolate, has been used since the 1930s to prevent bacteria and other organisms from contaminating vaccines, especially in opened multidose vials. With the possible exception of minor skin sensitivity reactions, no adverse effect following immunization has been attributed to thiomersal in all this time and its use over the years has made a valuable contribution to vaccine safety. Now, public opinion has turned firmly against the use of mercury of any sort, and there is a need to minimize exposure to this chemical from all sources.

WHO supports a statement made on 7 July 1999 by the American Academy of Pediatrics and the United States Public Health Service regarding the prospective phasing out of thiomersal. However, because there are currently no tested, efficacious and safe alternatives, WHO will continue recommending procurement of vaccines that contain the preservative. This approach has been endorsed by the WHO Expert Committee on Biological Standardization (see page 232). Although, thiomersal poses a theoretical risk of neurodevelopmental toxicity in infants, the known risk of morbidity and mortality from vaccine-preventable diseases and of contaminated multidose vaccine vials far outweighs any potential risk posed by thiomersal.

WHO and other agencies have begun the process of reducing and removing thiomersal from vaccines and within the next three years modifications to existing strategies will result in a reduction in exposure to thiomersal. Beyond three years, efforts will be focused on new vaccine-delivery technologies, alternative preservatives and combination vaccines. This will further reduce and possibly eliminate thiomersal from vaccines.

**Reference:** *Weekly Epidemiological Record*, **75**:12-16 (2000).