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Requirements for Yellow Fever Vaccine
(Requirements for Biological Substances No. 3)
Proposed Amendment

NOTE:

Adopted by the 59th meeting of the WHO Expert Committee on Biological Standardization, October 2009. A definitive version of this document, which will differ from this version in editorial but not scientific details, will be published in the WHO Technical Report Series.

ADDENDUM

Proposed Amendments to the Requirements for Yellow Fever Vaccine (Requirements for Biological Substances No.3, Annex 2 WHO Technical Report Series, No. 872. 1998)

General considerations

A collaborative study to assess the suitability of a candidate International Standard for Yellow Fever Vaccine indicated that the use of a standard with an arbitrary unitage in International Unit (IU) would markedly improve the agreement in the results between laboratories. The first International Standard for Yellow Fever Vaccine with an assigned potency of $10^{4.5}$ IU per ampoule was established in 2003 (4).

There are several reasons for expressing potencies in IU:

- The current minimum potency requirement is 1,000 mouse LD_{50} per dose and therefore requires periodic assay of reference vaccines in animals even though routine potency tests are performed in tissue culture.
- The results of an international collaborative study to assess the suitability of a candidate International Standard for Yellow Fever Vaccine indicated that potencies expressed as LD_{50}'s are variable between laboratories whereas potencies determined in plaque assays and expressed in IU are not so variable.
- Variability between laboratories is increased by the expression of potencies in LD_{50}s as the determination of the conversion factor from plaque forming units to LD_{50}s is highly variable.
- The release specifications for vaccines in current use produced by different manufacturers vary considerably. These could be made more comparable by expressing them in IU/dose.

Part A. Manufacturing requirements

A.1 Definitions

A.1 .3 *International reference materials*

An International Standard for Yellow Fever Vaccine and an International Reference Preparation of Anti-Yellow-Fever Serum are available from the National Institute for Biological Standards and Control (NIBSC), Potters Bar, England. A non-immune control serum is also available. Samples are distributed free of charge, on request, to National Control Laboratories.

A.4 Production control

A.4. 1.3 Monkey safety test

New master and working seed lots shall be tested for viscerotropism, immunogenicity and neurotropism in a group of 10 test monkeys. For the neurotropism test, the test monkeys inoculated with the virus seed lot shall be compared with a similar group of 10 monkeys injected with a reference virus. The reference virus shall be approved by the national control authority.

The monkeys shall be *Macaca mulatta* (i.e. rhesus monkeys) or *Macaca fascicularis* (i.e. cynomolgus monkeys) and shall have been demonstrated to be non-immune to yellow fever immediately prior to injection of the seed virus. They shall be healthy and shall not have been previously subjected to intracerebral or intraspinal inoculation. Furthermore, they shall not have been inoculated by any route with neurotropic viruses or antigens related to yellow fever.

The test dose shall consist of 0.25 ml containing the equivalent of not less than 5,000 and not more than 50,000 mouse LD50, as shown by a titration conducted by the method described in Appendix 3. The test dose shall be injected into one frontal lobe of each monkey under anaesthetic, and the monkeys shall be observed for a minimum of 30 days.

Viscerotropism test. The criterion of viscerotropism (indicated by the amount of circulating virus) shall be fulfilled as follows. Sera obtained from each of the test monkeys on the second, fourth and sixth days after injection of the test dose shall be inoculated at dilutions of 1:10, 1:100 and 1:1000 into at least 4 cell-culture vessels (or intracerebrally in 0.03 ml aliquots into at least 6 mice) per dilution, as specified in Appendix 3. In no case shall 0.03 ml of serum contain more than 500 mouse LD50 or the equivalent in PFU (see section A.6.2) and in no more than one case shall 0.03 ml of serum contain more than the equivalent of 100 mouse LD_{50} (appropriate techniques for potency testing are given in Appendix 3).

Immunogenicity test and ***Neurotropism test*** as described previously.

A.4.3.2 Virus titration

Live virus content shall be determined by titration in cell culture against a reference preparation of yellow fever vaccine.

A.4.4 Final bulk

A.4.4.5 Virus titration

The live virus content of each final bulk shall be determined by titration in cell culture against a reference preparation of yellow fever vaccine.

A.6 Control tests on final lot

A.6.1 Identity test

A.6.1.1 Test in mice

Delete

A. 6.1.2 Test in cell cultures (plaque reduction test)

The technique described in section A.4.1.3 *Immunogenicity test* (pp. 37—38) of the *Requirements for Yellow Fever Vaccine (1)* shall be used, with dilutions of vaccine with immune and non- immune serum. If a 50% reduction in plaque number at the 1:10 dilution is not observed for the vaccine mixed with immune serum compared with vaccine mixed with non-immune serum, the vaccine shall be rejected.

A.6.2 Potency test

Three final containers shall be selected at random from each filling lot and shall be individually tested on the same day against a reference preparation of yellow fever vaccine calibrated in IU, approved by the National Regulatory Authority. The containers shall be assayed in cell cultures demonstrated to be of adequate sensitivity and approved by the National Regulatory Authority.

Before assay and after reconstitution of the vaccine in the volume and diluents recommended by the manufacturer for preparation for human administration, the vaccine shall stand at a temperature between 20 °C and 30 °C for 20 minutes before further dilution. This material shall be considered as undiluted vaccine.

The dose recommended for use in humans shall not be less than 3.0 $\log_{10}$ IU at the end of shelf life. The release specification shall be approved by the National Regulatory Authority.

The following will apply if changes to the release specification or production should be proposed:

- Existing release specifications should not be changed unless justified by clinical data;
- Any changes to existing vaccines potentially impacting on safety or clinical efficacy e.g. during production or in formulation, should be justified by clinical data;
- Transfer of production from one manufacturer to another should include specifications in IU and not mouse LD_{50}; and
- Specifications for release and at the end of shelf life new manufacturers (including manufacturers with production transfer) should be based on by clinical trial and expressed in IU.

6.3 Thermal stability test

Three final containers from the freeze-dried final lot shall be incubated at 37 ^{o}C for 2 weeks. These containers shall be titrated in parallel with three containers that have been stored at or below the recommended storage temperature. A reference preparation calibrated in IU approved by the National Regulatory Authority shall be included in each assay. At the end of the incubation period the geometric mean infectious titre shall not have decreased by more than 1.0 $\log_{10}$ unit and shall be at least equal to the required minimum number of infectious units per human dose.

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References

1 Requirements for yellow fever vaccine (Requirements for Biological Substances No.3). In: World Health Organization Expert Committee on Biological Standardisation. 46th Report. Geneva. WHO Technical Report Series, No. 872. 1998.

2 Ferguson M and Heath AB. Collaborative study to assess the suitability of a candidate International Standard for Yellow Fever Vaccine. WHO/BS/03.1985 Rev.1.

3 Ferguson M and Heath A. (2004) Collaborative study to assess the suitability of a candidate International Standard for Yellow Fever Vaccine. Biologicals 32:195-205.

4 Yellow Fever Vaccine. In: World Health Organization Expert Committee on Biological Standardisation. 54th Report. Geneva. WHO Technical Report Series, No. 927. p 18.