Annex 5

Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines


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Acknowledgements

References
Recommendations published by WHO are intended to be scientific and advisory. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these recommendations be made only on condition that the modifications ensure that the vaccine is at least as safe and efficacious as that prepared in accordance with the recommendations set out below. The parts of each section printed in small type are comments for additional guidance intended for manufacturers and NRAs, which may benefit from these details.
Abbreviations

AEFI  adverse events following immunization
ALV  avian leukosis virus
ATCC  American Type Culture Collection
C  capsid (structural protein)
CMC  carboxymethyl cellulose
CSF  cerebrospinal fluid
E  envelope (structural protein)
EPI  Expanded Programme on Immunization
FNV  French neurotropic vaccine
GMT  geometric mean titre
HAI  haemagglutination inhibition
HI  haemagglutination inhibition
IgM  immunoglobulin M
IU  International Unit
LD₅₀  median lethal dose
LNI  log₁₀ neutralization index
M  membrane (structural protein)
MMR  measles–mumps–rubella
NAT  nucleic acid amplification technique
NISBC  National Institute for Biological Standards and Control
NRA  national regulatory authority
NS  non-structural (protein)
PCR  polymerase chain reaction
PFU  plaque-forming unit
PRNT  plaque-reduction neutralization test
PS  pig kidney epithelial cells (latently infected with swine fever virus)
RCD  reverse cumulative distribution
1. Introduction

Requirements for yellow fever vaccine were first formulated in 1958, by the WHO Study Group on Requirements for Yellow Fever Vaccine and Requirements for Cholera Vaccine, and were published as Requirements for yellow fever vaccine (1). The Requirements embodied recommendations made by the WHO Expert Committee on Yellow Fever Vaccine at its first meeting (2), and they applied to vaccine prepared from a suitable strain of yellow fever virus. The vaccine was intended to be given by subcutaneous injection. Conformity with these Requirements has been the basis for WHO approval of yellow fever vaccine used for vaccination and revaccination against yellow fever in connection with certification for the purposes of international travel (3). Such approval has been given only to vaccine prepared using seed derived from the 17D strain of yellow fever virus. Yellow fever continues to be the only disease for which a certificate of vaccination is required for entry into some countries, and the update of the International Health Regulations (4) increased attention to the need for such certificates. The Requirements have also been used by national regulatory authorities (NRAs) for the control and approval of yellow fever vaccine used in national immunization programmes.

In 1969, at its twenty-second meeting, the WHO Expert Committee on Biological Standardization agreed that developments in virology in general, and in the manufacture and control of yellow fever vaccine in particular, warranted a revision of the existing Requirements, with due consideration of their national and international applications (5). In 1975, at its twenty-seventh meeting, the Committee formulated revised Requirements for yellow fever vaccine (6). Much experience was gained with the preparation of yellow fever vaccine after 1975, and a further revision of the Requirements was approved by the Committee in 1995 (7).

A collaborative study to assess the suitability of a candidate International Standard for yellow fever vaccine indicated that the use of a standard for measuring potency, which has been assigned an arbitrary unitage in International Units (IU), would markedly improve the agreement between the results of different laboratories (8). The First International Standard for yellow fever vaccine with an assigned potency of $10^{1.5}$ IU per ampoule was established in 2003 (9). A proposal
to amend the Requirements for yellow fever vaccine so that the potency of such vaccines could be expressed in IU per dose, and the dose recommended for use in humans should be not less than 3.0 log₁₀ IU with no upper limit on the quantity of virus in a dose, was approved by the Committee in 2008 (10). The availability of an International Standard for yellow fever vaccine with an assigned potency in IU, such that assay in mice and expression of virus titres in median lethal dose (LD₅₀) are not required, also impacts on other sections of the Requirements established in 1995 (7).

In 2008, the Committee recommended that the Requirements for yellow fever vaccines be reviewed, as it was more than 10 years since they had been published and sections on nonclinical and clinical evaluation for new candidate yellow fever vaccines were required. To facilitate this process, WHO convened a meeting of experts, regulatory professionals and other stakeholders in Geneva in May 2009, to discuss the scientific basis for the present revision of the Requirements and to develop revised Recommendations for yellow fever vaccines (11).

The scope of the current Recommendations encompasses live attenuated yellow fever vaccines derived from strain 17D, including 17D-204 and 17DD substrains.

This document should be read in conjunction with the relevant WHO guidelines, including those on nonclinical (12) and clinical evaluation (13) of vaccines.

2. General considerations

The yellow fever virus is small (50 nm) and consists of a nucleocapsid with core protein (13 kDa) containing single-stranded, positive-sense RNA surrounded by a lipoprotein envelope (14). The lipoprotein envelope contains two proteins – a small membrane protein (8 kDa) and an envelope glycoprotein (53 kDa), which is the major target of neutralizing antibodies and has type- and group-specific antigenic determinants. Wild-type yellow fever viruses have genomes of similar length but vary depending on the size of the 3’ non-coding region (15, 16). On the basis of sequence analysis, wild-type yellow fever virus strains have been classified into at least seven genotypes: five in Africa and two in South America. The genotypic variation is not accompanied by significant antigenic differences across strains and there is a single serotype (17).

The genome of the yellow fever virus strain from which all 17D vaccines are derived has been completely sequenced and has been found to contain 10 862 nucleotides, which encode three structural and seven non-structural proteins (18). There are two substrains in use today for the manufacture of 17D vaccine, namely 17D-204 and 17DD. 17D-213 is a derivative of 17D-204 that has gained
a glycosylation site in the E protein but differs significantly in phenotype from 17D-204. It is sometimes considered to be a substrain of 17D and sometimes referred to as 17D-213. Genomic sequencing has been reported for many of the yellow fever vaccine viruses and their seeds that are currently used by different manufacturers. These studies show that there are very few nucleotide and amino acid differences between the vaccine strains. The yellow fever vaccine strains that have been and are being used for vaccine manufacture, and their history, are outlined in Appendix 1.

Yellow fever is a viral haemorrhagic fever that is endemic to 32 countries in Africa and 13 countries in Central and South America (19).

In 1900, a commission headed by the American physician Walter Reed confirmed that the disease was transmitted from human to human by the mosquito Aedes aegypti, a hypothesis proposed earlier by the Cuban physician Carlos Finlay in 1881 (20). There are two epidemiological patterns of yellow fever virus transmission: the urban cycle and the forest cycle (also known as the jungle or sylvan cycle). The two patterns of transmission lead to a clinically identical disease. In the Americas, the yellow fever virus circulates by means of an endemic forest cycle that results in as many as several hundred reports per year of infection, primarily in non-immune forest workers, with occasional reports of isolated cases of urban yellow fever. In Africa, the virus circulates by means of both urban and forest cycles and periodically breaks out of its endemic pattern to infect large numbers of non-immune persons in the course of major epidemics (21).

The case-fatality rate of yellow fever can reach as high as 20–80% in severely ill patients who are hospitalized (22). Case-fatality rates are highest among young children and the elderly. There are no antiviral drugs for any flavivirus infection, including yellow fever, so the availability of vaccines is important for both resident populations and travellers.

When 17D vaccine was first used in the late 1930s and early 1940s, some problems were observed that were associated with under- or over-attenuation of the 17D strain on passage. These problems were resolved by the establishment of a virus seed lot system in 1945. As of 2009, more than 500 million doses of 17D vaccine had been administered (23), so a large amount of information is available on vaccine safety. This vaccine has been shown to be very effective for the control of yellow fever both during outbreaks and between epidemics. In 1990, the Global Advisory Group of the Expanded Programme on Immunization (EPI) recommended that all countries at risk of yellow fever should incorporate the vaccine in their routine immunization programmes. In Africa, 22 countries have introduced yellow fever vaccine in routine childhood immunization. Routine vaccination coverage in countries at risk in Africa increased from 16% in 2000 (eight countries) to 43% in 2008. In the Americas, coverage rose from 64% to 91% (19). In this regard, it is of note that the limited data on vaccination
of individuals with immunosuppression associated with infection with HIV suggest that seroconversion is reduced without an increase in adverse events following immunization (AEFI) (24).

Serious adverse reactions that have been reported to be associated with the administration of 17D yellow fever vaccine and that are of particular note include the following:

- Hypersensitivity reactions, including anaphylaxis, are believed to be associated with egg protein, owing to the vaccine being grown in embryonated chicken eggs. However, gelatine used by some manufacturers may be implicated in some hypersensitivity reactions.

- Yellow fever vaccine-associated neurological disease (YEL-AND) is a term recently introduced to define neurological AEFIs that have occurred in temporal association with yellow fever vaccination since 2000 (25). Encephalitis following 17D vaccination in vaccinees of any age was first described in the 1940s (26). The incidence rate was dramatically reduced to background levels after introduction of the seed lot system for manufacture of 17D vaccines. However, in the 1950s, there were several individual case reports describing a self-limited encephalitis in infants and very young children that occurred in temporal association with 17D vaccines manufactured in accordance with the seed lot system (see section A.4.2.1). With one exception, these children recovered fully with no sequelae. Nevertheless, these reports led to the recommendation by WHO that infants aged 6 months and below should not be vaccinated (17). The adoption of this recommendation and possible unknown factors led to the virtual elimination of post-vaccinal encephalitis by the mid-1960s. However, since 2000, there have been rare case reports of a variety of neurological AEFIs in 17D vaccinees of all ages, and particularly in the elderly (25). Rates of YEL-AND varied in different studies undertaken in different populations, but were observed to range from 0.19 to 0.8 per 100 000 doses in studies in Europe and the United States of America (USA) (25, 27). Vaccines of both 17D-204 and 17DD substrains have been associated with YEL-AND.

- A total of 51 cases of yellow fever vaccine-associated viscerotropic disease (YEL-AVD) had been identified up to May 2009 (23). The estimated reporting rate is between 0.004 and 0.4 per 100 000 doses, with a case-fatality rate of up to 64%. All the reported cases occurred after the primary dose (23). The published index case is from Brazil in 1975 (28). The mechanism(s) responsible for the clinical picture of YEL-AVD, which can vary from multi-organ system failure without much evidence of hepatitis to a fulminant hepatitis resembling the
disease yellow fever, is currently unknown (29–32). Available data suggest that YEL-AVD is related to individual, genetically determined and currently unknown host factors, rather than to the vaccine virus itself. Molecular and animal studies performed to date provide no evidence that 17D vaccine virus mutations have contributed to YEL AVD (33, 34).

In 2007, a cluster of five YEL-AVD cases was reported after a yellow fever mass vaccination campaign in Peru, with four fatal cases that were confirmed virologically and clinically among 42 000 vaccinees who received vaccine from the same lot. This was the first (and so far the only) occasion that a cluster of YEL-AVD cases has been observed in association with a particular lot of vaccine, and it remains unexplained. No quality issues were identified in the manufacture of the vaccine, and the characterization of the working seed and batch records were satisfactory. There were no reported problems from nine batches prepared from the same final bulk as the lot associated with YEL-AVD. The virus isolated from one of the individuals was sequenced and found to be vaccine virus with no evidence that it had mutated (35, 36). An expert panel that was convened to investigate the reports found no features of the vaccine lot that would explain the cluster of cases (35), even though deaths were due to extensive replication of vaccine virus in multiple organs. No difference was identified between the quality of this lot and that of other lots of vaccine, so it has been concluded that cofactors must have led to these cases of YEL-AVD.

The rarity of YEL-AVD cases and the limited number of clinical samples make it difficult to substantiate hypotheses regarding the underlying pathological mechanisms. One potential hypothesis proposes a disconnection between the signalling of innate immune response and the timely activation of the adaptive immune response. Thus, future work that may lead to a more detailed understanding of the immune response induced by the vaccine may help to explain YEL-AVD pathology. Thus far, risk factors that may be associated with the development of YEL-AVD include age (60 years and above) and a history of thymus disease or ablation.

Between 2007 and 2009, three cases of encephalitis in neonates (aged 10 days to 5 weeks) were reported, in which infection in the infants appeared to have resulted from transmission of yellow fever vaccine virus from their recently vaccinated mothers through breastfeeding (37, 38). The onset of symptoms in those infants ranged from 8 to 25 days after maternal vaccination. One of the three cases was confirmed to be vaccine-associated, by detection of vaccine virus RNA in the cerebrospinal fluid (CSF) of the infant (37). Maternal breast milk was not tested for evidence of vaccine virus in any of the three cases. Direct blood-to-blood transmission, through a break in the maternal areola and the mucosa of the infant’s mouth, was thought to be the possible mode of infection. However,
no examination for possible breast lesions was made in any of the cases. These reports are in accordance with the known risk of encephalitis after vaccination of infants under 6 months of age. Based on these case reports, the potential risk of transmission of yellow fever vaccine virus from vaccinated mothers to breastfeeding infants was recently reviewed by the WHO Global Advisory Committee on Vaccine Safety, which concluded that further research is needed to quantify the potential risk, including the possibility of transmission through breast milk. Such studies might include testing breast milk from vaccinated mothers for the presence of vaccine virus and testing their infants for evidence of seroconversion to the vaccine virus. The committee also noted that the risk of potential transmission may vary according to whether mothers are primary vaccinees or have previously been vaccinated, and the age of the infant when exposed (39).

The first immunoglobulin M (IgM)-confirmed transmission of yellow fever vaccine virus through transfusion of blood donated by recently vaccinated military personnel in the USA was described in 2009 (40). Serological evidence of infection was confirmed in three out of five transfusion recipients, though no adverse events or clinical illness attributable to the infection were reported. This documented finding supports the current widely existing recommendations (previously based on a theoretical risk of vaccine virus transmission) that yellow fever vaccine recipients should defer donating blood products for a period (generally 2 weeks) after vaccination.

It is important to ensure that new master or working seeds are confirmed to exhibit levels of neurotropism and viscerotropism that are comparable with those documented for available 17D vaccines. Owing to the lack of suitable animal models for viscerotropic disease, much weight is currently placed on monkey neurovirulence studies, which have a long history. The relevant safety test, performed on monkeys, is therefore retained in these revised Recommendations.

There have been investigations into alternative animal models. A hamster model has been developed that shows viscerotropic disease (41). However, most wild-type strains (which need to be adapted to hamsters) and viruses from YEL-AVD cases do not show viscerotropic disease in this model. Another study reported results of a mouse model for studying viscerotropic disease caused by yellow fever virus infection, which may have some potential as a small-animal model for yellow fever virus (42). The applicability of these models will have to be established before they can be considered for use in the qualification of virus seeds (see Part B).

The thermostability test (see section A.7.4) is undertaken to demonstrate consistency of production and not as a predictive value of real-time stability (43). At the end of the incubation period, the geometric mean infectious titre in the incubated final containers should not have decreased by more than $1.0 \log_{10}$ IU but there is no requirement for the minimum specification to be met.
Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 International name and proper name

The international name should be “live attenuated yellow fever vaccine”. The proper name should be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the recommendations formulated below.

A.1.2 Descriptive definition

Yellow fever vaccine should consist of a freeze-dried preparation of viable, attenuated yellow fever virus (Flavivirus hominis, 17D strain). The preparation should satisfy all the recommendations formulated below.

A.1.3 International Standards

An International Standard for yellow fever vaccine is available from the National Institute for Biological Standards and Control (NIBSC), Potters Bar, England. This material is for use in the calibration of working reference materials for yellow fever vaccine, which are included in each potency test, so that the potency of vaccines is expressed in IU/dose.

NIBSC distributes the International Reference Preparation of anti-yellow-fever serum. Such a preparation is needed as a basis for comparison of antibody responses in the monkey neurovirulence test, and may also be used in antibody assays of clinical trial sera. Additionally, a non-immune control serum is available. These preparations are monkey sera.

WHO reference virus 168–73 is available from NIBSC (see Appendix 2).

A.1.4 Terminology

The definitions given below apply to the terms as used in these Recommendations. They may have different meanings in other contexts.

Adventitious agents: contaminating microorganisms, including bacteria, fungi, mycoplasmas and endogenous and exogenous viruses, that have been unintentionally introduced.

Final bulk: the material prepared from one or more single harvests in the container from which the final containers are filled.

Final lot: a collection of sealed final containers of finished vaccine that are homogeneous with respect to the risk of contamination during filling and freeze-drying. All final containers must, therefore, have been filled from a single container of final bulk in one working session and lyophilized under standardized conditions in a common chamber.
**International Unit (IU):** an IU is a unit of measurement of potency for the yellow fever vaccine, based on the determination of the infectivity of a virus preparation resulting in plaque formation in a suitable tissue culture monolayer in parallel with an accepted working standard, calibrated in IU against the International Standard for yellow fever vaccine.

**Single harvest:** a quantity of virus suspension, derived from tissues of the same origin that were inoculated with the same working seed lot, that has been collected and processed in a single production run.

**Specific-pathogen-free (SPF):** specific-pathogen-free refers to animals that have been shown by the use of appropriate tests to be free from specified pathogenic microorganisms, and also to eggs derived from SPF birds (44, 45).

**Virus master seed lot:** a quantity of virus suspension that has been processed at the same time to assure a uniform composition and that has been characterized to the extent necessary to support development of the virus working seed lot. The characterized virus master seed lot is used for the preparation of virus working seed lots.

**Virus working seed lot:** a quantity of virus of uniform composition, fully characterized and only one passage from a virus master seed lot. The virus working seed lot is used for inoculating embryonated chicken eggs in the preparation of vaccine.

**WHO primary seed virus (213-77):** a quantity of virus suspension of uniform composition produced for WHO by the Robert Koch Institute and available to manufacturers for use in the preparation of a virus master seed lot.

### A.2 Certification of the substrain of 17D virus for use in vaccine production

Currently used substrains 17D-204 and 17DD have a well-documented passage history (see Appendix 1) and safety records, from nonclinical and clinical studies. Any new candidate 17D virus to be used as a master seed for production would require supporting data to qualify it for use. Virus seed lots that have been certified previously can be used. A yellow fever virus primary seed (213-77) is available from WHO on request (previously known as “WHO master seed”) (46). Parts B and C of this document provide recommendations for evaluating new candidate 17D vaccine viruses. Only seed lots derived from viruses that are approved by the NRA should be used in the production of yellow fever vaccines.

### A.3 General manufacturing recommendations

The general manufacturing recommendations for manufacturing establishments contained in the WHO good manufacturing practices: main principles for pharmaceutical products (47) and the Good manufacturing practices for biological
products (48) should apply to establishments manufacturing yellow fever vaccine. Staff directly involved with the production and testing of yellow fever vaccine should be shown to be immune to yellow fever.

A.4  Control of source materials
A.4.1  Eggs used for seed virus growth and vaccine production

Virus for the preparation of virus master and working seed lots and all vaccine production should be grown in embryonated chicken eggs from a closed SPF flock monitored by methods approved by the NRA or the national animal health authority.

All chickens are bled when an SPF flock is established, and thereafter a percentage of the birds are bled at specified intervals to detect exposure of the flock to microbes with potential to cause quality failure in assessments for adventitious agents. In some countries, SPF flocks are monitored on a weekly basis for quality control. The sera are screened for antibodies to the relevant pathogens. The pathogens may also be detected in the flocks by culture or other detection methods, including polymerase chain reaction (PCR). Any chicken in an SPF flock that dies should be investigated, in order to determine the cause of death.

Microbes of interest in flock husbandry may vary by geographical region but should include as a minimum the following: avian adenoviruses, avian encephalomyelitis virus, avian infectious bronchitis viruses, avian infectious laryngotracheitis virus, avian leukosis viruses (ALVs), avian nephritis virus, avian orthoreoviruses, avian reticuloendotheliosis virus, chicken anaemia virus, egg drop syndrome virus, fowl pox virus, infectious bursal disease viruses, influenza A viruses, Marek disease virus, Newcastle disease virus, Mycobacterium avium, Mycoplasma gallisepticum, Mycoplasma synoviae, Salmonella gallinarum, Salmonella pullorum, Salmonella species and Haemophilus paragallinarum.

The flock must not have been vaccinated with live Newcastle disease virus vaccine. In addition, flocks should not be receiving any chemotherapeutic agents (e.g. antimicrobial agents and coccidiostats). It is also recommended that eggs be obtained from young hens.

A.4.2  Yellow fever virus

The substrain of 17D vaccine virus used in the production of vaccine should be certified as described in section A.2.

A.4.2.1  Virus seed lot system

The production of vaccine should be based on the virus master seed lot and virus working seed lot system.
Virus seed lots should be stored in a dedicated temperature-monitored freezer at a temperature that ensures stability, namely less than –60 °C. In some laboratories, the virus master and working seed lots are stored in more than one location.

The virus master and working seed lots must not contain any human protein or added serum or antibiotics.

The virus master and working seed lots should be free of ALVs, mycoplasmas or other adventitious agents, as shown by suitable tests (see sections A.4.2.2.3 and A.4.2.2.4).

The inoculum for infecting eggs used in the production of vaccine should be from a virus working seed lot without intervening passage, in order to ensure that no vaccine is manufactured that is more than one passage removed from a seed lot that has passed all safety tests.

A.4.2.2 Tests on virus master and working seeds

A.4.2.2.1 Identity

Each virus master and working seed lot should be identified as yellow fever virus, by immunological assay or by molecular methods and comparison to an appropriate published 17D vaccine virus. An identity test should be performed on at least one container from each virus master and working seed lot.

A.4.2.2.2 Genotype characterization

For any new virus master and working seed, it is recommended that the first three consecutive consistency vaccine lots be analysed for consensus sequence changes from the seed virus (total genome sequence). The sequence results should be used to demonstrate the consistency of the production process. Routine sequence analysis of final bulk vaccine is not recommended.

A.4.2.2.3 Tests for bacteria, fungi and mycoplasmas

Each virus master and working seed lot should be tested for bacterial, fungal and mycoplasmal contamination by appropriate tests, as specified in Part A, sections 5.2 (49) and 5.3 (50) of General requirements for the sterility of biological substances, or by a method approved by the NRA.

Nucleic acid amplification techniques (NATs) alone or in combination with cell culture, with an appropriate detection method, may be used as an alternative to one or both of the compendial mycoplasma detection methods, after suitable validation and agreement from the NRA (51).

A.4.2.2.4 Tests for adventitious agents

Each virus master and working seed lot should be tested for ALVs and other adventitious agents relevant to the passage history of the seed virus. In addition,
each virus working seed lot should be tested in both cells and eggs for the other adventitious agents.

Neutralization of yellow fever virus is necessary for many tests because the virus is cytopathogenic. Where antisera are used to neutralize yellow fever virus, the antigen used to generate the antisera should be produced in cell cultures (other than those derived from chickens) and should be free from extraneous agents. After neutralization of the yellow fever virus by hyperimmune antibody preparation, the virus pool should be inoculated on cell cultures of human cells, monkey cells and chicken cells. Following inoculation, the cell cultures should be observed microscopically for cytopathic changes. At the end of the observation period, the cells should be tested for haemadsorbing viruses. The cell cultures, the method of incubation and the period of observation should be approved by the NRA. A specific monoclonal antibody may be used instead of a hyperimmune polyclonal serum.

Each virus master or working seed lot should also be tested in animals that may include guinea-pigs, adult mice, suckling mice and embryonated chicken eggs, as appropriate. For test details, refer to the Requirements for measles vaccines (live) (52). See also section A.4.2.1.1.

New molecular methods with broad detection capabilities are being developed for the detection of adventitious agents. These methods include: degenerate NAT for whole virus families with analysis of the amplicons by hybridization, sequencing or mass spectrometry; NAT with random primers followed by analysis of the amplicons on large oligonucleotide microarrays of conserved viral sequencing or digital subtraction of expressed sequences; and high-throughput sequencing. These methods may be used in the future to supplement existing methods, or as alternative methods to both in vivo and in vitro tests, after appropriate validation and approval by the NRA (51).

Each virus master and working seed lot should be tested for, and shown to be free from, *Mycobacterium avium*, by an appropriate test approved by the NRA.

NAT may be used as an alternative to the mycobacteria microbiological culture method and/or to the in vivo guinea-pig test for the detection of mycobacteria, after suitable validation and approval by the NRA (51).

Additional testing for ALVs and adventitious agents may be performed on control eggs for the virus working seed lot (e.g. fowl pox, salmonella, mycobacteria).

A.4.2.5 Tests in nonhuman primates

Each virus master and working seed lot should be tested for neurotropism, viscerotropism and immunogenicity in nonhuman primates, as described in Appendix 2.
A.4.2.6 *Virus titration for infectivity*

Each virus master and working seed lot should be assayed for yellow fever virus infectivity in a sensitive assay in cell cultures, as described in Appendix 4.

A.5 **Control of vaccine production**

Penicillin and other beta-lactams should not be used at any stage of the manufacture because of their nature as highly sensitizing substances. Other antibiotics may be used if approved by the NRA, and provided that the quantity present in the final product is acceptable to the NRA.

A.5.1 **Tests on uninoculated control eggs**

If monitoring of the flocks supplying embryonated chicken eggs is not under the direct responsibility of the vaccine manufacturer, an SPF certificate and quality control certificate (with test results) should be available from the supplier. The test described next should be performed.

A sample of 2% (which should comprise at least 20 but no more than 80 eggs) of the uninoculated embryonated eggs from the batch used for vaccine production should be incubated under the same conditions as the inoculated embryonated eggs. At the time of virus harvest, the uninoculated embryonated eggs should be processed in the same manner as the inoculated embryonated eggs, and the extract from the control embryos must be shown to be free from haemagglutinating agents, ALVs and other adventitious agents, by methods approved by the NRA.

A.5.2 **Single harvests**

After inoculation and incubation at controlled temperature and humidity, living and normal chicken embryos only should be harvested. The age of embryos at the time of harvest should be calculated from the initial introduction of the eggs into the incubator and should be no more than 12 days. The number of rejected eggs may be estimated, to monitor the consistency of the production.

After homogenization and centrifugation, the embryonic extract should be kept at –60 °C or below until further processing.

All intermediates should be maintained under conditions shown by the manufacturer to retain the desired biological activity. Storage periods should be approved by the NRA.

A.5.3 **Tests on single harvests**

A.5.3.1 **Sampling**

Samples required for the testing of single harvests should be taken immediately on harvesting and prior to further processing. If the tests are not performed
immediately, the samples taken for tests on single harvests should be kept at a temperature of \(-60\,^\circ\text{C}\) or below and subjected to no more than one freeze/thaw cycle.

A.5.3.2 Identity
Each single harvest or group of single harvests from a daily production should be identified as yellow fever virus by immunological assay, on cell culture using specific antibodies or by molecular methods approved by the NRA.

A.5.3.3 Tests for bacteria, fungi and mycoplasmas
Each single harvest or group of single harvests from a daily production should be tested for bacterial, fungal and mycoplasmal contamination by appropriate tests, as specified in Part A, sections 5.2 (49) and 5.3 (50) of General requirements for the sterility of biological substances, or by a method approved by the NRA.

NAT alone or in combination with cell culture, with an appropriate detection method, may be used as an alternative to one or both of the compendial mycoplasma detection methods, after suitable validation and agreement by the NRA (51).

A.5.3.4 Tests for adventitious agents
Each single harvest or group of single harvests from a daily production should be tested for and shown to be free from *Mycobacterium avium* by an appropriate test approved by the NRA.

NAT may be used as an alternative to mycobacteria microbiological culture method and/or the in vivo guinea-pig test for the detection of mycobacteria, after suitable validation and approval by the NRA (51).

A.5.3.5 Virus titration
The live yellow fever virus content of each single harvest or group of single harvests from a daily production should be determined by titration in cell culture against a reference preparation and the titre should be expressed in IU/ml (see Appendix 4).

A.5.4 Final bulk
The final bulk should be prepared from one or several single harvests. The addition of any stabilizing agents should be approved by the NRA. The tests described next should be performed unless they have already been performed on each single harvest. The final bulk should, in any case, be tested for sterility. Samples that are
not tested immediately should be stored at or below –60 °C and subjected to no more than one freeze/thaw cycle.

A.5.4.1 Sterility tests for bacteria and fungi
Each final bulk should be tested for bacterial and fungal sterility as specified in Part A, section 5.2 of General requirements for the sterility of biological substances (49), or by the methods approved by the NRA.

A.5.4.2 Stabilizers
If a stabilizing agent is added, its concentration should be measured. The method used and permitted levels should be approved by the NRA.

A.5.4.3 Virus titration (if performed)
The live yellow fever virus content of each final bulk should be determined by titration in cell culture against a reference preparation, and the titre should be expressed in IU/ml (see Appendix 4).

A.6 Filling and containers
The general requirements concerning filling and containers given in Good manufacturing practices for biological products (48) should apply to yellow fever vaccine. Care must be taken to ensure that the materials of which the container, and if applicable the closure, are made do not adversely affect the virus content of the vaccine under the recommended conditions of storage. The vaccine should be freeze-dried.

Single- and multiple-dose containers may be used.

Failure to achieve adequate drying will result in a product that is susceptible to rapid deterioration even at 0 °C. Since yellow fever virus is extremely labile, unless the container is well sealed, variations in virus content may occur during storage. The manufacturer should ensure that the seal is satisfactory.

The manufacturer should provide the NRA with adequate data to prove the stability of the vaccine under appropriate conditions of storage and shipping (see section A.12).

A.7 Control tests on final lot
Samples should be taken from each final vaccine lot for testing and should fulfil the requirements of this section. All the tests and specifications, including the
methods used and the permissible limits for the different parameters listed under this section, unless otherwise specified, should be approved by the NRA.

A.7.1  **Inspection of final containers**

Every container in each final lot should be inspected visually, and those showing abnormalities should be discarded.

A7.1.1  **Appearance**

The appearance of the freeze-dried vaccine and the reconstituted vaccine should be described with respect to their form and colour. If reconstitution with the product diluent does not allow for the detection of particulates, an alternative diluent may be used.

If the glass used for the final containers does not permit inspection of the contents (e.g. with tinted glass), visual inspection should be performed on the reconstituted vaccine and the observations should comply with the specifications approved by the NRA.

A.7.2  **Identity**

An identity test should be performed on at least one container from each final lot after reconstitution of the vaccine according to the indications of the manufacturer for preparing the vaccine for human administration. A high-titre, monospecific immune serum or a monoclonal antibody known to be free from neutralizing agents that react with other flaviviruses should be used.

A sensitive test in cell cultures (plaque-reduction test) should be used for the identity test. Dilutions of vaccine are mixed with immune and non-immune serum. A suitable immunogenicity test is described in Appendix 2. 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 should be rejected.

Molecular tests may also be used after suitable validation and approval by the NRA.

A.7.3  **Potency**

Three final containers should be selected at random from each final lot and should be individually tested on the same day against a reference preparation of yellow fever vaccine calibrated in IU and approved by the NRA. The containers should be assayed in cell cultures demonstrated to be of adequate sensitivity and approved by the NRA (see Appendix 4).

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

The dose recommended for use in humans should not be less than $3.0 \log_{10}$ IU. The release specification should be approved by the NRA.

An internal upper limit may be established by each manufacturer to monitor the consistency of production (e.g. based on mean titre in IU/dose +3 standard deviations). The upper limit should be approved by the NRA.

Existing release specifications should not be changed unless justified by clinical data and approved by the NRA. Major changes to existing vaccines (e.g. during production or in formulation and which may have a potential impact on the efficacy of the vaccine) should be justified by clinical data and approved by the NRA.

Specifications for new manufacturers (including manufacturers with production transfer) should be set by clinical trial and expressed in IU.

A.7.4 Thermal stability
The thermostability test aims to demonstrate consistency of production. Additional guidance on the evaluation of vaccine stability is provided in WHO’s Guidelines for stability evaluation of vaccines (43).

Three final containers from the freeze-dried final lot should be incubated at 37 °C for 2 weeks. These containers should be titrated in parallel with three containers that have been stored at or below the recommended storage temperature. A reference preparation calibrated in IU and approved by the NRA should be included in each assay. At the end of the incubation period, the geometric mean infectious titre in the incubated final containers should not have decreased by more than $1.0 \log_{10}$ IU.

A.7.5 Sterility tests for bacteria and fungi
Each final lot should be tested for bacterial and fungal sterility as specified in Part A, section 5.2 of General requirements for the sterility of biological substances (49), or by the methods approved by the NRA.

A.7.6 General safety test
Each final lot should be tested for the absence of abnormal toxicity in mice and guinea-pigs, using a general safety (innocuity) test approved by the NRA, and should pass the test.

This test may be omitted for routine lot release once consistency of production has been established to the satisfaction of the NRA.
A.7.7  **Residual moisture**
The residual moisture in a representative sample of each freeze-dried final lot should be determined by a method approved by the NRA. The upper limit of the moisture content should be approved by the NRA on the basis of stability tests.

A.7.8  **Residual ovalbumin**
The content of residual ovalbumin should be determined and should be within limits approved by the NRA.

A.7.9  **Endotoxin content**
The vaccine in the final container should be tested for endotoxin with a *Limulus* amoebocyte lysate test. The endotoxin content should be consistent with levels found to be acceptable in vaccine lots used in clinical trials and approved by the NRA.

A.7.10  **Residual antibiotics (if applicable)**
If any antibiotics are added during vaccine production, the content of the residual antibiotics should be determined and must be within limits approved by the NRA.

A.8  **Records**
The requirements given in section 8 of Good manufacturing practices for biological products (48) should apply.

A.9  **Retained samples**
The requirements given in section 9.5 of Good manufacturing practices for biological products (48) should apply.

A.10  **Labelling**
The requirements given in section 7 of Good manufacturing practices for biological products (48) should apply, with the additions listed next.

The label on the carton or the leaflet accompanying the container should:

- state that the vaccine fulfils Part A of these Recommendations;
- state the nature of the preparation, specify the substrain of yellow fever virus in the vaccine and the minimum number of infectious units per human dose, and state that SPF eggs were used;
- state the nature and quantity of any residual antibiotic present in the vaccine;
- indicate that the vaccine contains proteins derived from eggs;
■ indicate that contact of the vaccine with disinfectants is to be avoided;
■ indicate that the dose should be the same for persons of all ages;
■ indicate the volume and nature of the diluent to be added to reconstitute the vaccine, and specify that only the diluent supplied by the manufacturer should be used;
■ state that the vaccine is contraindicated in children aged under 6 months and is not recommended for those aged 6–8 months (17), except in specific circumstances, and it should be in accordance with available official recommendations;
■ state that the reconstituted vaccine should be used without delay or, if not used immediately, that it should be stored between 2 °C and 8 °C, protected from direct light and used within six hours (53).

A.11 Distribution and shipping
The requirements given in section 8 of Good manufacturing practices for biological products (48) should apply. Further guidance is provided in WHO’s Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (54).

A.12 Stability, storage and expiry date
A.12.1 Stability testing
Adequate stability studies form an essential part of vaccine development. Current guidance on the evaluation of vaccine stability is provided in WHO’s Guidelines for stability evaluation of vaccines (43). Stability testing should be performed at different stages of production, namely on single harvests or pools of single harvests, final bulk and final lot. In addition, such studies should be undertaken on reconstituted vaccine. Stability-indicating parameters should be defined or selected appropriately, according to the stage of production. It is advisable to assign a shelf-life to all in-process materials during vaccine production – particularly intermediate materials such as single harvests and final bulk.

The stability of the vaccine in its final container and at the recommended storage temperatures should be demonstrated to the satisfaction of the NRA on at least three consecutive lots of final product. Accelerated thermal stability tests may be undertaken to give additional information on the overall stability of a vaccine.

The formulation of vaccine should be stable throughout its shelf-life. Acceptable limits for stability should be agreed with the NRA. Following licensure, ongoing monitoring of vaccine stability is recommended, in order to support shelf-life specifications and refine the stability profile (43). Data should be provided to the NRA, in accordance with regulatory requirements.
A.12.2  Storage conditions
Before being distributed by the manufacturing establishment, or before being issued from a depot for the maintenance of vaccine reserves, all vaccines should be kept, at all times, at a temperature approved by the NRA.

The manufacturer should recommend conditions of storage and shipping that will ensure that the vaccine conforms to the requirements of potency until the expiry date stated on the label. These conditions should be approved by the NRA. The vaccine should have been shown to meet the release specifications for a period equal to that between the date of release and the expiry date.

A.12.3  Expiry date
The expiry date should be defined on the basis of shelf-life and should be supported by the stability studies with the approval of the NRA.

A.12.4  Expiry of reconstituted vaccine
For single-dose containers, the reconstituted vaccine should be used immediately. For multidose containers, the container should be kept in the dark at 2–8 °C. The expiry time for use of an opened container should be defined by stability studies and approved by the NRA, but should not be more than six hours (54).

Part B. Nonclinical evaluation of live attenuated yellow fever vaccines

The nonclinical evaluation of candidate live attenuated yellow fever vaccines derived from substrains of the 17D strain should be based on the WHO guidelines on nonclinical evaluation of vaccines (12).

Any new candidate 17D strain that is not already in use by a manufacturer should be characterized with respect to immunogenicity and safety and should be compared to at least one strain in current use for the manufacture of a licensed vaccine. In the case of manufacturing changes for an existing vaccine, recharacterization of the vaccine strain may be required.

The specific issues discussed next should be considered.

B.1  Characterization of a new candidate yellow fever vaccine
Any new candidate virus used in the production of vaccine requires supporting data that would qualify it for use. The new candidate virus should be identified by historical records that include information on the origin of the virus, its method of attenuation, whether the virus has been biologically or genetically cloned prior to generation of the master seed, genetic sequence information and the passage level.
To assess genotypic and phenotypic stability, virus from each production passage level should be characterized by laboratory and animal tests, in comparison with a currently acceptable vaccine. These tests may include full genome sequencing, growth in permissive and semi-permissive cell cultures, plaque-size estimation by plaque assays, and mosquito infectivity and dissemination.

Seed viruses used in the manufacture of vaccine intended for clinical trials should be tested as described in Appendix 2, to demonstrate that the seed virus is suitable for use in vaccine production.

B.2 Immunogenicity and other pharmacodynamic studies

The nonclinical studies should indicate that the new candidate yellow fever vaccine induces neutralizing antibodies to yellow fever virus in mice and nonhuman primates. A currently licensed yellow fever vaccine should be included as a control in such studies.

B.3 Toxicity assessment

In the early development of a new candidate yellow fever vaccine, and prior to the initiation of clinical trials in humans, toxicity assessment – including systemic toxicity and local tolerance – should be considered in relevant species in accordance with WHO guidelines (12). The toxicology assessment should include an evaluation of neurotropism and viscerotropism. If the candidate vaccine is to be licensed to include women of childbearing potential, a reproductive toxicity study will need to be conducted at an appropriate point in development, in accordance with WHO guidelines (12), and would require administration of the vaccine to pregnant animals in the early phase of implantation/organogenesis, since this is the phase that is most at risk.

These studies must demonstrate that the new candidate yellow fever vaccine is safe and suitable for use in humans.

Appropriate safety characterization studies should be conducted, and should include an evaluation of neurotropism and viscerotropism, according to the accepted protocol, which suggests monkey as the relevant species and the use of the 17D vaccine as a comparator (see section A.4.2.2.5 and Appendix 2).

Part C. Clinical evaluation of live attenuated yellow fever vaccines

Clinical trials should adhere to the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (55) and to the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (13). All clinical trials should be approved by the relevant NRAs.
Some of the issues that are specific to the clinical evaluation of yellow fever vaccines derived from the 17D strain are discussed in the following sections. These sections should be read in conjunction with the general guidance mentioned above. It is also recommended that manufacturers should consult with relevant NRAs regarding the overall clinical development programme.

Part C considers the provision of clinical data required (i) when a new candidate live attenuated yellow fever vaccine derived from the 17D virus is developed; and (ii) when there have been major changes to the manufacturing process of an established vaccine (including preparation of a new virus master seed lot of an established strain). Clinical evaluation of vaccine manufactured using a new working seed lot is not required, provided that the passage level is not more than one from the master seed lot, the working seed has been characterized, and consistency of the manufacturing process has been demonstrated.

C.1 General considerations

Due to the success of 17D vaccines over the past 70 years, studies of vaccine efficacy are not feasible. Therefore, clinical studies should assess the safety and immunogenicity of a candidate yellow fever vaccine in comparison with at least one licensed vaccine. The assessment of immunogenicity should be based on the elicitation of neutralizing antibodies, which are thought to be the basis of protection (56), although the actual mechanism of protection is not known (57–59).

The relative risk of YEL-AVD and YEL-AND for a new candidate yellow fever vaccine versus approved vaccines cannot be estimated from pre-approval studies but should be addressed as part of post-marketing surveillance.

C.2 Safety and immunogenicity studies

C.2.1 Assessment of the immune response

The demonstration of an immune response to vaccination should be based on the measurement of neutralizing antibody titres both pre- and post-vaccination. Neutralizing antibody may be determined either by the plaque-reduction neutralization test (PRNT) or by using the log10 neutralization index (LNI). Geometric mean titres (GMTs), seroconversion rates and reverse cumulative distributions (RCDs) should be provided. Seroconversion may be defined as either a fourfold increase in neutralizing antibody or the induction of measurable neutralizing antibody in a previously seronegative individual. It is desirable to consider these two phenomena separately in the comparison between a novel 17D vaccine and a licensed one used as control.

The flavivirus haemagglutination inhibition (HAI) test may be used to determine whether or not individuals enrolled in vaccine studies are flavivirus naïve (see below). This test is not suitable for assessing responses to vaccination.
C.2.2 Immunogenicity studies

New candidate yellow fever vaccines (i.e. manufactured using a newly derived 17D strain) should be compared with at least one well-established and licensed 17D yellow fever vaccine. It is preferable that the comparative vaccine(s) selected should have been in widespread use for some years, so that some data on effectiveness are available as well as a reliable description of the safety profile.

If the candidate vaccine has been produced by an existing manufacturer from a new virus master seed lot, the comparison should be against a lot derived from the existing virus master seed.

C.2.3 Population

Safety and immunogenicity studies should be undertaken initially in healthy adults aged 18–60 years, preferably in need of vaccination against yellow fever. Subjects may be resident in non-endemic or endemic areas and should have no history of yellow fever or vaccination against yellow fever.

Studies in children should be undertaken only after adult studies have demonstrated that the safety profile is acceptable. In accordance with national and regional recommendations, it is likely that inclusion of children aged 9 months or more would be possible and desirable in endemic countries. However, some NRAs have agreed that studies in children are not always required, provided that the studies in adults are satisfactory and the overall experience with the use of 17D vaccines in children is taken into account.

The study exclusion criteria should reflect the current contraindications to administration of live attenuated yellow fever vaccines (e.g. pregnancy, known allergy to vaccine components, and immunosuppression).

C.2.4 End-points and analyses

The protocol should state the primary objective(s) of the study. The neutralizing antibody response to the candidate vaccine should be demonstrated to be non-inferior compared to an appropriate licensed yellow fever vaccine based primarily on GMTs and/or seroconversion rates. The primary end-point should be selected according to the study population and the anticipated immune response. For example, very high seroconversion rates are expected in healthy adults, and this has implications for the selection of the non-inferiority margin and thus the sample size calculation. Further details on demonstrating non-inferiority are described in WHO’s Guidelines on clinical evaluation of vaccines: regulatory expectations (13).

The primary analysis should be conducted in subjects who are flavivirus-naive. If the HAI results are obtained only after vaccination (rather than being used to screen subjects for study eligibility before enrolment), the results for neutralizing antibody against yellow fever should be analysed both overall and
separately for those who were flavivirus-naive or non-naive, in order to assess any effect of pre-existing antibody to a heterologous flavivirus (e.g. dengue or West Nile viruses) on the response to yellow fever vaccine.

Other immunological parameters should be compared in planned secondary analyses (e.g. percentages reaching predefined titres).

C.2.5  **Dose-ranging studies**

Dose-ranging studies may be undertaken for new vaccines based on a 17D virus seed, to determine the minimum dose of virus (in IU) required to provide adequate immune responses. These data can also be used to support the derivation of the minimum viral titre that should be present in the vaccine at the end of its shelf-life. The assessment of safety of a 17D yellow fever vaccine during clinical studies should be in accordance with WHO’s Guidelines on clinical evaluation of vaccines: regulatory expectations (13).

C.2.6  **Concomitant administration with other vaccines**

An evaluation of the effects of co-administration of a yellow fever vaccine with other vaccines should be considered, taking into account which vaccines are most likely to be given concomitantly in different age groups and populations.

If a yellow fever vaccine is to be used in an EPI programme simultaneously with other vaccines, it is particularly important that the effects of co-administration should be evaluated. For example, some studies in children have shown that co-administration with measles–mumps–rubella (MMR) combined vaccines has resulted in lowered serological responses to yellow fever vaccines (60).

Immune responses to all other antigens co-administered with the yellow fever vaccine should be measured at least in subsets. While the study will usually be powered only to demonstrate non-inferiority with respect to neutralizing antibody against yellow fever, the protocols should at least include planned secondary analyses of antigen-specific responses. If these analyses indicate that immune responses are lower on co-administration with a new yellow fever vaccine compared to the licensed vaccine(s), NRAs will need to consider the potential clinical consequences on a case-by-case basis.

C.2.7  **Viraemia**

Assessment of viraemia is not routinely required for a 17D-derived vaccine because it is usual that recipients of yellow fever vaccines have a transient viraemia.

A low-level viraemia is known to occur after 17D vaccination. Titres of virus in blood have traditionally been determined by counting plaques in tissue culture monolayers that have been infected with serial dilutions of serum samples. More recently, reverse transcriptase polymerase chain reaction (RT-PCR) and quantitative RT-PCR have been used instead of the plaque assay. Quantitative
RT-PCR assays should include the generation of a standard curve using quantitative RT-PCR of 17D vaccine virus, so that the results can be expressed as plaque-forming unit (PFU) equivalents or genomic equivalents. There is currently no International Standard available for quantitative RT-PCR assays.

C.2.8 Pre-licensure safety data

The general approach to the assessment of safety of a new 17D yellow fever vaccine during clinical studies should be in accordance with WHO’s Guidelines on clinical evaluation of vaccines: regulatory expectations (13). Planned safety studies should be supported by a clear scientific rationale. However, given the long history of the use of 17D vaccines, the NRA may decide that sufficient data can be obtained from the immunogenicity studies in relatively small numbers. Where a new 17D seed that has not been used previously is investigated, larger-scale studies may be needed.

An appropriate pharmacovigilance plan should be developed and approved by the NRA prior to licensure.

C.3 Post-marketing studies and surveillance

Enhanced safety surveillance (particularly for the detection of YEL-AND and YEL-AVD) should be undertaken during the initial post-approval years, in collaboration with NRAs. The total duration of enhanced surveillance should be regularly reviewed by the NRA. Case definitions for YEL-AVD are being developed by the Brighton Collaboration and should be used when they are finalized (61, 62).

If particular issues arise during pre-licensure studies or during post-licensure safety surveillance, it may be necessary to conduct specific post-licensure safety studies.

Part D. Recommendations for national regulatory authorities

D.1 General

The general recommendations for control laboratories given in the Guidelines for national authorities on quality assurance for biological products (63) and Guidelines for independent lot release of vaccines by regulatory authorities (64) should apply. These Guidelines specify that no new biological substance should be released until consistency of manufacturing and quality, as demonstrated by a consistent release of batches, has been established. The detailed production and control procedures, and any significant changes in them that may affect the quality, safety and efficacy of yellow fever vaccine, should be discussed with,
and approved by, the NRA. For control purposes, the NRA should obtain the International Standard for potency testing and, where necessary, should establish national working reference preparation(s) calibrated against the International Standard.

D.2 Release and certification

A vaccine lot should be released only if it fulfils the national requirements and/or Part A of the current Recommendations. A protocol based on the model given in Appendix 5, and signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA in support of a request for release of a vaccine for use. A statement signed by the appropriate official of the NRA should be provided, if requested by a manufacturing establishment, and should certify whether or not the lot of vaccine in question meets all national requirements, as well as Part A of these Recommendations. The certificate should also state the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, the date of the last satisfactory potency test, as well as the assigned expiry date on the basis of shelf-life, should be stated. A copy of the official national release document should be attached. The certificate should be based on the model given in Appendix 6. The purpose of the certificate is to facilitate the exchange of vaccines between countries.

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References


Appendix 1

Genealogy of 17D yellow fever vaccine strains

Two live attenuated yellow fever vaccines were developed in the 1930s: the French neurotropic vaccine (FNV) prepared from wild-type strain French viscerotropic virus passaged in mouse brain, and the 17D vaccine, which was prepared from wild-type strain Asibi virus passaged in embryonated chicken eggs. Today, the 17D vaccine is the only type of yellow fever vaccine produced, since the use of FNV was found to be associated with a high incidence of encephalitic reactions in children (1).

The 17D vaccine was developed by Theiler and Smith in 1937 and has been shown to protect against all seven known genotypes of wild-type yellow fever virus.

Two substrains of the 17D vaccine are used in vaccine production today (see Figure 1) (2), namely 17D-204 and 17DD. Some vaccines are also prepared from a distinct substrain of 17D-204 (17D-213) using seed viruses 112/95 and 213/77.

The 17D-204 vaccine substrain is utilized in all countries, except Brazil, where the 17DD vaccine substrain is used. The 17D-204 vaccine was developed from the original attenuated 17D by continued chick embryo passage (without neuronal tissue) from passage 176 to passage 204. Subsequently, the virus was passaged in embryonated chicken eggs, and most currently manufactured vaccines are manufactured at passage levels between 235 and 240. The 17DD vaccine was derived by passage in whole-chick embryonic tissue with the neuronal tissue removed from passage 176–195; however, subsequent passages were undertaken independently in Brazil, such that its development differed from 17D-204. This vaccine was passaged in embryonated chicken eggs and all currently manufactured vaccines are at passage level 287. During the 1970s and 1980s it became apparent that some vaccines had been prepared in eggs contaminated with ALV and therefore a number of manufacturers prepared ALV-free seeds of 17D virus, in order to remove the endogenous retrovirus. The Robert Koch Institute in Germany, on behalf of WHO, established a new seed lot from the 17D-204 substrate at passage 237, termed 213-77. This was certified free of ALV contamination and is used at passage 239–240 in embryonated chicken eggs (2, 3). 213-77 is considered by some (though not all) scientists as a substrain of 17D, owing to acquisition of an envelope protein glycosylation site compared to the 17D-204 substrate, and is sometimes referred to as 17D-213 (3).

Over the years, there have been many manufacturers of yellow fever vaccines (see Figure 1). The 17D-204 substrate vaccine has been manufactured
in France, Germany, India, the Netherlands, Senegal, South Africa, the United Kingdom and the USA, while the 17DD substrain vaccine has been manufactured in Brazil and Colombia. The 17D-213 substrain vaccine has been manufactured in Nigeria and the Russian Federation, and Berna Biotech (now Crucell) in Switzerland has developed a vaccine derived from seed virus 112/95, though it had not yet been marketed by Crucell at the time this report was prepared.

At present, there are only six producers: Sanofi Pasteur in France and the USA (17D-204); Institut Pasteur in Dakar, Senegal (17D-204); the Chumakov Institute of Poliomyelitis and Viral Encephalitides of the Russian Academy of Medical Sciences, Russian Federation (17D-213); Beijing Tiantan Biological Products Co. Ltd, China (17D-204); and Bio-Manguinhos/FioCruz of Rio de Janeiro, Brazil (17DD). Currently, four manufacturers are prequalified by WHO to provide yellow fever vaccine for use in developing countries. The other manufacturers produce yellow fever vaccine for domestic use.

The 17D-204 vaccine virus genome is 10,862 nucleotides in length and encodes a 3411 amino acid polyprotein, which is flanked by a 5’ non-coding region of 118 nucleotides and a 3’ non-coding region of 511 nucleotides (4,5). The 5’ terminus has a type 1 cap followed by two conserved nucleotides (AG) and the 3’ terminus lacks a poly A tract (4). The polyprotein encodes 10 proteins: the structural proteins capsid (C), membrane (M) and envelope (E) proteins are encoded by the N-terminal one third of the polyprotein; and the non-structural (NS) proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 are encoded by the C-terminal two thirds of the polyprotein. The major immunogen is the E protein, which encodes epitopes, inducing neutralizing antibodies that are primarily responsible for the protective immune response. Monoclonal antibodies have identified a number of overlapping epitopes on the E protein (6,7). Physically, these epitopes are either yellow fever strain-specific, yellow fever type-specific, complex-specific or flavivirus genus common, while biologically some of the epitopes are associated with haemagglutination inhibition (HI), which may or may not be associated with neutralization (6–17). Overall, few epitopes are involved in neutralization and very few elicit high-titre neutralization (6,12,16). Monoclonal antibodies have been generated against yellow fever wild-type and vaccine strains. Wild-type-specific (6,8,10,11,14,15,17) and vaccine-specific epitopes (i.e. recognizing 17D and FNV viruses only) (7,8,10,11,14,15,17), and 17D-204 and 17DD substrain-specific epitopes (8,9,13,14) have all been identified on the E protein. To date, few epitopes have been mapped to specific amino acids on the E protein: two yellow fever type-specific epitopes have been mapped to amino acids 71/72 and 153/155, a wild-type epitope to amino acid 173, and a 17D-204 substrain-specific epitope to amino acids 305 and 325 (18–20). Human cytotoxic T cell epitopes are found on the E structural protein and the NS1, NS2B and NS3 non-structural proteins (21–22).
Figure 1
History and genealogy of 17D vaccines and reference viruses: status as of October 2010*
The genomes of 17DD (23, 24), 17D-204 (4, 5) and 17D-213 (23, 24) vaccine viruses and parent wild-type Asibi virus have been sequenced (25). Unfortunately, the original 17D virus is not available. The three substrains differ slightly in sequence, thus justifying their classification as substrains (24), but they share 20 amino acid substitutions and four nucleotide changes in the 3’ non-coding region. The capsid gene and 5’ non-coding region of wild-type Asibi and 17D vaccine viruses were identical in sequence (see Table 1). At present, the molecular basis of attenuation of 17D vaccine is not known. Mouse models indicate that multiple mutations may be responsible for the attenuated phenotype.

Genomic sequences have been published for various 17D vaccines, some by manufacturers and some by academic laboratories. These include vaccines prepared in Brazil (23, 24), China (unpublished Genbank accession # FJ654700), France (5, 26), Senegal (27), South Africa (28), USA (29) and the American Type Culture Collection (ATCC) (4). The original published sequence of 17D-204 vaccine (4) is based on the virus obtained from ATCC.

### Table 1

Amino acid differences and nucleotide differences in the 3’ non-coding region between wild-type Asibi virus and attenuated 17D vaccines

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Gene</th>
<th>Amino acid&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Asibi</th>
<th>17D-204, 17D-213 and 17DD vaccine viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>854</td>
<td>M</td>
<td>36</td>
<td>Leu</td>
<td>Phe</td>
</tr>
<tr>
<td>1127</td>
<td>E</td>
<td>52</td>
<td>Gly</td>
<td>Arg</td>
</tr>
<tr>
<td>1482</td>
<td></td>
<td>170</td>
<td>Ala</td>
<td>Val</td>
</tr>
<tr>
<td>1491</td>
<td></td>
<td>173</td>
<td>Thr</td>
<td>Ile</td>
</tr>
<tr>
<td>1572</td>
<td></td>
<td>200</td>
<td>Lys</td>
<td>Thr</td>
</tr>
<tr>
<td>1870</td>
<td></td>
<td>299</td>
<td>Met</td>
<td>Ile</td>
</tr>
</tbody>
</table>

<sup>a</sup> This diagram provides information on a historical overview of the use of strains derived from the 17D yellow fever vaccine strain (as of October 2010). It does not indicate any WHO qualification or approval of the strains or vaccines in the context of this document.

The First International Standard for yellow fever vaccine (Code 99/616) was derived from a bulk vaccine derived from seed S2 YFS/10-11 (England).

The 17D-204 vaccines from Australia, Colombia, Germany, India, the Netherlands and South Africa, plus the 17DD vaccines from Colombia and Senegal, are not manufactured today.

Source: reproduced, with the permission of the publisher, from reference 2.
Table 1 continued

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Gene</th>
<th>Amino acid*</th>
<th>Asibi</th>
<th>17D-204, 17D-213 and 17DD vaccine viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1887</td>
<td></td>
<td>305</td>
<td>Ser</td>
<td>Phe</td>
</tr>
<tr>
<td>2112</td>
<td></td>
<td>380</td>
<td>Thr</td>
<td>Arg</td>
</tr>
<tr>
<td>2193</td>
<td></td>
<td>407</td>
<td>Ala</td>
<td>Val</td>
</tr>
<tr>
<td>3371</td>
<td>NS1</td>
<td>307</td>
<td>Ile</td>
<td>Val</td>
</tr>
<tr>
<td>3860</td>
<td>NS2A</td>
<td>118</td>
<td>Met</td>
<td>Val</td>
</tr>
<tr>
<td>4007</td>
<td></td>
<td>167</td>
<td>Thr</td>
<td>Ala</td>
</tr>
<tr>
<td>4022</td>
<td></td>
<td>172</td>
<td>Thr</td>
<td>Ala</td>
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<td>Phe</td>
</tr>
<tr>
<td>4505</td>
<td>NS2B</td>
<td>109</td>
<td>Ile</td>
<td>Leu</td>
</tr>
<tr>
<td>6023</td>
<td>NS3</td>
<td>485</td>
<td>Asp</td>
<td>Asn</td>
</tr>
<tr>
<td>6876</td>
<td>NS4A</td>
<td>146</td>
<td>Val</td>
<td>Ala</td>
</tr>
<tr>
<td>7171</td>
<td>NS4B</td>
<td>95</td>
<td>Ile</td>
<td>Met</td>
</tr>
<tr>
<td>10 142</td>
<td>NS5</td>
<td>836</td>
<td>Glu</td>
<td>Lys</td>
</tr>
<tr>
<td>10 338</td>
<td></td>
<td>900</td>
<td>Pro</td>
<td>Leu</td>
</tr>
<tr>
<td>10 367</td>
<td>(3’NCR)</td>
<td>—</td>
<td>U</td>
<td>C</td>
</tr>
<tr>
<td>10 418</td>
<td></td>
<td>—</td>
<td>U</td>
<td>C</td>
</tr>
<tr>
<td>10 800</td>
<td></td>
<td>—</td>
<td>G</td>
<td>A</td>
</tr>
<tr>
<td>10 847</td>
<td></td>
<td>—</td>
<td>A</td>
<td>C</td>
</tr>
</tbody>
</table>

* The 20 amino acids and 4 nucleotide changes in the 3’ non-coding region identified in this table are conserved in any vaccine virus derived from the 17D strain.

References


Appendix 2

Tests in nonhuman primates of new virus master and working seeds

Each virus master and working seed lot should be tested for viscerotropism, immunogenicity and neurotropism, in a group of 10 test monkeys. Animals that are in the test vaccine and reference groups should be blinded to the operators throughout the experiment. For the neurotropism test, the test monkeys inoculated with the virus seed lot should be compared with a similar group of 10 monkeys injected with a reference virus.

A WHO reference virus, 168-73, is available from the National Institute for Biological Standards and Control, Potters Bar, England. This virus is of the same lineage as the WHO primary seed 213-77 (see Appendix 1, Figure 1), and unpublished data indicate that it is less neurovirulent in monkeys than strains of at least one other lineage known to produce an acceptable vaccine. Existing manufacturers should use a homologous reference; for instance, where their existing working seed is to be replaced by another derived from the same master seed, the existing seed can be used as the reference material, provided it has been shown to produce a vaccine with satisfactory properties. It is recommended that sufficient stocks of such a reference are kept for all future anticipated replacements of the working seeds.

It is likely, though unproven, that 168-73 will be a satisfactory reference for seeds of the 213-77 lineage.

A new manufacturer with a new seed should use a homologous preparation known to produce a satisfactory product as reference. The inclusion of 168-73 as a common material would make it possible to compare different tests and one lineage with another for information. The reference virus should be approved by the NRA.

The monkeys should be Macaca mulatta (i.e. rhesus monkeys) or Macaca fascicularis (i.e. cynomolgus monkeys) and should have been demonstrated to be non-immune to yellow fever virus by the haemagglutination inhibition test immediately prior to injection of the seed virus. They should be healthy and should not have been previously subjected to any experimentation. The test dose should be injected into one frontal lobe of each monkey, under anaesthetic, and the monkeys should be observed for a minimum of 30 days.

The test dose should consist of 0.25 ml containing not less than 5000 (3.7 log10) IU and not more than 50 000 (4.7 log10) IU, as shown by titration in
cell culture. In addition, the virus titre of the test virus seed lot and the reference virus should be as close as possible.

Historically, the test dose should consist of 0.25 ml containing the equivalent of not less than 5000 and not more than 50 000 mouse LD$_{50}$, as shown by a titration in cell culture.

1. Viscerotropism test

The criterion of viscerotropism (indicated by the amount of circulating virus) should 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 should be inoculated at dilutions of 1:10, 1:100 and 1:1000 into at least four cell culture vessels per dilution. In no case should 0.03 ml of serum contain more than 500 (2.7 log$_{10}$) IU and in no more than one case should 0.03 ml of serum contain more than 100 (2.0 log$_{10}$) IU.

2. Immunogenicity test

The criterion of sufficient virus-neutralizing antibody in the sera (immunogenicity) should be fulfilled as follows: at least 90% of the test monkeys should be shown to have become immune within 30 days following injection of the test dose, as determined by examining their sera in the test for neutralization of yellow fever virus described below. In some countries it has been shown that, at low dilutions, some sera contain non-specific inhibitors that interfere with this test. The NRA may require sera to be treated to remove such substances.

Dilutions of 1:10, 1:40 and 1:160 of serum from each test monkey should be mixed with an equal volume of strain 17D vaccine virus at a dilution that has been shown to yield an optimum number of plaques when assayed according to one of the cell culture methods given in Appendix 4. These serum–virus mixtures should be incubated in a water bath at 37 °C for 1 h and then chilled in an ice-water bath before inoculation of 0.2 ml aliquots of each mixture into each of four separate cell culture vessels. The vessels should be handled according to one of the cell culture techniques described in Appendix 4. In addition, 10 vessels should be similarly inoculated with virus as above, and with an equal volume of a 1:10 dilution of monkey serum known to contain no neutralizing antibodies to yellow fever virus. At the end of the observation period, the mean number of plaques in the vessels receiving virus and non-immune serum should be compared with the mean number of plaques in the vessels receiving virus and serum from test monkeys. For the immunogenicity test to be satisfied, serum at the 1:10 dilution from no more than 10% of the test monkeys should fail to reduce the mean number of plaques by 50% as compared with the vessels containing non-immune serum.
3. Neurotropism test

Monkeys in the test group should be compared with 10 monkeys injected with the reference virus, with respect to both the clinical evidence of encephalitis and the severity of histological lesions of the nervous system (1, 2).

The onset and duration of the febrile reaction should not differ between monkeys injected with the test virus or with the reference virus.

3.1 Clinical evaluation

The monkeys should be examined daily for 30 days by personnel familiar with the clinical signs of encephalitis in primates.

If necessary, the monkeys may be removed from their cages and examined for signs of motor weakness or spasticity, as described elsewhere (2).

Signs of encephalitis – such as paresis, incoordination, lethargy, tremors or spasticity – should be assigned numerical values for severity by the following grading method. Each day each monkey should be given a numerical score based on the scale:

1: rough coat, not eating;
2: high-pitched voice, inactive, slow moving;
3: shaky movements, tremors, incoordination, limb weakness;
4: inability to stand, limb paralysis or death.

A monkey that dies receives the score “4” from the day of death until day 30.

The clinical score for a monkey is the average of its daily scores; the clinical score for a group is the arithmetic mean of the individual scores. For the clinical criterion of the neurotropism test to be satisfied, the clinical score of the monkeys injected with the virus being tested should not exceed the clinical score of the monkeys injected with the reference virus.

3.2 Histological evaluation

The cervical and lumbar enlargements of the spinal cord and specific structures at five levels of the brain should be examined (2) (see Appendix 3). The cervical and lumbar enlargements should each be divided equally into six blocks. The blocks should be dehydrated and embedded in paraffin wax; 15-µm sections should be cut and stained with gallocyanin. One section, consisting of two hemisections, should be cut from each block.

Tissue blocks 3–4 mm thick should be taken from the brain by making the following frontal cuts:
Block I: the corpus striatum at the level of the optic chiasma;
Block II: the thalamus at the level of the mamillary bodies;
Block III: the mesencephalon at the level of the superior colliculi;
Block IV: the pons and cerebellum at the level of the superior olives;
Block V: the medulla oblongata at the midlevel of the inferior olives.

These blocks should be dehydrated and embedded in paraffin wax, and 15 µm sections should be cut and stained with gallocyanin. A single section, consisting of two hemisections, should be cut from each block.

Sections should be examined microscopically and numerical scores should be given to each hemisection of the lumbar and cervical cord enlargements and to each anatomical structure (see Appendix 3) within each hemisection of the brain blocks, according to the following grading system:

1 (minimal): 1–3 small, focal inflammatory infiltrates. A few neurons may be changed or lost;
2 (moderate): more extensive focal inflammatory infiltrates (neuronal changes or loss affects no more than one third of neurons);
3 (severe): neuronal changes or loss of 33–90% of neurons, with moderate focal or diffuse inflammatory infiltration;
4 (overwhelming): more than 90% of neurons are changed or lost, with variable, but frequently severe, inflammatory infiltration.

Each brain block contains several anatomical structures, which contribute in different ways to the assessment of a test sample. For example, certain structures differentiate more reproducibly than others between acceptable and unacceptable yellow fever seed lots and vaccines (2). These are called discriminator areas, whereas structures that are more susceptible to yellow fever virus replication are called target areas. Though both rhesus and cynomolgus monkeys are acceptable, the discriminator and target areas are different for the two species. The major difference is that in cynomolgus monkeys the cervical and lumbar cord are target areas, whereas in rhesus monkeys they are discriminator areas. The footnotes to the worksheets (Appendix 3) indicate in more detail the discriminator and target areas for the two species. The worksheets also list other anatomical structures that will be present in the brain sections but that are not included in the evaluation of a test sample because they are rarely affected (spared areas).

Three separate scores should be calculated for each monkey: discriminator areas only, target areas only and discriminator plus target areas. These scores should be calculated as shown in the sample worksheets provided in Appendix 3.
Overall mean scores should also be calculated for each group of monkeys as the arithmetic mean of individual monkey scores for discriminator areas only and for discriminator plus target areas. Both overall mean scores should be considered when determining virus seed lot acceptability. For the histological criterion of the neurotropism test to be satisfied, both overall mean scores for the test monkeys should not be significantly greater (at the 5% significance level) than the overall mean scores for the monkeys injected with the reference virus.

Both the clinical and histological criteria of the neurotropism test should be satisfied, in order for the virus seed lot to meet the requirement for neurotropism.

References


Appendix 3

Example, for guidance, of a summary protocol for the testing of yellow fever vaccine in the monkey safety test as described in Appendix 2

Species: 
Number of monkeys inoculated: 
Master virus seed lot number: 
Reference virus lot number: 
Date of serology tests before inoculation: 
Dilution of yellow fever virus used for the inoculation: 
Volume and route of inoculation: 
Date of inoculation: 
Number of IU inoculated: 
Date of end of the test: 
Viscerotropism test (virus master seed lot)
Specify cell line used for virus titration.

<table>
<thead>
<tr>
<th>Monkey no.</th>
<th>Titre of circulating virus on:</th>
<th>Maximum titre of circulating virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 2</td>
<td>Day 4</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
<td></td>
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<tr>
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<tr>
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</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Result (pass or fail) __________________________________________
**Immunogenicity test (virus master seed lot)**

Specify cell line used for virus titration.

<table>
<thead>
<tr>
<th>Monkey no.</th>
<th>Seroneutralization titre</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
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<td></td>
</tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Result (pass or fail) ___________________________________________________________
Neurotropism test (virus master seed lot)

Summary clinical results

Date of inoculation: _________________

<table>
<thead>
<tr>
<th>Master virus seed lot no.</th>
<th>Monkey no.</th>
<th>Clinical score</th>
<th>Reference virus lot no.</th>
<th>Monkey no.</th>
<th>Clinical score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td>11</td>
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</tr>
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<td></td>
<td>Group mean</td>
<td></td>
<td></td>
<td>Group mean</td>
<td></td>
</tr>
</tbody>
</table>

Result (pass or fail) _________________________________
**Histological worksheet**

The worksheets below are provided as examples of how the histological score is calculated for a cynomolgus monkey with lesions graded as shown.

Species: cynomolgus
Pathology no: 
Monkey no: 

<table>
<thead>
<tr>
<th>Corpus striatum and thalamus</th>
<th>Block I</th>
<th>Block II</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>R</td>
<td>L</td>
</tr>
<tr>
<td>N. caudatus&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Globus pallidus&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Putamen&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>N. ant./med. thalami&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>N. lat. thalami&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Discriminator area for rhesus.  
<sup>b</sup> Discriminator area for cynomolgus.

<table>
<thead>
<tr>
<th>Mesencephalon (Block III)</th>
<th>L</th>
<th>R</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>Corpus geniculatum med.</td>
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</tr>
<tr>
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</tr>
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<td>N. ruber</td>
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<tr>
<td>Substantia nigra&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2</td>
<td>2</td>
<td>(4/2 =) 2.00</td>
</tr>
</tbody>
</table>

<sup>c</sup> Target area for rhesus and cynomolgus.

<table>
<thead>
<tr>
<th>Pons (Block IV)</th>
<th>L</th>
<th>R</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. abducens</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N. vestibularis</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N. trigeminus</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N. facialis</td>
<td>0</td>
<td>1</td>
<td>(1/2 =) 0.5</td>
</tr>
<tr>
<td>Table continued</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>-----------------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pons (Block IV)</strong></td>
<td>L</td>
<td>R</td>
<td>Total</td>
</tr>
<tr>
<td>Formatio reticularis</td>
<td>1</td>
<td>0</td>
<td>(1/2 =) 0.5</td>
</tr>
<tr>
<td>Oliva superior</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

| **Medulla oblongata (Block V)** | L | R | Total |
| N. hypoglossus | 0 | 0 | 0 |
| N. glossopharyngeus | 0 | 0 | 0 |
| N. vestibularis | 0 | 0 | 0 |
| N. trigeminus | 0 | 0 | 0 |
| N. ambiguus | 0 | 0 | 0 |
| Formatio reticularis | 0 | 0 | 0 |
| Oliva inferior | 0 | 0 | 0 |

| **Cerebellum (Blocks IV and V)** | L | R | Total |
| N. dentatus | 0 | 0 | 0 |
| Other nuclei | 0 | 0 | 0 |

<table>
<thead>
<tr>
<th><strong>Spinal cord</strong></th>
<th>I</th>
<th>II</th>
<th>II</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>R</td>
<td>L</td>
<td>R</td>
<td>L</td>
<td>R</td>
<td>L</td>
</tr>
<tr>
<td>Cervical enlargement&lt;sup&gt;a,d&lt;/sup&gt;</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Lumbar enlargement&lt;sup&gt;a,d&lt;/sup&gt;</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Discriminator area for rhesus.
<sup>d</sup> Target area for cynomolgus.
Calculations:

Discriminator areas (globus pallidus, putamen, n. ant./med. thalami, n. lat. thalami):

\[
\text{Lesion score} = \frac{0.75 + 1.00 + 0.75 + 1.25}{4} = 0.94
\]

Target areas (s. nigra, cervical enlargement, lumbar enlargement):

\[
\text{Lesion score} = \frac{2.00 + 2.08 + 1.33}{3} = 1.80
\]

Discriminator plus target areas:

\[
\text{Lesion score} = \frac{0.94 + 1.80}{2} = 1.37
\]
## Summary of histopathology results

Date of inoculation: ________________  Species: ________________

<table>
<thead>
<tr>
<th>Monkey no.</th>
<th>Discriminator area score</th>
<th>Discriminator plus target area score</th>
<th>Monkey no.</th>
<th>Discriminator area score</th>
<th>Discriminator plus target area score</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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<td>10</td>
<td></td>
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</tr>
<tr>
<td><strong>Group mean</strong></td>
<td></td>
<td></td>
<td><strong>Group mean</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Result (pass or fail) ____________________________________________
Appendix 4

Example, for guidance, of cell culture techniques for the potency evaluation of yellow fever vaccine

Vero cells or pig kidney epithelial (PS) cells (1) may be used. (Note that PS cells are latently infected with swine fever virus and their importation is prohibited in certain countries.) Vero cell seed and a description of a method for Vero cell cultivation may be obtained from WHO.

A reference vaccine calibrated in IU should be included in all assays, with potency expressed as IU/dose.

Monolayers of the cell substrate are prepared in six-well (35 mm) tissue culture plates. Serial fourfold dilutions of the reconstituted test and reference vaccine are prepared and inoculated in duplicate in the plate wells and incubated at 36 °C for 1 h. After this incubation period, the inoculum is replaced by 3 ml of agarose or 3.3% carboxymethyl cellulose (CMC) overlay, and the plates are further incubated at 36 °C for 7 days. The agarose or CMC overlay is removed and the cell cultures are stained with either naphthalene black or crystal violet, washed and air-dried. The virus plaques are counted. In calculating the titre, all dilutions should be considered in which the average number of plaques per well is between 1 and 30. The potency in IU/dose is calculated relative to the standard vaccine.

For the test to be considered valid:

- the control cells should not show any plaque-forming or other cytopathic effect;
- the reference vaccine should be within 100.5 (0.5 log10) IU of its established mean titre.

Since yellow fever virus is light sensitive, the vaccine should be protected from direct light during storage and testing.

Further detailed guidance is available in the *Manual of laboratory methods for testing vaccines used in the WHO Expanded Programme on Immunization* (2).

References

Appendix 5

Model summary protocol for manufacturing and control of live attenuated yellow fever vaccines

The following protocol is intended for guidance. It indicates the minimum information that should be provided by the manufacturer to the NRA. Information and tests may be added or deleted as required by the NRA, if applicable.

It is possible that a protocol for a specific product may differ in detail from the model provided. The essential point is that all relevant details demonstrating compliance with the licence and with the relevant WHO recommendations on a particular product should be given in the protocol submitted.

The section concerning the final product must be accompanied by a sample of the label from the vaccine container and a copy of the leaflet that accompanies it. If the protocol is submitted in support of a request to permit importation, it should also be accompanied by a lot release certificate from the NRA of the country in which the vaccine was produced/released, stating that the product meets national requirements as well as Part A of the WHO Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines.

Summary information on the finished product (final lot)

International name: ________________________________
Trade name: ________________________________
Product licence (marketing authorization) number: __________________
Country: __________________
Name and address of manufacturer: ________________________________
Site of manufacture of final lot: __________________
Name and address of licence-holder
   (if different from manufacturer): ________________________________
Virus strain: ________________________________
Origin and short history: ________________________________
Authority that approved virus strain: ________________________________
Date approved: ________________________________
Final lot number: ________________________________
Final bulk number: ________________________________
Volume of final bulk: ________________________________
Final product: ________________________________
Type of container: ________________________________
Number of doses per container: ________________________________
Number of filled containers in this final lot: ________________________________
Date of manufacture of final lot (filling or lyophilizing, if applicable): ________________________________
Date on which last determination of virus concentration was started, or date of start of period of validity: ________________________________
Shelf-life approved (months): ________________________________
Expiry date: ________________________________
Diluent: ________________________________
Storage conditions: ________________________________
Volume of single human dose: ________________________________
Volume of vaccine per container: ________________________________
Number of doses per container: ________________________________
Prescribed virus concentration per single human dose: ________________________________
Antibiotics added: ________________________________
Release date: ________________________________

Production information
A genealogy of the lot numbers of all vaccine components used in the formulation of the final product will be informative.

The following sections are intended for reporting the results of the tests performed during the production of the vaccine.

Starting materials
The information requested below is to be presented on each submission. Full details on master and working seed lots should be provided upon first submission only and whenever a change has been introduced.

Virus master seed lot
Source of 17D substrain: ________________________________
Master virus seed lot number: ________________________________
Name and address of manufacturer: ________________________________
Passage level: ________________________________
Date of inoculation of embryos: ________________________________
Date of harvest: ________________________________
Age of embryos (at harvest): ________________________________
Number of containers: ________________________________
Conditions of storage: ________________________________
Date virus master seed lot was established: ________________________________
Date approved by the NRA: ________________________________
Information on source materials

Source of eggs

Is the flock under direct control of the manufacturer?  
Is the flock monitored for compliance with these recommendations?  

Tests on virus master seed lot production (A.4.2.2)

Identity test (A.4.2.2.1)
Method used:  
Date test started:  
Date test ended:  
Results:  
Lot number of Reference Reagents:  

Genotype characterization (A.4.2.2.2)
Method used:  
Dates test started and ended:  
Results:  
Lot number of Reference Reagents:  

Freedom from bacteria, fungi and mycoplasmas (A.4.2.2.3)

Tests for bacteria and fungi
Method used: 
Number of vials tested:  
Volume of inoculum per vial:  
Volume of medium per vial:  
Observation period (specification):  

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Media used</th>
<th>Inoculum</th>
<th>Date test began</th>
<th>Date test ended</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–25 °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30–36 °C</td>
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<td></td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Tests for mycoplasmas
Method used:  
Volume tested:  
Media used:  


Temperature of incubation: 
Observation period (specification): 
Positive controls (list of species used and results): 

<table>
<thead>
<tr>
<th>Subcultures at day 3</th>
<th>Date test began</th>
<th>Date test ended</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Subcultures at day 7</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Subcultures at day 17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcultures at day 21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Indicator cell culture method (if applicable)**

Cell substrate used: 
Inoculum: 
Date of test: 
Passage number: 
Negative control: 
Positive controls: 
Date of staining: 
Results: 

Tests for ALVs and other adventitious agents (A.4.2.2.4)

Method: 
Volume tested: 
Date test started: 
Date test ended: 
Result: 

Tests for avian mycobacteria

Method: 
Media used: 
Temperature of incubation: 
Volume tested: 
Date test started: 
Date test ended: 
Result: 

Safety test on animals (guinea-pigs, mice and embryonated chicken eggs)

Species used: 
Number of animals inoculated: 
Volume injected per animal: 
Inoculation route: ________________________________
Date test started: ________________________________
Date test ended: _________________________________
Result: _________________________________________________________________________________

Testing in nonhuman primates (A.4.2.2.5)
See Appendix 2

Virus titration for infectivity (A.4.2.2.6)
Method: _______________________________________________________________________________
Date: _________________________________________________________________________________
Result: _______________________________________________________________________________

Virus working seed lot
Working virus seed lot number: ________________________________
Name and address of manufacturer: ________________________________
Passage level: ________________________________
Date of inoculation of embryos: ________________________________
Temperature of incubation: ________________________________
Date of harvest: ________________________________
Age of embryos (at harvest): ________________________________
Date of filling: ________________________________
Date of lyophilization (if appropriate): ________________________________
Number of containers: ________________________________
Conditions of storage: ________________________________
Date virus working seed lot was established: ________________________________
Date approved by the NRA: ________________________________

Information on source materials
Source of eggs ________________________________
Is the flock under direct control of manufacturer? ________________________________
Is the flock monitored for compliance with these recommendations? ________________________________

Tests on virus working seed lot production (A.4.2.2)
Identity test (A.4.2.2.1)
Method used: ________________________________
Date test started: ________________________________
Date test ended: ________________________________
Results: ____________________________
Lot number of Reference Reagents: ____________________________

Genotype characterization (A.4.2.2.2)
Method used: ____________________________
Date test began and ended: ____________________________
Results: ____________________________
Lot number of Reference Reagents: ____________________________

Freedom from bacteria, fungi and mycoplasmas (A.4.2.2.3)
Tests for bacteria and fungi
Method used: ____________________________
Number of vials tested: ____________________________
Volume of inoculum per vial: ____________________________
Volume of medium per vial: ____________________________
Observation period (specification): ____________________________

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Media used</th>
<th>Inoculum</th>
<th>Date test began</th>
<th>Date test ended</th>
<th>Results</th>
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<tbody>
<tr>
<td>20–25 °C</td>
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<tr>
<td>30–36 °C</td>
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</tr>
<tr>
<td>Negative control</td>
<td></td>
<td></td>
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</tbody>
</table>

Tests for mycoplasmas
Method used: ____________________________
Volume tested: ____________________________
Media used: ____________________________
Temperature of incubation: ____________________________
Observation period (specification): ____________________________
Positive controls (list of species used and results): ____________________________

<table>
<thead>
<tr>
<th></th>
<th>Date test began</th>
<th>Date test ended</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcultures at day 3</td>
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<td></td>
</tr>
<tr>
<td>Subcultures at day 7</td>
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</tr>
<tr>
<td>Subcultures at day 17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcultures at day 21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Indicator cell culture method (if applicable)

Cell substrate used: ________________________________
Inoculum: ________________________________
Date of test: ________________________________
Passage number: ________________________________
Negative control: ________________________________
Positive controls: ________________________________
Date of staining: ________________________________
Results: ______________________________________

Tests for ALVs and other adventitious agents (A.4.2.2.4)

Method: ________________________________
Volume tested: ________________________________
Date test started: ________________________________
Date test ended: ________________________________
Result: ______________________________________

Tests for avian mycobacteria

Method: ________________________________
Media used: ________________________________
Temperature of incubation: ________________________________
Volume tested: ________________________________
Date test started: ________________________________
Date test ended: ________________________________
Result: ______________________________________

Tests for other adventitious agents on cell culture

Human diploid cells/monkey kidney cells/primary chick embryo fibroblast cells

Method used: ________________________________
Test on cell culture: ________________________________
Type of cells: ________________________________
Cell strain: ________________________________
Lot number of antiserum: ________________________________
Volume tested: ________________________________
Temperature of incubation: ________________________________
Date test started: ________________________________
Date test ended: ________________________________
Date of haemadsorption (if applicable): ________________________________
Result: ______________________________________
Test for adventitious agents on eggs (avian viruses)

Allantoic cavity

- Lot number of antiserum: ________________________________
- Number of eggs inoculated: _______________________________
- Volume inoculated per egg: ______________________________
- Temperature of incubation: ______________________________
- Inoculation date: ______________________________
- Date of harvest: ______________________________
- Date of haemagglutination test: ______________________________
- Result: ______________________________

Yolk sac

- Number of eggs inoculated: ______________________________
- Volume inoculated per egg: ______________________________
- Temperature of incubation: ______________________________
- Inoculation date: ______________________________
- Date of collection of embryo for observation: ______________________________
- Result: ______________________________

Safety test on animals (guinea-pigs, mice and embryonated chicken eggs)

- Species used: ______________________________
- Number of animals inoculated: ______________________________
- Volume injected per animal: ______________________________
- Inoculation route: ______________________________
- Date test started: ______________________________
- Date test ended: ______________________________
- Result: ______________________________

Testing in nonhuman primates (A.4.2.2.5)

See Appendix 2

Virus titration for infectivity (A.4.2.2.6)

- Method: ______________________________
- Date: ______________________________
- Result: ______________________________

Control of vaccine production (A.5)

Information on source materials ______________________________

- Source of eggs: ______________________________
- Is the flock under direct control of manufacturer? ______________________________
Is the flock monitored for compliance with these recommendations? 

**Virus used to inoculate embryos**
- Derived from master seed virus lot number: 
- Working virus seed lot, reference number and source: 
- Passage level of working virus seed lot: 

**Information on manufacture**
- Date of inoculation of embryos: 
- Quantity of inoculated embryos: 
- Temperature of incubation: 
- Date of harvest: 
- Age of embryos (at time of harvest): 
- Quantity of harvested embryos: 
- Number of rejected eggs (ratio): 
- Number of containers: 
- Conditions of storage: 
- Expiry date: 

**Tests on uninoculated control eggs (A.5.1)**
- Number of eggs used: 

**Test for haemagglutinating agents**

**Directly on allantoic fluid**
- Method: 
- Volume tested: 
- Date of test: 
- Result: 

**After a passage in SPF eggs**
- Method: 
- Volume tested: 
- Route of inoculation: 
- Date test started: 
- Date test ended: 
- Result: 

**Test for other adventitious agents on cell culture**
- Human diploid cells/monkey kidney cells/primary chick embryo fibroblast cells
Cell type: ________________________________
Volume tested: ________________________________
Temperature of incubation: ________________________________
Date test started: ________________________________
Date test ended: ________________________________
Result: ________________________________

**Test for ALVs**

Method: ________________________________
Volume tested: ________________________________
Date test started: ________________________________
Date test ended: ________________________________
Result: ________________________________

**Tests on control tissues**

**Test for Salmonella**

Method: ________________________________
Volume tested: ________________________________
Date test started: ________________________________
Date test ended: ________________________________

**Tests for avian mycobacteria**

Method: ________________________________
Media used: ________________________________
Temperature of incubation: ________________________________
Volume tested: ________________________________
Date test started: ________________________________
Date test ended: ________________________________
Result: ________________________________

**Test for fowl pox virus**

Method: ________________________________
Volume tested: ________________________________
Volume of negative controls: ________________________________
Date test started: ________________________________
Date test ended: ________________________________
Result: ________________________________

**Tests for ALVs (if applicable)**

Method used: ________________________________
Volume tested: ____________________________________________
Temperature: ____________________________________________
Date test started: _________________________________________
Date test ended: __________________________________________
Result: __________________________________________________

Test for haemagglutinating agents on embryonated hen eggs (avian viruses)

**Allantoic cavity**
- Number of eggs inoculated: ________________________________
- Volume inoculated per egg: ________________________________
- Temperature of incubation: ________________________________
- Inoculation date: _________________________________________
- Date of harvest: _________________________________________
- Date of haemagglutination test: _____________________________
- Result: _________________________________________________

**Yolk sac**
- Number of eggs inoculated: ________________________________
- Volume inoculated per egg: ________________________________
- Temperature of incubation: ________________________________
- Inoculation date: _________________________________________
- Date of collection of embryo for observation: ________________
- Result: _________________________________________________

Test for other extraneous agents on cell culture

Human diploid cells/monkey kidney cells/primary chick embryo fibroblast cells

- Cell type: ______________________________________________
- Volume tested: __________________________________________
- Temperature of incubation: ________________________________
- Date test started: _________________________________________
- Date test ended: _________________________________________
- Result: _________________________________________________

Tests on single harvests (A.5.3)

**Identity test (A.5.3.2)**
- Date test started: _________________________________________
- Date test ended: _________________________________________
- Result: _________________________________________________
Freedom from bacteria, fungi and mycoplasmas (A.5.3.3)

Tests for bacteria and fungi

Method used: ________________________________
Number of vials tested: ________________________________
Volume of inoculum per vial: ________________________________
Volume of medium per vial: ________________________________
Observation period (specification): ________________________________

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Media used</th>
<th>Inoculum</th>
<th>Date test began</th>
<th>Date test ended</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–25 °C</td>
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<td>30–36 °C</td>
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<tr>
<td>Negative control</td>
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</table>

Tests for mycoplasmas

Method used: ________________________________
Volume tested: ________________________________
Media used: ________________________________
Temperature of incubation: ________________________________
Observation period (specification): ________________________________
Positive controls (list of species used and results): ________________________________

<table>
<thead>
<tr>
<th>Subcultures at day 3</th>
<th>Date test began</th>
<th>Date test ended</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>Subcultures at day 7</td>
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<tr>
<td>Subcultures at day 17</td>
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<tr>
<td>Subcultures at day 21</td>
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<td></td>
</tr>
</tbody>
</table>

Indicator cell culture method (if applicable)

Cell substrate used: ________________________________
Inoculum: ________________________________
Date of test: ________________________________
Passage number: ________________________________
Negative control: ________________________________
Positive controls: ________________________________
Date of staining: ________________________________
Results: ________________________________
Tests for adventitious agents (A5.3.4)

Tests for Mycobacterium avium

Method: 
Media used: 
Temperature of incubation: 
Volume tested: 
Date test started: 
Date test ended: 
Result: 

Virus titration (A.5.3.5)

Method: 
Date: 
Result: 

Control of final bulk (A.5.4)

Sterility tests (A.5.4.1)

Tests for bacteria and fungi

Method used: 
Number of vials tested: 
Volume of inoculum per vial: 
Volume of medium per vial: 
Observation period (specification): 

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Media used</th>
<th>Inoculum</th>
<th>Date test began</th>
<th>Date test ended</th>
<th>Results</th>
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<td>20–25 °C</td>
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<tr>
<td>30–36 °C</td>
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<tr>
<td>Negative control</td>
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</tbody>
</table>

Stabilizers (if added) (A.5.4.2)

Name of stabilizer: 
Quantity or percentage: 
Date: 

Virus titration (if performed) (A.5.4.3)

Method: 
Date: 
Result: 
Filling and containers (A.6)
Lot number: ________________________________
Date of filling: ______________________________
Volume of final bulk filled: ______________________________
Filling volume per container: ______________________________
Number of containers filled (gross): ______________________________
Date of lyophilization: ______________________________
Number of containers rejected during inspection: ______________________________
Number of containers sampled: ______________________________
Total number of containers (net): ______________________________
Maximum period of storage approved: ______________________________
Storage temperature and period: ______________________________

Control tests on final lot (A.7)

Inspection of final containers (A.7.1)
Appearance: ______________________________
Date of test: ______________________________
Results: ______________________________
Before reconstitution: ______________________________
After reconstitution: ______________________________
Diluent used: ______________________________
Lot number of diluent used: ______________________________

Identity test (A.7.2)
Method used: ______________________________
Date test started: ______________________________
Date test ended: ______________________________
Results: ______________________________
Lot number of Reference Reagents: ______________________________

Potency test (A.7.3)
Date of test: ______________________________
Reference batch number: ______________________________
Specification: ______________________________
Titre of reference batch (IU/0.5 ml): ______________________________
Vaccine: Virus concentration (IU/human dose)
Vial 1: ______________________________
Vial 2: ______________________________
Vial 3: ______________________________
Mean virus titre per human dose, with 95% fiducial limits: ______________________________
Thermal stability test (A.7.4)

Date of test: ________________________________

Reference batch number: ________________________________

Titre of reference batch (IU/0.5 ml): ________________________________

Vaccine held at 37 °C for 14 days: ________________________________

Vaccine: Virus concentration (IU/human dose)

Vial 1: ________________________________

Vial 2: ________________________________

Vial 3: ________________________________

Mean virus titre per human dose, with 95% fiducial limits: ________________________________

Loss in titre (in \log_{10} IU): ________________________________

Sterility tests (A.7.5)

Tests for bacteria and fungi

Method used: ________________________________

Number of vials tested: ________________________________

Volume of inoculum per vial: ________________________________

Volume of medium per vial: ________________________________

Observation period (specification): ________________________________

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Media used</th>
<th>Inoculum</th>
<th>Date test began</th>
<th>Date test ended</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–25 °C</td>
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<tr>
<td>Negative control</td>
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</tbody>
</table>

General safety test (if performed) (A.7.6)

Tests in mice

Date of inoculation: ________________________________

Number of mice tested: ________________________________

Volume and route of injection: ________________________________

Observation period: ________________________________

Results (give details of deaths): ________________________________

Tests in guinea-pigs

Date of inoculation: ________________________________

Number of guinea-pigs tested: ________________________________

Volume and route of injection: ________________________________

Observation period: ________________________________

Results (give details of deaths): ________________________________
Residual moisture (A.7.7)
Method: ____________________________
Specification: ____________________________
Date: ____________________________
Result: ____________________________

Residual ovalbumin (A.7.8)
Method: ____________________________
Specification: ____________________________
Date: ____________________________
Result: ____________________________

Endotoxin (A.7.9)
Method: ____________________________
Specification: ____________________________
Date: ____________________________
Result: ____________________________

Residual antibiotics (if applicable) (A.7.10)
Method: ____________________________
Specification: ____________________________
Date: ____________________________
Result: ____________________________

Submission addressed to national regulatory authority

Name of Head of Production (typed) ____________________________

Certification by the person from the control laboratory of the manufacturing company taking over responsibility for the production and control of the vaccine:

I certify that lot no. ______________ of yellow fever vaccine, whose number appears on the label of the final container, meets all national requirements and/or satisfies Part A of the WHO Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines.

Signature: ____________________________
Name (typed): ____________________________
Date: ____________________________
Appendix 6

Model certificate for the release of live attenuated yellow fever vaccine by a national regulatory authority

LOT-RELEASE CERTIFICATE

The following lot(s) of yellow fever vaccine produced by ___________________\(^1\) in ___________________\(^2\), whose numbers appear on the labels of the final containers, meet all national requirements\(^3\) and Part A\(^4\) of the WHO Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines (___________________),\(^5\) and comply with WHO Good manufacturing practices: main principles for pharmaceutical products\(^6\) and Good manufacturing practices for biological products.\(^7\)

As a minimum, this certificate is based on examination of the summary protocol of manufacturing and control.

The certificate may include the following information:

- name and address of manufacturer;
- site(s) of manufacturing;
- trade name and/or common name of product;
- marketing authorization number;
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary);
- type of container;
- number of doses per container;
- number of containers/lot size;
- date of start of period of validity (e.g. manufacturing date) and/or expiry date;

\(^1\) Name of manufacturer.

\(^2\) Country of origin.

\(^3\) If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the national regulatory authority.

\(^4\) With the exception of provisions on distribution and shipping, which the national regulatory authority may not be in a position to assess.


- storage condition;
- signature and function of the authorized person and authorized agent to issue the certificate;
- date of issue of certificate;
- certificate number.

The Director of the national regulatory authority (or authority as appropriate):

Name (typed) ________________________________
Signature  ________________________________
Date  ________________________________