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Meeting Report

WHO Working Group on Technical Specifications for Manufacture and Evaluation of Yellow Fever Vaccines

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Summary

In May 2009, WHO convened a meeting of Working Group on Technical Specifications for Manufacturing and Evaluating Yellow Fever (YF) Vaccines, Geneva, Switzerland to initiate revision of the WHO Recommendations (formerly, Requirements) for YF vaccine published in WHO Technical Report Series number 872 (1998). The Working Group, consisting of experts from academia, industry, national regulatory authorities and national control laboratories, reviewed the latest issues of safety, efficacy and quality of YF vaccines and agreed that i) the revision should focus on live attenuated YF vaccine virus 17D lineage; and that ii) nonclinical and clinical guidelines for new vaccines prepared from 17D lineage be developed.

Suggested Keywords: Yellow fever vaccine; World Health Organization; Recommendations

1. Introduction

Dr David Wood, Coordinator of the Quality, Safety and Standards (QSS) team, World Health Organization (WHO), welcomed participants to the meeting. He reminded them that yellow fever (YF) is a disease of major public health importance in Africa and South America and that there are still outbreaks, such as those recently in Paraguay. In countries at risk, vaccines of assured quality are essential for routine immunization and mass campaigns. He emphasized the importance of this consultation in view of the adverse events including yellow fever vaccine-associated neurologic disease (YEL-AND and yellow fever vaccine-associated viscerotropic disease (YEL-AVD) that had occurred in the past few years. A cluster of adverse events had occurred after a mass vaccination campaign in Peru but an expert panel convened to investigate the reports has found no link to the vaccine production lot. However, they did recommend that the guidelines for YF vaccines be reviewed as it is over 10 years since they were published. Dr Wood also informed the group that YF vaccines are important in regulating international travel and that it is the only disease for which a certificate of vaccination is required for entry into some countries. The updates of the International Health Regulations (IHR) [1] increase attention to the need for such certificates. YF vaccines that have gone through the approval process established by WHO are on a published list but some vaccines are not listed and they therefore now need to

go through the approval process before they can be used in the context of the IHR. The current Recommendations (formerly, Requirements) for YF vaccine were adopted in 1995 and published in WHO Technical Report Series (TRS) No. 872 in 1998 [2] and this working group should look at current specifications to see what needs to be changed.

Dr Ivana Knezevic (QSS/WHO) presented an update on biological standardization and positioned the discussion in terms of the work of QSS and the issues of importance in the area of YF vaccine. The activities of the QSS group within WHO include ensuring that all vaccines are of assured quality through regulatory guidance and the implementation of new WHO standards. Goals of immunization programmes include increasing and sustaining vaccine coverage, reducing morbidity and mortality through the use of vaccines of assured quality, and introducing new vaccines and vaccines from new manufacturers or with modified vaccine production. The strengthening of National Regulatory Authorities (NRA) is also important as NRA functionality forms an essential part of quality assurance of vaccines. The Expert Committee on Biological Standards (ECBS) has played essential roles in WHO's normative function, e.g. establishment of standards, both written and physical. Written standards are produced as a form of guidelines or recommendations and used for the regulation of vaccines worldwide and also for the prequalification of vaccines. The established written standards must be evidence-based and only experts in the field can provide the evidence. The written standards now include nonclinical and clinical issues. The work of ECBS is supported by an Expert Advisory Panel and Collaborating Centres including the WHO International Laboratory for Biological Standards at the National Institute for Biological Standards and Control (NIBSC), the Paul-Ehrlich-Institut (PEI) and the Center for Biologics Evaluation and Research (CBER) of the US Food and Drug Administration (FDA).

Key issues that have stimulated revising the recommendations for YF vaccines are: i) the implementation of a new potency specification in International Units (IU), ii) the availability of molecular methods applicable to detecting microbial agents and testing for identity, and iii) the need to review and update as necessary the established safety tests, guiding principles on clinical and nonclinical evaluation, such as efficacy endpoint, dose-ranging studies, clinical minimum potency in IU and safety considerations. Once such standards are fully adopted by the NRAs and implemented into national regulation, they will be implemented into manufacturer's procedures and considered by National Pharmacopoeias who will, where appropriate, incorporate them into

general or specific monographs. Dr Knezevic informed that WHO TRS 878 Annex 1 guidelines on cell substrates is under revision [3]. If new cell-based YF vaccines are under development, the revision of YF recommendations should be read in conjunction with them.

In the discussion, Dr Minor asked whether any manufacturer or research group is planning to develop a YF vaccine in Vero cells as historically cell culture methods have not been found suitable for use in routine production in terms of the quality of the product. As there was no indication that this was the case, the group agreed that the scope of discussions should be limited to embryonated chicken egg-based production.

Dr Jinho Shin (QSS/WHO) presented the objectives and expected outcomes of this meeting. ECBS endorsed a proposal to update the entire document of Recommendations for YF vaccines upon noting key issues as described above by Dr Knezevic. He acknowledged that the expression of YF vaccine potency in IU was a great achievement over two decades of international collaborative efforts and that it was the first case for live vaccines. The first collaborative study on YF vaccine potency was undertaken in 1983 [4] but at that time, which was shortly after cell culture assays were implemented, the availability of a common standard did not improve reproducibility between laboratories. This issue was addressed twenty years later and an International Standard for YF vaccine for use in potency tests established [5]. The objectives of this May 2009 consultation were to: i) initiate revision of the WHO Recommendations for YF vaccine published in WHO TRS No. 872 in 1998 [2]; ii) discuss scientific basis of setting manufacture & quality control specifications for YF vaccines; and iii) develop regulatory expectations for evaluating nonclinical and clinical studies of YF vaccines.

In the discussion, Dr Barrett highlighted a controversial issue which had been proposed by scientists not involved in production and regulation of vaccines, namely, sequencing of every batch. Dr Minor agreed that this would be addressed and that non-clinical tests were crucial even though YF vaccines are the oldest live viral vaccine on the market and have been very successful.

2. International effort to control YF

Dr Gilles Pomeroy (IHR/WHO) updated the group on IHR and YF. The IHR was first issued in 1969. An update was approved by all member states in 2005 and implemented in June 2007. The severe acute respiratory syndrome (SARS) outbreak had stimulated this revision to ensure that health security should minimize interference with health and trade. There are 1 billion air travellers each year and an issue in one area of the world can affect entire world. These regulations are comprehensive and useful. These regulations are considerably changed from the previous version and include: i) change from control of borders to containment at source; ii) change from disease list to all public health threats; and iii) change from preset measures to adaptive responses.

There is now a national IHR focal point in each state and WHO IHR contact points from which information may be obtained or exchanged. The rapid exchange of information on the current outbreak of influenza A/H1N1 would be a good example of how this system is working.

YF is the only disease specifically cited in the IHR as requiring a certificate of vaccination for access to some countries. The vaccines used must be of suitable quality and approved by WHO presumably through the prequalification process because this is the only existing mechanism at WHO for evaluating the quality of vaccines that are supplied to countries for mainly mass vaccination purposes. Vaccine shall be administered by designated vaccination centres and specific vaccination certificates issued. IHR require sharing information on global serious public health events and risk mapping for YF in Article 36 annexes 6 and 7. Vaccination centres are subject to national regulation. The international certificate of vaccination or of contraindication should be signed by a physician.

Dissemination of key public health information is crucial and WHO must send out relevant information as soon as possible to enable member states to respond to public health risks. YF risk mapping and vaccination recommendations are detailed and updated in the publication for international travel and health [6]. A map of 'areas at risk' indicates low, medium and high risk. In the new revision WHO does not recommend YF vaccination in low risk areas and countries that fall into this group will be under constant review. Encephalitis has been reported following vaccination of infants under 7 months of age, albeit as a rare event; as a result, the vaccine is contraindicated in children aged under 6 months and is not recommended for those aged 6–8 months.

A WHO website on international travel and health has proved very successful with 25,000 hits per week. It includes an interactive map to provide information on recommendations for YF vaccination and malaria prophylaxis. A YF expert group advises WHO on practical criteria for defining areas for vaccine recommendation for visitors and the areas/countries at risk of YF transmission which triggers potential certificate requirements.

Dr Rosamund Lewis from the Epidemic Readiness and Intervention team of WHO reminded the group of the history of YF when it had been endemic in the US and Europe and that now the zones of transmission are South America and Africa. In the mid-20th century, YF had declined dramatically in Africa following a number of mass vaccination campaigns and the decreasing number of cases of YF and success of the vaccine resulted in lack of interest in YF surveillance, and, as a consequence, immunization was progressively neglected. YF reappeared in the late 1980s in many African countries. Outbreaks occur with recurring periodicity depending on geographic location with frequent outbreaks in West Africa, outbreaks often separated by 4 to 7 years in South America, and rare outbreaks in East Africa. The Yellow Fever Initiative is a partnership working to prevent yellow fever epidemics across Africa and Latin America through preventive vaccination and emergency response. The objective of the Yellow Fever Initiative is to secure vaccine production capacity and to vaccinate people at high risk to boost population immunity. A stockpile of 6 million doses has been established as an emergency response capability, although routine vaccine supply may be diverted to emergency response if required. Vaccines supplied through the United Nations Children's Fund (UNICEF) are WHO pre-qualified but not all producer's vaccines are prequalified. Procured vaccine is used and replaced annually. In addition to emergency response, routine infant immunization and preventive vaccination campaigns are being implemented for populations at highest risk. For routine immunization, children are immunized at 9 months of age in Africa and at 9 months (Brazil) and 12 months in the Americas. In Africa, no boosters are offered but preventive campaigns serve as catch-up to vaccinate those unreached by routine programmes. In Brazil, boosters are mandatory each 10 years in endemic areas. Country level risk assessments are performed, which consider the history of cases, the presence of the vector, and population immunity (recent vaccination) to select districts at highest risk. Funding support has included 103 million dollars from the Global Alliance for Vaccines and Immunisation (GAVI), funds from the European Commission Humanitarian Aid (ECHO) for emergency response, and funds from the EU for surveillance.

Vaccination strategies are adapted to country needs and vaccine supply. Vaccine demand has risen from 34 million doses in 2001 to 105 million doses in 2009. In the same period, theoretical vaccine supply tripled from 40 million to 115 million doses but production in 2009 was only 75 million doses due to presentations selected (fewer 50-dose vials). Surveillance of adverse events following immunization (AEFI) is also undertaken during preventive mass campaigns, but some countries have also established a regular system for continuous surveillance of AEFIs. Suspected serious AEFI cases reported per 100,000 persons vaccinated were 0.67 in Togo, 0.06 in Senegal, and 0.02 Mali. However, no case of serious AEFI has yet been classified as confirmed and there were no deaths or virological studies undertaken in these countries. Dr Lewis indicated that obtaining samples from potential AEFIs was complex but improving with experience. Dr Reinaldo Martins (Bio-Manguinhos) added that sample collection had been problematic in Brazil, but this was improved using collection kits for blood samples.

3. New insights on YF vaccine and safety

Dr Alan Barrett (UTMB - University of Texas Medical Branch) summarized the outcome of a workshop of the World Congress on Medicine and Health in the Tropics held in Marseilles France in 2005. During the workshop, YF epidemiology, vaccines and vaccination were discussed [7].

At the time of the meeting there was an outbreak of YF in Trinidad. An outbreak in Paraguay in early 2008 provided evidence of urban YF for the first time since 1942. There are no antiviral drugs for any flavivirus infection including YF so the availability of vaccine is important for resident populations and travellers. Risks for vaccine recipients are low, but currently available data suggest that the rare cases of YF vaccine-associated viscerotropic disease (YEL-AVD) are related to individual, genetically-determined, and currently unknown host factors that regulate cellular susceptibility to the attenuated YF virus rather than reversion of the vaccine virus to a virulent phenotype. Molecular and animal studies performed to date provide no evidence that mutations of 17D vaccine virus have contributed to YEL-AVD [7].

A cluster of cases of viscerotropic disease occurred in 2007 in Ica, Peru, when four out of 42,000 recipients of the same lot of vaccine died. However, the geographical dispersion of subjects

makes it unlikely that this was related to their genetic background. The virus isolated from these individuals was sequenced and found to be vaccine virus with no evidence that it had mutated. The conclusions of the extensive investigations were that the adverse events observed were not vaccine batch-related or production-related even though it was vaccine virus that caused disease. There was no difference between this and other lots of vaccine suggesting there must be cofactors that lead to disease. The immune responses induced by 17D virus have been investigated in a large number of studies. Neutralization antibody is a correlate of protection. Although the actual mechanism of protection is not known there have been recent studies investigating the immune response induced by the vaccine [8-10], including studies on T and B cells, but assays on neutralizing antibodies are still the gold standard; there is no internationally-recognized protective neutralizing antibody titre. Querec et al. (2009) identified C1qB and ETIF2ak4 as a 90% predictor of CD8+ T-cell responses and TNFRSF17 (which encodes a receptor for growth factor BLyS-BAFF) as a 100% predictor of the neutralizing antibody response induced by the vaccine [10]. Although such studies on cytokines will help understand adverse events, they will not contribute to the quality control of vaccine.

Serious AEFI following YF immunization are poorly understood although advances in understanding the immune response induced by the vaccine may help elucidate their mechanism. While many papers have been published on adverse events the mechanism(s) of death by viscerotropic disease is unknown [11-14]. A comprehensive study of a fatal YEL-AVD in a young female patient in the United States identified heterozygous genetic polymorphisms in chemokine receptor CCR5 and its ligand RANTES, but OAS1, OAS2, TLR3 and DC-SIGN were wild-type. A potential hypothesis centres on a disconnection between the signalling of innate immune response and the timely activation of the adaptive immune response.

Following the cluster of adverse events observed at Ica in Peru in 2007 it has been suggested, mainly by physicians, that the 17D virus genome should be sequenced from each lot of vaccine. However, this is unlikely to be helpful as sequencing deals with majority populations and heterogeneity is not necessarily detected.

Much weight is currently placed on monkey studies and there have been investigations into alternative animal models. A hamster model has been developed at the University of Texas Medical Branch (UTMB) and Instituto Evandro Chagas (IEC, Brazil) which shows viscerotropic disease. However, wild-type strains need to be adapted to hamsters and viruses from YEL-AVD

cases do not show viscerotropic disease in this model. A new small animal model in which non-adapted wild-type YFV shows viscerotropic disease is said to show promise, and has been published subsequent to the meeting [15].

The molecular basis of attenuation has been investigated by comparing known sequences of the wild-type Asibi virus and its attenuated 17D vaccine derivatives (17D-204, 17DD and 17D-213), but to date the mechanism of attenuation has not been elucidated.. The mouse model indicates a multigenic cause for attenuation of neurotropism. Nothing is known about the basis of attenuation of viscerotropism in either the non-human primates or the hamster model. Structural information may be useful in non clinical studies as has been shown for dengue virus where neutralizing epitopes on the surface are critical for the virulent genotype.

Dr Adwoa Bentsi-Enchill (QSS/WHO) reported on the recent review of YF vaccine safety data and discussions of the Global Advisory Committee on Vaccine Safety (GACVS). Serious AEs include hypersensitivity and anaphylaxis, YEL-AVD and YF vaccine-associated neurotropic disease (YEL-AND). Risk factors – either known or potential - for vaccination include age, compromised immunity including HIV/AIDS, pregnancy, and non-adherence to indications for use. GACVS undertook a risk benefit consideration in endemic and non-endemic populations and obtained information from prequalified manufacturers relating to viscerotropic and non-viscerotropic disease so that risk factors identified and estimates could be made. Issues relating to the incident in Peru were reviewed in detail.

The first report of YEL-AVD was published in 2001. Over the past eight years there have been several incidents. The frequency is hard to determine accurately because i) it is low; ii) it is difficult to obtain reliable figures for the number of doses used in most countries; and iii) the figures may be affected by population related factors, including exposure to other flaviviruses. Generally estimates range from 0.004 per 100,000 in Brazil to 0.21 for travellers from non-endemic areas . Differences in incidence in endemic regions versus naïve populations are probably related to population differences such as previous vaccination and exposure to wild virus.

In the Ica, Peru incident, 4 fatal cases of YEL-AVD observed in 42,000 recipients of a single lot of vaccine (Lot 05OVFA121Z) were confirmed virologically and clinically. This gives rates of YEL-AVD of 7.9-11.7 per 100,000 for this lot of vaccine.

Extensive investigations were undertaken by WHO Regional Office for the Americas (AMRO) and WHO headquarters, and an expert panel was convened to assist the Peruvian government. A visit to the manufacturing site revealed no quality issues and the characterization of the secondary seed and batch records were satisfactory. There were no reported problems from 9 batches prepared from the same final bulk as the lot which had caused problems. Potency data was available from various laboratories on samples of the batch used at release, end of shelf life and recovered from the field. Although the potencies from the different laboratories are not directly comparable as the methodology used was different, vaccine Lot 05OVFA121Z had retained potency even though it had moved around South America prior to use in Peru. In addition, the results of these potency tests indicated that it was indistinguishable from sister lots of vaccine not associated with YEL-AVD. The results of potency tests of Lot 05OVFA121Z therefore gave no explanation for the higher frequency of viscerotropic disease of this batch. .

The incident remains unexplained. While the cases were clearly vaccine associated, extensive studies on the vaccine itself revealed no features that would account for them; the bulk had been used in other batches which gave no problems, and there was nothing about the specific batch, for example very high titre, that distinguished it from the other batches. An account of the investigations has been published subsequent to the meeting [16].

The incidence of YEL-AVDs was 20 times higher than previously reported for 17D vaccines. The cause of death appeared to be overwhelming disease caused by the 17DD strain possibly associated with the host immune response. The vaccine virus from a vial of vaccine, the seed virus and virus isolated from a patient were sequenced but no significant difference identified. Possible host factors were advanced age (1 case was 79 years old), autoimmune disease (systemic lupus erythematosus and rheumatoid arthritis in 1 case) and potentially immunosuppressive medication may have been administered after vaccination in two cases. Although autoimmune disease is not a recognized risk factor, it warrants further attention. There may also have been other coincident infections which contributed to disease or some unknown agents. Age and thymus disease are recognized as risk factors for viscerotropic disease

The higher incidence of YEL-AVD in Ica, Peru in 2007 is unexplained. Nevertheless, the current recommendations for use of YF vaccine should not be changed so long as the indications for use are adhered to. There needs to be a careful assessment of risk benefit with respect to age.

Rates of YEL-AND vary in different studies undertaken in different populations – Europe, UK and US travellers. There is limited information on adverse events YEL-AND in campaign settings. Rates of YEL-AND vary based on study/population but were observed to range from 0.19 to 0.8 per 100,000 in studies in Europe and the US [17, 18].

During the ensuing discussion, Dr Minor emphasized that there is no explanation for what happened in Peru and emphasized that the risk of viscerotropic disease is 0.4 per 100,000 from known vaccine doses used but in older individuals with known thymus disease, the risk is 3-4 times higher. A total of 46 cases of viscerotropic disease had been identified up to the end of 2008. The first case described in Brazil, other than a 1975 case identified retrospectively, was in 1999, in a 5 years of age female child. All cases of viscerotropic disease were in primary vaccinees with more than 50% fatalities. In Brazil, there is no clearcut age distribution and 1 million infants are vaccinated every year. There are no reports of YEL-AVD associated with the YF vaccine produced in Russia but there has been one case reported in 2004 associated with the YF vaccine produced in China.. This individual had previously had cancer so immunosuppression may have predisposed YEL-AVD.

Isolates from adverse events are indistinguishable from vaccine virus based on the assays used. The Peruvian cases are the best characterized to date. Other cases investigated gave non-significant heterogeneity. Sequences identical to 17DD were reported [19]. Several cases have been described clinically and the genome of the recovered viruses were partially or fully sequenced. Additional studies in monkeys have also been done [20-22].

Several YEL-AVD cases had a very high viremia. However this may depend on the samples taken and the time at which they were taken, which may be before or after death of the patient. There are also examples of high anti-YF virus immune responses in some cases. There is a need for standardized sampling in all instances of adverse events.

The group discussed whether the occurrence of adverse events following YF vaccination is new. There are three collections of YF viruses totalling 600-800 wild type viruses but only three vaccine-related strains have been identified. One isolate from a fatal case in 1975 was retrospectively found to be 17DD [23].

Viruses were tested by Theiler in monkeys for viscerotropism in the course of derivation of the 17D strain. The question of whether the viscerotropic test described in the current Recommendations gives relevant data must be addressed. In addition, the data recorded by manufacturers when this test is undertaken should be defined.

Minor sequence changes in non-coding regions of manufacturer's seeds have been observed but their significance is not known. Dr Barrett from UTMB observed sequence changes in seeds in the US as the strain was transferred to manufacturers but these were not considered significant. Significantly, amino acid substitutions from wild-type Asibi to 17D vaccine substrains 17D-204, 17DD and 17D-213 are always conserved in the virus, although there are amino acid changes characteristic for each of them [24].

4. Quality and safety of YF vaccines

4.1 Change of minimum potency specification

Dr Morag Ferguson (NIBSC) summarized quality and safety issues in the technical specifications which need to be addressed in the revision of the Recommendations. The current Recommendations for the seed virus state that it should be 'identified by historical records that include information on the origin of the sub-strain, its method of attenuation and the passage level at which attenuation and immunogenicity were demonstrated by clinical evaluation'. It was not clear if all strains are acceptable and whether additional testing by molecular methods be included to characterize strains would be useful.

The Recommendations state that the master and working seed lots of YF vaccine virus shall be shown to be safe and immunogenic by appropriate laboratory tests 'which include tests for

viscerotropism, immunogenicity and neurotropism in a group of 10 test monkeys'. The group were asked whether or not these tests were still appropriate and required. Previous discussions at an International Association of Biologicals (IABS) symposium concluded that the WHO seed virus has a very low score and consequently is not a good control virus and that the WHO Recommendations on test procedures could be clarified [25]. A minimum potency specification expressed in IU per dose was approved by ECBS in 2008. In addition it was agreed that any changes to the release specification of existing vaccines should be justified by clinical data, e.g.:

- Transfer of production from one manufacturer to another should include specifications in IU, not mouse LD₅₀;
- Specifications for new manufacturers should be set by clinical trial, expressed in IU; and
- The specification should be no less than 3.0 log₁₀ IU per dose.

This change also impacts on the expression of potency in IU on other sections namely section 6.2 (the potency specifications) so that assay in mice and expression of virus titres in LD₅₀ is not required, and A.4.1.3 <Monkey neurovirulence test> where the dose of virus used is given in LD₅₀; A.4.3.2 <Single harvests> - Virus titration, A.4.4.5 <Final bulk> - Virus titration, and A.6.1 <Final container>. The identity test in mice has been deleted and identity is now only shown in cell culture

The need for an upper limit of potency had also been discussed at a GACVS meeting in June 2008. In addition, the need for a thermal stability test should be considered in the context of other stability data and establishment of expiry dates.

During the discussion, the group agreed that historic records and charts on derivation of seed virus need to be revisited and updated. Such diagrams were published in 1975 and a more recent one by Susan Robertson in late 1980s.

Manufacturers were asked to document the source of their seeds and their passage levels. Strains had been passaged to get avian leucosis virus (ALV)-free virus and the virus genealogy and historic records and the chain of custody should be documented. It was agreed that production in specific pathogen free (SPF) eggs was achievable by all manufacturers and would now be required. It was suggested that a molecular test be included in the characterization of new seeds.

It was also reported that studies by Dr I. Levenbook were discussed during an IABS meeting on neurovirulence. Different seeds were compared. However, the utility of the test without an appropriate positive control was questioned. Pass/fail criteria were not established and the significance of the results obtained was not clear. However, as the test was one of the few possible measures of pathogenicity it should be included in the revised Recommendations.

The upper potency limit of YF vaccines is not defined at present. The relationships between median lethal dose (LD_{50}), plaque forming unit (PFU) and IU are variable and this is the main justification for the use of IU. The establishment of an upper potency limit will be difficult to establish other than by clinical trial. Dr Barrett commented that the amount of virus in vaccines has gone up over the 25 years he has been assaying vaccines. Release specifications vary and as there is currently a shortage of vaccine perhaps virus content in a dose could be reduced so that more doses could be produced from the same number of eggs. However, release and end of shelf-life specifications may differ for each manufacturer as this is related to stability of the vaccine. It was also noted for further discussion that published clinical trial data use either 50% plaque reduction neutralization ($PRNT_{50}$) or log neutralization index (LNI) for the measurement of antibody levels so comparisons are difficult.

4.2 Perspectives from manufacturers

Dr Darcy Hokama (Bio-Manguinhos, Brazil) reported that they produce 5-, 10- and 50-dose presentations of YF vaccine all of which have been prequalified by WHO. The company's minimum potency limit is $3.0 \log_{10}$ IU set following the WHO consultation to discuss minimum potency requirements. The range of potencies observed for 5-dose vial stored at the recommended temperature was $3.81 - 4.92 \log_{10}$ IU/human dose. For the 50-dose vial, the range is $3.56-4.39 \log_{10}$ IU/human dose and $3.28-4.11 \log_{10}$ IU/human dose in the thermostability test. These presentations contain different stabilizers. Batches from 2000-2009 had a mean titre $>4.0 \log_{10}$ IU and the company considered that the minimum WHO requirement endorsed by ECBS in 2008 is therefore appropriate. A dose-response trial is being undertaken at Bio-Manguinhos and it should be helpful to establish the upper potency limit. Four thousand batches have been tested in the general safety test with no failures and the company recommends omitting this test from lot

release testing. Dr Hokama also presented data on molecular detection of mycoplasma instead of a culture method. A polymerase chain reaction (PCR) detection method has been validated on 286 samples with 100% specificity and sensitivity. The limit of detection for a PCR method used was approximately 3.1 colony forming unit (CFU), which is much lower than that of a culture method. Their current YF seed (993FB013Z) of 17DD strain was produced in 1999 and was derived from the secondary seed lot no. 102/84, which was used as working seed lot until 2000. Four vials of master seed remain, and 40 vials of the secondary seed. Working seed lot 993FB013Z comprising 12,861 vials is available and should last 19 years, assuming an annual production of 50 million doses. The molecular characterization of this working seed lot was performed in the Biochemistry and Molecular Biology Department of Oswaldo Cruz Institute/Fiocruz by Dr Ricardo Galler. The full genome of the working seed lot 993FB013Z was compared with the genome of the seed lot 102/84. The derivation of the seed virus has been documented. SPF eggs are used in production and neurovirulence tests performed on the seed virus with lot no.102/84 included as reference virus [26]. The working seed was also tested in clinical trials [27, 28] , without evidence of viscerotropism.

In discussion it was noted that a PCR-based mycoplasma detection test is under discussion at the European Pharmacopoeia (EP).

Dr Bing Zeng (CNBG - China National Biotech Group) reported that YF vaccine has been marketed in China since 1953. A new plant with a capacity of 2 million doses is expected to be operational in 2010. Actual production is approximately 200,000-300,000 doses/year. This vaccine is recommended for travellers from China. The seed virus originated from the Rockefeller Institute in 1942 and is derived from the 17D strain. The master seed was established after 51 passages in chick embryos. The seeds were tested for neurovirulence and immunogenicity as required in WHO Recommendations. They have different minimum levels for potency tests in mice and cells, namely 5.5 and 5.8 log₁₀/ml, respectively. However the specification given in the product insert is 'each single human dose is 0.5 ml containing not less than 4.2 log PFU of live yellow fever virus'. The vaccine has a shelf-life of 24 months and it is stable with titres of 5.5 – 6.0 log₁₀. Production in the new facility will result in an improved vaccine with lower ovalbumin content and single dose presentation. This vaccine is not pre-qualified as the functionality of Chinese NRA has yet to be approved through WHO NRA assessment. They may request the virus seed from WHO for production which would result in a

new product. During discussion Dr Zeng indicated that the virus is ALV free and production is in SPF eggs. The sequence has also been published.

Dr Alexandra Sinyugina (Chumakov Institute, Russia) reported that YF vaccines have been produced since the 1970s in Russia. This vaccine is now WHO pre-qualified. SPF eggs have been used since 2005 and the facilities recently renovated. The use of SPF eggs resulted in increased harvest titre and a decrease in rejection rates. The institute hopes to be able to extend the shelf-life to 36 months. The working seeds for this vaccine was prepared from the WHO 17D primary seed (213/77). Master and working seeds were tested in the monkey neurovirulence and viscerotropism tests with the WHO virus being used as reference standard. The Russian standard has been calibrated in IU and the minimum titre is $3.0 \log_{10}$ although titres are generally $\geq 4.2 \log_{10}$ and after 2 years, no less than $3.0 \log_{10}$ IU.

Dr Lionel Gerentes (Sanofi Pasteur, France) reported that the company includes an in-house standard (U5217) in every assay and has run the IS (NIBSC code 99/616) in parallel in 50 tests between 2004 and 2009. They indicated that their data suggest that the IS is losing potency as the mean titre for the IS in 2004 was $10^{4.52}$ PFU/ 0.5 ml and $10^{4.39}$ PFU/ 0.5 ml in 2009. No other user has observed or reported such a loss of potency to NIBSC. The variability in titres of the IS is higher than with the Sanofi standard respectively 0.11 log compared to 0.06 log. Dr Gerentes did not consider an upper potency limit to be appropriate, because of the variability of the mean titer from a vaccine to another. However, an internal upper limit is essential for the follow-up of the consistency of production. As a consequence, it was suggested that this internal upper limit: (i) should be specific for each YF vaccine; (ii) has to be defined by the manufacturer according to its trend analysis of its product (mean titre in IU/dose + 3 standard deviations); and (iii) needs to be approved by the National Regulatory Authority.

The thermostability specification is appropriate. Other modifications of the TRS No. 872 proposed by Sanofi Pasteur are suggested: i) section A.1.4 Terminology - delete « Median mouse lethal dose (mouse LD₅₀) »; ii) section A.4.1.3 Monkey safety test - modify the test dose expressed in mouse LD₅₀; and iii) section A.4.2 Tests on uninoculated eggs - modify the number of eggs from 50 to 80 to harmonize with EP.

Dr Pascale Cottin (Sanofi Pasteur, France) then described safety issues and investigations on production lots produced over a 12-year period from 1990 to 2002 [29]. These studies included viral plaque size distribution which demonstrated a consistent potency, homogeneity of the viral population and genetic stability. Reverse transcriptase PCR (RT-PCR) estimation on the total viral load in the bulks was found to be highly homogeneous. This study reinforces the robustness of the process of production and the safety profile of StamarilTM. Quality and manufacturing investigations have been routinely conducted since 2006 and no specific action has been taken for StamarilTM to date. This investigation is undertaken in case of serious adverse event notification and involves review of batch records, non-conformities, changes, and technical complaints. The procedures also allow for the possibility of an evaluation of the retained samples (visual or physical testing). With respect to YEL-AVD, a total of 9 cases, 5 fatal and 4 non-fatal, have been reported in the Pharmacovigilance database of Sanofi Pasteur since 1994. A fatal case of YEL-AVD in Spain was investigated regarding vaccine quality. There were no difference evidenced between this lot and other vaccines doses analysed in parallel in terms of potency, genomic titer or plaque size profile [30].

Dr Nicola Boschetti (Crucell Berna, Switzerland) updated the group on the development of a YF vaccine by Crucell. This vaccine is currently in the licensing process. The production virus (17D-213/77 strain) was transferred from the Robert Koch Institute (RKI) and the virus is grown in SPF eggs. The release specification for Flavimun is ≥ 4.7 log PFU/dose and the end of shelf-life specification is ≥ 4.2 log PFU/dose which is identical to that of RKI. The relationship established by Crucell is 1,000 mouse LD₅₀/dose \approx 15,000 PFU/dose (= 4.2 log PFU/dose). The genetic sequence of the vaccine seed virus has been published. Animal safety tests with working seed lot 112/95 have been performed including monkey tests for immunogenicity, viscerotropism, neurotropism, guinea pig, adult mice tests for extraneous agents and guinea pig, adult mice tests for abnormal toxicity (done on three consecutive manufacturing lots). All gave satisfactory results.

Dr Antoine Diatta (Institut-Pasteur Dakar, Senegal) reported on YF vaccine in Senegal that has been produced since 1966 using the 17D strain. Production capacity is 10 million doses per year but it is a challenge to supply high quality and safe YF vaccine in compliance with GMP and WHO TRS recommendations. SPF eggs sourced from Germany are used in production. He emphasized the need to define good minimum potency and that the thermal stability test is

important when vaccine is to be used in tropical areas. Only 1 vial of master seed (passage 233) produced in 1974 remains and there is a limited stock of the working seed lot produced in 1985 (passage 235). If there is to be an increase in production capacity, a new working seed is required and Dr Diatta asked whether a vaccine batch (passage 235) could be used as a vaccine seed lot if it was characterized according to the TRS recommendations. It was noted that this level is less than WHO seed passage level. He also queried which laboratories are able to undertake the animal tests and noted that if the tests were performed in a limited number of laboratories, there would be less variation. A WHO reference strain is also required to train staff on this test. He agreed that an upper limit of potency should be documented but queried how this could be determined. In addition more information is required about the effect of the increase of YF titer on the quality and safety of the vaccine. The group agreed that clinical data will be required whatever virus is used for a new seed.

Some cases of viscerotropic disease had occurred following use of the Institut-Pasteur Dakar YF vaccine but no link to the specific vaccine batch had been identified. This may be because of previous exposure of vaccinees and flavivirus antibodies in recipients. It was also noted that at least some of the campaigns undertaken with this vaccine had good surveillance for AEFI.

4.3 Perspectives from national control laboratories

Dr Sylvie Morgeaux (AFSSAPS - Agence Française de Sécurité Sanitaire des Produits de Santé, France) presented their data on the testing of the Sanofi Pasteur vaccine. The manufacturer's corrective factor was $\text{Titer in LD}_{50}/\text{dose} = \text{Titer in PFU}/\text{dose} - 0.3 \log_{10}$ and the correlation between IU and PFU of AFSSAPS was $\text{Log}_{10} \text{ IU}/\text{dose} = \text{Log}_{10} \text{ PFU} - 0.1$. AFSSAPS have calibrated their in-house standard (IHS) in IU and the mean from 29 values was $4.4 \log_{10} \text{ IU}/0.5 \text{ ml}$. The mean of 43 assays performed on the IS 99/616 from 2004 to 2009 was $4.3 \log_{10} \text{ IU}/0.5 \text{ ml}$ which was the same as that obtained in the collaborative study. Fifty two batches have been tested for potency and thermal stability in assays in which the IS and in-house standard included. The titers calculated against the in-house standard were $4.2 \log_{10} \text{ IU}/\text{human dose}$ and $4.3 \log_{10} \text{ IU}/\text{human dose}$ against the IS. Similar results were obtained in thermostability tests namely $4.0 \log_{10} \text{ IU}/\text{human dose}$ against the IHS and $4.1 \log_{10} \text{ IU}/\text{human dose}$ against the IS. AFSSAPS will continue to express potency as LD_{50} until the European Pharmacopoeia and marketing

authorization are changed but will use IHS to validate each assay and use periodically the IS to check standard deviations of IHS. Dr Morgeaux noted that the IS looks stable in their assays. They had, however, noted a decline in the titres of vaccine batches relative to both IHS and IS. This had been discussed with manufacturers and they had concluded that this was an issue with the vaccine titre and not the standards.

Dr Guanmu Dong (NICPBP - National Institute for the Control of Pharmaceutical & Biological Products, China) reported the history of monitoring for adverse events in China. A national adverse drug reaction reporting system was initiated in the 1990s by doctors and hospitals with a passive reporting system and a new network is in place from 2009. He reported the minor side effects in a peacekeeping force going to Sudan. Of 60 people immunized with both YF vaccine and inactivated JE vaccine, 9 (15%) had headache and fever. The only case report of a serious adverse event was in 2004 in a 60 year-old American Chinese [31]. He had previously had rectal cancer and chemotherapy in 2002 so may have been immunocompromised. The patient was discharged from hospital following recovery. No virological confirmation was obtained. At that time the virus seed Beijing 17D strain was compared with the WHO reference 17D virus strain. The strains shared 99% homology of the published sequence in Genbank database. A putative amino acid residue 173 of the Beijing strain E protein had a likelihood of reversion to wild-type Asibi virus (from isoleucine to threonine) and which may be associated with virulence. Specification and quality improvements have been implemented with the Chinese vaccine leading to a lower content of ovalbumin, <30 ug/dose, a lower amount of impurity and single dose vials. In addition, tests for residual albumin and endotoxin in the final product will be performed. A candidate national reference vaccine is in preparation. Twenty three thousand ampoules are available with a titre of 6.76 log PFU/ml. This is a 1.0 ml fill with a coefficient of variation of 3.87% and residual moisture of 1.4%. It will be stored at -20 °C. The stability of this preparation at different temperatures appears to be very good. This needs to be calibrated against the IS. China would like to develop a new seed lot from the WHO seed and vaccine produced from any new seed would be assessed in clinical trials. They also think that an upper limit of virus titre should be set and implemented.

Dr Jurg Stalder (Swissmedic, Switzerland) described the results of virus titrations involving the IS over a 5 year period at the OMCL Biologika. The mean was 4.5 log₁₀ IU/ampoule with an SD of 0.1. In assays performed at Swissmedic, 1 PFU equals to 2.5 IU whereas Crucell has 1 PFU =

5 IU. Despite this discrepancy, the IU of batches was quite similar with values differing by 0.4 log. There was little loss of potency in thermostability studies. Dr Stalder concluded that conversion of PFU into IU improves comparability of results between different laboratories.

Dr Maya Vorobieva (Tarashevich State Research Institute, Russia) described studies to calibrate a national standard in IU. Their correlation between LD₅₀ and PFU is 0.36 (ranging from 0.0027 to 1.06), established in 1997 and between PFU and IU is 0.30. They performed 9 assays using the national standard (NS) and IS. The Russian NS has a titer of 3.95 log PFU/0.5 mL (ranging from 3.64 to 4.24). From June 2006 to October 2007, the range of potencies in IU of routine vaccine batches was 4.12 to 4.38 and in the stability test, the range was 3.38 to 4.4. The minimum potency specification is usually expressed in IU. Dr Vorobieva agreed that clinical studies are important to establish the minimum potency requirements. The thermostability test data indicated that the vaccine was very stable. She also commented that it was difficult to perform tests in monkeys as it was difficult to get suitable animals. So far there have been no cases of viscerotropic disease with Russian vaccine in the 6 million doses administered since 1978. This vaccine has been used in Russia, South America and Africa.

5. Nonclinical & clinical evaluation

Dr Barrett presented key points, specific to YF vaccine, which need to be considered in the nonclinical section. These included the strain of virus to be cited as suitable for use in the manufacture of YF vaccines and whether this should be restricted to 17D. Such viruses should be ALV-free and the passage history/records and genealogy of vaccine known and documented. The tests required on master seed, secondary seed and vaccine lots need to be reviewed. Genomic sequence should be determined and criteria established as to whether to proceed with the use of a virus preparation as seed virus if there were amino acid substitutions. The degree of genetic heterogeneity/stability and the use of RT-PCR should also be reviewed. The biology of the virus and tests to be conducted in mice, non-human primates and mosquitoes should be considered. Cell culture assays and molecular characterization would also be addressed and consideration given as to whether a virus strain or batch of vaccine might cause neurotropic or viscerotropic disease. Tests currently undertaken are in non-human primates for neurovirulence (histopathological studies) and viscerotropism (viremia). Neuroinvasiveness can be assayed for in

5- or 6-day old mice and a new small animal model for viscerotropism is being developed and was subsequently published [15]. The testing required could cascade into assays for mosquito competence if a seed virus has relevant amino acid substitutions. The cell types used in assays and whether an assessment of plaque size and heterogeneity was a useful consistency criterion should be considered.

During the discussion, it was noted that the producers in Brazil, France, Switzerland, Senegal and USA use 17D strain and that the sequences are in a central database and have been shown to have good homogeneity. It was agreed that all master and working seeds, including all subsequent working seeds, should be sequenced but that no useful information would come from sequencing each vaccine lots. It was suggested that if a high number of amino acid changes compared to the 17D consensus sequence were observed, then mosquito competence tests should be undertaken. However, as for the non-human primate studies, very few laboratories are able to perform such tests.

Dr Mair Powell (MHRA - Medicines and Healthcare Products Regulatory Agency, UK) outlined the information needed in the clinical section of the document. This should include guidance to companies and NRAs that is specific to the clinical evaluation of YF vaccines. The section needs to include some consideration to the provision of clinical data to support major changes to established manufacturing processes.

It was discussed and agreed that the Recommendations under revision would only apply 17D live attenuated vaccines.

It was agreed that the approval of a new YF 17D vaccine would be based on clinical studies of safety and immunogenicity. Therefore the total database would not allow for any estimation of the possible risk of YEL-AND and YEL-AVD during pre-approval studies. In addition, the population in which the studies are conducted needs consideration i.e. vaccine naïve or previously vaccinated individuals and residents of endemic or non-endemic areas. Studies conducted in non-endemic areas will be restricted to those considered to be in need of YF vaccine and most likely will be conducted in adults presenting to travel clinics. Elderly persons may be included if they are travelling to high risk areas and there are no other contraindications to vaccination. Data on children are most likely to be obtained from studies in endemic areas where

routine vaccination is performed. In accordance with national and regional recommendations it is likely that inclusion of children aged 6-9 months would be possible and desirable in endemic countries. The exclusion criteria in trials would include all the usual contraindications to receipt of live attenuated vaccines plus individuals with thymus disease.

In terms of relevant past experience it was commented that in Brazil, studies in children in endemic areas were done only after adult studies had demonstrated that the safety profile was acceptable. However, some NRAs have agreed that specific studies in children are not required provided that the studies in adults are satisfactory and approval has been based on the experience with the use of 17D vaccines in children. However, the effects of co-administration with MMR in children should be evaluated in clinical studies if it seems likely that this practice will be desirable.

At the end of this part of the discussion the group agreed that it is preferred that initial studies for safety and immunogenicity should be undertaken in healthy young adults who are naïve to YF vaccines and resident in non-endemic areas. If these subjects show an adequate immune response and a comparable safety profile in comparison with a licensed YF vaccine then it would be expected that similar findings would be obtained in endemic areas. It may or may not be considered necessary to obtain some experience of use in children, which for practical reasons would likely have to come from studies in endemic areas.

It was agreed that dose ranging studies to determine the minimum PFU required to provide adequate immune responses would be valuable for new vaccines based on a 17D seed. These data could be used to set an acceptable end of shelf-life PFU and could also potentially increase vaccine production by estimating the minimum initial virus titre per dose.

In each study, the choice of the comparator vaccine should depend on the study objectives. In most cases a well-established and licensed comparator may be selected that is acceptable to as many NRAs as possible. It is desirable that the comparator 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. However a vaccine manufactured with the existing seed should be used as the comparator in studies that assess vaccine manufactured from a new seed lot.

The group agreed that virus neutralization assays should be used to compare the immunogenicity of the test and reference vaccines and either PRNT assays or LNI assays are acceptable. The primary comparison should be based on the geometric mean titre (GMT) and appropriate acceptance criteria should be pre-defined in the study protocol. However, seroconversion rates and reverse cumulative distributions should also be provided and reviewed and should be compared for consistency with the general experience gained with 17D YF vaccines. The group also agreed that a flavivirus hemagglutinin inhibition test may be used to demonstrate that an individual is flavivirus-naïve.

Assessment of viremia is not routinely required because it is usual that recipients of YF vaccines have a temporary viremia.

Enhanced safety surveillance should be undertaken after marketing for a pre-defined period and data should be kept under review according to the extent of the experience gained (i.e. reflecting use) during the initial post-approval years. It is preferable that the enhanced safety surveillance should involve a collaboration with national authorities. The Brighton Collaboration case definitions for YEL-AVD and other events should be used for describing adverse events once they are available. It was noted that WHO is preparing a new protocol for investigating serious adverse events following YF vaccination. It is hoped that the incidence of YEL-AVD and YEL-AND may be reduced through identification of factors that predispose individuals to these reactions. Also, that AEFI associated with YEL-AVD and YEL-AND may be more quickly identified leading to more rapid institution of adequate management measures.

The meeting group noted the following additional reports and comments.

Dr Reinaldo Martins (Bio-Manguinhos, Brazil) reported that molecular and animal tests, as well as clinical trials, undertaken on a new 17DD seed virus lot 13Z produced in 1999 gave identical results to the secondary seed lot 102/84 used since 1984 [26].

The batch of vaccine associated with reports of serious adverse reactions in Peru had a titre of 106,898 PFU/dose when tested. Although the titre at release two years before was greater than this; there were no indications of quality problems or differences in manufacture of the batch compared to others.

There is some evidence that the current PFU doses used in YF vaccines may be higher than those needed to achieve acceptable seroconversion rates. For example: i) 32 volunteers given 100-200 PFU had 93.7% SC [32]; ii) 13 volunteers given 200 PFU had 85% SC [33]; and iii) 15 volunteers given 200 PFU had 93% SC [34]. Bio-Manguinhos had therefore initiated a dose-response study in Brazil, with doses ranging from 250 PFU/dose to 60,000 PFU/dose.

Dr Nicola Boschetti (Crucell Berna, Switzerland) described the bridging study undertaken on their YF vaccine following the transfer of production from the Robert Koch Institute to Crucell [35]. This vaccine is produced from the WHO strain of virus and Stamaril was the comparator vaccine. The aim of the study was to demonstrate non-inferiority of immune responses as well as monitoring safety. Flavimune was shown to be non-inferior to both YFV RKI and Stamaril regarding immunogenicity. No significant adverse events were observed. The genetic stability of the Crucell vaccine had been demonstrated by the sequencing of virus isolated from vaccine recipients by amplification of E protein gene in 6 out of 15 subjects. Sequence analysis demonstrated that these viruses were identical to the vaccine virus.

Dr Rémy Teyssou (Sanofi Pasteur, France) described the Sanofi Pasteur experience with Stamaril and various studies undertaken on vaccine lots and the virus seeds. Since January 1993, 9 cases classified as YEL-AVD and 18 as YEL-AND had been reported with Stamaril and initiatives had been developed by Sanofi Pasteur to address potential safety concerns.

Preclinical studies had been undertaken retrospectively on 12 archive bulk lots derived from the same secondary seed lot, produced from 1990 to 2002. These were tested for consensus sequence analysis and plaque size phenotypes [29]. Compared to the published 17D-204 Sanofi Pasteur sequence, only four nucleotides substitutions were present in the secondary seed lot and conserved in all production batches, with no incidence at amino-acid level. Plaque phenotypes were studied to detect any phenotypic difference between populations but no differences observed. The potency of each lot was also tested by RT-PCR to determine viral load and this was also consistent. These data reinforced the stability of the virus used to manufacture Stamaril. Should Sanofi produce a new seed, it will be sequenced. For the future analysis of seed lots, there was a need to develop new models for monitoring viscerotropism and neurotropism. Dr Teyssou considered that, when a new working seed is developed, clinical trials are not needed provided

that bridging characterization studies have been performed. Should mutations between master and working seed lots be observed, it is important to know whether these mutations lead to phenotypic alteration and affect immunogenicity.

6. Conclusions

The group reviewed the main points of the discussion and agreed on the key changes in the scope of the revision of the current Recommendations, namely:

- The section of general considerations should include reference to YEL-AND and YEL-AVD. The genealogy diagram published in 1993 [36] should also be reviewed and updated as appropriate;
- Production and quality assessment should express vaccine virus titres in IU and specify the use of SPF eggs; and
- Monkey tests should be transferred to the non-clinical section. Monkey test should remain as currently written but be transferred to an appendix. The dose to be used in monkeys should be equivalent to human doses. Preclinical assessment should include virus history and identified on the genealogy diagram. The master and working seeds should be sequenced. In addition the seeds should be characterized in animal and plaque tests to demonstrate consistency.
- Consideration should be given to the inclusion of a requirement for all manufacturers to have upper limit of potency depending on the clinical experience of their product

The clinical section should detail when a clinical trial is needed. This will include when there is a new master seed, even if it is prepared from the WHO seed. The maximum allowable level passage from master seed to working seed and from working seed to production should be limited as one pass, respectively. If a new working seed is at the same passage as previously, no clinical trial is required.

Specific examples which are under consideration at present were discussed:

- IP Dakar wishes to prepare a new master seed which will be one pass from current master seed. Then working seed will again be one passage further on. In this instance it would be appropriate for a clinical trial to be a comparison of the old and new vaccines.

- Bio-Manguinhos has prepared a new master which is one more passage in egg and a new working seed. A clinical trial on immunogenicity and safety was completed and published, and gave satisfactory results [27, 28].
- Crucell used WHO YF vaccine seed virus to make a master and then working seed and a clinical trial has been undertaken.
- In China, there are three passages between the master and working seeds.

The group were informed that the EP states that there should be one passage level between master to working seed. The group agreed that the process of revision focuses on updating of the current Recommendations and not a complete rewrite.

References

- [1] World Health Organization. International Health Regulations (2005). In: Geneva, World Health Organization, 2008, [<http://www.who.int/ihr/9789241596664/en/index.html>].
- [2] World Health Organization. Requirements for yellow fever vaccine (Requirements for biological substances no.3). In: WHO Expert Committee on Biological Standardization. Forty-sixth report. Geneva, World Health Organization, 1998, Annex 2 (WHO Technical Report Series No. 872) [http://www.who.int/biologicals/publications/trs/areas/vaccines/yellow_fever/en/index.html].
- [3] Knezevic I, Stacey G, Petricciani J. WHO Study Group on cell substrates for production of biologicals, Geneva, Switzerland, 11-12 June 2007. Biologicals 2008 May;36(3):203-11.
- [4] Seagroatt V, Magrath DI. A W.H.O. collaborative study of in vitro and in vivo methods for the assay of yellow fever vaccines. J Biol Stand 1983 Jan;11(1):47-54.
- [5] Ferguson M, Heath A. Collaborative study to assess the suitability of a candidate International Standard for yellow fever vaccine. Biologicals 2004 Dec;32(4):195-205.

- [6] World Health Organization. International Travel and Health 2009. In: Geneva, World Health Organization, 2009, [http://www.who.int/ith/ITH_2009.pdf; or <http://www.who.int/ith/chapters/en/index.html>].
- [7] Barrett AD, Monath TP, Barban V, Niedrig M, Teuwen DE. 17D yellow fever vaccines: new insights. A report of a workshop held during the World Congress on medicine and health in the tropics, Marseille, France, Monday 12 September 2005. *Vaccine* 2007 Apr 12;25(15):2758-65.
- [8] Gaucher D, Therrien R, Kettaf N, Angermann BR, Boucher G, Filali-Mouhim A, et al. Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. *J Exp Med* 2008 Dec 22;205(13):3119-31.
- [9] Miller JD, van der Most RG, Akondy RS, Glidewell JT, Albott S, Masopust D, et al. Human effector and memory CD8+ T cell responses to smallpox and yellow fever vaccines. *Immunity* 2008 May;28(5):710-22.
- [10] Querec TD, Akondy RS, Lee EK, Cao W, Nakaya HI, Teuwen D, et al. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. *Nat Immunol* 2009 Jan;10(1):116-25.
- [11] Bae HG, Domingo C, Tenorio A, de Ory F, Munoz J, Weber P, et al. Immune response during adverse events after 17D-derived yellow fever vaccination in Europe. *J Infect Dis* 2008 Jun 1;197(11):1577-84.
- [12] Belsher JL, Gay P, Brinton M, DellaValla J, Ridenour R, Lanciotti R, et al. Fatal multiorgan failure due to yellow fever vaccine-associated viscerotropic disease. *Vaccine* 2007 Dec 5;25(50):8480-5.
- [13] Hayes EB. Acute viscerotropic disease following vaccination against yellow fever. *Trans R Soc Trop Med Hyg* 2007 Oct;101(10):967-71.
- [14] Pulendran B, Miller J, Querec TD, Akondy R, Moseley N, Laur O, et al. Case of yellow fever vaccine--associated viscerotropic disease with prolonged viremia, robust adaptive immune responses, and polymorphisms in CCR5 and RANTES genes. *J Infect Dis* 2008 Aug 15;198(4):500-7.
- [15] Meier KC, Gardner CL, Khoretonenko MV, Klimstra WB, Ryman KD. A mouse model for studying viscerotropic disease caused by yellow fever virus infection. *PLoS Pathog* 2009 Oct;5(10):e1000614.

- [16] Whittembury A, Ramirez G, Hernandez H, Roper AM, Waterman S, Ticona M, et al. Viscerotropic disease following yellow fever vaccination in Peru. *Vaccine* 2009 Oct 9;27(43):5974-81.
- [17] Kitchener S. Viscerotropic and neurotropic disease following vaccination with the 17D yellow fever vaccine, ARILVAX. *Vaccine* 2004 Jun 2;22(17-18):2103-5.
- [18] Lindsey NP, Schroeder BA, Miller ER, Braun MM, Hinckley AF, Marano N, et al. Adverse event reports following yellow fever vaccination. *Vaccine* 2008 Nov 11;26(48):6077-82.
- [19] World Health Organization. Meeting of Global Advisory Committee on Vaccine Safety, 18-19 June 2008. *Wkly Epidemiol Rec* 2008 Aug 8;83(32):287-92.
- [20] Struchiner CJ, Luz PM, Dourado I, Sato HK, Aguiar SG, Ribeiro JG, et al. Risk of fatal adverse events associated with 17DD yellow fever vaccine. *Epidemiol Infect* 2004 Oct;132(5):939-46.
- [21] Vasconcelos PF, Luna EJ, Galler R, Silva LJ, Coimbra TL, Barros VL, et al. Serious adverse events associated with yellow fever 17DD vaccine in Brazil: a report of two cases. *Lancet* 2001 Jul 14;358(9276):91-7.
- [22] Galler R, Pugachev KV, Santos CL, Ocran SW, Jabor AV, Rodrigues SG, et al. Phenotypic and molecular analyses of yellow fever 17DD vaccine viruses associated with serious adverse events in Brazil. *Virology* 2001 Nov 25;290(2):309-19.
- [23] Engel AR, Vasconcelos PF, McArthur MA, Barrett AD. Characterization of a viscerotropic yellow fever vaccine variant from a patient in Brazil. *Vaccine* 2006 Apr 5;24(15):2803-9.
- [24] Galler R, Post PR, Santos CN, Ferreira, II. Genetic variability among yellow fever virus 17D substrains. *Vaccine* 1998 May-Jun;16(9-10):1024-8.
- [25] [Anonymous]. IABs scientific workshop on neurovirulence tests for live virus vaccines, January 31-February 1, 2005, Geneva. *Biologicals* 2006 Sep;34(3):233-6.
- [26] Marchevsky RS, da Luz Leal M, Homma A, Coutinho ES, Camacho LA, Jabor AV, et al. Molecular and phenotypic analysis of a working seed lot of yellow fever virus 17DD vaccine strain produced from the secondary seed lot 102/84 with an additional passage in chicken embryos. *Biologicals* 2006 Sep;34(3):191-7.

- [27] Camacho LA, de Aguiar SG, Freire Mda S, Leal Mda L, do Nascimento JP, Iguchi T, et al. Reactogenicity of yellow fever vaccines in a randomized, placebo-controlled trial. *Rev Saude Publica* 2005 Jun;39(3):413-20.
- [28] Camacho LA, Freire Mda S, Leal Mda L, Aguiar SG, Nascimento JP, Iguchi T, et al. Immunogenicity of WHO-17D and Brazilian 17DD yellow fever vaccines: a randomized trial. *Rev Saude Publica* 2004 Oct;38(5):671-8.
- [29] Barban V, Girerd Y, Aguirre M, Gulia S, Petiard F, Riou P, et al. High stability of yellow fever 17D-204 vaccine: a 12-year retrospective analysis of large-scale production. *Vaccine* 2007 Apr 12;25(15):2941-50.
- [30] Doblas A, Domingo C, Bae HG, Bohorquez CL, de Ory F, Niedrig M, et al. Yellow fever vaccine-associated viscerotropic disease and death in Spain. *J Clin Virol* 2006 Jun;36(2):156-8.
- [31] Huang H, Xu ZJ. One case of severe adverse event following to yellow fever vaccination. *Chin J Intern Med* 2005;44:460-1.
- [32] Lopes OS, Guimarães SS, de Carvalho R. Studies on yellow fever vaccine. III. Dose response in volunteers. 1988;16(2):77-82.
- [33] Freestone DS, Ferris RD, Weinberg AL, Kelly A. Stabilized 17D strain yellow fever vaccine: dose response studies, clinical reactions and effects on hepatic function. *J Biol Stand* 1977;5(3):181-6.
- [34] Monath TP, Cetron MS, Teuwen DE. Yellow fever vaccines. In: Plotkin S, Orenstein W, Offit P, editors. *Vaccines*. 5th ed. New York: Elsevier Inc., 2008: 959-1055.
- [35] Pfister M, Kursteiner O, Hilfiker H, Favre D, Durrer P, Ennaji A, et al. Immunogenicity and safety of BERNAL-YF compared with two other 17D yellow fever vaccines in a phase 3 clinical trial. *Am J Trop Med Hyg* 2005 Mar;72(3):339-46.
- [36] World Health Organization. Module 8: Yellow fever. In: *The Immunological Basis for Immunization Series*. WHO/EPI/GEN/93.18. Geneva, World Health Organization, 1993, [http://www.who.int/immunization/documents/WHO_EPI_GEN_93.18/en/index.html].

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