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## **Meeting Report**

### **WHO Informal Consultation of the Minimum Potency Specification for Yellow Fever Vaccines**

**National Institute for Biological Standards and Control  
(NIBSC), Potters Bar, UK**

**19-20 November 2007**

## Executive Summary

The objective of this report is to document the outcome of a WHO informal consultation on the minimum potency specification for yellow fever vaccine held in Potters Bar, UK on 19-20 November 2007.

Conclusions drawn by the participants were as follows:

- The current potency requirement is 3 log<sup>1</sup> mouse LD<sub>50</sub> per dose.
- The relationship between LD<sub>50</sub> and PFU is variable between laboratories
- The expression of potency relative to a standard calibrated in IU is less variable.
- The release specifications for current products vary and may be related to the variability in the measurement of LD<sub>50</sub> between laboratories.
- Specifications would be made more comparable if potencies were expressed in IU.
- All existing products are believed to be satisfactory based on clinical use of vaccines at the approved potency.
- Existing specifications should not be changed unless justified by clinical data.
- The minimum specification in current use corresponds to 3.0 log IU per dose following thermostability tests.

Participants proposed the following recommendations in amending the current WHO recommendations for the clinical minimum potency specification:

- Potency of yellow fever vaccines should be expressed in IU.
- For existing vaccines, changes from already established specifications to new specifications should be justified by clinical data.
- Transfer of production from one manufacturer to another should include specifications in IU, not mouse LD<sub>50</sub>.

Specifications of new products should be set by clinical trial and expressed in IU. The specification should be equal to or greater than 3.0 log IU per dose.

As a follow-up of the proposal, a draft amendment of the current WHO recommendations on the minimum potency specification will be reported to the Expert Committee on Biological Standardization at the 59<sup>th</sup> meeting in October 2008 for formal adoption.

**Keywords:** International Unit, Potency test, Vaccine, Yellow fever

## 1. Introduction

Yellow fever, a viral hemorrhagic fever transmitted by mosquitoes, continues to be a public health concern in many African and South American countries. There are 33 countries in Africa with a population of 508 millions at risk of yellow fever. In addition,

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<sup>1</sup> In this report, log always refers to the base-10 logarithm.

nine countries in South America plus several Caribbean islands are at risk. It is estimated that 200 000 cases and 30 000 deaths are attributable to yellow fever annually, most of them occurring in sub-Saharan Africa, although far fewer cases than this are reported.

Yellow fever vaccines are an important component of global control strategies for yellow fever. In all countries at risk, they are part of the routine national immunization programmes, whereas in high-risk areas mass campaigns are taking place as a way of preventing outbreaks. Moreover, these vaccines also play an important role in regulating international travel. The Global Alliance for Vaccines and Immunization (GAVI) started a yellow fever control initiative upon the resurgence of the disease in African countries in early 2000. The International Health Regulations revised in 2005 mandate vaccination certification for international travellers.

The potency of live virus vaccines frequently correlates to virus infectivity titers in animals (e.g. 50% lethal dose) or cell cultures (e.g. plaque forming unit). Compared to batch protection test in animals, virus infectivity titration in cell cultures is relatively simple and inexpensive, and there is a rapid quality control test that can be easily applied to all batches of live vaccines.

Virus infectivity titers measured in cell cultures vary within and between laboratories. A proposal to address this problem was that a standard preparation should be made so that the results of virus infectivity titers of vaccine batches could be expressed in terms of the result for the standard vaccine tested in parallel. This proposal was based on the results of a collaborative study on yellow fever vaccine reported in 1983. However, the first WHO international standard for yellow fever vaccine was not established until 2003.

The WHO recommendations for potency of yellow fever vaccine specify that the titer of the vaccine shall not be less than 1,000 mouse LD<sub>50</sub> or its equivalent in PFU.

Accordingly, most manufacturers' QC laboratories established the relationship between LD<sub>50</sub> and PFU potency tests many years ago, but the relationship is not necessarily valid today.

At the time of the collaborative study that established the suitability of the first international standard for yellow fever vaccine in 2003, it was suggested that the use of the standard would markedly improve agreement in results between laboratories, re-confirming the previous observation reported in 1983. Although it appeared from the study data that 10<sup>4</sup> IU/dose was equivalent to 10<sup>3</sup> LD<sub>50</sub>, ECBS recommended that this would have to be confirmed in assays of a larger number of batches of vaccine from all manufacturers tested in plaque assays in which a standard calibrated against the first international standard is included. Subsequent to this recommendation, follow-up data on the expression of yellow fever vaccine potency in IU was reported in 2004. Again, ECBS requested additional data from manufacturers on stability and clinical trial and information on the minimum PFU or LD<sub>50</sub> which results in seroconversion.

An informal WHO consultation was organized (i) to review potency data on yellow fever vaccines in current production; (ii) to review potency data expressed as IU; and (iii) to develop a proposal for a minimum potency specification in IU/dose.

The meeting was hosted by the National Institute for Biological Standards and Control (NIBSC) and attended by representatives from seven yellow fever vaccine manufacturers

and six national vaccine control laboratories. All of them, except two manufacturers, had participated in the collaborative studies on the first International Standard for yellow fever vaccine. Dr Philip Minor (NIBSC) was elected as Chair and Dr Robin Levis (CBER) as Rapporteur.

This report describes (i) the experience of manufacturers and national vaccine control laboratories with the use of the WHO International Standard for yellow fever vaccine (NIBSC 99/616), (ii) questionnaire responses on release and shelf-life specifications, the use of IS, correlation between different potency assay measurements, range of potency in IU of routine vaccine batch, proposed minimum potency in IU/dose, and 1,000 LD<sub>50</sub> in IU compared to proposed minimum potency in IU; and (iii) conclusions including a proposal for amending the current WHO recommendations on the minimum potency specification.

## **2. Experience of manufacturers with use of WHO International Standard for YF**

### **2.1 Beijing Tiantan Biological Products, China (Dr Chenglin Xu)**

To vaccinate diplomatic agents and travellers who visit disease endemic areas, the Chinese government authorized the National Vaccine & Serum Institute, its predecessor company, as the only manufacturer of yellow fever vaccine in China since 1952. The vaccine production seed is a 17D yellow fever virus, obtained by propagation in embryonated eggs from Lot1028 yellow fever vaccine produced in a laboratory of the International Health Division of Rockefeller Foundation in 1942. After 39 passages in eggs, the seed lot was constructed and passed monkey neurovirulence and viscerotropism tests in 1952.

Here is a brief description of the vaccine production process: (i) the seed viruses are inoculated into 7 to 8-day-old SPF embryonated eggs; (ii) after incubation for 70-80 hours under 37 °C, the infected embryos are harvested, homogenized, and clarified by centrifugation; (iii) the supernatants are pooled as a bulk vaccine; (iv) the bulk vaccine is then diluted with buffer, and stabilizer is added to formulate the final bulk; (v) then it is filled into ampoules and lyophilized. The final vaccine contains six doses per ampoule. Normally, the company produces 100,000-200,000 doses every year since 1952. These vaccines are used exclusively in China for travellers.

The internal release specification of the potency is, if tested in mice, that the titer of the vaccine shall be not less than 3.9 log LD<sub>50</sub>/dose, or if tested in cell culture, the titer shall be not less than 4.2 log PFU/dose. This specification was established during the 1950s. This specification is admitted by the Chinese minimum requirement for yellow fever vaccine. Before the year 2001, the mouse LD<sub>50</sub> test was used for virus titration. The vaccine sample was diluted 10 times serially, and each dilution of 10<sup>-1</sup> to 10<sup>-6</sup> was inoculated intracerebrally into six mice weighing 10-12 g, 0.03 ml/mouse. LD<sub>50</sub> was calculated after 21 days of observation.

Yearly data from 1973 - 2000 on 153 lots ranged from 6.5 to 3.5 log LD<sub>50</sub>/dose with an average of 5.5 log LD<sub>50</sub>/dose at release potency. Only one lot showed below 3.9 log LD<sub>50</sub>/dose, the release potency specification.

Data on forty-three lots from 2001-2006 showed 4.5 to 6.1 log PFU/dose with an average of 5.5 PFU/dose. Based on studies of fourteen vaccine lots produced in 1997-1999, the conversion factor of PFU/dose over LD<sub>50</sub>/dose was 5.7 (i.e. 0.75 log difference). Similar studies on WHO IS 99/616 showed the range of 1.3 – 5.4 while in house working standard calibrated against 99/616 showed 0.6 – 14.8.

In the collaborative study of the candidate IS (99/616) in 2005, the results of PFU were compared with the labelled potency of 4.5 log IU/ml. The conversion factor of PFU/IU was 0.27 (0.2-0.4) in six independent assays, which meant the labelled titer in IU is actually 3.7 times higher than the titer obtained by PFU assay (i.e.  $3.93 \log \text{PFU/ml} = 4.5 \log \text{IU/ml}$ ). An in-house reference was calibrated against the WHO IS (99/616) and the estimated titer was 7.4 log IU/ml in six independent assays. Follow-up studies on seven lots produced in 2006 revealed that the potency ranged from 5.3 to 6.4 log IU/0.5 ml dose and that the stability test resulted in 5.2 to 5.9 log IU/dose.

## **2.2 Berna Biotech, Switzerland (Dr Peter Durrer)**

Live-attenuated yellow fever vaccine, YFV Berna (Flavimun<sup>®</sup>), was derived from strain 17D-204 (working seed 112/95). The seed was acquired from Robert Koch-Institute (RKI) in Berlin.

The RKI vaccine, produced in 1963-2001, showed proven safety and efficacy record over 2.5 million doses. In 2001, RKI transferred the strain and technology to Berna Biotech which is planning to obtain marketing authorization.

Both RKI and Berna Biotech established the relationship between mouse LD<sub>50</sub> and PFU and this has been approved by the competent authority. The relationship established by RKI is: 1000 mouse LD<sub>50</sub>/dose = 15,000 PFU/dose ( 4.2 log PFU/dose). The relationship established by Berna Biotech is identical.

As a bridging trial, the Berna Biotech-produced YF vaccine (Flavimun<sup>®</sup>) was compared with the original RKI vaccine and a licensed comparator vaccine (Stamaril<sup>®</sup>). The objective of the trial was to prove non-inferiority with respect to seroprotection (primary objective)) and tolerability (secondary objective). There were no differences in seroprotection rates between consecutive production batches of Flavimun<sup>®</sup>. The results suggest that Flavimun<sup>®</sup> is non-inferior to both the RKI vaccine and Stamaril<sup>®</sup> regarding immunogenicity and safety [1].

## **2.3 Bio-Manguinhos, Brazil (Dr Darcy Hokama)**

Currently, Bio-Manguinhos produces 5-, 10- and 50-dose presentations of YF vaccine. Of these, the 5- and 50-dose presentations were prequalified by WHO.

The internal minimum potency specification of the YF vaccine at the end of shelf life was set as 1000 LD<sub>50</sub> or its equivalent in PFU (WHO, 1997). Our correlation study resulted in  $3.00 \log \text{LD}_{50}/\text{dose} = 3.73 \log 10 \text{PFU}/\text{dose}$ .

The correlation between PFU and IU on WHO IS (99/616) is:  $4.48 \log \text{PFU}/\text{dose} = 4.20 \log \text{IU}/\text{dose}$ . Using this correlation, release potency and thermostability test data were converted from PFU to IU retrospectively on released batches. Not all 5- and 50-dose lots in 1999-2007 met 4.0 log IU/dose, an estimate corresponding to 3.0 log LD<sub>50</sub>/dose in the collaborative study (WHO/BS/04.1993 and WHO TRS No.932 pp29-30).

The proposal of expressing the minimum specification for YF vaccine in IU/dose instead of LD<sub>50</sub> is an excellent choice for lot release. Considering that the YF vaccine produced in Brazil has demonstrated good efficacy from the 1940s until now, Bio-Manguinhos proposes 3.5 log IU/dose as the minimum potency for YF vaccines measured in plaque assays.

#### **2.4 Institute Pasteur Dakar, Senegal (Dr Antoine Diatta)**

Institute Pasteur Dakar used WHO IS 99/616, In House Standard (IHS) (17D strain), and commercial vaccine lots (10- and 20-dose presentations) produced between 2004 and 2007 (17D strain) (n = 145). IS, IHS and vaccine lots were stored at -20°C until use. For the stability test, vials were kept at 37 °C for 14 days and then frozen at -20°C. PS cells were used for plaque assay. Virus titration was performed as described in the WHO Technical Report Series 872, 1998. Briefly, 1 ampoule of IS, 1 vial of IHS, 3 vials per lot stored at -20 °C, and 3 vials per lot previously kept at 37 °C for 14 days were tested.

There was no significant difference between IS and our HIS in IU and PFU ( $P=0.92$  ; Non paired t test). The correlation of IS was  $4.48 \log \text{PFU/dose} = 4.20 \log \text{IU/dose}$ , therefore, the correlation factor between IU and log PFU was  $\log \text{IU/dose} = \log \text{PFU/dose} - 0.28$ .

Lots presented an IU value ranging from 3.98 to 4.92. Only 1 out of 145 lots showed a log IU value less than 4.0. All lots presented LD<sub>50</sub> values greater than 1,000. Log IU value in the thermostability test ranged from 3.58 to 4.59. Over 50% of the lots presented a log IU per dose less than 4.0.

There was good homogeneity between IS and our IHS. There is a need to determine the minimum requirement in IU (i) to avoid correlating plaque assays with mouse LD<sub>50</sub> assays, (ii) to reduce inter-laboratory variability. However, IP Dakar cautions against assigning a high level of minimum potency requirement calculated in IU, which will result in a high proportion of non-conformity lots in spite of the conformity of LD<sub>50</sub> activity. More information based on dose-ranging studies, like the work published by Grachev and his colleagues [2] will better establish relevant information about the immunological effectiveness of yellow fever vaccines.

#### **2.5 Chumakov Institute of Poliomyelitis, Russia (Dr Victor Grachev)**

To re-register the titre of the YF vaccine in IU, the Chumakov Institute of Poliomyelitis first calibrates the house standard (HS) by titrating IS 99/616 and HS in parallel, then by calculating the titre of IS and HS in PFU/dose, and then, by calculating the mean value for the each standard in PFU. This is carried out once per year. The PFU titre of HS is converted to IU using the following formula:  $\text{Titer of HS (log IU)} = \text{Titer of HS (log PFU)} - \text{Mean Titer of IS (Mean log PFU)} + 4.2$ , where 4.2 is the labelled potency of IS in log IU per dose. A value of HS in log IU is calculated for the period one year.

Next, the Institute titrates a sample of the YFV and HS in parallel, then, calculates the titre YFV and HS in PFU/dose, and then re-registers the titre of vaccine in IU using the following formula:  $\text{Titre of vaccine (log IU)} = \text{Titre of vaccine (log PFU)} - \text{Titre of HS (log PFU)} + \text{Titer of HS (log IU)}$ . For example, if the titre of vaccine (log PFU/dose) = 4.2, titre of HS (log PFU/dose) = 3.94, and then, the titre of vaccine (log IU/dose) = 4.2 –

$3.94 + 4.06 = 4.32$  IU. The assigned potency of HS in log IU in 2007 was 4.06 because IS and HS in log PFU/dose were 4.10 and 3.96, respectively.

Calculated titres in log IU per dose of the YF vaccine produced in 2004-2007 at the Institute were 4.29 (3.87~4.70) log IU/dose before heating and 3.73 (3.30 ~ 4.26) after heating.

## **2.6 Sanofi Pasteur, France (Dr Pascale Gonnet)**

Internal release potency specifications of Sanofi Pasteur (France) are  $\geq 3.3$  log mouse LD50 /dose (0.5 ml) and  $\geq 3$  log mouse LD50/dose after 14 days at 37 °C. Decrease in potency after heating should not be greater than 1.0 in the log scale. The correlation between mouse LD50 and PFU was established as "log LD50 on mice/dose = log PFU/dose – 0.8". This correlation was established in 1989 and 2000 by tests performed in parallel on PS (1989) or Vero cells (2000) and mice. Tests included in-house standard (IHS) in every plaque assay. Batch titres were adjusted according to IHS.

WHO IS (NIBSC 99/616) was tested between February and June 2004. Eighteen independent potency tests were performed in parallel with IHS. NIBSC 99/616 titre averaged 4.54 log PFU/0.5ml, which was 0.34 higher than the labelled potency 4.2 log IU/0.5 ml stated in the certificate of the IS. Therefore, "titre in log IU/0.5 ml = titer in log PFU/0.5ml – 0.34". IHS U5217(sp) titre averaged 4.72 log PFU/dose, which was converted to 4.38 log IU/dose.

Results on multi-dose batches released in January 2006 to September 2007 were expressed in log IU/dose using the established PFU and IU correlation as follows: (i) the potency was 3.90~ 4.31; (ii) Thermostability test potency was 3.48~4.03. All these batches met the internal release potency specifications (i.e.  $\geq 3.3$  log LD50 on mice/0.5 ml dose).

Cell-based plaque assay is easier and the its variability is lower than assay performed on mice. IS for cell-based plaque assay allows the laboratory to validate each test using a control chart. There may be, however, risks of increasing the chances of out-of-specification in applying the conversion factor to obtain a calculated estimate in IU, even though vaccines meet the release criteria defined by LD50 and/or its equivalent PFU. In addition, the study with NIBSC 99/616 indicates that adjusting the titre of release batches against the standard results in a decrease of 0.34 in the common log scale.

The minimum potency specification proposed in 2004, i.e. 4.0 log IU/0.5 ml dose, is too high. Sanofi Pasteur (France) proposes that it should be  $\geq 3.7$  log IU/ 0.5 ml dose, and that the potency after 14 days at 37 °C should be  $\geq 3.4$  log IU/ 0.5 ml dose.

## **2.7 Sanofi Pasteur, USA (Dr William Lapps)**

Sanofi Pasteur (USA) releases YF vaccine at the potency  $\geq 5.04$  log PFU/0.5 ml (1 log safety margin from 4.04 value proposed and supported by clinical study data). The potency should not be less than 4.74 log PFU/0.5ml throughout the life of the product. The production substrate is embryonated SPF eggs. The product of Sanofi Pasteur (USA) is licensed in USA and Canada is a traveller vaccine. Strain 17D-204 was passaged more than 232 times. In 2006, the potency in log PFU/dose ranged between 4.99 and 6.28 at the release, and 4.87~6.17 after 12 months.

It will be useful to cross-validate in-house standard and to trend assays with WHO IS. There are, however, many questions and points to consider before introducing an IU standard. How to apply IU adjustment to individual replicates and GMT? What is the impact of applying IU standard to YF products? Is inter- and intra-assay variability still high? Are clinical studies needed for changes? Are vaccines with different manufacturing techniques equivalent, for instance, passage history, cell bank history, production, stabilizers, excipients, egg substrate SPF or not, distribution and cold chain maintenance, and stability difference?

### **3. Experience of National Control Laboratories with use of WHO International Standard for YF**

#### **3.1 AFSSAPS, France (Drs Sylvie Morgeaux & Bertrand Poirier)**

Since 1996, AFSSAPS has carried out YF vaccine control and batch release for Europe and exportation to other countries through the United Nations Children's Fund. From 1996 to 2006, the Agency acted as the subcontractor NCL for the Senegalese NRA too. Since 2000, the Agency has been involved in the WHO Technical Service Agreement for vaccine quality testing.

AFSSAPS performs in vitro potency assay, thermal stability and appearance testing on official batch releases of sanofi pasteur's YF vaccines. The Agency has tested 580 batches over the last 10 years. Correlation between LD<sub>50</sub> and PFU on Vero cells was established and a corrective factor of 0.3 was adopted in 2001. The routine method of control was validated in 2000. Validity criteria was set by the control chart of the Agency's in-house standard. The pass/fail decision on the compliance of tested batches was given on the titer expressed as mouse LD<sub>50</sub>/dose, i.e. potency:  $\geq 3.3 \log \text{LD}_{50}/\text{dose}$ ; and thermal stability:  $\geq 3.0 \log \text{LD}_{50}/\text{dose}$  and loss of potency cannot exceed 1 log.

As Subcontractor NCL for the Senegalese NRA, AFSSAPS has tested 330 batches over the last 10 years. The corrective factor was "Titer in log LD<sub>50</sub>/dose = Titer in log PFU/dose – 0.7 ". Subject to WHO Technical Service Agreement, AFSSAPS tested 32 batches from 4 manufacturers since 2001.

In view of replacing vaccine potency in mouse LD50 with IU in plaque assay, AFSSAPS has been requested to use the IS in routine assays to check if the assigned specification could be met by all vaccines produced in worldwide. AFSSAPS set up control charts for WHO IS NIBSC 99/616, in-house reference, and vaccines from 3 manufacturers in 2004-2007. The Agency extrapolated potency and thermal-stability in PFU to IU for vaccines from 3 manufacturers. The average potency of IS 99/616 was 4.3 log PFU/dose. Most of the potency results from the 4 manufacturers were greater than 3.6 log IU/dose. The thermostability test resulted in a titer below 4.0 log IU/dose in many lots of all three manufacturers.

To replace LD50 unitage, the specification should be set based on correlation between IU and LD50. The Agency proposes specification for the potency as 3.6 log IU/dose. It will be necessary to assign a specification for the thermal stability. More concrete data should be available before confirming the specification in the TRS for adoption at the next ECBS meeting.



### **3.2 INCQS, Brazil (Dr Lucia Werneck)**

The National Institute for Quality Control in Health (INCQS), a technical scientific unit of Oswaldo Cruz Foundation, was inaugurated in 1981. Since 1983, INCQS has analysed about 30,000 lots of immunobiologicals for the official immunization program. Since 1983, the Institute has done quality control assays on 1,400 batches of YF vaccine.

National quality control tests for YF vaccine include visual inspection, a sterility test, a potency test by cell culture titration, a thermal stability test (loss after 14 days at 37 °C), residual ovalbumin, endotoxin, and moisture content by thermogravimetry. Diluent is also tested for pH, volume, conductivity, and chloride concentration.

Studies on correlation between LD50 and PFU was carried out in animals (Swiss Webster mice) and in cell cultures (Vero) at the same time. Each animal was intracerebrally inoculated with 0.03 ml, using 10-fold serial dilutions from 1:10 to 1:10,000, with ten mice per dilution. Plaque assay in cell cultures was carried out using 4-fold serial dilutions from 1:10 to 1:2,560. The latest study was performed in 2005. The difference between the log-transformed titres of PFU and LD50 was 0.99 (0.51~1.54), i.e.  $1000 \text{ LD50/dose} = 3.99 \text{ log PFU/dose}$ .

Validity criteria include: (i) the monolayer preserves the original morphological characteristics at the end of the test; (ii) the reference vaccine titer variation does not exceed 0.5 log PFU/dose compared to the pre-established reference titer; and (iii) the vaccine titer must be  $\geq 3.99 \text{ log PFU/dose}$  ( $\approx 3.00 \text{ log LD50/dose}$ ).

INCQS participated in the 2004 collaborative study on WHO IS for YF vaccine to correlated PFU with IU. The Institute included the in-house standard in each assay to monitor the sensitivity of the vaccine potency tests in IU. Conversion of IHS potency from log PFU to log IU per dose was done as follows:  $\text{log PFU of IS} - \text{log PFU of IHS} + 4.2$ . The Institute did fifteen independent assays, and obtained this correlation:  $4.86 \text{ log PFU/dose} = 4.2 \text{ log IU/dose}$ .

As suggested by the Study Coordinator at NIBSC, the potency and thermal stability were calculated in IU retrospectively. INCQS has analysed 110 lots from March 2006 to September 2007. The mean potency in log IU/dose before and after heating was 4.17 (2SD: 4.28~4.06) and 3.76 (2SD: 3.86~3.66), respectively. INCQS suggests a 3.5 log IU/dose as the minimum potency for YF vaccines.

### **3.3 NIBSC, UK (Dr Gillian Cooper)**

NIBSC no longer performs routine batch release because the UK producer stopped production in 2004. But the Institute still tests YF for WHO as part of the prequalification programme.

Correlation of mouse LD50 with PFU was established in 1983 in a WHO collaborative study. The relationship was 1 mouse LD50 = 2.7 PFU. The release specification at the end of shelf life was  $\geq 3.4 \text{ log PFU/dose}$  in the Institute, while the manufacturer's specification was  $\geq 3.7 \text{ log PFU/dose}$ .

The Institute has performed 55 assays with the IS and in-house reference YF20/2. The mean titre of the reference YF20/2 was 4.50 log PFU/dose. The mean titre of the IS was

4.87 log PFU/dose. The correlation factor is 0.67 log, with 1 IU = 4.7 PFU. The assigned titre for in-house reference YF20/2 was 3.9 log IU/dose.

The Institute investigated the potency expressed in IU, of YF vaccines produced in 2003-2005 by four different manufacturers (coded A to D). All lots from three of the manufacturers (A, B, and C) showed the potency  $\geq 3.5$  log IU/dose. The three manufacturers lots resulted in  $> 3.0$  after heating at 37 °C for 14 days. Several lots from one manufacturer (D) fell below these levels for an unknown reason. The proposed minimum potency is 3.0 log IU/dose.

### **3.4 NICPBP, China (Dr Guanmu Dong)**

Control tests on final products, as national requirements for YF vaccine in China, include an identity test, visual inspection of final containers, moisture content, virus titration, a thermostability test, a sterility test, and abnormal toxicity tests. The titer at lot release shall be not less than 3.9 log LD<sub>50</sub>/dose by mouse method or not less than 4.2 log PFU/dose by plaque assay. The vaccine shall be incubated at 37 °C for 2 weeks and the virus titers before or after heat exposure shall be not less than 3.9 log LD<sub>50</sub>/dose by mouse method or not less than 4.2 log PFU/dose by plaque assay. The loss of virus titer of the heat exposed vaccine shall be not more than 1.0 Log PFU/ml.

The Institute received 50 ampoules of WHO IS for YF vaccine in 2005. Two institutes participated to titrate virus titer, which are NICPBP and the National Vaccine and Serum Institute in Beijing (now Beijing Tiantan Biological Products), which manufactures YF vaccine in China. Virus titration for IS and in-house standard was carried out by two methods: one is “plaque forming unit method on Vero cells” and the other one is “LD<sub>50</sub> method in mice”. The relationship was established between both methods by 5 independent assays in parallel. The relationship between log PFU and log LD<sub>50</sub> tests of IS batch NIBSC 99/616 was 3.58 log LD<sub>50</sub>/ml = 4.33 log PFU/ml.

To express potency in IU of routine vaccine batches in 2005-2007, the Institute analysed 13 batches of domestic or imported products using the established relationship either (i) 1 PFU = 1.48 IU or (ii) log IU/dose = log PFU/dose + 0.17. The results of 13 batches ranged from 4.80-6.87 log IU/dose.

### **3.5 Swissmedic, Switzerland (Dr Jürg Stalder)**

Swissmedic compared 3 lots of YF vaccine candidate Flavimun® from Berna Biotech which resulted in a higher titer in log PFU/0.5 ml dose: 5.5~5.8 before heating and 5.1~5.4 after heating. The results of Berna Biotech were 4.9~5.2 before heating and 4.8~5.0 after heating.

The mean potency of IS 99/616 tested in 2002-2006 (n=17) showed 3.8 (3.7~4.0) log PFU/dose; therefore, 4.2 log IU/dose = 3.8 log PFU/dose.

### **3.6 Tarashevich, Russia (Prof Maya Vorobieva)**

The correlation between LD<sub>50</sub> and PFU was 0.36 (0.0027 – 1.06). This correlation was established in 1997, in Tarashevich Institute. The Institute took the volume necessary to prepare the dilutions of the same vial to inoculate animals (CBA mice) and cell culture (Vero or PS) at the same time. The Institute used 10-fold serial dilutions from 1:10 to 1:1,000,000 for animals, and from 1:100 to 1:10,000 for Vero, and 1:100 to 1:1000 for

PS cells. Animals were inoculated with 0.03 ml IC in 6 mice per dilution. The last animal test was done in 1997.

The correlation between PFU and IU was 0.30. The Institute followed the protocol provided at the 2004 collaborative studies. The Institute has done 9 assays using National Standard (NS) and IS in IU. NS was calibrated in IU: NS = 3.95 log PFU/0.5 mL (3.64~4.24) = 4.13 log IU/0.5 mL. IS = 4.02 log PFU/0.5 mL. NS was included in every plaque assay. In May 2006 – October 2007, the range of potencies in IU of routine vaccine batches was 4.12~4.38 (NS ranged 3.48~3.84). Thermostability tests ranged from 3.38~4.40. For Russian YF vaccine, the Institute usually uses IU for a minimum potency specification. The Institute proposes 3.2 log PFU/0.5 mL for minimum specification as the equivalent of 3.0 log LD50/0.5 mL. But it is important to do a clinical study to establish the minimum requirements of the potency of YF vaccine.

#### 4. Review of questionnaire responses

Dr Morag Ferguson explained that questionnaires had been sent to all participants from both manufacturers and NCLs, and that responses were obtained from almost everyone from 6 manufacturers and 6 NCLs before this meeting.

##### 4.1 Responses to questions specifically for manufacturers

	M1	M2	M3	M4	M5	M6
What is your internal release specification?	At release: $\geq 4.7 \log$ PFU/dose  End of shelf life: $\geq 4.2 \log$ PFU/dose	$> 3.8 \log$ PFU/dose	$3.9 \log$ LD50/dose, or $4.2 \log$ PFU/dose.	$3.73 \log$ PFU/dose	As per WHO, 1000 LD50 which converts to 1600 PFU	$10^{3.3}$ LD50 per dose
How was this established?	Established by initial manufacturer prior to transfer	By correlation study between plaque assay and mouse LD50	It is not known how it was established – in 1950s	1999 to 2000 using 5 and 50 dose vials	WHO	?

#### 4.2 Use of IS

Manufacturer	M1	M2	M3	M4	M5	M6
Has your in-house standard been calibrated in IU?	Yes	Yes	No	Yes	Yes	Yes
Do you include your in-house standard in every plaque assay?	Yes	Yes	Yes	Yes	Yes	Yes
Do you have any clinical data which would support your minimum potency proposal?	No dose ranging study	No	No	Yes	Yes	Clinical data from 2 production batches

NCL	L1	L2	L3	L4	L5	L6
Has your in-house standard been calibrated in IU	No (IS used to date)	Yes	Yes	Yes	No	Yes
Do you include your in-house standard in every plaque assay	No (IS used to date)	Yes	Yes	Yes	No	Yes
Do you have any clinical data which would support your minimum potency proposal?	No	No	No	No	No	yes

#### 4.3 Correlation between MLD<sub>50</sub>, PFU, and IU

Manufacturers	M1	M2	M3	M4	M5	M6
LD50 and pfu	1 LD50 = 15 pfuU	1 LD50 = 6.3 pfu	1 LD50 = 5.8 pfu or 6.6pfu Mean 6.3pfu	1 LD50 = 5.4 pfu	1 LD50 = 1.6 pfu	1 LD50 = 6.3 pfu
pfu and IU	1 pfu = 5 IU	1 pfu = 1.9 IU	1 pfu= 4 IU	1 pfu = 1.9 IU	1 pfu = 1.3 IU	1 pfu = 2.2 IU

NCLs	L1	L2	L3	L4	L5	L6
LD50 and pfu		1 LD50 = 2.7 pfu.	1 LD50= 10 PFU	1 LD50 = 2 pfu	1 LD50= 5.2 pfu	1 LD50 = 1.6 pfu
pfu and IU	1 pfu = 2.5 IU	1 pfu = 0.21 IU	1 pfu = 0.2 IU	1 pfu = 1.6 IU	1 pfu = 1.48 IU	1 pfu = 2 IU

#### 4.4 Range of potency in IU of routine vaccine batch

Manufacturers	M1	M2	M3	M4	M5	M6
Routine vaccine batches	None	3.98-4.92	5.3 -6.4	4.22 – 5.07	3.87 – 4.70	Multidose 3.9-4.31 Monodose 3.93-4.50
Routine vaccine batches on stability test?	None	3.58-4.59	5.2 - 5.9	3.60 - 4.85	3.30 – 4.26	Multidose 3.48-4.03 Monodose 3.57-4.25

NCLs	L1	L2	L3	L4	L5	L6
Routine vaccine batches			4.04 – 4.38.	4.05 - 4.70	4.80-6.87	4.12-4.38
Routine vaccine batches on stability test?			3.66-3.97	3.80-4.45	5.15-6.47	3.38-4.4

#### 4.5 Proposed minimum potency in IU/dose

M1	M2	M3	M4	M5	M6
4.9	3.55	4.3	3.50	3.0	3.4
L1	L2	L3	L4	L5	L6
At release >4.7 Log / dose and >4.2 Log pfu at end of shelf life		3.0	Approx 3.6	4.5	3.2 (pfu)

#### 4.6 1000 LD50 in IU compared to proposed minimum potency in IU

Manufacturers	M1	M2	M3	M4	M5	M6
1000 LD50 in IU	75000	11970	12600	10260	2080	13860
Proposed minimum potency in IU	79432	3548	19952	3162	1000	2511

NCLS	L1	L2	L3	L4	L5	L6
1000 LD50 in IU		567	2000	3200	7696	3200
Proposed minimum potency	50118		1000	3981	31622	3.2 (pfu)

## 5. Conclusions

The participants agreed on the following conclusions: The current potency requirement is 3 log mouse LD<sub>50</sub> per dose. LD<sub>50</sub> is variable between laboratories and IU is not as variable. Specifications for current products now vary. Variability is increased by variability in the measurement of LD<sub>50</sub> between laboratories. Specifications could be made more comparable by expressing them in IU. All existing products are believed to be satisfactory based on clinical use of vaccines at the approved potency. Existing specifications should not be changed unless justified by clinical data. The minimum specification in current use corresponds to 3 log IU per dose.

The participants agreed in principle on the following proposal for amending the WHO recommendations for the clinical minimum potency specification: The potency of yellow fever vaccines should be expressed in IU. In existing vaccines, changes from already established specifications to new specifications should be justified by clinical data.

Transfer of production from one manufacturer to another should include specifications in IU, not mouse LD<sub>50</sub>. Specifications of new products should be set by clinical trial and expressed in IU. The specification should be equal to or greater than 3.0 log IU per dose.

During the meeting, the participants requested WHO to provide detailed guidance on calibrating the working standard against the first WHO International Standard for yellow fever vaccine, partly because this is the first live attenuated vaccine whose potency is proposed to be expressed in IU and partly because discrepancies in practice were recognized in this discussion.

Accordingly, the proposal and need for further guidance will be reported to the WHO Expert Committee on Biological Standardization at the 59<sup>th</sup> meeting in October 2008, to be considered for adoption and further developments.

## References

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