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# WHO COLLABORATIVE STUDIES - YELLOW FEVER VACCINE POTENCY TEST

by

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## 1. PURPOSE

The purpose of this test is to estimate the amount of active virus contained in the vaccine.

There are several methods of conducting virus particle counts. Among biological methods two are currently used:

inoculation of susceptible hosts with varied dilutions of the test suspension and recording for each dilution of the number of positive reactions. The $LD_{50}$ is determined statistically;

the plaque method, which has the advantage of being extremely simple and highly reproducible. The average number of plaques formed per dilution gives an indication of the virulence of the suspension to be titrated. This is expressed in plaque units per unit volume or PFU/ml.

Up till now the potency of the 17D vaccine has been defined as the number of mouse $LD_{50}$s per volume of the recommended human dose.

For the reasons mentioned above and also because it is easy to standardize a cell system, the use of the plaque method for titrating 17D seems highly recommendable. It is necessary however to conduct trials beforehand in order to determine whether the method can be used in all the control laboratories and to define the new potency standards in relation to the new method. That is why a collaborative study has been conducted by WHO on the basis of a protocol drawn up by Dr F. T. Perkins. The protocol envisages titration of four vaccine preparations distributed to all the participating laboratories and a vaccine prepared locally which will subsequently be used as a secondary reference batch. Each preparation will be titrated four days in succession both in mice and in cell culture.

To begin with it is wished to recall briefly the method of titration in mice; the plaque method as laid down for the collaborative study will then be discussed.

## 2. MOUSE TEST

### 2.1 Material and methods

2.1.1 Vaccine. Three final containers are selected at random and rehydrated to the concentration indicated for use by means of the diluent advocated by the manufacturer. After 20 minutes' diffusion at 20-30°C, serial tenfold dilutions are made from each ampoule, using as diluent a solution consisting of 0.75% bovine albumin, fraction V, in isotonic sodium chloride solution buffered with phosphate to a pH of 7.4, or any other equivalent diluent (phosphate buffer containing 10% normal rabbit serum). The dilutions are carried out in an ice-water bath and stored there until the mouse is inoculated.

2.1.2 Mouse. A highly susceptible strain must be used. Inoculations are given intracutaneously to at least six 4-6 weeks old mice for each dilution. The mice are kept under observation for 21 days.

### 2.2 Results

The results are expressed in $LD_{50}$.

Calculations should establish that the ready-to-use vaccine does not contain less than 1000 mouse $LD_{50}$s per human dose.

### 2.3 Discussion

An important criticism that can be made of this method is that it depends essentially on the condition of the mice. In fact, even when a highly susceptible strain is used variations are observed in response to climatic conditions, susceptibility being less during very hot weather.

## 3. TEST IN CELL CULTURES

### 3.1 Material and methods

3.1.1 Vaccine. The vaccine in the ampoules is rehydrated in culture medium (Leibovitz L15 medium containing 3% foetal calf serum). Fourfold dilutions are made (1 ml of virus suspension in 3 ml of diluent) at the rate of five dilutions per vaccine.

3.1.2 Cell cultures. For the titration of yellow fever virus the PS cell line is the most sensitive, although it is chronically contaminated with swine fever virus. VERO cells can also be used. If for each laboratory a bank is established containing cells preserved in liquid nitrogen, it is possible always to use cells which have undergone the same number of passages. The techniques of cell propagation used are the routine ones.

3.1.3 Titration. The technique used is an application of the method of De Madrid & Porterfield, 1969. When confluent cell growth has been obtained, the cells are trypsinized and suspended in Leibovitz L15 medium in such a way as to obtain about $6 \times 10^5$ cells per ml.

Equal volumes (0.2 ml) of cell suspension and virus dilution are placed in the wells of a Linbro FB 16 24 TC tray, four wells being used for each dilution.

The trays are covered and incubated for four hours at 36°C. After this period of incubation 0.4 ml of overlay medium is added to each well. This medium is made up of:

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| Leibovitz L15 medium | 47% |
| Foetal calf serum | 3% |
| Carboxymethyl cellulose, 3.2% | 50% |

The trays are covered and incubated at 36°C for five days.

After this incubation the preparation is washed with saline, stained in naphthalene black for 30 minutes then rinsed in tap water and dried.

3.2 Results

3.2.1 Qualitative. Under the conditions described the yellow fever virus produced easily countable plaques, 2.3 mm in diameter.

3.2.2 Quantitative. The purpose of the method is of course titration of the infective particles. The use of fourfold instead of tenfold dilutions makes it possible to arrive more easily at the exact titre by using dilutions in which the plaques are clearly countable. Calculation of the titre, i.e. of the number of plaque units per unit volume (PFU/ml), is carried out taking into account:

the dilution of the virus suspension C;
the number of cells counted n;
the total number of plaques obtained N.

The relative error is inversely proportionate to the square root of the total number of plaques counted i.e. $\frac{1}{\sqrt{N}}$ .

3.3 Discussion

There are numerous advantages in using a cell culture system for the titration of virus particles. Among them is the fact that all laboratories can use the same cell culture with the same number of passages in the same medium and this eliminates variations resulting from the use of different strains of mice.

The use of PS cells, which have been shown to be regularly sensitive to yellow fever virus, and their ready growth in Leibovitz L15 medium without the use of a $CO_2$ incubator, together with substitution of carboxymethyl cellulose for agar, are all simplifications which should make the plaque-counting technique the method of choice for titrating the potency of yellow fever virus.

REFERENCES

1. De Madrid, A. T. & Porterfield, J. S. (1969) Bull. Wld Hlth Org., 40, 113-121

2. Inoue, Y. K. & Ogura, R. (1962) Virology, 16, 205-207

3. Wld Hlth Org. techn. Rep. Ser. (1957) No. 136

4. Wld Hlth Org. techn. Rep. Ser. (1959) No. 179

5. Salim, A. R. (1967) Trans. roy. Soc. trop. Med. Hyg., 61, 259-264