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REPORT OF THE CONSULTATIVE GROUP ON LABORATORY INVESTIGATION
OF DRIED SMALLPOX VACCINE

A meeting was held, from 23-25 June 1952, in Geneva attended by the Directors of four laboratories which had agreed to co-operate with WHO in subjecting to test specimens of dried smallpox vaccine. The following members comprised the Consultative Group:

1. Miss K. Andersen (representing Professor Ørskov), State Serum Institute, Copenhagen, Denmark.
2. Dr. G.D. Cummings, Division of Laboratories, Department of Health, Lansing, Michigan.
3. Professor R. Fasquelle, Institut de Vaccine Animale, 8 rue Ballu, Paris IX^e.
4. Dr. D. McClean, Lister Institute of Preventive Medicine, Elstree, Herts, England.

Dr. R. Muckenfuss, Assistant Commissioner of Health, 125 Worth Street, New York City, attended as an observer.

Dr. McClean was elected Chairman.

The Consultative Group of laboratory directors was convened to define and adopt practical details for the laboratory investigation of the rate of loss of potency of dried smallpox vaccines when kept at various temperatures. The investigation was planned to ensure, by the adoption of uniform procedures, that the results of the tests carried out by the several laboratories would be comparable.

The Group met five times and the following decisions and recommendations were made:

1. TEMPERATURES AT WHICH VACCINES UNDER TEST ARE TO BE STORED

It was agreed that the temperatures at which the vaccines should be stored would be

0°C 22°C 37°C 45°C

0°C was held to be a temperature within the range 0° to 4°; and
22°C was held to be a temperature within the range 19° to 23°C.

2. TIME INTERVALS AT WHICH POTENCY TESTS WILL BE CARRIED OUT

The following time schedule was agreed upon:

<u>Material stored at</u>	<u>To be tested</u>
0°C (0° to 4°)	On receipt and at 4, 12, 24 and 52 weeks
22°C (19° to 23°)	2, and 4 weeks, then at 4-week intervals
37°	2, 4, 6 and 8 weeks, then at 4-week intervals
45°	1, 2, 4, 6 and 8 weeks, then at 4-week intervals.

This test schedule will be adhered to as far as is practicable but if a laboratory is not able to test a vaccine at the proper time the sample may be removed from the temperature at which it has been stored and placed at 0°C or below until the test can be carried out. It is important that such a test should be carried out as near as possible to the stipulated time.

3. TIME INTERVALS AT WHICH PURITY TESTS WILL BE CARRIED OUT

A purity test will be carried out by the testing laboratory on receipt of each vaccine. A purity test will only be carried out again if a vaccine does not conform to the standard laid down (namely, that it is free from anaerobic and aerobic pathogens and that it does not contain more than a total of 1,000 bacteria per ml.). Should this occur the sample kept at 22°C will be tested for purity when its potency has fallen below the level defined in paragraph 4. A sample which, on arrival, fails to reach the purity standard laid down will nevertheless be subjected to tests for maintenance of potency and retested for purity as described above.

4. THE MINIMUM POTENCY STANDARD BELOW WHICH A SAMPLE WOULD NOT BE TESTED FURTHER

Although it was agreed that the minimum potency standard to which a vaccine should conform and below which it should not be issued for use was that it shall give confluent lesions at a dilution of 1 in 1,000 and shall produce not less than 10 vesicles at 1 in 9,000, it was decided that potency tests should be continued on each vaccine until it fails to give confluent lesions at a dilution of 1 in 100. When a vaccine fails to achieve the potency at which it is suitable for issue and when it fails to produce confluent lesions at a dilution of 1 in 100 the final tests should be repeated at least once.

5. STANDARDIZED TECHNIQUE FOR POTENCY TESTING

The standardized technique for potency testing will be by scarification on the skin of a rabbit. The following dilutions will be used:

1 in 100
1 in 1,000
1 in 3,000
1 in 9,000
(if necessary 1 in 27,000)

A quantity of 0.2 ml of the appropriate dilution will be spread over an area of 10 sq.cm.

or

A quantity of 0.1 ml of the appropriate dilution will be spread over an area of 5 sq.cm.

Two rabbits will be used for each titration. In order to control the susceptibility of the test animal, appropriate dilutions of a laboratory standard lymph should be similarly inserted by scarification on each rabbit used. The diluent must be an isotonic buffered solution at or near neutrality. A fresh pipette will be used for each dilution. It was decided that it would not be practicable to use a pock count on the chorio allantoic membrane of the embryonated egg as a standard method in the experiment but that this method may be used as an additional method for checking results.

6. STANDARDIZED TECHNIQUE FOR PURITY TESTING

After detailed explanations had been given by each of the directors of the methods for purity testing in use in their laboratories, it was decided that no standardized procedure should be defined for purity testing. The Group approved, for the purpose of the experiment, the methods in use in the different laboratories. It was decided that in addition to determining the total bacterial content the purity test should be designated to detect the presence of:

Cl. tetani, Bac. anthracis, B-haemolytic streptococci, Staphylococcus aureus.

Any strain of Staph. aureus isolated should be regarded as potentially pathogenic if it produces coagulase.

7. DETAILS CONCERNING SUPPLY OF VACCINES BY PRODUCING LABORATORIES

(a) Amount of vaccine in each ampoule and number of ampoules to be sent:

Each "testing" laboratory would require from each producing laboratory 80 ampoules, each containing not less than 1 ml of the wet glycerinated preparation and 80 ampoules containing an equivalent amount of the dried preparation. An ampoule, once it is opened for test, must be discarded unless the contents are to be used for another observation after the same storage period; in which case it may be retained at 0°C or below until the further observation is carried out.

It was agreed that the producing laboratories should be asked not to supply their samples for test in capillary tubing in view of the difficulty of removing the contents completely from this type of container.

(b) Information to be included in the protocol supplied to the testing laboratories:

It was agreed that the producing laboratories should be asked, when submitting their samples for test, to state:

- (i) Identifying symbols of the vaccine and laboratory of origin.
- (ii) The method of preparation including whether any antibiotic or any bacteriostatic or bacteriocidal substance has been used.

- (iii) The method of dispensing the vaccine in containers (whether in air, vacuo or nitrogen).
- (iv) Instructions for its use as issued with the vaccine.
- (v) The results of purity tests and potency tests carried out on the vaccine, and stated in terms normally used by the laboratory.
- (vi) A statement of the titre below which a vaccine would not be issued for general use.

It was agreed that the producing laboratories would be requested to furnish information on the differences in temperature resistance of the various batches of dry vaccine which they have made. These results, if made available, may help to shorten any subsequent investigation undertaken by testing laboratories into temperature resistance of individual batches.

(c) Method of transport and packing:

It was agreed that prepaid air freight would be the method of transport of the samples and that steps should be taken by the producing laboratories to ensure that the samples were maintained during transit as near 0°C as possible. The directors of the producing laboratories present agreed to investigate appropriate air routes in order to select a method of carriage to enable the samples to be received in the shortest possible time. In each case the vaccines should be packed with dry ice and arrangements made, where necessary, for replacement of the dry ice during transit.

It is important in this connexion that the directors of testing laboratories ensure that appropriate import licences are obtained from their national governments in order to obviate any delay through formalities on landing.

Producing laboratories should warn the testing laboratories by cable of the date and time of despatch, the estimated time of arrival together with the airline and flight number of the aircraft carrying the samples for test. Directors of the testing laboratories, when the import licence has been received, should notify the producing laboratories of this fact, together with details of the licence, including the name of the holder, so that appropriate mention may

be made on the outside of the package. A statement that the contents are of no commercial value and are for research purposes should appear on a label on the package. Consignments for the Institut de Vaccine Animale, Paris, should be labelled in French.

8. DATE ON WHICH LABORATORY INVESTIGATION WILL COMMENCE

It was agreed that the laboratory investigations would not commence before 1 November 1952 but that in any case all samples should be in the hands of the testing laboratories before the end of that month, preferably earlier. Where a laboratory decides, for convenience, to commence all investigations on the dry and wet preparations at the same time, there would be no objection to a preliminary storage of the consignment at or below 0°C from the date of arrival until the appropriate time.

9. METHOD OF RECORDING RESULTS

(a) It was agreed that a mimeograph form would be designed which would be circulated to the directors of the testing laboratories. This form will be designed to permit the results to be presented in summary form adequate for statistical analysis. The method of recording results will include adequate identification of each vaccine tested, date of receipt of the vaccine, the date on which the experiment is started, the temperatures of storage and time intervals of tests, and the separate recording of each potency test and each test for bacterial purity. Any repeat tests which are performed are to be separately recorded.

In addition, information will be given of the area vaccinated and amount of inoculum used in all tests.

(b) Terminology to be used for the description of the lesions:

- | | | |
|-----|-------------------|--|
| c | = confluent | = lesions covering the whole area |
| sc+ | = semi-confluent+ | = lesions covering 70-80% of the area |
| sc | = semi-confluent | = lesions coalescing but covering 50-70% of the area |

sc- = semi-confluent- = lesions coalescing but covering substantially less than 50% of the area.

Discreet and counted pustules will be indicated by the appropriate figure.

10. NUMBER OF DRIED VACCINES AND THEIR CORRESPONDING WET PREPARATIONS WHICH EACH LABORATORY WILL SUBJECT TO TEST

Four "producing" laboratories have agreed to co-operate and supply, for test, samples of their dried smallpox vaccine and of a wet preparation prepared from the same strain of vaccinia -

1. Institut de Vaccine Animale, 8 rue Ballu, Paris IX (Professor Fasquelle)
2. Division of Laboratories, Department of Health, Lansing, Michigan (Dr. Cummings)
3. Staatliche Impfanstalt und Staatliches Serumprüfungsinstitut, Possingergasse 38, Vienna XVI/107 (Dr. Puntigam)
4. Institut Pasteur, Djalan Pasteur No. 9, Postbox 47, Bandung (Prof. R. Md. Djuhana Wiradikarta)

In addition to the following four laboratories which have agreed to act as "testing" laboratories, Dr. R. Muckenfuss, Assistant Commissioner of Health of New York City, offered to co-operate, thus reducing the load of work on some of the laboratories.

The testing laboratories therefore are:

1. State Serum Institute, Copenhagen
2. Division of Laboratories, Department of Health, Michigan
3. Institut de Vaccine Animale, Paris
4. Lister Institute of Preventive Medicine, Elstree, Herts., England
5. Bureau of Laboratories, New York City Department of Health

(Names and postal addresses are given in the Introduction to this report).

11. ALLOCATION OF MATERIAL FOR TEST

The following allocation of material to be tested was agreed:

- (a) the Lister Institute test the dried and wet preparations produced by the Institut de Vaccine Animale, Paris, and the Michigan Department of Health;
- (b) the Institut de Vaccine, Paris, test the dried and wet preparations produced by the Staatliche Impfanstalt und Staatliches Serumprüfungsinstitut in Vienna, the Pasteur Institut, Bandung, and the Michigan Department of Health;
- (c) the Michigan Department of Health test the dried and wet preparations produced by the Institut de Vaccine Animale, Paris, the Staatliche Impfanstalt und Staatliches Serumprüfungsinstitut in Vienna and the Pasteur Institut, Bandung.
- (d) the State Serum Institute, Copenhagen, test the dried and wet preparations produced by the Institut de Vaccine Animale, Paris, and the Pasteur Institut, Bandung.
- (e) New York City Laboratory test the dried and wet preparations produced by the Staatliche Impfanstalt und Staatliches Serumprüfungsinstitut in Vienna and the Michigan Department of Health.

12. GRANT TOWARDS THE COST OF THE INVESTIGATION

The terms of a contract to allocate the funds at the disposal of the Organization for the purpose of the investigation were placed before the Group. The members of the Group agreed, prior to signature, to discuss final details with the directors of the Institutes concerned. They stated that they did not anticipate any difficulties in obtaining signatures to the proposed contract.

13. RECOMMENDATION FOR FURTHER RESEARCH

As the laboratory investigation is only of a preliminary nature, the Group is of the opinion that, at the termination of the experiment, and after a statistical analysis of the results has been made, the findings should be subjected to a critical review by a group of experts in order that recommendations for further research in the use of dried smallpox vaccine may be made.