

World Health Organization

Technical Report Series

No. 2

EXPERT COMMITTEE ON
BIOLOGICAL STANDARDIZATION

Report on the Third Session

London, 2-7 May 1949

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WORLD HEALTH ORGANIZATION

PALAIS DES NATIONS

GENEVA

FEBRUARY 1950

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Third Session

Members :

- Professeur E. Grasset, Directeur de l'Institut d'Hygiène, Geneva, Switzerland
- Dr A. A. Miles, Director, Department of Biological Standards, National Institute for Medical Research (Medical Research Council), London, United Kingdom
- Dr J. Ørskov, Director, State Serum Institute, Copenhagen, Denmark
- Major-General Sir Sahib Singh Sokhey, Director, Haffkine Institute, Bombay, India
- Dr W. Aeg. Timmerman, Director, Rijks Instituut voor de Volksgezondheid, Utrecht, Netherlands (*Chairman*)
- Professeur J. Tréfouël, Directeur de l'Institut Pasteur, Paris, France
- Dr M. V. Veldee, Chief, Biologics Control Laboratory, National Institutes of Health (US Public Health Service), Bethesda, Md., USA

Co-opted Members :

- Dr J. Bretey, Chef de la Division de la Tuberculose, Institut Pasteur, Paris, France
- Dr J. Chevé, Directeur de l'Annexe de l'Institut Pasteur, Laroche-Beaulieu (Dordogne), France
- Dr N. K. Jerne, Acting Chief, Department of Biological Standardization, State Serum Institute, Copenhagen, Denmark

Secretary :

- Dr R. Gautier, Assistant Director-General, WHO

The report on the third session of this committee was originally issued in mimeographed form as document WHO/BS/70, 13 May 1949.

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Report on the Third Session¹

The Expert Committee on Biological Standardization held its third session in London on the premises of the Medical Research Council, 26 Old Queen Street, from 2 to 7 May 1949.

In addition to the members and co-opted members, one expert on blood groups (Dr R. R. Race, Director, Blood-Group Research Unit, Lister Institute of Preventive Medicine, London), two experts on endocrinology (A. S. Parkes, National Institute for Medical Research, London, and C. Hewett, Organon Laboratories, Glasgow), two experts on immunology (Dr A. Felix, Director, Central Enteric Reference Laboratory and Bureau, Public Health Laboratory Service, London, and Dr D. A. Long, National Institute for Medical Research, London), one expert on pharmacology (Dr W. L. M. Perry, National Institute for Medical Research, London), and one expert on vitamin B₁₂ (E. Lester Smith, Glaxo Laboratories, Greenford, Middlesex) attended part of the session.

The Assistant Director-General outlined the steps taken to implement the decision of the First World Health Assembly² regarding the establishment of an expert committee on biological standardization.

Dr Timmerman was elected chairman and Dr Miles rapporteur.

The chairman felt sure that the committee would first wish to express its sorrow for the recent death of Mr P. Bruce White, who had attended the second session of the Interim Commission's expert committee as a specialist on cholera. The committee would remember Mr Bruce White not only as an outstanding bacteriologist and serologist, but also as a good friend.

¹ The Executive Board at its fourth session adopted the following resolution:

“The Executive Board (1) NOTES the report of the Expert Committee on Biological Standardization on its third session and the report of its Subcommittee on Fat-Soluble Vitamins, and (2) AUTHORIZES their publication.” *Off. Rec. World Hlth Org.* 22, 3

² *Off. Rec. World Hlth Org.* 13, 307

1. Ogawa and Inaba Cholera Vaccines and Diagnostic Antisera ³

The committee noted that the recommendations made by the Expert Committee on Biological Standardization of the Interim Commission of the World Health Organization at its second session, regarding the provision of reference prophylactic vaccines, of freeze-dried living cultures, and of freeze-dried antigens for the production of diagnostic antisera in rabbits, had been implemented.⁴ In the light of recent advances in the antigenic analysis of the cholera and other vibrios, however, the committee decided to defer the establishment of the freeze-dried antigens for rabbit immunization as reference preparations, pending a reinvestigation of the strains employed.

The committee recommends that the suitability of the Ogawa and Inaba prophylactic vaccines as reference preparations should be tested by comparative assay against three "unknown" vaccines of different potencies. Sir Sahib Singh Sokhey agreed to prepare the unknown vaccines. Laboratories in five countries will take part in the assay.

2. Anti-Pertussis Vaccine ⁵

The committee decided to defer the establishment of an international standard preparation for an *Haemophilus pertussis* vaccine until sufficient information was available on the following points:

(1) The relation between immunizing potency in laboratory animals and in man. It was noted that information on this point would probably be forthcoming within a year.

(2) The consistency of results obtained by independent workers when several vaccines are compared with a standard preparation.

Among the difficulties in reaching agreement about the potency of pertussis vaccines as measured by current methods are the variable interpretations of turbidity of vaccines in terms of bacterial content, and the variable results obtained in the intracerebral tests in mice.

Dr Veldee agreed to place at the disposal of the interested laboratories samples of the US National Institutes of Health (NIH) turbidity standard, which is a suspension of particles of Pyrex glass in water, together with samples of the NIH reference vaccine measured against this standard,

³ White, P. Bruce, WHO/BS/52; Sokhey, S.S., WHO/BS/66; Gallut, J., WHO/BS/69; unpublished working documents

⁴ *Off. Rec. World Hlth Org.* 11, 8

⁵ Veldee, M. V., WHO/BS/54; Evans, D. G., WHO/BS/62; unpublished working documents

with a view to obtaining opinions in various countries regarding the suitability of a Pyrex glass suspension as a turbidity standard; and to make available the strains of *H. pertussis* and of mice used in the NIH laboratories for the intracerebral test.

3. Smallpox Vaccine

The committee considered the investigations necessary for the definition of minimum requirements for smallpox vaccines. To this end it recommended that the seed vaccinia virus used in different countries for the preparation of smallpox vaccines be tested in rabbits for its immunizing potency against freshly isolated human variola virus.

The committee considered that India was a suitable country for such investigations and, at the suggestion of Sir Sahib Singh Sokhey, it recommended that WHO should ask the Government of India to arrange for the testing of seed virus in this manner through the mediation of WHO.

4. Diphtheria and Tetanus Toxoids ⁶

The committee decided that the purified diphtheria and tetanus toxoids intended to serve as international standard preparations should be compared with the plain toxoids currently prepared in different countries for the immunization of man, with a view to ascertaining the amount of the proposed standard preparations to which convenient unit immunizing potency might be assigned.

5. Streptococcus Antitoxin ⁷

A suitable preparation of streptococcus antitoxin has been procured by the Department of Biological Standards, National Institute for Medical Research (NIMR), London. Preliminary tests indicate that the preparation is suitable for an international standard. Estimation of the potency of this preparation in terms of the US National Institutes of Health standard streptococcus antitoxin is to be made by laboratories in four countries.

6. Tetanus Antitoxin

The committee noted that informal opinion in various countries was in favour of unifying the dual notation of potency—the international and US units—in current use. Having regard to the circumstances of the original definition of the first international unit for tetanus antitoxin, it

⁶ Department of Biological Standardization, State Serum Institute, Copenhagen, WHO/BS/48; Jerne, N. K., WHO/BS/68; unpublished working documents

⁷ Department of Biological Standards, National Institute for Medical Research, London, unpublished working document WHO/BS/60

recommended that the weight of the international standard preparation, to which international unit potency is at present assigned, should be doubled, so that in future the international unit for tetanus antitoxin will be equal to the US (NIH) unit. The committee recommended that this change be announced as soon as possible and that this notation should be universally adopted for new issues not later than 1 July 1950.

7. Detection of Tubercle Bacilli ⁸

The committee considered that the problem of devising standard techniques for the detection of tubercle bacilli in pathological material should be studied in close collaboration with the Expert Committee on Tuberculosis, preferably by a joint subcommittee.

8. Serodiagnosis of Typhoid and Paratyphoid Infections ⁹

The committee accepted the proposal of Dr A. Felix to prepare eight horse antisera for the specification of agglutinating suspensions for use in the serodiagnosis of typhoid and paratyphoid infections. Antisera specific for the following antigens will be made: *Salmonella typhi* (H); *Salmonella typhi* (O); *Salmonella typhi* (Vi); *Salmonella paratyphi A* (H); *Salmonella paratyphi A* (O); *Salmonella paratyphi B* (H); *Salmonella paratyphi B* (O); *Salmonella paratyphi*, non-specific (H).

The committee recommended that the antisera should be examined in laboratories in six countries for their suitability as standard preparations, and that, if suitable, they should be adopted as international standard preparations. It decided to defer the definition of agglutinating potency until its next meeting.

9. Serodiagnosis of Rickettsial Infections ¹⁰

The committee accepted the proposal of Dr A. Felix to prepare horse antisera for the specification of the X strains of *Proteus* for specifying agglutinating suspensions to be used in the serodiagnosis of rickettsial infections. Antisera for the following antigens will be made: *P. OX19*; *P. OXK*; *P. OX2*. The committee recommended that the antisera should be examined in laboratories in six countries for their suitability as international standard preparations.

⁸ Timmerman, W. Aeg., unpublished working document WHO/BS/57

⁹ Felix, A., document WHO/BS/53; to be published in *Bull. World Hlth Org.* 1950, 2, No. 3

¹⁰ Felix, A., document WHO/BS/63; to be published in *Bull. World Hlth Org.* 1950, 2, No. 3

The committee recommended that interested workers be asked for their opinion about the desirability of standard antisera for specifying rickettsial suspensions.

10. Serodiagnosis of Syphilis

The committee noted that the question of the serodiagnosis of syphilis was being considered by a special subcommittee of the Expert Committee on Venereal Infections¹¹ and expressed a wish that a member of the Expert Committee on Biological Standardization should be invited to attend the meetings of the subcommittee and the planned International Serological Laboratory Conference.

11. PPD¹²

Further tests of various preparations of PPD have shown that their sensitizing properties do not debar them from use as standard preparations. The reaction they produce in hypersensitive animals and man, however, are qualitatively different in a significant degree. Moreover, PPD and similar materials prepared in different ways contain variable proportions of active fractions of different molecular weight. The committee therefore decided to defer the establishment of a reference preparation until its next session, and meanwhile recommended and arranged for a comparative biological and physicochemical examination in five laboratories of representative types of purified tuberculo-proteins.

12. BCG¹³

The committee approved the reports submitted at the request of the United Nations International Children's Emergency Fund (UNICEF) by Drs Timmerman & Gautier, Dr Timmerman, and Sir Sahib Singh Sokhey on the preparation of BCG at the State Serum Institute, Copenhagen, the Institut Pasteur, Paris, the Institut Pasteur d'Algérie and the King Institute, Madras.¹⁴

The committee discussed the conditions necessary for the production of safe and effective BCG vaccine and defined them (see Annex 1, page 14).

¹¹ Expert Committee on Venereal Infections. Report of the Subcommittee on Serology and Laboratory Aspects (unpublished working document WHO/VD/38). After approval by the Executive Board, this report will be published as *World Health Organization: Technical Report Series*, no. 15.

¹² Grasset, E., WHO/BS/59; Long, D. A., WHO/BS/64; unpublished working documents

¹³ Holm, J., unpublished working document WHO/BS/45

¹⁴ Timmerman, W. Aeg. & Gautier, R., JC2/UNICEF/WHO/1, JC3/UNICEF/WHO/2; Timmerman, W. Aeg., JC3/UNICEF/WHO/32; Sokhey, S. S., JC3/UNICEF/WHO/1; unpublished working documents. See also *Off. Rec. World Hlth Org.* 22, 42, 44

It recommended that laboratories manufacturing BCG on behalf of UNICEF should conform to the specifications contained in this document.

The committee noted that the Institut Pasteur, Paris, was now ready to serve as the world centre for the preparation and distribution of freeze-dried cultures of living BCG for use as seed culture for the preparation of BCG vaccine. The distribution will be made monthly, direct to the laboratories producing BCG for the UNICEF vaccination campaign and to other interested laboratories on the recommendation of the national control centres.

13. Digitalis¹⁵

Fifteen of the 17 participants in the comparison of the proposed third international standard for digitalis with the second international standard have submitted their results. The proposed standard preparation is of suitable quality and its potency is almost identical with that of the second international standard. The exact designation of potency must await the completion of the assays. The committee recommended that the Department of Biological Standards, National Institute for Medical Research, London, be authorized, after due consultation with participating laboratories, to assign to this standard the potency indicated by the combined results of the 17 assays.

14. Sulfarsphenamine

A suitable batch of sulfarsphenamine has been procured by the Department of Biological Standards, National Institute for Medical Research, London. Preliminary comparisons with the second international standard sulfarsphenamine indicate that the new preparation is suitable for the third international standard. A final comparison of the two preparations is to be made by laboratories in five countries.

15. Anti-Pernicious-Anaemia Factor^{16,17}

A standard preparation for the measurement of the anti-pernicious-anaemia potency of extracts of liver and other animal tissues is urgently required. The recent isolation of vitamin B₁₂ provides a substance which may prove to be suitable for such a preparation. The scarcity and high

¹⁵ Department of Biological Standards, National Institute for Medical Research, London, unpublished working document WHO/BS/51

¹⁶ Smith, E. Lester, document WHO/BS/61; to be published in *Bull. World Hlth Org.* 1950, 2, No. 3

¹⁷ Hampshire, C. H., unpublished working document WHO/BS/58

potency of these materials would entail dispensing the standard preparation in exceedingly small quantities. Tests of the stability of dry vitamin B₁₂ in these conditions are in progress.

In the meantime, the committee recommended that the Department of Biological Standards, National Institute for Medical Research, London, be authorized to procure pure vitamin B₁₂ in a sufficient quantity to serve as a standard preparation for anti-pernicious-anaemia factors and to ascertain the opinion of interested workers about the preferred size of a unit of activity.

16. Hormones

16.1 *Oestrone and oestradiol monobenzoate*¹⁸

The committee recognized that preparations of oestradiol monobenzoate and of oestrone were no longer necessary as biological standards and recommended that their issue as international biological standards should cease on 1 January 1951. In the meantime, users of the standards should be warned of this decision.

16.2 *Thyrotrophin and corticotrophin*

The committee considered the need for continuing with the preparation of a standard for thyrotrophin recommended by the Third International Conference on the Standardization of Hormones (1938),¹⁹ and interrupted by the war, and the need for a standard for corticotrophin. The committee recommended that the opinion of interested workers should be sought on these matters.

16.3 *Androsterone*²⁰

The committee recognized that the international standard preparation of androsterone is mainly used as a pure chemical standard and, as such, is obsolete as a biological standard. However, since the standard is still to some extent used in biological assay, the committee recommended that the international standard androsterone, the stock of which is nearing exhaustion, should be replenished, but that users should be warned that the distribution of this standard, when stocks again become low, will be restricted to those using it for biological assay.

¹⁸ Department of Biological Standards, National Institute for Medical Research, London, unpublished working document WHO/BS/50

¹⁹ *Bull. Hlth Org. L.o.N.* 1938, 7, 887

²⁰ Department of Biological Standards, National Institute for Medical Research, London, unpublished working document WHO/BS/50

The committee authorized the Department of Biological Standards, National Institute for Medical Research, London, to proceed with the replenishment of the standard preparation.

16.4 *Secretin*

The committee considered the proposal for the establishment of an international standard for secretin and recommended that more information about the demand for this standard should be obtained.

17. Fat-Soluble Vitamins

The committee adopted parts I, II and III of the Report of the Subcommittee on Fat-Soluble Vitamins²¹ and noted the proposal in part IV concerning the estimation of vitamin content in foodstuffs.

The committee recommended that the international standard for vitamin A shall be crystalline all-*trans* vitamin A acetate, having the characteristics and potency described in Part I of the Report; and that the existing standard preparation of pure all-*trans* β -carotene ceases to be the international standard for vitamin A and becomes the international standard for provitamin A, having the potency indicated in the report.

The committee recommended that the international standard for vitamin D shall be the preparation of crystalline vitamin D₃, having the characteristics and potency described in Part III of the report; and that the existing standard preparation of irradiated ergosterol ceases to be the international standard for vitamin D but is maintained as a reference preparation.

18. Antibiotics

18.1 *Penicillin*²²

The stocks of the international standard for penicillin are running low. The committee therefore decided to procure a preparation of pure penicillin G (II) to serve as the second international standard for penicillin. The Department of Biological Standards, National Institute for Medical Research, London, was authorized to institute an assay of the proposed second international standard for penicillin in terms of the existing standard. The committee suggested that laboratories in six countries should participate in this work.

As regards the recommendation made at the first session of the Expert Committee on Biological Standardization of the Interim Commission of

²¹ *World Hlth Org. techn. Rep. Ser.* 1950, 3

²² Welch, H., unpublished working document WHO/BS/67

WHO (1947)²³ that a reference preparation of penicillin K (IV) should be set up, the committee noted that there was at present little demand for such a preparation as a standard in the differential biological assay of the penicillins and recommended that no immediate action be taken.

18.2 *Streptomycin*²⁴

The committee decided that the reference preparation of streptomycin held by the Department of Biological Standards, National Institute for Medical Research, London, since April 1948, should now be established as the international standard for streptomycin, and recommended that a preparation of dihydrostreptomycin should be obtained and held as a reference preparation.

The committee recommended that the potency of the international standard should be expressed either as units of activity or as gram-equivalents of streptomycin base or as both.

Unit activity should, in the first place, be assigned to that weight of the standard preparation which, as far as can be ascertained, contains one microgram of streptomycin base, thereby making the international unit as nearly as possible equivalent to the original Waksman unit.

19. Blood Groups

19.1 *ABO*²⁵

Eight of the 11 participants in an examination of the preparation of anti-A and anti-B sera proposed as standards for A and B blood-grouping sera have submitted their results. The proposed standard preparations are of suitable quality and potency. The exact designation of potency must await the completion of the examination.

The committee recommended the establishment of the international anti-A and anti-B agglutinating-serum standards and that the Department of Biological Standards, National Institute for Medical Research, London, be authorized to assign to these standards potencies indicated by the modal macroscopic agglutination titres obtained in the 11 assays, the unit of agglutinating potency in each case to be the reciprocal of the agglutination titre.

19.2 *Rh*²⁶

The committee did not consider that at present it was expedient to establish international standard preparations of the various anti-Rh sera

²³ *Off. Rec. World Hlth Org.* 8, 7

²⁴ Welch, H., unpublished working document WHO/BS/67

²⁵ Unpublished working document WHO/BS/49

²⁶ Loghem, J. J. van, jr. (1949) *Bull. World Hlth Org.* 2, 215

or to make any recommendations about the notation of the Rh groups.

The committee decided that a universally acceptable definition of the term "Rh-negative" was desirable and recommended that the following definitions should be adopted internationally.

The term "Rh-negative" should, whenever possible, be applied to a human blood *donor* only if his erythrocytes are not agglutinated by three anti-Rh sera, namely:

- (1) anti-Rh₀ (Wiener notation); anti-D (Fisher-Race notation)
- (2) anti-Rh' (Wiener notation); anti-C (Fisher-Race notation)
- (3) anti-Rh'' (Wiener notation); anti-E (Fisher-Race notation)

Tests for the antigens represented by antisera (1) and (2) are conveniently made by the mixed serum anti-Rh₀' (Wiener)—anti-D + C (Fisher-Race)—and for the antigens represented by antisera (1) and (3), by the mixed serum anti-Rh₀'' (Wiener)—anti-D + E (Fisher-Race).

The antisera (2) and (3), however, are not always available. In this case, tests with antiserum (1) may be considered sufficient for specifying an Rh-negative donor, but it should be realized that the risk of incompatibility is thereby slightly increased. Tests with antiserum (1) anti-Rh₀ (Wiener)—anti-D (Fisher-Race)—are indispensable.

The term "Rh-negative" should be applied to a human blood *recipient* when his erythrocytes are not agglutinated by an anti-Rh₀ (Wiener)—anti-D (Fisher-Race)—serum. An Rh-negative recipient is not necessarily an Rh-negative donor.

20. Request of the Expert Committee on the Unification of Pharmacopoeias²⁷

The committee agreed to collaborate with the Expert Committee on the Unification of Pharmacopoeias in preparing appendices on the determination of therapeutic potency of sulfarsphenamine, neoarsphenamine, digitalis, the vitamins, antitoxins, and Old Tuberculin.

21. Notation of Measures of Potency²⁸

The committee considered the circumstances in which the three current methods of designating the potency of biological substances are valid.

²⁷ Unpublished working document WHO/BS/47

²⁸ Miles, A. A. (1949) *Bull. World Hlth Org.* 2, 205

These circumstances are :

(1) Whether or not the active principles in biological substances have been characterized by chemical and physical means, the potency of preparations of such substances should be expressed in *units* whenever the active principle in both standard preparation and preparations to be assayed may be heterogeneous.

(2) The expression of potency in *gram-equivalents* is valid, although not always desirable, when the active principle in the standard preparation is known to be homogeneous and free from inert material, and the active principles in preparations to be assayed may be heterogeneous.

(3) Designation of potency in *grams* is justified only when the active principle in the standard preparation is known to be homogeneous and free from inert material and when the active principle in the preparations to be assayed is known to be homogeneous. In such circumstances, however, biological assay will be necessary only when expense prohibits the routine characterization of preparations by physical and chemical means.

Annex 1**REQUIREMENTS FOR LABORATORIES ENGAGED
IN THE PREPARATION OF BCG VACCINE FOR THE UNICEF
VACCINATION CAMPAIGN****1. General Observations**

In the present state of our knowledge, it is unfortunately unavoidable that vaccination with BCG should have to be performed on human subjects before all the necessary precautionary testing of the vaccine has been completed. This position is unsatisfactory, and a reliable drying technique, including a sufficiently accurate estimate of the viable bacilli together with their properties in the dried product is an urgent necessity. Meanwhile, in order to eliminate as far as possible the dangers resulting from this situation, rigorous precautionary measures must be taken for safeguarding the purity of the vaccine. Contamination with other micro-organisms may take place at any stage in the process of preparation.

In the following, the factors which seem of importance are discussed in detail.

2. Staff

It must be emphasized that, however ideal may be the conditions and precautionary measures adopted, an incompetent staff will imperil the safety and efficacy of the vaccine. Therefore, the staff must be very carefully selected, special attention being paid both to its technical ability and to its sense of responsibility, the latter being as important as the former.

The number of workers on the staff should be sufficient to ensure that, in the case of the absence of one of its members, the duties can be carried out by one of the others.

All staff members, including the persons who clean the building, should be in good health and not suffering from infectious disease or carrying virulent micro-organisms. They should be medically examined by competent specialists before entering the BCG service, great attention being paid to the absence of tubercular infection in any form. These medical examinations should be repeated every three months.

Staff members should be immediately excused from duty on falling ill and should not re-enter the BCG laboratories until a careful medical inspection has proved the absence of infectious disease.

It is highly desirable that all members of the staff work only on BCG. However, under special circumstances it may be unavoidable that other part-time activities are performed. Permission to undertake other work should receive consideration only when it is certain that it in no way entails the danger of infection with viruses or virulent micro-organisms, especially spore-bearing and tubercle bacilli.

Visitors should not be permitted to enter any of the laboratories concerned in the production of BCG, unless proved free from tubercular infection.

3. The Building

The preparation of BCG vaccine should be carried out in special rooms forming a topographically separate, and self-contained, unit.

An ideal BCG-vaccine-producing laboratory should be housed in a separate building, used exclusively for BCG work. However, a unit on a separate floor of a larger building or at the blind end of a corridor of such a building might be acceptable, provided the location is such as to render negligible any risk of contamination with virulent tubercle bacilli or other agents. Each situation as it occurs should be studied according to its merits. Glassware, utensils, and other instruments should never leave the BCG unit. The preparation of all media and other materials and all sterilizations should take place in the BCG unit.

The unit should consist of at least :

- (1) a laboratory for the maintenance of the BCG strains and for the inoculation of media to be used for the vaccine ;
- (2) a laboratory for the preparation of the vaccine ;
- (3) a laboratory for ampouling the vaccine ;
- (4) a laboratory for the preparation of the media and for sterilization ;
- (5) an office for administrative purposes ;
- (6) adequate changing rooms, lavatories and water closets.

4. Animal Quarters

No animals should be permitted in the unit ; the animal inoculation and postmortem examinations necessary for the testing of the parent strain of BCG and the batches of vaccine should be carried out in animal quarters topographically and functionally separate from the unit in which vaccine is prepared.

5. Special Precautions against Contamination

Full precautions should be taken to prevent the introduction of bacterial contamination through the agency of air, water, equipment, material or personnel. All workers in the unit should wear laboratory gowns and laboratory footwear.

Workers immediately engaged in the direct preparation of the vaccine should wear clean cap, mask, gown and footwear specially kept for the purpose in a room guarding the entrance to the rooms where the vaccine is handled. The cleaning and maintenance of these rooms should be under the direct supervision of a qualified technician. All rooms in the unit shall be kept locked when not in use. Incubators should be kept locked and the keys of both rooms and incubators should be kept in the custody of responsible workers.

6. Methods of Cultivating the BCG Strains and of Vaccine Production

The methods of cultivating the BCG strains and of preparing the vaccine should be such that at no stage could contamination with other micro-organisms or viruses occur. The adequate fulfilment of this requirement depends on the staff, the location of the building, the laboratories, the animal house, the equipment, etc., all of which have already been discussed. A rigid prescription of techniques to be used is not recommendable, owing to the variety of methods possible. The following data should be recorded in a permanent form under the supervision of the director :

- (1) origin and date of reception of the seed strain ; the date of all subcultures of the strain and the method of subcultivation employed ;
- (2) morphological character of the culture at all relevant stages during manufacture of the vaccine ;
- (3) method of preparation of each batch of vaccine ;
- (4) tests for sterility, innocuity, infective and skin-sensitizing potency.

The vaccine should be stored at a suitable temperature.

7. Control Tests

The following tests should be applied :

- (1) *Sterility test.* Suitable aerobic and anaerobic methods should be used.
- (2) *Innocuity test.* At least 5 mg. of BCG bacilli of the prepared vaccine are injected subcutaneously or intraperitoneally into one or more guinea-pigs. After 6 months' observation the animals are sacrificed and examined.

An innocuity test should also be carried out with a subculture to serve as a step in the preparation of a particular batch of vaccine, at least 3 weeks before the batch is ready for use. One or more guinea-pigs should be injected intraperitoneally with 10 mg. of bacilli from this subculture and should be sacrificed and examined before the vaccine prepared from this subculture is approved.

(3) *Test for skin-sensitizing potency.* 5 mg. of BCG bacilli of the prepared vaccine are injected subcutaneously or intraperitoneally into one or more guinea-pigs. After 4 weeks the intracutaneous injection of 0.1 ml. containing 100 International Units Old Tuberculin should produce an area of induration and oedema not less than 5 mm. in diameter.

(4) *Potency test.* Graded doses of the finished vaccine should be injected intradermally into one or more albino guinea-pigs. One of these doses should be equal to the human dose. The potency of the vaccine should also be compared with the results obtained in children. All control tests and their results should be carefully recorded.

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BULLETIN OF THE WORLD HEALTH ORGANIZATION

The *Bulletin of the World Health Organization*, published quarterly in separate editions in English and in French, is the principal scientific organ of WHO, and is a successor to the *Bulletin mensuel de l'Office International d'Hygiène Publique* and the *Bulletin of the Health Organization of the League of Nations*. It is intended to bring to the knowledge of governments, health administrations, and the medical profession communications submitted by the representatives of Member States, technical papers considered or prepared by expert committees, original articles on scientific and public-health subjects of international significance, and bibliographical data.

Volume 2, number 2 of the *Bulletin* contains, in addition to summaries of a Symposium on the Tubercle Bacillus held in Lausanne, April 1949, and a bibliographical section, the following articles:

- Disinsectization of aircraft — *J. Duguet*
- On pethidine and methadone derivatives — *P. O. Wolff*
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