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**EXPERT COMMITTEE  
ON VENEREAL INFECTIONS  
AND TREPONEMATOSES**

**SUBCOMMITTEE ON  
SEROLOGY AND LABORATORY ASPECTS**

**Third Report**

	Page
1. Introduction . . . . .	3
2. Developments and perspectives . . . . .	3
3. Cardiolipin antigen . . . . .	7
4. Use of freeze-dried sera in the serology of syphilis . . . . .	13
5. National and international serodiagnostic laboratory activities . . . . .	16
6. Recommended diagnostic methods . . . . .	19
7. International Serodiagnostic Laboratory Conference . . . . .	20
8. Studies on treponemata and treponematoses . . . . .	20
9. Work of WHO field teams . . . . .	22
10. Terminology . . . . .	23
11. Relations with other bodies . . . . .	25
Annex 1. Continuation of pilot experiment . . . . .	27
Annex 2. Suggestions for reports on evaluation of serological results . . . . .	28
Annex 3. Results of storage experiment on freeze-dried sera (pilot experiment) . . . . .	30
Annex 4. Results of preliminary testing of 25 freeze-dried sera, May-July 1953 . . . . .	34
Annex 5. Information on serological laboratories in Member States and on the tests used for serodiagnosis of syphilis . . . . .	37
Annex 6. Serological methods used in WHO field-team laboratories . . . . .	50

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**EXPERT COMMITTEE ON VENEREAL INFECTIONS  
AND TREPONEMATOSES  
SUBCOMMITTEE ON SEROLOGY AND LABORATORY ASPECTS**

**Third Session**

*Copenhagen, 31 August-5 September 1953*

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The report on the third session of this committee was originally issued in mimeographed form as document WHO/VD/110, 19 November 1953.

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\* Attended two meetings.

# EXPERT COMMITTEE ON VENEREAL INFECTIONS AND TREPONEMATOSES

## SUBCOMMITTEE ON SEROLOGY AND LABORATORY ASPECTS

### Third Report \*

#### 1. Introduction

The Subcommittee on Serology and Laboratory Aspects, of the WHO Expert Committee on Venereal Infections and Treponematoses, held its third session in Copenhagen, Denmark, from 31 August to 5 September 1953.

The subcommittee unanimously elected Dr. K. V. Venkatraman as Chairman, Dr. F. Márquez as Vice-Chairman, and Dr. I. N. Orpwood Price as Rapporteur. The proposed agenda was adopted with minor modifications at the opening meeting. Eleven meetings were held and the report was approved by all members.

Dr. P. V. Marcussen, Chief, Dermatology Department, Finsen-institutet og Radiumstationen, Copenhagen, Denmark, attended two meetings as an observer from the Expert Committee on Venereal Infections and Treponematoses.

#### 2. Developments and Perspectives

The role of the laboratory in the management and control of the treponematoses (syphilis, bejel, yaws, pinta) and the non-treponemal venereal infections (gonorrhoea, non-gonococcal urethritis, chancroid, lymphogranuloma venereum, granuloma inguinale), following the introduction of antibiotic therapy, has been outlined in the reports on the

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\* The Executive Board, at its thirteenth session, adopted the following resolution:  
The Executive Board

1. NOTES the third report of the Subcommittee on Serology and Laboratory Aspects of Venereal Infections and Treponematoses;

2. THANKS the members of the subcommittee for their work; and

3. AUTHORIZES publication of the report.

(Resolution EB13.R8, *Off. Rec. Wld Hlth Org.* 52, 4)

third and fourth sessions of the Expert Committee on Venereal Infections and Treponematoses<sup>1</sup> and in the reports on the first and second sessions of the Subcommittee on Serology and Laboratory Aspects.<sup>2</sup> From an international viewpoint, the nature and magnitude of the problem of treponemal infections overshadow by far that encountered in the non-treponemal venereal infections, and major emphasis continues to be placed by the subcommittee on the serology and laboratory aspects of the treponematoses.

Almost three years have elapsed since the second session of the Subcommittee on Serology and Laboratory Aspects. The report on the second session and the comments of the WHO Executive Board on that report were noted by the members of the present session.<sup>3</sup> It was noted that advice had been obtained by correspondence from the participants in the second session of the subcommittee, and other members of the Expert Advisory Panel on Serology and Laboratory Aspects, for the further development of the planned programme. This programme had been fully approved by the Expert Committee on Venereal Infections and Treponematoses at its fourth session,<sup>4</sup> the report on which indicated in general terms the current outlook on the role of the laboratory in the treponematoses programme (a) from the point of view of diagnosis and therapy, and (b) in regard to mass control of these infections. This report also stressed the necessity for further international efforts to standardize antigens and sera as well as the continued need for national and international inter-laboratory co-operation and exchange of data and comparative research. The subcommittee was of the opinion that the considerations of the main committee, particularly the recommendations to health administrations concerning the stimulation of training of professional and auxiliary personnel and the importance of collaboration with the International Treponematoses Laboratory Center (for isolation of further strains of treponemes for comparative investigations, in order to advance the studies of the biological relationships between the treponematoses), were very useful indeed. The subcommittee welcomed the approach made to define serological specificity on the basis of treponematoses rather than on that of syphilis alone, as has previously been the tradition. Full consideration was given by the subcommittee in its deliberations to the comments of the main committee on studies on treponemata and the serological work of field laboratories in WHO-assisted national programmes, and details of the subcommittee's discussions on these points will be found under the

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.* 1950, 13 ; 1953, 63

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.* 1950, 14 ; 1951, 33

<sup>3</sup> Resolution EB7. R66, *Off. Rec. Wld Hlth Org.* 32, 28

<sup>4</sup> *Wld Hlth Org. techn. Rep. Ser.* 1953, 63, 25

relevant sections in this report. It was noted that certain matters had been actively taken up by the Secretariat since the last session of the subcommittee in view of the urgency of the problems concerned. Thus, the need for the establishment of minimum international requirements for preparations containing procaine penicillin G in oil with 2% aluminium monostearate (PAM), and the inclusion of details of standard microbiological assay techniques for the definition of duration of effective penicillinaemia in treponematoses therapy, had been well defined in various WHO documents and publications, as well as in the report of the main committee; and the subcommittee welcomed the eventual inclusion of appropriate techniques in volume II of the *Pharmacopoea Internationalis*. It was also noted that the work of the International Treponematoses Laboratory Center was inherently more closely connected with the field work of WHO-assisted treponematoses projects than with the work of national institutions or local laboratories directed by members of the Expert Advisory Panel on Serology and Laboratory Aspects; a considerable interchange of information is taking place between these experts, who are conducting comparative investigations on standardization of antigens and of serological methods for the detection of reagin ("reagin tests"), and on whom WHO is dependent for much of its accumulating information. It is therefore logical that the work of the International Treponematoses Laboratory Center should be considered in greater detail by the main committee than by the subcommittee; accordingly only marginal reference to this important activity has been made in the present report.

The development of the treponema-immobilization technique (TPI test) and the discovery of treponema-immobilizing antibodies in the serum, as distinct from the reagins measured by the use of cardiolipin antigens or less purified antigens ("lipoidal antigens"), were recent achievements at the time of the second session of the subcommittee (1950). Since that time, further definition of the place that this new technique might hold in serology has taken place. Although originally the technique was essentially a research tool for the study of immunobiological aspects of syphilis, it is gradually gaining place in the comparative investigations between the treponematoses as a group. It is also being gradually accepted as a useful confirmatory method and for the exclusion of suspected false-positive serological reactions in reagin tests. Studies on the TPI test have been initiated by more than 20 laboratories in Europe and elsewhere, and research on the immunology of the treponematoses is in progress. One health administration and some local laboratories have made the TPI test available as a routine measure to practising physicians as a supplement to the routine battery of reagin tests in syphilis. A co-operative study between laboratories now carrying out the TPI test has recently been initiated by WHO.

With the advancement of techniques, permitting the manufacture of antigens composed of dead treponemes in relatively pure, concentrated form, it has recently been possible to utilize specific antigens composed of dead treponemes in agglutination procedures. Initial experience with such treponema agglutination tests has been encouraging, and an additional tool for immunological study of the treponematoses has become available—*Treponema pallidum* agglutination tests (TPA). Considerable time and extensive investigations are, however, required before the place of this procedure in the serodiagnosis of the treponematoses can be evaluated.

While both the TPI test and the TPA test represent valuable supplements to available laboratory techniques, reliance must continue to be placed on the use of routine serological reagin tests, and further work towards the standardization of antigens and serological methods is necessary. The compilation of extensive data by WHO from the major laboratories of Member States in all regions indicates that there is great variation in the types of tests used, and in the manner in which they are interpreted. The statistical studies on the variability in testing results initiated by WHO, with a view to comparing antigens and sera on a more rational basis, should therefore be encouraged. The steps taken by WHO to create a framework for the future control of cardioliipin antigens by the publication of a monograph on cardioliipin,<sup>5</sup> by the inclusion in the *Pharmacopoea Internationalis* of an annex on solutions of cardioliipin and lecithins for serological tests, and by the establishment of Provisional International Reference Preparations (PIRPs)<sup>6</sup> on these substances have been welcomed by all major laboratories. An increasing number of laboratories have taken up the use of cardioliipin antigens and descriptions of new antigens of the cardioliipin type have been published by their authors. The Division of Laboratories and Research, New York State Department of Health, has prepared substances for the PIRPs, while the Statens Seruminstitut in Copenhagen, acting as the WHO International Serological Reference Laboratory, has continued the investigation of the problems involved in establishing reference preparations of durable freeze-dried syphilitic sera at different levels of reactivity. The results of the studies so far carried out are encouraging.

Laboratories directed by members of the Expert Advisory Panel on Serology and Laboratory Aspects, as well as some of the laboratories in WHO-assisted field projects, have taken part in the following activities, all of which were planned at the second session of the subcommittee

<sup>5</sup> Pangborn, M. C., Maltaner, F., Tompkins, V. N., Beecher, T., Thompson, W. R. & Flynn, M. R. (1951) *Cardioliipin antigens: preparation and chemical and serological control*, Geneva (*World Health Organization: Monograph Series*, No. 6)

<sup>6</sup> See *Wld Hlth Org. techn. Rep. Ser.* 1952, 56, 8 (section 10.2).

(1950): a study of the stability of blood samples in postal transmission (see section 5.1, page 16), inter-laboratory exchange of blood and serum samples (see section 5.2, page 16), and a study of the conservation of freeze-dried sera (see section 4, page 13). Laboratories in four of the WHO Regions are assisting in a further collection of freeze-dried sera, and the studies begun in 1952 on the classification of freeze-dried sera and comparison of cardioliipin antigens are continuing.

### 3. Cardioliipin Antigen

#### 3.1 Observations

Having referred to the various recommendations on production and control of cardioliipin and lecithins contained in the report on the second session, the subcommittee first studied the information on production of these substances collected by the WHO Secretariat in 1953.<sup>7</sup> It is observed that since 1950 production has increased considerably, not only in national serological laboratories (which produce mainly for local supply), but also in commercial plants. As some of the latter are at present in an early stage of production, a further increase can be expected. It was stated that, in spite of this increase in production, certain factors, such as lack of adequate information on sources of supply, currency exchange restrictions, and administrative difficulties in ordering, prevent many laboratories from obtaining cardioliipin antigens.

The subcommittee was of the opinion that increased production makes it even more essential to try to put into effect an adequate system which would ensure that only reliable cardioliipin antigens are put on the market and used in laboratories. The subcommittee welcomed the following action taken by WHO since the last session to implement its recommendations:

(1) A monograph entitled *Cardioliipin antigens*, by Pangborn et al.,<sup>8</sup> was published in 1951, and a second impression with numerous additions and corrections was issued in 1953. This manual describes a method by which reliable cardioliipin, lecithins, and cardioliipin antigens can be produced and tested by the methods used in the Division of Laboratories and Research, New York State Department of Health, Albany, N.Y.

(2) The Expert Committee on the International Pharmacopoeia agreed that an annex on solutions of cardioliipin, lecithins, and cardioliipin antigens

<sup>7</sup> Unpublished working document WHO/VD/SERO/39

<sup>8</sup> Pangborn, M. C., Maltaner, F., Tompkins, V. N., Beecher, T., Thompson, W. R. & Flynn, M. R. (1951) *Cardioliipin antigens: preparation and chemical and serological control*, Geneva (World Health Organization: Monograph Series, No. 6)

should be inserted in volume II of the *Pharmacopoea Internationalis*. The text of this annex, which is based upon the principles in the above monograph, was prepared in collaboration with the Expert Committee on Biological Standardization and with advice from those members of the Expert Advisory Panel on Serology and Laboratory Aspects who had attended the second session of the subcommittee. The text gives chemical and serological criteria by which cardioliipin and lecithins should be judged.

(3) The Expert Committee on Biological Standardization was not able to establish International Standards for cardioliipin and for the lecithins in the manner applied to biological substances, as the former may have a shorter lifetime (chemical or serological) than the latter. In 1951, the Expert Committee on Biological Standardization established PIRPs of cardioliipin and lecithins.<sup>9</sup> These will provide producers of cardioliipin and lecithins with reference preparations against which to check whether newly produced batches of cardioliipin and lecithins have the same reactivity, thus avoiding the supply of bad preparations. As no guarantee could be given that the PIRPs would be stable for more than two years, the Expert Committee on Biological Standardization arranged in 1953 for the production of a second batch.<sup>10</sup> The PIRPs are kept in the Standards Department of the Statens Serum Institut, Copenhagen, and are issued to recognized laboratories on request.

In 1950, the subcommittee recommended that "in addition to the Division of Laboratories and Research, New York State Department of Health, USA, a limited number of other laboratories be approached with a view to controlling the purity of cardioliipin and lecithin".<sup>11</sup> Since August 1952, the WHO International Serological Reference Laboratory, Copenhagen, has been engaged, in close collaboration with the New York State Department of Health, Albany, on preparations for taking up this control work.<sup>12</sup> The subcommittee considered that this was a step in the right direction, and that in due course certain designated area laboratories working in close contact with each other and with the Copenhagen and Albany laboratories could help to ensure that the products would be kept up to standard. Such a control system could enable the purchasers of cardioliipin antigens to require that the products be accompanied by a statement that their components are in accordance with the criteria given in the *Pharmacopoea Internationalis*.

The subcommittee was pleased to note that the National Serology Advisory Council, USA, had agreed at its 1953 session that action similar

<sup>9</sup> *Wld Hlth Org. techn. Rep. Ser.* 1952, 56, 8 (section 10.2)

<sup>10</sup> *Wld Hlth Org. techn. Rep. Ser.* 1954, 86, 11 (section 11)

<sup>11</sup> *Wld Hlth Org. techn. Rep. Ser.* 1950, 33, 19 (section 5.2, I.5)

<sup>12</sup> Unpublished working document WHO/VD/SERO/40

to that recommended by WHO should be taken in regard to serological reagents sold in the USA. The recommendation of the second session of the Subcommittee on Serology and Laboratory Aspects reads as follows: "that all samples of antigens produced for serological tests for syphilis be accompanied by or labelled with special information regarding composition and characteristics".<sup>13</sup>

With reference to this recommendation the subcommittee studied a collection of cardioliipin antigens from various producers, their labels, and accompanying literature.<sup>14</sup> It was observed that most of the antigens were not accompanied by all the information which the subcommittee had recommended should be supplied.

### 3.2 *Provisional International Reference Preparations*

These preparations, which are under the authority of the Expert Committee on Biological Standardization, consist of cardioliipin, beef-heart lecithin, and egg lecithin. All these substances were produced by the Division of Laboratories and Research, New York State Department of Health, USA, and were subjected to tests there before issue.

The subcommittee reviewed the comparative study on antigens prepared from the first and second PIRPs.<sup>15</sup> In this study, which was undertaken at the request of the Expert Committee on Biological Standardization,<sup>16</sup> 13 laboratories are comparing five antigens, made up from PIRPs, by testing on several days both reactive and non-reactive sera. An example of the way in which the five antigens were composed is given below.

In order to compare the 1951 cardioliipin with the 1953 cardioliipin, two antigens were prepared—one from the 1951 cardioliipin and the 1951 egg lecithin, the other from the 1953 cardioliipin and the 1951 egg lecithin. In both antigens the preparations were taken in the same proportions, and the same lots of cholesterol and alcohol were used for both preparations.

The study is not yet complete, but the subcommittee considered the reports which had come in from seven of the laboratories, and agreed that no major differences could be detected between the 1951 PIRPs and those of 1953. The subcommittee was of the opinion that valuable information both on experimental error and on the comparison of cardioliipin and lecithins by means of different serological methods will probably appear when the proposed statistical evaluation of the results has been made.

<sup>13</sup> *Wld Hlth Org. techn. Rep. Ser.* 1950, 33, 19 (section 5.2, II)

<sup>14</sup> Unpublished working document WHO/VD/SERO/39

<sup>15</sup> Unpublished working document WHO/VD/SERO/44

<sup>16</sup> See *Wld Hlth Org. techn. Rep. Ser.* 1953, 68, 9 (section 7).

The subcommittee expressed the hope that this evaluation would take place at an early date.

The subcommittee observed that preliminary studies on the keeping quality of cardiolipin, lecithins, and cardiolipin antigens at 37°C and 56°C had shown that no change in reactivity had been demonstrated after two weeks' storage,<sup>17</sup> but it welcomed the information that further studies on this subject were to be undertaken.

The subcommittee realized that all work on the comparison of antigens and on checking the reactivity of cardiolipin and lecithins is very time-consuming, since great care is needed at all stages of the testing procedure. Coded duplicates are needed to judge the experimental error, and it is preferable to have technicians specially trained for the checking work.

The subcommittee suggested that freeze-dried sera stored at -10°C might be valuable in studies on the keeping quality of cardiolipin antigens.

### 3.3 Considerations

The subcommittee agreed that the sensitivity of cardiolipin antigen prepared with egg lecithin was likely to be of the same order as that of cardiolipin antigen prepared with beef-heart lecithin.<sup>18</sup> At the same time it was felt that further studies on the specificity of cardiolipin antigens prepared with the two different types of lecithin would be useful.

The subcommittee noted the importance of good-quality cholesterol as a component of antigens,<sup>19</sup> and suggested that a description of this product might be included in the next edition of the monograph entitled *Cardiolipin antigens*. It recommended the use of chemically pure cholesterol, recrystallized from alcohol and stored under such conditions as to prevent any change in reactivity.

The subcommittee noted that experiments are being conducted using physical tests for the detection of unsatisfactory lots of lecithins, and stated that it would await with interest the outcome of these experiments.<sup>20</sup>

The subcommittee expressed the wish that care be taken in all comparisons of serological results to specify not only the cardiolipin antigen used and its lot number, but also the type of the lecithin component. This was necessary in view of recent reports that, when the cardiolipin antigen for a certain test was prepared, some six different lots of lecithin were found to be unsatisfactory (previously, it had not been difficult to

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<sup>17</sup> Unpublished working documents WHO/VD/SERO/22, WHO/VD/SERO/27

<sup>18</sup> Unpublished working documents WHO/VD/SERO/32, WHO/VD/SERO/33, WHO/VD/SERO/34

<sup>19</sup> Unpublished working document WHO/VD/SERO/22

<sup>20</sup> Unpublished working document WHO/VD/SERO/42

find a satisfactory lecithin for this particular test), although they were quite suitable when used in antigens for several other tests.

It was also stated that lecithins produced during the past few years with improved methods have shown more consistent reactivity.

The subcommittee discussed fully the recent work, an account of which had been made available to the members in the documentation<sup>21</sup> relating to the maturation phenomenon of cardioliipin antigens, the factors influencing its progress, and the experience of some workers concerning the effect on the maturation phenomenon of lecithins prepared from different sources or obtained by different methods of preparation. The subcommittee considered the subject to be of sufficient importance to merit further study, with a view to obtaining a better understanding of all the factors involved. Such study might result in a more accurate description of lecithins and in due course facilitate the standardization of cardioliipin antigens.

The subcommittee discussed at length the limits for acceptance and rejection of cardioliipin and lecithins, and the consequences of setting narrow or broad limits.<sup>22</sup>

The discussion also touched on the question of whether it was possible to use a fixed antigen formula for each of the different tests which employ cardioliipin antigens and on how great the variations would need to be, especially in lecithin percentage, in order to obtain the standard of reactivity demanded by authors of the various serological methods. Opinion was divided on this matter, and it was felt that insufficient information was available at present on the effect of minor variations in lecithin percentage for any decision to be taken. The subcommittee hoped that information from laboratories which have been preparing cardioliipin antigens for many years would be published, and that further research would be undertaken with a view to verifying the effect of various lots of lecithin on the results of different serological tests. It would also like to see published information on the experimental error for various serological tests.

The subcommittee noted with interest the research being conducted in several laboratories on the preparation and analysis of phospholipid acids from "vegetable" sources.<sup>23</sup> These studies, as well as those on synthetic cardioliipin and lecithins, may be of value for the understanding of the chemical composition of antigens. The subcommittee considered that, although a technique for producing sitoliipin was now available, and

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<sup>21</sup> Unpublished working document WHO/VD/SERO/36

<sup>22</sup> Unpublished working document WHO/VD/SERO/40

<sup>23</sup> Unpublished working documents WHO/VD/SERO/22, WHO/VD/SERO/23, WHO/VD/SERO/24, WHO/VD/SERO/25

although some laboratories have published results obtained with sitolipin antigens, it would be premature to recommend that sitolipin be used as a substitute for cardiolipin. The subcommittee was also of the opinion that although large-scale research on treponemal antibodies was more likely to promote progress in serology than work on standardization of phospholipids prepared from vegetable sources, tests using this type of antigen should in due course be included in the studies on freeze-dried sera.

The subcommittee recommended that, with a view to giving members of the Expert Advisory Panel on Serology and Laboratory Aspects up-to-date information on progress, the Secretariat keep in contact with laboratories engaged in studies of cardiolipin antigens.

The subcommittee noted with interest the proposed comparative study in two European and two American laboratories on the influence of the percentage of lecithin in the American Public Health Association's reference antigens for the microflocculation test.

The subcommittee noted that the determination of concentrations of cardiolipin and lecithins in the alcoholic solutions must be performed with great accuracy, because an error may influence the sensitivity of any antigen using a fixed formula, or may make necessary an extra adjustment of the lecithin percentage in antigens which are being brought to a standard reactivity.<sup>24</sup>

Studies on the factors promoting better keeping quality of cardiolipin antigens should be undertaken.

#### 3.4 *Distribution of cardiolipin antigens*

The subcommittee considered that only large-scale production should be encouraged, as small-scale production is uneconomical, from the point of view of both the work of production and the use of PIRPs for control purposes.

The subcommittee agreed that it is generally accepted that two years represent a safe margin for storage of cardiolipin, lecithins, and cardiolipin antigens when these products are kept in sealed ampoules and under selected storage conditions.

The subcommittee recommended that antigen producers should retain aliquots of each lot of antigen produced for periodical testing during the period of certification. Antigens found to have become defective should be recalled.

The subcommittee underlined the necessity for ensuring that the quality of these controlled products is not changed by inadequate packaging or bad storage conditions.

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<sup>24</sup> Unpublished working document WHO/VD/SERO/53

It was noted that some cardioliipin antigens are placed on the market by commercial firms which are not themselves producing cardioliipin and lecithins.

The subcommittee recommended that health authorities in non-producing countries should consider the central purchase of antigens based upon the advice of their leading laboratories, which should check that samples from the lots to be purchased are of a reactivity useful in that area.

The subcommittee noted that it is difficult to buy solutions of cardioliipin and of lecithins which would allow non-producing laboratories to prepare their own antigens.

The Subcommittee on Serology and Laboratory Aspects,

Having studied the documentation made available on production and control of cardioliipin ; and

Having noted the developments which have taken place since the last meeting of the subcommittee,

RECOMMENDS

- (1) that WHO should maintain contact with laboratories engaged in studies of cardioliipin antigens so that members of the Expert Advisory Panel on Serology and Laboratory Aspects may be sent up-to-date information on the progress made ;
- (2) that antigen producers should retain aliquots of each lot of antigen produced for periodical testing during the period of certification ;
- (3) that health authorities in non-producing countries should consider the central purchase of antigens based upon the advice of their leading laboratories, which should check that samples from the lots to be purchased are of a reactivity useful in that area.

#### 4. Use of Freeze-Dried Sera in the Serology of Syphilis <sup>25</sup>

At its second session, in 1950, the subcommittee recommended that the value of freeze-dried sera for work in the serology of syphilis should be studied, and drafted a detailed plan for the first phase of these investigations as a pilot experiment.<sup>26</sup>

At the present session, the subcommittee studied with great interest the final report on this experiment in which six laboratories had tested

<sup>25</sup> Unpublished working documents WHO/VD/SERO/20, WHO/VD/SERO/28

<sup>26</sup> *Wld Hlth Org. techn. Rep. Ser.* 1951, 33, 23

freeze-dried reactive sera from ten syphilitics and one non-syphilitic, and non-reactive sera from three normal persons. These freeze-dried sera had been stored at  $-10^{\circ}\text{C}$ ,  $20^{\circ}\text{C}$ , and  $37^{\circ}\text{C}$  for three months in order to ascertain their keeping quality. A preliminary report had been issued in 1951 on the basis of which the members attending the second session of the subcommittee had agreed, by letter, that a further 80 sera, mainly reactive sera from non-syphilitics, should be collected. The final statistical evaluation of the pilot experiment was completed in 1953.<sup>27</sup>

Having examined all these reports on the pilot experiment, the subcommittee endorsed the conclusions reached by correspondence on the successful outcome of the experiment. The subcommittee expressed the wish that studies on the keeping quality of reactive freeze-dried sera from non-syphilitic persons should be undertaken on the lines of the plan used for the pilot experiment.

The subcommittee noted that steps had been taken to begin the collection of some 80 freeze-dried reactive sera from 15 syphilitic donors and 57 non-syphilitics, and freeze-dried non-reactive sera from 8 normal donors, but was sorry to learn that unforeseen difficulties had delayed completion of the collection. (30 sera were obtained by 1 January 1953 and a further 17 by 1 September 1953.) The subcommittee was of the opinion that action should be taken to obtain at least another 30 sera, and several members offered to help in this collection when required.

The subcommittee stressed the fact that individual reactive sera from non-syphilitics, rather than pools of such sera, would be useful for the studies. Sera from treponematoses other than syphilis should not be included in the group of sera from non-syphilitics. The subcommittee also stressed the necessity for obtaining, in consultation with members of the Expert Advisory Panel on Venereal Infections and Treponematoses and the Expert Advisory Panel on Serology and Laboratory Aspects, definite criteria for selection of non-syphilitics with reactive sera.

The subcommittee studied the preliminary report on the results obtained from the testing of the first 30 freeze-dried sera. These sera (some duplicated) had been distributed under code numbers to 18 laboratories. The report contains the results from 13 laboratories in which altogether 19 serological methods were used.<sup>28</sup> A preliminary statistical evaluation has been undertaken on two-thirds of these results.

The subcommittee considered that the system used for distribution, the working instructions, and the ways of reporting were quite satisfactory.

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<sup>27</sup> See Annex 3, page 30.

<sup>28</sup> See Annex 4, page 34.

The subcommittee expressed the wish that the occurrence of zone phenomena among test results on freeze-dried sera be studied. It was observed that the test results on the first 30 freeze-dried sera were all sufficiently good to make it unnecessary to discard any of the serum.

The subcommittee expressed the wish that the pilot experiment be continued by testing a sufficient number of reactive syphilitic and non-syphilitic sera. The outline for the continued pilot experiment is given in Annex 1 (see page 27). The subcommittee considered that this experiment should be performed, even though some 70 ampoules of each serum will be required, because it is only in this way that the keeping quality of reactive sera from non-syphilitic persons can be ascertained; and it is essential that this information should be available before sera are sent to laboratories where they are to be used for testing the specificity of serological techniques.

The subcommittee recommended further that all the laboratories which have already examined the 30 freeze-dried sera be asked to repeat their experiments on a new batch of the 30 sera which will be distributed under fresh code numbers. It was realized that these two experiments will reduce the quantity of freeze-dried sera to such an extent that only 80-150 ampoules will remain for evaluating the sensitivity and specificity of serological methods, and that this number of ampoules will only allow single testing of some 60, or duplicate testing of some 30, serological methods. It is therefore imperative that, if this experiment is to be completed with satisfactory results, steps should immediately be taken to establish another collection of freeze-dried serum samples as soon as possible. Funds would be necessary for this project.

The subcommittee was of the opinion that freeze-dried sera are useful, in serological work, for many purposes, such as daily intra-laboratory check of sensitivity, inter-laboratory check of sensitivity, testing of reference antigens and their components, preliminary evaluation of serological techniques (before selection of participants in laboratory conferences), and evaluation of serological methods. For the first three of these purposes it is necessary to have sera from syphilitics, while for the last two, reactive sera from non-syphilitics are also needed.

The use of freeze-dried sera for nationwide checking of the sensitivity of serological technique (in the USA) was discussed. Diluted reactive serum from syphilitics is freeze-dried in ampoules and then distributed to major laboratories together with the information on the testing results obtained in the issuing laboratory. The major laboratories prepare from the content of an ampoule small portions of this control serum which are kept frozen until tested on a day when an experiment is carried out. This process is repeated until the frozen aliquots from the ampoule are used

up. The system allows laboratories to keep a daily check on the sensitivity of their serological methods and to compare the level of sensitivity of these methods with that of the issuing laboratory.

The Subcommittee on Serology and Laboratory Aspects,

Having considered the report on the study of freeze-dried reactive sera at different levels of sensitivity,

RECOMMENDS

- (1) that each series of freeze-dried sera be tested under code number on two different occasions in a number of laboratories ;
- (2) that the keeping quality of freeze-dried sera from non-syphilitics be examined at different temperatures ;
- (3) that the experiment be continued by establishing a collection of a greater number of syphilitic and non-syphilitic sera, and that criteria for the selection of non-syphilitic sera should be determined in consultation with the members of the Expert Advisory Panel on Venereal Infections and Treponematoses and the Expert Advisory Panel on Serology and Laboratory Aspects.

## 5. National and International Serodiagnostic Laboratory Activities

### 5.1 *Stability of blood samples in postal transmission*<sup>29</sup>

The subcommittee studied the report on the experiment to test the stability of blood samples in postal transmission. The experiment had been performed in order to ascertain whether blood samples sent from countries distant from the eventual site of a serological laboratory conference would reach that laboratory in a fit condition for serological testing. The experiment showed that although haemolysis had appeared in a large number of specimens which had been in transit for more than four days the attendant reduction in titre had been small. The subcommittee realized, however, that haemolysed samples might well be unacceptable to many of the participants in a laboratory conference, and that for this reason it would be desirable to send serum rather than blood.

### 5.2 *Inter-laboratory test evaluation*<sup>30</sup>

Twenty-nine laboratories participated in this experiment, which lasted for three months. Each laboratory sent weekly samples of blood or serum from different donors to each of three other laboratories, itself keeping

<sup>29</sup> Unpublished working document WHO/VD/SERO/19 ; see also *Wld Hlth Org. techn. Rep. Ser.* 1951, 33, 13 (section 4.2).

<sup>30</sup> Unpublished working document WHO/VD/SERO/21

control samples for testing after storage at room temperature for from three to five days. Altogether the study included samples from some 1,300 donors. The serum from each donor was tested with from two to six tests in the collecting laboratory and in each of three receiving laboratories.

In considering the report on the experiment, the subcommittee realized that three factors prevented the results from being utilized for statistical evaluation. These factors were the differences in the type of sample sent, the many variations in transport times and conditions, and the relatively small number of samples from each donor laboratory which were examined in the appropriate receiving laboratories. The subcommittee expressed the opinion that the WHO Secretariat should, in whichever way it might find most convenient and suitable, make available the basic documentation on the results to the various groups of laboratories which had taken part in the experiment.

The subcommittee agreed that vacutainers (which could be used several times) had proved their value in many areas, as less haemolysis occurred in blood samples sent in this way than in blood taken by syringe and sent in ordinary tubes.

The subcommittee considered also the value of merthiolate as a bacteriostatic agent in serum,<sup>31</sup> and agreed that for long travel, or for short travel under difficult conditions, it might be better to send sterile serum, or serum with added merthiolate, than to send whole blood.

### 5.3 *Transit of samples*

The subcommittee briefly discussed difficulties occurring in transit of samples of antigens, blood, or sera due to delay in customs, and was of the opinion that the dispatch of such samples should not be made until the customs regulations in the receiving country had been ascertained. It is advisable to inform the receiver of the date of dispatch and the transit route. The subcommittee expressed the hope that the Secretariat might explore ways of improving facilities for rapid consignment and receipt of serological samples, although it realized that many of the difficulties could be overcome only by the efforts of the local serologists.

### 5.4 *WHO International Serological Reference Laboratory, Copenhagen* <sup>32</sup>

The subcommittee expressed its satisfaction with the establishment of the WHO International Serological Reference Laboratory in Copenhagen

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<sup>31</sup> Unpublished working document WHO/VD/SERO/46

<sup>32</sup> Unpublished working document WHO/VD/SERO/18

and was of the opinion that one or more similar centres should be set up elsewhere in due course. It requested that not only the yearly reports from the Reference Laboratory, but also any intermediate reports of a scientific nature be distributed to all members of the Expert Advisory Panel on Serology and Laboratory Aspects.

The subcommittee considered that, in addition to taking up the control of cardiolipin and lecithins, the Reference Laboratory should also investigate and develop means for distributing reference reagents to recognized laboratories. The subcommittee stated that these extended services requested of the Reference Laboratory would require an increased yearly grant from the Organization.

The Subcommittee on Serology and Laboratory Aspects,

Having noted with interest the report of the work done by the WHO International Serological Reference Laboratory,

#### RECOMMENDS

- (1) that the Reference Laboratory should be asked to investigate and develop means for distributing reference reagents to recognized laboratories, and that consideration should be given to increasing the grant to the Reference Laboratory should such a service be possible; and
- (2) that the Reference Laboratory should aim at retaining reference preparations for all antigens used by WHO field teams and in due course for recognized tests used in large groups of laboratories.

#### 5.5 *Control of serological work*

The subcommittee was of the opinion that an adequate national system for control of serological work, such as exists in some countries, calls for a manual on serology, training facilities for technicians, distribution of reference reagents, collaboration among laboratories, interchange of samples, and a yearly check of the sensitivity and specificity of the serological methods used, the latter to be made by distributing samples for testing purposes.

The subcommittee also considered that WHO should try to find ways and means of adapting such a system for the purpose of inter-laboratory collaboration on an international scale.

#### 5.6 *Reference antigens and sera*

The subcommittee agreed that the establishment and distribution of reference preparations of antigens would be a very valuable method of promoting more uniformity among antigens used. This might well be an appropriate task for the WHO International Serological Reference

Laboratory. The subcommittee recommended that the Reference Laboratory should aim at retaining reference preparations for all antigens used by WHO field teams and also, in due course, for recognized tests used in large groups of laboratories. The Reference Laboratory should obtain the reference antigens from the laboratories of author serologists, or from other suitable sources, or should itself prepare them. All reference antigens would be checked in at least one other laboratory before distribution. The subcommittee agreed that a collection of reference sera should also be established when they have proved to be satisfactory in the continued pilot experiment. The composition and number of this collection of reference sera should depend on the experience gained from the current testing of freeze-dried sera.

#### 5.7 *Information on serological laboratories in Member States*<sup>33</sup>

The subcommittee studied with interest the information, comprising the serological methods used and the number of samples examined, collected during the past three years from laboratories in Member States. The great variety in methods used even in a single country strengthened the subcommittee's opinion that more uniformity in testing procedures is desirable both nationally and internationally (see also Annex 5, page 37).

### 6. Recommended Diagnostic Methods<sup>34</sup>

The subcommittee reviewed the replies to questionnaires which had been distributed to the members of the Expert Advisory Panel on Serology and Laboratory Aspects by the Secretariat, in accordance with the request of the Expert Committee on Biological Standardization,<sup>35</sup> relating to the publication of recommended diagnostic methods in the serology of syphilis, and agreed that the publication of a manual of selected procedures for the serology of syphilis would serve as a useful guide for WHO field-team operations.

The subcommittee recommends that :

1. Such a publication should be clearly defined as only a working manual for field operations and not as a list of recommended procedures, unless and until adequate information about the relative specificity and sensitivity of the many currently available methods in complement-fixation, flocculation, immobilization, and agglutination testing is obtained through a serological congress or other suitable means.

<sup>33</sup> Unpublished working documents WHO/VD/SERO/30, WHO/VD/SERO/45

<sup>34</sup> Unpublished working document WHO/VD/SERO/26

<sup>35</sup> See *Wld Hlth Org. techn. Rep. Ser.* 1953, **68**, 21 (section 45).

2. This manual should contain an annotated bibliography on recent use of the selected procedures.

3. Cognizance should be taken of the areas into which WHO field teams will probably be sent during the future five years so that selection of procedures may be based on conditions existing in these areas.

4. A group of practising serologists should be charged with the responsibility of selecting testing procedures for this manual and of preparing a detailed text for each test.

5. The final text of this manual should be submitted to the Expert Advisory Panel on Serology and Laboratory Aspects for comments before release.

### 7. International Serodiagnostic Laboratory Conference

The subcommittee reviewed the considerations of its previous session on the postponement of the International Serodiagnostic Laboratory Conference.<sup>36</sup> It considered that if it could be shown that the testing of freeze-dried serum by various techniques would yield accurate and reliable information as to sensitivity and specificity, the necessity for the holding of a serological conference would be obviated. If this proves to be the case, much time, labour, and money will be saved. At the same time, the subcommittee stressed the importance and necessity of subsequently holding a conference of the participants in such a scheme in order to discuss the results and the value of the various techniques employed.

### 8. Studies on Treponemata and Treponematoses

#### 8.1 *International Treponematoses Laboratory Center*<sup>37</sup>

The subcommittee studied with great interest the report on the technical work performed in the International Treponematoses Laboratory Center, and considered that, in view of the magnitude of the treponematoses problem, the international scientific investigations into the biological relationship between species and strains of treponemes isolated from various parts of the world were of considerable importance. The studies on the effect of environmental temperature on different species of treponemes and the question of penicillin sensitivity and resistance of strains were new developments in this field. It was hoped that WHO and the International Treponematoses Laboratory Center would be in a position to assist in investigating the applicability of the agglutination test to each

<sup>36</sup> *Wld Hlth Org. techn. Rep. Ser.* 1951, 33, 6 (section 3)

<sup>37</sup> Unpublished working document WHO/VD/109

of the human treponematoses. Further details of the work of the Center are given in the fourth report of the Expert Committee on Venereal Infections and Treponematoses.<sup>38</sup>

### 8.2 Tests using *treponemata*<sup>39</sup>

The subcommittee reviewed the developments in the TPI test which had taken place since 1950. Experimental work had been carried out in laboratories in the Americas as well as in Europe. In several laboratories, the results of the TPI test are now used to supplement the test results obtained with ordinary serological methods. The subcommittee is aware that the technique for this test is time-consuming, expensive, and difficult to perform. It is now known that variations in test results, in cases with low immobilizing titre, may lead to difficulties, and that quantitative results are likely to vary from one testing day to another to a much greater extent than is found in results from usual serological tests.

The subcommittee agreed that the TPI test might well be a valuable addition to the serology of syphilis. It was stated that it is mainly useful for testing sera from cases with no clinical signs or history of treponematoses but which are reactive with the usual serological methods, and for testing cases with weak seroreactions and suspicious clinical signs of syphilis. This test helps in the detection of another type of antibody different from the "reagin", but sufficient knowledge on the interpretation of the results of this test is not yet available.

It was reported that in a random population survey a larger percentage of positive reactions was obtained with the TPI test than with other tests for syphilis. It was also reported that some positive reactions in the TPI test were associated with the absence of clinical or historical evidence of treponemal infection. Studies on the relation between the incidence of treponematoses and the occurrence of such reactors should be undertaken.

The subcommittee notes with great interest the recent information on the use of *T. pallidum* in an agglutination test and will await developments. When sufficient antigen of a suitable suspension of killed treponemata is available and testing conditions are standardized, it is very possible that a valuable tool in the serodiagnosis of the treponematoses will have been created.

The subcommittee noted the steps taken by WHO to promote co-operation among laboratories using the TPI test, and agreed that such efforts should be continued. It was considered that the co-operating laboratories should report to the Secretariat yearly instead of quarterly as

<sup>38</sup> See *Wld Hlth Org. techn. Rep. Ser.* 1953, 63.

<sup>39</sup> Unpublished working documents WHO/VD/SERO/31, WHO/VD/SERO/37, WHO/VD/SERO/50, WHO/VD/SERO/55, WHO/VD/SERO/56

at present. These yearly reports should be supplemented by intermediate reports when any results of a specially interesting character are encountered. Reports on quantitative results with the WHO reference serum should be sent at frequent intervals, and the distribution by the WHO International Serological Reference Laboratory of such control serum should be continued. The subcommittee envisaged that other tests, such as the agglutination test, using *T. pallidum* might be studied in due course.

The subcommittee considered that future yearly reports, but not the intermediate reports, should be distributed to all members of the Expert Advisory Panel on Serology and Laboratory Aspects.

The subcommittee also noted the serological experiments with cultured treponemata and derivatives, and stated that, although some of the results had been difficult to interpret, it felt certain that members of the Panel would follow this type of work with the greatest interest, since it may be a guide for future studies on antigenic preparations from treponemata.

The Subcommittee on Serology and Laboratory Aspects,

Having noted the information available on tests using treponemata and the steps taken by WHO to promote co-operation among laboratories using the *Treponema pallidum* immobilization test,

RECOMMENDS that the study of the *Treponema pallidum* immobilization test and the distribution of reference sera should be continued, and that yearly reports on the progress made should be distributed to all members of the Expert Advisory Panel on Serology and Laboratory Aspects.

### 8.3 Pinta and yaws<sup>40</sup>

The subcommittee noted the reports on the serological results from cases of pinta and yaws, and expressed its interest in these results, which were obtained by means of the serological tests for syphilis. At present there appears to be no way of differentiating serologically, by means of the usual tests for syphilis, between pinta, yaws, and syphilis.

## 9. Work of WHO Field Teams<sup>41</sup>

The subcommittee reviewed the information on the work performed by WHO field teams which are engaged in demonstration projects or in mass campaigns of diagnosis and treatment. Although the subcommittee was in favour of uniformity of testing procedures in these projects, it

<sup>40</sup> Unpublished working documents WHO/VD/SERO/29, WHO/VD/SERO/35, WHO/VD/SERO/49, WHO/VD/SERO/51, WHO/VD/SERO/57

<sup>41</sup> Unpublished working documents WHO/VD/SERO/54, WHO/VD/SERO/55; see also Annex 6, page 50.

realized that the choice of tests for a particular operation must depend upon the previous experience of the serologist and upon the experience gained by the team in the course of its daily work. The subcommittee felt that it was not possible to choose a single diagnostic test for use in all campaigns, and referred to the considerations on recommended diagnostic methods contained in section 6 (see page 19). It noted that steps had been taken to test a specially designed field unit for laboratory work.

The subcommittee felt that it was important to use mass testing in mass treatment campaigns, but realized that serological examination was not performed in several of these campaigns because of practical difficulties.

The subcommittee recommended that, at the outset, the laboratory serving a control area should use several techniques (one of these being a quantitative test) in an endeavour to find out the most suitable serological method or methods for that particular area. A thorough clinical examination of each case should be undertaken in the control area, and the serological tests chosen should be used to evaluate the effect of treatment by means of serological results.

The subcommittee noted that a comparison was being made between case selection based on mass serodiagnosis and case selection based on clinical findings plus known contacts.

The subcommittee was informed that it is planned to inaugurate an experiment to assess the value of skin tests using treponemal antigens.

The Subcommittee on Serology and Laboratory Aspects,

Having studied the information presented to the meeting on the work performed by the field teams and discussed the importance of testing procedures in these projects,

Realizes that it is not possible to choose a single diagnostic test for use in all campaigns, but

RECOMMENDS that, at the outset of a project, the laboratory serving a control area should use several techniques (one of these to be a quantitative test) in an endeavour to find out the most suitable serological method or methods for the particular area, and that the tests chosen should be used to evaluate the effect of treatment by means of serological results.

## 10. Terminology

### 10.1 *Reactive sera from non-syphilitics*<sup>42</sup>

The subcommittee discussed briefly whether it was possible to find a substitute for the term "biologically false-positive". It was obvious that

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<sup>42</sup> Unpublished working document WHO/VD/SERO/38

the expression had no relation to the wording of laboratory reports, which should give only a serological appraisal of the sera examined. The expression is valid only in studies on specificity, examination of groups of populations, and other combined clinical-serological studies. Several suggestions for substitutes were made, such as "non-syphilitic reactive sera" or "reactive non-treponemetic sera". The latter expression was theoretically the better but was rather clumsy; it could be abbreviated to "reactive non-t.p. sera". The committee was of the opinion that a great deal would be gained if it were found possible to replace the expression by a description of the particular disease or condition which was thought to be the reason for the occurrence of a reactive serum in a person without any treponematosi.

### 10.2 *Reports on evaluation of serological results*

The subcommittee studied in detail the various considerations on the manner of reporting evaluation results,<sup>43</sup> and agreed that a calculation based on an overall percentage of agreement between serological results was often misleading. It was of the opinion that it would be preferable to use the percentage of agreement among reactors.

The subcommittee was in favour of the use of correlation diagrams for comparison of qualitative or quantitative results, and stated that this reporting technique could be used for comparison of results obtained with two tests on the same group of sera as well as for serological evaluations of the treatment results. The importance of including in the reports on serological studies full details of the antigens used as well as of the sera tested was stressed. It was considered that the determination of the experimental error in routine use of serological tests would be of great value.

### 10.3 *Screening procedure*<sup>44</sup>

The subcommittee felt that the improvement obtained in sensitivity and specificity of serological methods using cardiolipin antigens might outmode the previous screening procedures, since it is now possible to choose between several tests with sensitivities which are the same as or higher than those of tests previously designated "screening tests". It was noted that some laboratories with a large number of samples perform preliminary tests (e.g., a one-tube complement-fixation test) in order to eliminate non-reactive sera, and retest later in the day (with the same technique) all reactive sera, using a serum control and quantitative test.

<sup>43</sup> Unpublished working document WHO/VD/SERO/52

<sup>44</sup> Unpublished working documents WHO/VD/SERO/47, WHO/VD/SERO/55

#### 10.4 *Microtest*<sup>45</sup>

The subcommittee felt that the term "microtest" was unsatisfactory and should not be used, as a great number of tests, including refined complement-fixation tests and relatively simple flocculation tests, use very small amounts of serum.

The subcommittee discussed briefly some of the so-called "micro-methods" published during the past three years, as well as the experience gained with previously known tests. It was agreed that although slide flocculation tests may be used in hot climates when adequate measures are taken to prevent evaporation, it might be as well to consider the use in certain areas of a simple tube flocculation test.

### 11. Relations with Other Bodies

#### 11.1 WHO expert committees

##### 11.1.1 *Expert Committee on Biological Standardization*

The subcommittee noted with interest the work undertaken by the Expert Committee on Biological Standardization, leading up to the establishment of PIRPs. This work has been dealt with in section 3 of this report (see page 7) in the consideration of cardiolipin antigen.

The subcommittee noted further that the Expert Committee on Biological Standardization had shown interest in the work on freeze-dried sera, especially on their keeping quality, and also that it had requested that studies on the latter be performed at and above 37°C. The subcommittee agreed that the laboratories in Chamblee, Copenhagen, and London should, in consultation with each other, investigate this problem on a small number of freeze-dried sera prepared by themselves for this purpose. For guidance the three laboratories will in due course receive information on the outcome of similar experiments on antitoxic sera now being undertaken in the Department of Biological Standards of the Statens Serum-institut, Copenhagen.

The subcommittee discussed at some length the desirability of publishing recommended diagnostic methods, and its considerations are given in section 6 of this report (see page 19).

##### 11.1.2 *Expert Committee on the International Pharmacopoeia*

The subcommittee noted the work done by the Expert Committee on the International Pharmacopoeia which has been referred to in this report under section 3 (see page 7).

<sup>45</sup> Unpublished working document WHO/VD/SERO/43

## 11.2 Other committees or groups

### 11.2.1 *Meeting of serologists in South-East Asia, 1951*

The subcommittee reviewed with interest the report of the meeting of serologists in South-East Asia held at the end of 1951,<sup>46</sup> and expressed the opinion that such meetings should be held from time to time. Similar meetings should take place in other regions or areas with a view to promoting co-operation between laboratories, improvements in testing work, and uniformity in techniques.

### 11.2.2 *Rotterdam Serological Study-Group*

In reviewing the report from the Serological Study-Group in Rotterdam, the subcommittee observed that for use in laboratories serving clinics in port areas the study-group had selected 4 tests—1 tube flocculation test, 1 complement-fixation test, and 2 slide flocculation tests—and that it had recommended that quantitative testing be performed by the use of one of the last three. The subcommittee felt that the purpose of selecting tests for laboratories serving port clinics should be to obtain such uniformity in testing procedure that it would be possible to compare test results in blood samples taken from a sailor visiting different ports, and it considered therefore that it might be possible for the study-group to designate one single quantitative test for this special purpose. The subcommittee also desired to bring to the notice of the study-group the recommendations contained in section 6 of this report (see page 19) on recommended diagnostic methods.

The Subcommittee on Serology and Laboratory Aspects,

Having reviewed the report of the Serological Study-Group attached to the Rotterdam Port Demonstration Project,

#### RECOMMENDS

- (1) that the study-group should be asked to consider the possible designation of one single quantitative test for the testing of seamen with a view to facilitating the comparison of results on blood samples taken from sailors in different ports ;
- (2) that the attention of the study-group be drawn to the considerations of the subcommittee on recommended diagnostic methods.

### 11.2.3 *American Public Health Association*

The Serological Subcommittee of the American Public Health Association had offered to co-operate with WHO on a formal or informal basis.<sup>47</sup>

<sup>46</sup> Unpublished working document SSA-52-170

<sup>47</sup> Unpublished working document WHO/VD/SERO/48

While expressing its appreciation of this offer, the WHO subcommittee felt that as under the present regulations it existed as a subcommittee only while in session it was not in a position to deal with this offer. The subcommittee expressed the opinion that good co-operation should already exist by virtue of the fact that three members of the WHO Expert Advisory Panel on Serology and Laboratory Aspects are members of the Serological Subcommittee of the American Public Health Association.

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### Annex 1

#### CONTINUATION OF PILOT EXPERIMENT \*

A sufficient number of ampoules from the reactive non-syphilitic sera and from some 10 reactive sera from syphilitics, all belonging to the 80 sera which are in the process of being collected, should be taken for this experiment. Half of the ampoules should be stored at 37°C and the rest at -10°C during three months in the WHO International Serological Reference Laboratory, Copenhagen. After storage, these sera should be distributed under code numbers to some six laboratories and then tested by means of at least 2 complement-fixation tests, 2 tube flocculation tests, and 2 slide flocculation tests, one or two tests being performed in each laboratory. The choice of laboratories and tests should be made bearing in mind that several of these sera are reactive with only a few tests. Sera known to be reactive with only one or two tests should be studied to demonstrate their reactivity with other tests. Samples of the same sera (kept at -10°C and 37°C) should be examined in the same serological experiment. The details of the continuation of the pilot experiment should be planned in consultation with a bio-statistician.

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\* See section 4 of this report (page 13) and *Wld Hlth Org. techn. Rep. Ser.* 1951, 33, 23.

## Annex 2

**SUGGESTIONS FOR REPORTS ON EVALUATION  
OF SEROLOGICAL RESULTS \***

Study of the many reports on comparison between serological methods, between antigens, and between groups of population has made it clear that some general recommendations on the manner of reporting serological results would be of value.

When results from two serological tests performed on the same samples are reported, the expression "percentage of agreement" is very often used; the calculation of this percentage includes all sera which give the same or practically the same results, as well as non-reactive sera, of which there are frequently a great many. The more non-reactive sera there are, the higher the percentage of agreement found. Examples are given below.

**AGREEMENT BETWEEN TEST RESULTS**

Example	Number of cases reacting with				Percentage of agreement	Percentage of reactors	Percentage of agreement among reactors	
	Test A Test B	+	-	+				-
1.		50	20	30	400	$\frac{50 + 400}{500} = 90\%$	$\frac{100}{500} = 20\%$	$\frac{50}{100} = 50\%$
2.		50	20	30	900	$\frac{50 + 900}{1,000} = 95\%$	$\frac{100}{1,000} = 10\%$	$\frac{50}{100} = 50\%$
3.		50	45	55	850	$\frac{50 + 850}{1,000} = 90\%$	$\frac{150}{1,000} = 15\%$	$\frac{50}{150} = 33\%$

The percentage of agreement among reactors gives a better description of the findings than the plain percentage of agreement, as it would be wrong to characterize the results in examples 1 and 3 with the same percentage expression. Examples 1 and 2 show the effect on the percentage of agreement when there are more sero-negative samples in a study.

If a great many strongly reactive sera are added to studies such as the above they will have the same effect as the non-reactive sera, increasing

\* Taken from unpublished working document WHO/VD/SERO/52.

the percentage of agreement, since it is seldom that two tests show disagreement in strongly reactive sera. It follows therefore that the number and titre of all strongly reacting sera used in such studies should always be stated.

When qualitative or quantitative results are reported for two tests or for other comparative purposes it may be of value to use correlation diagrams of such size and detail that it is possible to see whether the results are grouping themselves along the identity line of a diagram or not.

When serological results from the examination of the same sera with different tests or from the examination of different groups of sera with the same test are evaluated, the clinical diagnosis and stage of disease should be stated. The comparison of the above results should be undertaken with due regard to the clinical grouping.

When evaluating treatment results, the changes in titre before and after treatment should be studied for each clinical group (or stage of disease) with due regard to the height of the initial titre.

The reader of results from a serological comparison of antigens should be unaware of the lot number of the antigen used in the various tubes. This can be done by giving the tubes code numbers and postponing de-coding until all readings have been performed.

It is important for laboratories to study the magnitude of error found in routine testing. This can be done by testing duplicate samples which are placed under code number among the routine samples. Thus the variations on one experimental day can be seen. Control sera tested every day under code numbers will demonstrate the variations from day to day.

## Annex 3

RESULTS OF STORAGE EXPERIMENT ON FREEZE-DRIED SERA  
(PILOT EXPERIMENT) \*

Seven sera from syphilitic donors were tested after storage at  $-10^{\circ}\text{C}$ ,  $20^{\circ}\text{C}$ , and  $37^{\circ}\text{C}$  for three months (three ampoules for each serum at each temperature). Log values express the number of tubes reacting in dilution rows—such as : undiluted, 1/2, 1/4, etc. ; or 1/4, 1/8, 1/16, etc. Displacements from the total average results are given below for each temperature and type of test :

Test group	Storage temperature		
	$-10^{\circ}\text{C}$	$20^{\circ}\text{C}$	$37^{\circ}\text{C}$
I. Complement fixation . . . . .	0.31	0.01	- 0.32
II. Tube precipitation . . . . .	0.05	0.03	- 0.09
III. Slide precipitation . . . . .	0.00	0.05	- 0.06

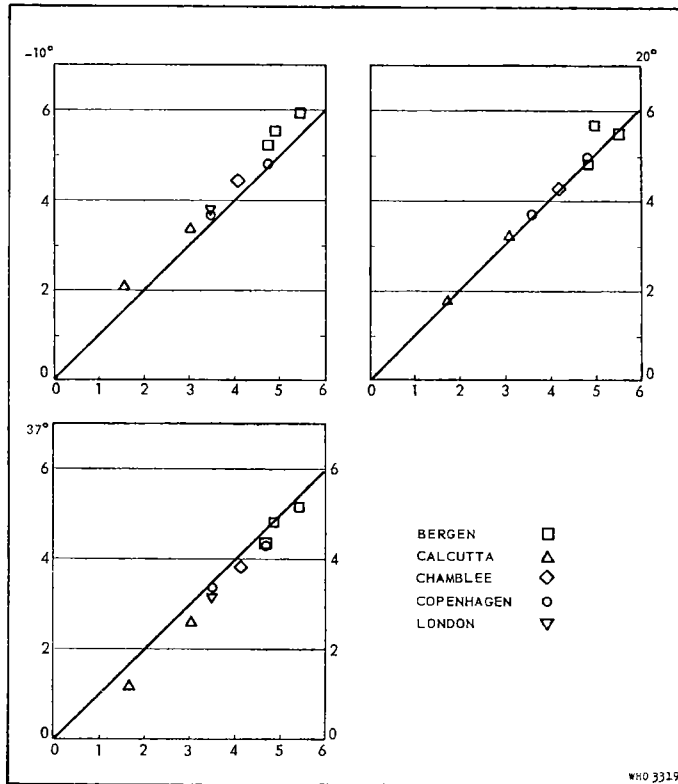
In fig. 1 (a), 1 (b), and 1 (c), are shown the average log value for each test and temperature compared with the overall average value for each test.

## SEROLOGICAL TESTS USED

Laboratory	Test		
	complement fixation	tube flocculation	slide flocculation
Bergen	Ox-heart Cardiolipin Sitolipin	Meinicke VDRL (Venereal Disease Research Laboratory)	
Calcutta	Ox-heart MRC (Medical Research Council)		VDRL
Chamblee	Kolmer	Kahn	VDRL
Copenhagen	Ox-heart Cardiolipin	Kahn	VDRL
London	Ox-heart	Kahn PPR (Price Precipitation Reaction)	
Tel Aviv		Kahn Meinicke cardiolipin Rappaport-Eichhorn Rapid cardiolipin	Rein-Bossak

\* Taken from unpublished working document WHO/VD/SERO/20 (and its Annexes I-III).

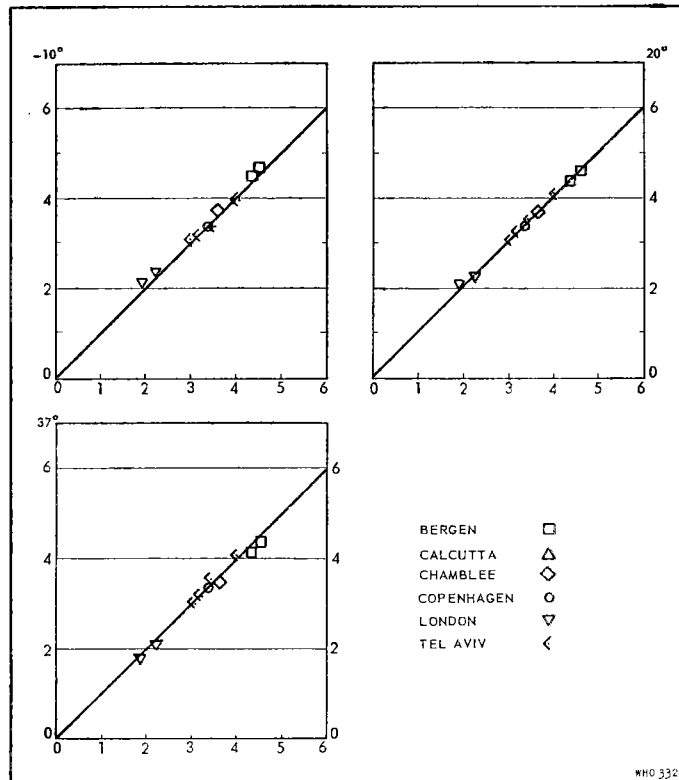
**FIG. 1 (a). COMPLEMENT-FIXATION TESTS.  
COMPARISON OF AVERAGE TITRES PER STORING TEMPERATURE WITH  
TOTAL AVERAGE TITRES FOR THE APRIL EXPERIMENTS, THE AVERAGES  
BEING TAKEN OVER ALL SERA ANALYSED**



Ordinates : mean titres

Abcissae : mean titres April

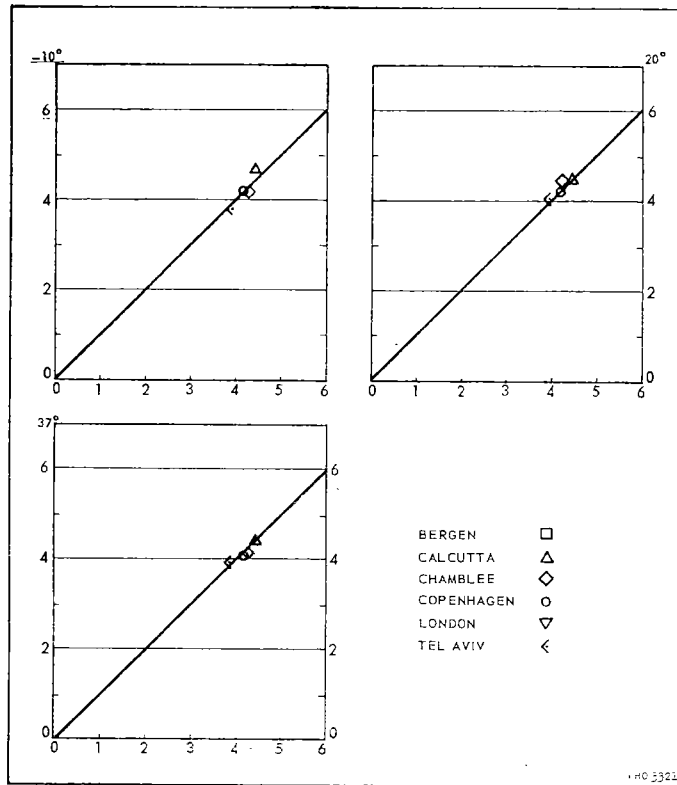
**FIG. 1 (b). TUBE PRECIPITATION TESTS.  
COMPARISON OF AVERAGE TITRES PER STORING TEMPERATURE WITH  
TOTAL AVERAGE TITRES FOR THE APRIL EXPERIMENTS, THE AVERAGES  
BEING TAKEN OVER ALL SERA ANALYSED**



Ordinates: mean titres

Abcissae: mean titres April

**FIG. 1 (c). SLIDE PRECIPITATION TESTS.  
COMPARISON OF AVERAGE TITRES PER STORING TEMPERATURE WITH  
TOTAL AVERAGE TITRES FOR THE APRIL EXPERIMENTS, THE AVERAGES  
BEING TAKEN OVER ALL SERA ANALYSED**



Ordinates : mean titres

Abcissae : mean titres April

## Annex 4

**RESULTS OF PRELIMINARY TESTING  
OF 25 FREEZE-DRIED SERA, MAY-JULY 1953 \***

Sera were tested under code numbers in 16 laboratories by 22 methods (10 complement-fixation tests, 6 tube flocculation test, and 6 slide flocculation tests). In one of the complement-fixation tests the titration was not continued to an end-point.

Six sera from presumed non-reactive normal donors were tested; only four of these were partially reactive, each with one or two tests only, as shown in table I.

**TABLE I. TITRATION RESULTS FOR SERA FROM  
PRESUMED NON-REACTIVE NORMAL DONORS**

Laboratory	Test	Serum	Log value <sup>a</sup>
Bergen	Meinicke	Coonoor 2	0.5
Bombay	VDRL	Copenhagen 13 <sup>b</sup>	0.5 <sup>b</sup>
Calcutta	MRC	London 1	AC
Johannesburg	Kolmer	Bombay 1 <sup>b</sup>	4.0 <sup>b</sup>
New York	Rein-Bossak	London 1	0.5
New York	Rein-Bossak	Bombay 1 <sup>b</sup>	1.25 <sup>b</sup>

<sup>a</sup> Log values express the number of tubes reacting in dilution rows—such as: undiluted, 1/2, 1/4, etc.; or 1/4, 1/8, 1/16, etc.

<sup>b</sup> These sera were tested on two days: on the first testing day they were non-reactive; on the second the log values were as given above.

Tables II and III show the results (in log values) for sera from presumed reactive non-syphilitic donors (9) and presumed reactive syphilitic donors (10).

\* Taken from unpublished working documents WHO/VD/SERO/28 (and its Annexes I-IV), WHO/VD/SERO/28 Add. 1 (and Annex I), and WHO/VD/SERO/28 Add. 2 (and Annex I).

TABLE II. TITRATION RESULTS (IN LOG VALUES \*) FOR SERA FROM DONORS WITH DISEASES LIKELY TO PRODUCE FALSE REACTIONS

Laboratory	Test	Serum												
		Coonoor		Copenhagen						Bombay				
		1	2	8	11	7	9	10	12	2				
	Complement fixation													
Albany	Cardiolipin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cairo	MRC	0.0	—	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Calcutta	MRC	0.0	0.0	0.0	2.0	4.5	2.0	1.75?	1.5	0.0	0.0	0.0	0.0	6.5
Coonoor	MRC	0.0	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0	0.0	3.5	?	0.0
"	Cardiolipin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Copenhagen	Cardiolipin	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
"	Ox-heart	0.6	1.8	2.3	4.0	5.2	4.8	4.8	4.7	4.7	4.7	4.7	7.9	?
Johannesburg	Kolmer	0.0	0.0	1.3	4.55	0.0	0.0	0.0	5.05	0.0	0.0	0.0	?	0.0
Naples	Kolmer	0.0	0.0	0.0	1.75	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Tube flocculation													
Bergen	Meinicke	1.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
London	PPR	0.0	0.0	3.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Osaka	Taniguchi	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tel Aviv	Rappaport-Eichhorn	0.0	0.0	0.0	0.25	0.0	0.0	0.25	0.0	0.0	0.0	0.0	0.0	0.0
"	Cardiolipin unstained	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
"	Cardiolipin stained	0.0	0.0	0.0	0.25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Slide flocculation													
Bombay	VDRL	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Calcutta	VDRL	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0
Caracas	VDRL	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
Chamblee	VDRL	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
New York	Rein-Bossak	0.0	0.0	0.0	1.75	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Trondheim	Kvittingen	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

\* Log values express the number of tubes reacting in dilution rows — such as : undiluted, 1/2, 1/4, etc. ; or 1/4, 1/8, 1/16, etc.

TABLE III. TITRATION RESULTS (IN LOG VALUES \*) FOR SERA FROM SYPHILITIC DONORS

Laboratory	Test	Serum															
		Copenhagen			Naples			Copenhagen			Coonoor			Bombay		Copenhagen	
		6	4	4	3	4	3	2	5	3	3	3	3	3	3	14	
	Complement fixation																
Albany	Cardiolipin	0.0	2.8	5.7	?	3.3	6.1	3.3	7.4**	7.1**	8.0**						
Cairo	MRC	0.0	0.25	4.75	1.5	6.0	5.0**	6.0	7.0	7.75	7.0						
Calcutta	MRC	0.0	0.0	6.5	3.5	7.5	5.5	5.5	8.5	6.5	8.5						
Coonoor	MRC	0.0	0.0	3.0	0.0	1.0	3.0	1.0	6.0	4.5	6.0?						
"	Cardiolipin	0.0	0.0	3.0	0.0	2.0?	4.0	3.5	6.0	5.0	6.0?						
Copenhagen	Cardiolipin	0.0	4.3	6.55	3.4	6.4	6.0	6.9	8.4	7.6	8.1						
Johannesburg	Ox-heart	4.2	5.75	9.8	1.8	8.3	6.0	7.6	8.8	7.5	7.6						
Naples	Kolmer	0.0	9.8	9.8	6.8	8.05	7.8	8.8	3.55	9.8	10.8						
	Kolmer	0.5	3.5	7.75	5.25	6.5	7.5	8.5	8.5	7.75	8.5						
	Tube flocculation																
Bergen	Meinicke	0.0	1.75	5.5	2.5	7.5	4.5	4.5	8.0	6.0	6.5						
London	PPR	0.0	0.0	5.0	2.5	6.5	5.5	5.0	8.5	6.0	7.5						
Osaka	Taniguchi	0.0	1.0	6.0	4.5	6.75	5.5	6.5	6.75	6.5	6.5						
Tel Aviv	Rappaport-Eichhorn	0.0	0.0	5.5	4.5	7.75	5.75	5.75	8.25	7.75	8.0						
"	Cardiolipin unstained	0.0	0.0	5.75	4.25	7.75	5.5	6.0	8.5	8.25	8.25						
"	Cardiolipin stained	0.0	0.0	6.0	5.0	8.25	5.5	6.0	8.25	8.0	8.25						
	Slide flocculation																
Bombay	VDRL	0.0	1.0	5.0	3.0	5.25	5.5	4.5	7.25	6.5	7.0						
Calcutta	VDRL	0.0	1.5	6.5	3.5	8.0	7.0	7.0	9.0	7.5	9.0						
Caracas	VDRL	3.0?	4.75	6.0	4.0	7.5	7.0	6.5	8.5	9.25	8.5						
Chambles	VDRL	0.0	2.0	6.75	1.5	6.5	5.5	5.5	7.5	7.75	7.75						
New York	Rein-Bossek	0.0	3.0	7.5	4.25	8.0	7.5	5.5	8.5	7.5	9.0						
Trondheim	Kvittingen	0.0	0.0	2.75	1.5	3.5	3.5	3.5	5.5	4.0	6.0						

\* Log values express the number of tubes reacting in dilution rows—such as: undiluted, 1/2, 1/4, etc.; or 1/4, 1/8, 1/16, etc.

\*\* Examined on a later date.

## Annex 5

**INFORMATION ON SEROLOGICAL LABORATORIES  
IN MEMBER STATES AND ON THE TESTS USED  
FOR SERODIAGNOSIS OF SYPHILIS \***

At the first session of the Subcommittee on Serology and Laboratory Aspects, which was held in 1949, it was decided that information should be collected on the serological tests for the diagnosis of syphilis used in laboratories in all Member States, and on the number of samples tested in these laboratories.

Collection of this information was begun in April 1950 when a letter, accompanied by the annex given below, was sent to all national health authorities in Member States.

**WHO STUDY OF SERODIAGNOSIS IN SYPHILIS:  
INFORMATION ON LABORATORIES  
PERFORMING SERODIAGNOSTIC TESTS**

- A. For important laboratories receiving more than 10,000 samples to be examined with seroreactions for syphilis, the following information is required :
1. Name of laboratory :
  2. Address :
  3. Name of Director :
  4. Name and position of supervisor of seroreactions :
  5. Number of samples received in 1947 :  
each of the last three years : 1948 :  
1949 :
  6. Seroreactions employed at present (with specific reference to published techniques) :
  7. To what extent are " screening " procedures used :
  8. Seroreactions discontinued or taken up since 1 January, 1949 :
- 
- B. For important smaller laboratories receiving less than 10,000 samples a year, the following information is required :
1. Number of smaller laboratories :
  2. Total approximate number of samples received in these laboratories taken together :

\* Taken from unpublished working document WHO/VD/SERO/45.

## 3. Techniques used :

3.1 Number of seroreactions used for each sample  
and number of laboratories using this routine :

	1	2	3	4
	test	tests	tests	tests

3.2 The names of all seroreactions used in  
these small laboratories :

\_\_\_\_\_

C. In the case of an author serologist working in a laboratory receiving less than 10,000 samples a year, information concerning that laboratory is required as for (A) above.

\_\_\_\_\_

D. In the case of decentralization of serological work and where only a few or no laboratories at all have 10,000 samples a year, information as for (A) above is required for the 10 most important laboratories in each country.

\_\_\_\_\_

By October 1950, when the second session of the subcommittee was held, only a few reports had been received.

Further reports continued to arrive up to March 1953. Among these were reports from new Member States which had in the interval been asked to supply the necessary information.

Details were given for laboratories testing 10,000 samples or more a year, and/or for laboratories directed by author serologists. In countries where laboratory work is decentralized the same information in respect of the ten most important laboratories was given.

The above information will be referred to as "A" information.

Less detailed information was given for the remaining laboratories in each country. This was more difficult to obtain, because many countries stated that it was impossible for them to obtain from minor laboratories sufficient information to enable them to answer the questions adequately.

This information will be referred to as "B" information.

It should be noted that the figures in the tables which follow may not give an entirely accurate picture of the serological work being performed in the various countries, as it has not been possible to check whether the national health authorities were able to obtain information from all the laboratories in their countries. In addition there may have been a certain

amount of progress in laboratory service in some countries since the first reports were sent to WHO in 1950.

Tables I-XII (two for each WHO Region) show the general outline of the system for serology in each country.

The odd-numbered tables give for each country one line summarizing "A" information and one line summarizing "B" information (where this is available). In each line are given the number of laboratories, the number (in thousands) of samples tested yearly, and the number of laboratories using one, two, three, and four or more tests. The lines containing "A" information also give the number of laboratories using three different combinations of complement-fixation and flocculation tests, the number of laboratories using one slide flocculation test only, and the number of laboratories which use a screening procedure.

From these tables it is possible to calculate the number of laboratories which are not using complement-fixation tests but only two or more flocculation tests.

The even-numbered tables show the serological tests used in the various countries. Tests without specific names, and national standard tests without an author name are recorded in these tables under "name?" in each group of tests. Tests which are used in only one or two laboratories in a Region are, for some Regions, recorded together under the expressions "other c.f. tests"; "other t.f. tests"; "other s.f. tests".

It is obvious that, when the reports were written, standardization of methods was far from having been achieved, although a certain pattern of uniformity is seen in countries where one or more of the following conditions exist:

- (a) a system of national control of antigens;
- (b) a minimum requirement for the number and types of tests to be used in recognized laboratories; and
- (c) distribution of certain antigens free of charge to all laboratories.

The number of modifications of some of the universally used tests is rather large, and it is very likely that in the case of many of these modifications no description has been published.

The main difference between Europe and the Americas—which are both Regions with many laboratories performing a great number of examinations yearly—lies in the fact that the majority of the European laboratories examine every sample with three or more tests, which often include one or two complement-fixation tests, whereas the majority of the laboratories in the Americas examine every sample with only one or two tests, which as a rule are flocculation tests.

TABLE I. AFRICAN REGION

Country	Number of laboratories	Annual number of samples tested (in thousands)	Number of laboratories performing 1, 2, 3, or $\geq 4$ tests per sample				Number of laboratories performing				
			1	2	3	$\geq 4$	1 c.f. & 1 floc.*	1 c.f. & $\geq 2$ floc.	2 c.f. & $\geq 1$ floc.	only 1 slide	screening procedure
Union of South Africa	7	494	2	2	1	2	1	3			4

\* c.f. = complement fixation  
floc. = flocculation

TABLE II. AFRICAN REGION

Test	Union of South Africa
<b>Complement fixation</b>	
Harrison-Wyler	2
Kolmer	2
Name ?	2
<b>Tube flocculation</b>	
Eagle	3
Kahn standard	4
Kahn verification or modification	2
<b>Slide flocculation</b>	
Macnab-Levin	3

TABLE III. REGION FOR THE AMERICAS

Country	Number of laboratories	Annual number of samples tested (in thousands)	Number of laboratories performing 1, 2, 3, or $\geq 4$ tests per sample				Number of laboratories performing					
			1	2	3	$\geq 4$	1 c.f. & 1 floc.	1 c.f. & $\geq 2$ floc.	$\geq 2$ c.f. & 1 floc.	only 1 slide	screening procedure	
Argentina	A 7	128			6	1		7				
Canada	A 16 B 13	1,238 57	8	1 2	12 3	3	1	15				15
Chile	A 1	101			3							
Costa Rica	A 3 B 18	73 17	6	2 9	1 2	1		1				
Cuba	A 1	60				1						1
Dominican Republic	A 1 B 20	38 9	13	1 6		1						
El Salvador	A 2 B 8	68 31	3	1 4	1	1						2
Guatemala	A 1	43		1			1					1
Honduras	A 1	15		1								
Mexico	A 1	32				1		1				1
Nicaragua	A 1	31	1									
Panama	A 1	24		1								
Paraguay	A 5	37	2	2		1		1				1
Peru	A 1	42			1			1				
USA	A 154 B 1,954	12,533 > 2,727	23 639	52 747	51 37	26 2	22	65	3	8		48
Venezuela	A 10 B 41	255 98	41	9		1			1			Yes
Total	A 206 B 2,054	14,718 > 2,939	26 710	71 768	74 43	35 4	24	91	4	8		69

TABLE IV. REGION FOR THE AMERICAS

Test	Argentina	Canada	Chile	Costa Rica	Cuba	Dominican Republic	El Salvador	Guatemala	Honduras	Mexico	Nicaragua	Panama	Paraguay	Peru	USA	Venezuela	Total
<b>Complement fixation</b>																	
Eagle	1	15		1				1						1	3	1	5
Koerner															81	1	101
Michigan	1														4		4
Other c.f. tests <sup>a</sup>	6	1								1					6		8
Name ?																	7
<b>Tube flocculation</b>																	
Eagle	7	16	1	3	1	1	2		1	1	1	1	5	1	5		7
Hinton and modification	7	13	1			2	2			1			2		13		13
Kahn standard															95	10	146
Kahn presumptive															11		35
Kahn verification															3		4
Other t.f. tests <sup>b</sup>			1		1		1								4		5
<b>Slide flocculation</b>																	
Kline	1	1			1	1									36		40
Mazzini		4			1	1				1			1	1	46		54
Rein-Bossak					1	1				1			1	1	1		3
VDRL					1	1				1			1	1	52	10	73
Other s.f. tests <sup>c</sup>		2		3	1		1	1	1	1		1	1	3			5

<sup>a</sup> Other complement-fixation tests :

- Boerner-Lukens
- Kent
- Marquez
- Miravent
- Nebraska
- New York State
- South Carolina

<sup>b</sup> Other tube flocculation tests :

- Boerner-Lukens
- Meinicke
- Nebraska
- VDRL

<sup>c</sup> Other slide flocculation tests :

- Chediak
- Filter-paper method
- Marquez
- New York State
- Wisconsin Psychiatry Institute

TABLE V. EASTERN MEDITERRANEAN REGION

Country	Number of laboratories	Annual number of samples tested (in thousands)	Number of laboratories performing 1, 2, 3, or $\geq 4$ tests per sample				Number of laboratories performing				
			1	2	3	$\geq 4$	1 c.f. & 1 floc.	1 c.f. & $\geq 2$ floc.	2 c.f. & $\geq 1$ floc.	only 1 slide	screening procedure
Egypt	A B	1 several	95 116	1 not mentioned							1
Ethiopia	A A	1 1	2 33	1 1			1				
Iraq	A A	1 5	33 70	1 2	1 2		1	1			1
Israel	A B	5 4	70 14	1 2	2 2		1	1		1	2
Jordan, Hashemite Kingdom of the	A B	1 4	1 1	1 4							
Lebanon	A A	2 4	12 9		1 1			1			
Pakistan	A B	4 8	> 9 19	2 8	1		1				
<b>Total</b>	A B	> 15 > 16	> 222 150	4 14*	5 2*	5	3	4		1	4

\* Partial information

TABLE VI. EASTERN MEDITERRANEAN REGION

Test	Egypt	Ethio- pia	Iraq	Israel	Jordan	Leba- non	Paki- stan	Total
<b>Complement fixation</b>								
Harrison			1					1
Sachs				1				1
Kolmer						1		1
Hecht						1		1
Calmette-Massol						1	1	2
Name ?	1	1		1				4
<b>Tube flocculation</b>								
Citochol				1				1
Kahn	1	1	1	4	1	1	3	12
Meinicke				1				1
PPR		1						1
Rappaport				2				2
" Screen " test	1							1
VDRL						1		1
<b>Slide flocculation</b>								
Laughlen			1					1
Mazzini				1				1

TABLE VII. EUROPEAN REGION

Country	Number of laboratories	Annual number of samples tested (in thousands)	Number of laboratories performing 1, 2, 3, or $\geq 4$ tests per sample				Number of laboratories performing					
			1	2	3	$\geq 4$	1 c.f. & 1 floc.	1 c.f. & $\geq 2$ floc.	2 c.f. & $\geq 1$ floc.	only 1 slide	screening procedure	
Austria	A	10			1	9		9	1			3
Belgium	A	12		6	4	2	6	5	1			
Denmark	A	1				1		1				1
England and Wales	A	22		14	7	1	14	7	1			6
Finland	A	6			5	1			6			
France	A	28		1	7	20		8	19			3
	B	402	3	48	214	137						
Germany, Federal Republic of	A	72			3	69		20	52			20
	B	61	1	3	14	43						
Greece	A	5		5			5					
Iceland	A	1		1								
Ireland	A	3		2	1		2	1				1
Italy	A	12			1	11			12			2
Luxembourg	A	1			1			1				
Monaco	A	1				1		1				
Netherlands	A	12		1	7	4	1	8	2			3
	B	11	*	*	*	*						
Northern Ireland	A	4		1	3		1	3				2
Norway	A	6			4	2		5	1			2
Portugal	A	10		7	3		7	3				
	B	103		100	3	3						
Scotland	A	6		3	2	1	3	3				3
Spain	A	8			4	4		7	1			
	B	several	*	*	*	*						
Sweden	A	9		1	1	7		3	5			1
Switzerland	A	8		3	2	3	3	4	1			1
Turkey	A	1		1			1					
	B	6		6								
Yugoslavia	A	6			3	3		6				2
	B	several	*	*	*	*						
Total	A	244		4	46	59	139	43	95	102		50
	B	583		157	231	180						

\* No information available

TABLE VIII.

Test	Austria	Belgium	Denmark	England and Wales	Finland	France	Germany	Greece	Iceland	Ireland	Italy
<b>Complement fixation</b>											
Bordet		8				3					
" modification		3				1					
Calmette-Massol						6					
Debains (& modification)						8					
Demanche						7					
Eagle											
Fildes McIntosh & modification				2						1	
Harrison				1							
" -Wyler				10							
" modification				4						2	
Hecht		1				6					
" modification						4					
Jacobsthal							1				
Kaup	1						3				
Kolmer	1			1		11					
" original							17				
" cardiolipin											
" modification											
Moersch			1								
" cardiolipin			1								
Mütermilch						2					
Pallida							1				10
Rubenstein						1					
Sitolipin					1						
Sormani											
WR name ?	5			5	6		12				3
" cardiolipin name ?							38				9
" original or classic	4				6	1	2	5			2
WR 2 antigen name ?							23				2
WR 3							30				
Other c.f. tests <sup>a</sup>		1		1		2					2
<b>Tube flocculation</b>											
Citochol	3					1	61				11
Kahn	9	11	1	19	6	25	41	5	1	1	9
" other	1	1		1		5					
" modification				2						2	
Meinicke II	10		1			5	43				6
" other							9				
" without specification		5		1		10	20				
Müller Ballung	7										
PPR				4							
Sachs-Georgi							3				
Sitolipin					1						
Sigma											
VDRL			1	1							
Vernes						7					
Other t.f. tests <sup>b</sup>		1				2			1		1
<b>Slide flocculation</b>											
Chediak and modification	1						12				
Kline		2		1		10					
Ko Da Gua						2	2				
Laughlen											
Meinicke-Kvittingen											
Microflocculation cardiolipin							6				1
Rein-Bossak						1					1
VDRL			1	1		3	2			1	3
Other s.f. tests <sup>c</sup>						1	2				1

<sup>a</sup> Other complement-fixation tests :Donald           Kaipo  
Eschbach-Duhot   OkoloffSordelli-Miravant  
SuchetWR 1922 Copenhagen  
Treponemics proteico purif.  
Spicca<sup>b</sup> Other tube flocculation tests :Eagle  
Ford-Robertson-ColquhounRappaport-Eichhorn  
Wadsworth-BrownSuchet flocculation  
Suchet conglomeration  
Spicca

EUROPEAN REGION

Luxembourg	Monaco	Netherlands	Northern Ireland	Norway	Portugal	Scotland	Spain	Sweden	Switzerland	Turkey	Yugoslavia	Total
1	1	1	3	1		1 1 2 1	2		3			14 4 6 8 7 2 4 3 16 7 8 7 2 2 7 19 18 2 6 6 2 2 12 2 1 2 60 51 20 25 30 9
1	1	3 7	3 1	1 2	2 9	4	5 7	8	2 5	1	4 6	93 182 8 6 92 11 49 10 4 6 1 2 3 10 7
		2 4	1	2		4		3				15 20 4 5 2 7 3 13 5

Other slide flocculation tests :

- Chromotest
- Giacardy
- Meinicke-Fischer
- Mazzini

TABLE IX. SOUTH-EAST ASIA REGION

Country	Number of laboratories	Annual number of samples tested (in thousands)	Number of laboratories performing 1, 2, 3, or $\geq 4$ tests per sample				Number of laboratories performing				
			1	2	3	$\geq 4$	1 c.f. & 1 floc.	1 c.f. & $\geq 2$ floc.	2 c.f. & $\geq 1$ floc.	only 1 slide	screening procedure
Burma	A	1			1			1			
Ceylon	A	1			1			1			
India	A	18	4	9	5		9	2			2
	B	34	21	6	7						
Indonesia	A	8		2	5	1	2	5	1		
Thailand	A	5	3	2			2				
Total	A	33	7	13	12	1	13	9	1		2
	B	34	21	6	7						

TABLE X. SOUTH-EAST ASIA REGION

Test	Burma	Ceylon	India	Indonesia	Thailand	Total
<b>Complement fixation</b>						
MRC 4			3			3
Harrison-Wyler		1	3			4
Hinton			1			1
Kolmer			1	4	1	6
Name ?	1		5	5	1	12
<b>Tube flocculation</b>						
Citochol				1		1
Hinton			1			1
Kahn	1	1	15	7	5	29
Kirschner				1		1
Meinicke				1		1
Murata				3		3
PPR			1			1
Rapid test			1			1
Sachs-Georgi				1		1
<b>Slide flocculation</b>						
Kline		1				1
Kvittingen-Meinicke			2			2
VDRL	1		4			5

TABLE XI. WESTERN PACIFIC REGION

Country	Number of laboratories	Annual number of samples tested (in thousands)	Number of laboratories performing 1, 2, 3, or $\geq 4$ tests per sample				Number of laboratories performing					
			1	2	3	$\geq 4$	1 c.f. & 1 floc.	1 c.f. & $\geq 2$ floc.	2 c.f. & $\geq 1$ floc.	only 1 slide	screening procedure	
Australia	A B	11 11	1 *	8 *	2 *	*	8	2			6	
Cambodia	A	1		1				1				
China	A B	5 19	2 12	1 5	1 2	1	1	2			2	
Japan	A B	44 272	977 1,094	3 31	12 159	20 68	9 14	7	25	3	1	1
New Zealand	A B	5 4	28 6		2 4	3		2	2	1		2
Philippines	A B	1 10	25 17		1				1			
Viet Nam	A	3	15	10	2		1			1		
Total	A B	70 316	1,205 1,166	6 53	25 168	28 70	11 14	18	33	5	1	11

\* No information available

TABLE XII. WESTERN PACIFIC REGION

Test	Australia	Cambodia	China	Japan	New Zealand	Philippines	Viet Nam	Total
<b>Complement fixation</b>								
Browning				10				10
" Taniguchi				4				4
Eagle	2							2
Harrison-Wyler	1				1			2
Kitasato				11				11
Kolmer	1		1		1	1		4
MRC 4	3							3
Ogata				9				9
Richardson					2			2
Name ?	2		3	6				11
Other c.f. tests <sup>a</sup>	1	1			2		2	6
<b>Tube flocculation</b>								
Kahn and modification	6	1	3	15	4	1	2	32
Kitasato				19				19
Meinicke		1					1	2
Murata			3	36				39
Sachs-Georgi				4				4
Other t.f. tests <sup>b</sup>	1						1	2
<b>Slide flocculation</b>								
Ide				10				10
Kline	6		1				1	8
Laughlen					2			2
VDRL					1	1		2

<sup>a</sup> Other complement-fixation tests :

Boerner-Lukens  
Calmette-Massol  
Debains  
Griffith & Scott  
Hecht-Muttermilch  
MRC 1

<sup>b</sup> Other tube flocculation tests :

Eagle and modification  
Vernes

## Annex 6

**SEROLOGICAL METHODS  
USED IN WHO FIELD-TEAM LABORATORIES \***

Country	Test										
	complement fixation			tube flocculation				slide flocculation			
	Kol-mer	Harrison-Wyler	New York State	Eagle	Kahn	Meinicke	VDRL	Che-diak	Kvit-tingen-Meinicke	Kline	VDRL
Ecuador					+						+
Guatemala					+						+
Haiti					+						+
Paraguay											+
Egypt	+					+					+
Ethiopia	+				+			+++		+++	+
Iraq	+++		+++		+	+++			+++	+++	+
Saudi Arabia					+	+					+
Afghanistan	+++				+				+	+++	+
Burma	+++	+++			+				+++		+
Ceylon					+			+++		+	
India											
Madras (General Hospital)									+++	+++	+
(public-health laboratories)					+						+
Simla					+				+		+
Kulu									+		+
Bombay					+				+		+
Indonesia					+					+	+
Thailand	+++	+++		+++	+		+++		+	+	+

\* Taken from unpublished working document WHO/VD/SERO/54.

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