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# EXPERT COMMITTEE ON VENEREAL INFECTIONS AND TREPONEMATOSES

## Report on the Second Session of the Subcommittee on Serology and Laboratory Aspects

*Paris, 23 September — 2 October 1950*



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## EXPERT COMMITTEE ON VENEREAL INFECTIONS AND TREPONEMATOSES

### Second Session of the Subcommittee on Serology and Laboratory Aspects

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Dr. R. Laporte, Chef du Service de la Sérologie, Institut Pasteur, Paris, France

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Dr. F. Rappaport, Chief of Laboratories, Municipal and Beilinson Hospitals, Tel Aviv, Israel

Dr. K. V. Venkatraman, Serologist to the Government of India, School of Tropical Medicine, Calcutta, India (*Rapporteur*)

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Dr. T. Guthe, Chief, Venereal-disease Section, WHO

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The report on the second session of this subcommittee was originally issued in mimeographed form as document WHO/VD/73, 28 November 1950.

# EXPERT COMMITTEE ON VENEREAL INFECTIONS AND TREPONEMATOSES

## Report on the Second Session of the Subcommittee on Serology and Laboratory Aspects [1] [2]

### 1. Introduction

By invitation of the Chairman, Dr. Mary Pangborn, Division of Laboratories and Research, New York State Department of Health, Albany, N.Y., USA, and Dr. C. Rein, Associate Clinical Professor of Dermatology and Syphilology, New York University Bellevue Medical Center, N.Y., USA, attended several meetings. These consultants were participating in the WHO Syphilis Seminar held concurrently in Paris.

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[1] The Executive Board, at its seventh session, adopted the following resolution:

The Executive Board,

Having considered the report of the Subcommittee on Serology and Laboratory Aspects of the Expert Committee on Venereal Infections and Treponematoses on its second session and the recommendations presented by the expert committee itself,

1. THANKS the members of the committee for their work;
2. AUTHORIZES the publication of the report;
3. ENDORSES the recommendations that descriptions of cardiolipin and lecithin be included in the *Pharmacopoea Internationalis* and that preliminary standards for cardiolipin and lecithin be established;
4. WELCOMES the appropriate steps taken by the Expert Committees on the Unification of Pharmacopoeias and on Biological Standardization in this respect;
5. ACCEPTS postponement of the International Serodiagnostic Laboratory Conference until pilot experiments on the usefulness of freeze-dried sera for evaluation of serological tests have been studied, realizing that the plans for the conference might be changed if dried reference sera of various levels of sensitivity from syphilitics and non-syphilitics are proved to be valuable for an estimate of the merit of serological tests;
6. NOTES that the pilot study is under way;
7. DRAWS ATTENTION to the progress of the inter-laboratory work on exchange of samples, and
8. ENDORSES the convening of the Subcommittee on Serology and Laboratory Aspects in 1952.

(Resolution EB7.R66, *Off. Rec. World Hlth Org.* 32)

[2] In order to avoid delays in the consideration of the report, the members of the Expert Committee on Venereal Infections and Treponematoses were consulted by mail, and unanimously agreed upon the resolutions contained in Annex 2, page 27.

Dr. E. H. Hermans, Medical Director, Anti-Venereal-Disease Association, Rotterdam, Netherlands, attended one meeting as an observer from the Expert Committee on Venereal Infections and Treponematoses.

Dr. A. A. Miles, Director, Department of Biological Standards, National Institute for Medical Research (Medical Research Council), London, United Kingdom, attended several meetings as representative of the Expert Committee on Biological Standardization.

Dr. J. A. Lorenzo, Director, Central Laboratory of Serology, Ministry of Public Health, Montevideo, Uruguay, appointed by the Uruguayan Ministry of Public Health as its observer, attended several meetings.

The subcommittee unanimously elected Mr. A. Harris as Chairman, Dr. I. N. Orpwood Price as Vice-Chairman, and Dr. K. V. Venkatraman as Rapporteur. The proposed agenda was adopted at the opening meeting. Fifteen meetings were held and the report was approved by all members.

## 2. Developments and Perspectives

The Expert Committee on Venereal Infections reiterated in 1949 [3] its previous opinion that any sound venereal-disease-control programme is dependent to a major degree on the efficient conduct of serological tests for syphilis, and pointed out the great lack of uniformity of procedure, technique, and the manner of reporting results in serological studies. The Subcommittee on Serology and Laboratory Aspects of the above committee endorsed this view at its first session.[4] After having re-examined the situation, the subcommittee agreed that its work should proceed on the basis of this declaration and that the chief work in the coming year should be to advise WHO on such further activities as could contribute to more efficient and uniform performance of serology in syphilis, nationally, as well as internationally.

The preparatory work for the holding of the International Serodiagnostic Laboratory Conference in 1951 had proceeded in 1949 and 1950 along the lines originally drawn up by the main committee [5] and developed in further detail by the subcommittee at its first session,[6] as approved by the fifth session of the Executive Board [7] and the Third World Health Assembly.[8]

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[3] *World Hlth Org. techn. Rep. Ser.* 1950, **13**, 23
[4] *World Hlth Org. techn. Rep. Ser.* 1950, **14**, 6
[5] *Off. Rec. World Hlth Org.* **8**, 63; **15**, 24
[6] *World Hlth Org. techn. Rep. Ser.* 1950, **14**, 6
[7] *Off. Rec. World Hlth Org.* **25**, 12
[8] Resolution WHA3.37, *Off. Rec. World Hlth Org.* **28**, 28

Certain developments have, however, taken place, and freeze-drying techniques are currently being used in the preparation of standard sera used in the international standardization of various methods and products. These refined techniques have not, as yet, been applied in an effort to establish a collection of syphilitic sera at various levels of reactivity from different stages of syphilis, or from different types of false-positive reactors. Should it be possible to use such sera as preliminary standards, this might alter several important problems connected with the planning of the International Serodiagnostic Laboratory Conference. Until preliminary studies on the possibility of establishing such a collection of dried sera have been carried out, the further detailed planning and organizational work for the conference might therefore be postponed.

The subcommittee also reviewed briefly the technical advances relating to treponematoses other than syphilis, as well as those pertaining to gonorrhoea and the minor venereal infections, which have taken place since the first session of the subcommittee. It was observed that several developments might merit consideration by the subcommittee at a later stage :

(1) the laboratory aspects of lymphogranuloma venereum ;

(2) the maintenance of live *Treponema pallidum* outside of human or animal hosts : recent claims for successful cultivation of treponema on artificial media ; studies of the life-cycle of *T. pallidum* ; and the results obtained in the differentiation studies carried out by means of the electron microscope technique ;

(3) further results available on immunological relationship between the treponemes of syphilis, yaws, bejel, and pinta, and between strains of *T. pallidum* itself ;

(4) the applicability of the Nelson test to studies on immunological changes in man and animal in relation to treatment ;

(5) the discovery of phosphatidic acid antigens other than cardiolipin.

The subcommittee reviewed further the serology and laboratory aspects of venereal diseases and treponematoses as applied to the WHO programme and activities in 1949 and 1950, and considered carefully the documentation made available to the members before and during the second session. Much of this information had been requested by the subcommittee at its first session as supporting documentation to the agenda of the second session.

This documentation included data on the inter-laboratory test evaluation being carried out between the Statens Seruminstitut, Copenhagen, Denmark, the Institut Pasteur, Paris, France, the Venereal Diseases

Reference Laboratory (Public Health Laboratory Service), St. Peter's Hospital, London, United Kingdom, and the Venereal Disease Research Laboratory (US Public Health Service), Communicable Diseases Center, Chamblee, Ga., USA (formerly Staten Island, N.Y.); the serological work of the WHO-supported venereal disease demonstration team in Simla, India; the deteriorating effect of transportation on samples; laboratory errors; as well as the plans for the studies of the investigative International Treponematosis Laboratory Centre, Johns Hopkins University, Baltimore, Md., USA.

Several WHO teams have started their activities during the last few months or preliminary surveys have been undertaken for the establishment of team centres. During the next few years, as techniques are adapted to tropical conditions and environments, serological methods will be used under difficult conditions which have not as yet been completely explored; factors giving rise to false-positive reactions will be further studied. These teams will make available technical data not only on the serological aspects of syphilis under varied and varying conditions, but will also provide WHO with information on yaws and bejel, furnishing—at little extra cost—valuable basic material for the comparative study of the biological and immunological characteristics and relationship of the treponemal diseases and their causative agents. Initial experience has shown that laboratory specimens and animals can now be sent from teams in different regions to the International Treponematosis Laboratory Centre in Baltimore for treponema immobilizing antibody tests, thus enabling the international treponematoses study to proceed along the lines recommended during the first session of the subcommittee,[9] and approved by the Expert Committee on Venereal Infections during its third session in 1949.[10]

### 3. International Serodiagnostic Laboratory Conference

The subcommittee discussed in detail the report on its first session [11] and the comments of the Expert Committee on Venereal Infections on this report.[12] Since the first session of the subcommittee, WHO had collected extensive information on the possible accommodation for the International Serodiagnostic Laboratory Conference. WHO had also notified Member States of the holding of the conference in 1951 in accordance with the resolution adopted by the Executive Board at its fifth session,[13] and on

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[9] *World Hlth Org. techn. Rep. Ser.* 1950, **14**, 17
[10] *World Hlth Org. techn. Rep. Ser.* 1950, **13**, 16
[11] *World Hlth Org. techn. Rep. Ser.* 1950, **14**
[12] *World Hlth Org. techn. Rep. Ser.* 1950, **13**, 23
[13] *Off. Rec. World Hlth Org.* **25**, 12

the basis of the inclusion of this activity in the 1951 budget as approved by the Third World Health Assembly [14] and the sixth session of the Executive Board.[15] An outline of the conference had been published by the medical press and preliminary applications for participation in the conference had now been received by WHO.

It was further noted that the need for sound statistical considerations during the planning stages of the conference and in the evaluation of the results obtained had been met by the Director-General.

3.1 *Considerations*

The subcommittee wished to reiterate its previous opinion that a conference would be a valuable means of evaluating the relative efficiency of serological tests for syphilis. A conference, aiming at the international standardization of serodiagnostic laboratory procedures, would also provide an opportunity for the rapid and easy exchange of scientific information by demonstrations and discussions of the attending author serologists.

The subcommittee noted that:

(1) funds at present available would allow the participation of only 15 testing teams while approximately 27 preliminary applications, representing 40 different methods, had been received from test authors;

(2) it would be difficult to evolve an acceptable scientific basis for limiting the number of participants and selecting the most desirable tests for the conference;

(3) although the use of cardiolipin antigens had increased during the past year, many laboratories had not as yet acquired experience in their use, and several new tests, or modifications of old tests, could be expected to be published during the year, which indicated that the difficulties in selecting tests for the conference would be greater than foreseen a year ago;

(4) freeze-dried serum samples might serve as suitable controls or substitutes for whole blood in evaluating relative test efficiencies; the capacity of these preparations to serve in this way could be ascertained with little expense within six months by preliminary investigations.

The subcommittee considered point (4) in detail and discussed the possible usefulness of establishing a collection of freeze-dried sera at various levels of reactivity from various stages of syphilis and from different

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[14] Resolution WHA3.64, *Off. Rec. World Hlth Org.* **28**, 39

[15] Resolution EB6.R22, *Off. Rec. World Hlth Org.* **29**, 10

types of false-positive reactors. The use of samples of such sera might serve as a means for the selection of the soundest and most suitable tests for participation in the conference.

It has been difficult with previous technical procedures to maintain the stability of weakly positive sera and many types of false-positive sera in dried form. The representative of the Expert Committee on Biological Standardization advised that methods for freeze-drying of sera were now available which had not so far been applied to syphilitic sera. The sub-committee welcomed the suggestion that a pilot study be established to determine whether serum samples, dehydrated according to such methods, will meet the primary requirements of complement-fixation and flocculation tests for syphilis. An outline for the conduct of such a pilot study is attached to this report.[16] This outline was prepared and recommended by the representative of the Expert Committee on Biological Standardization on the understanding that it be carried out in co-operation with the Statens Seruminstitut, Copenhagen, and the following six laboratories:

Institut Pasteur, Paris, France

School of Tropical Medicine, Calcutta, India

Municipal and Beilinson Hospitals, Tel Aviv, Israel

Gade's Institute, University of Bergen, Norway

Venereal Diseases Reference Laboratory, St. Peter's Hospital, London, United Kingdom

Venereal Disease Research Laboratory, Communicable Diseases Center, Chamblee, Ga., USA.

The pilot study should be restricted to 12 donor sera and the findings and reports on the project made available within the next six months. The pertinent observations are to be returned to the Secretary of the sub-committee for analysis and for distribution to the members of the sub-committee and participating laboratories, to the members of the Expert Committee on Venereal Infections and Treponematoses, and to the members of the Expert Committee on Biological Standardization, for further consideration.

Should this pilot study yield *encouraging*, but not entirely *satisfactory*, results, a further study should be undertaken to investigate the factors adversely affecting the study and the possible improvements in technique which could be made to establish stable dried syphilitic sera of various reactivity levels.

Should the results obtained be *satisfactory*, they would justify a large-scale collection of freeze-dried sera from different stages of treated and

[16] Annex 1, page 23

untreated syphilis at various levels of reactivity, plus sera of false-positive reactors from several diseases and conditions in various parts of the world. The subcommittee would then study this matter further at its next meeting with a view to the establishment of preliminary standards of sera in dried form. It was suggested that 80 sera, with approximately 500 ampoules of each serum, might be required. Such an undertaking would need considerable time but, if successful, a suitable repository would be established from which sera could be made available on demand for the rapid investigation and evaluation of newer testing procedures. Such dried sera might then ultimately serve as suitable substitutes for whole-blood specimens for evaluating the relative efficiency of serological tests.

In view of these considerations, the subcommittee suggested that the final arrangements for the serodiagnostic laboratory conference be postponed until the results of the pilot study become available early in 1951. The relative value of a single central conference, several regional conferences, or the selection of laboratories in several parts of the world for evaluating newly published serological testing procedures, may then be more clearly ascertained. The value of dehydrated sera as a means for the selection of tests to participate in the laboratory conference, and the overall value of this study in connexion with the long-range standardization activities of WHO in the serology of syphilis, should be ascertained at a meeting of the subcommittee early in 1951.

### 3.2 *Planning of the conference*

The administrative plans for a central serodiagnostic conference were not discussed in detail as the final outline and practical organization would be largely dependent on the results of the above-mentioned pilot study.

The following general observations were made:

(1) The subcommittee felt that it would be desirable for the participating serologists to be assisted by a technician to ensure that more than 100 sera were examined daily so as not to allow the time factor to influence the reliability of the tests performed. Duplicate and quantitative testing would involve more work with each single serological test than in previous international conferences.

(2) The subcommittee, having received (as requested during its first session[17]) a report on the accommodation available in Copenhagen, London, and Paris, felt that, at present, the accommodation to be found in Paris in the autumn of 1951 was better than that available in Copenhagen, while no accommodation was available in London. Large laboratories with at

[17] *World Hlth Org. techn. Rep. Ser.* 1950, **14**, 8

least 4 m of working table and more than 20 $m^2$ of floor space for each participant would be a minimum requirement.

(3) The subcommittee was of the opinion that technical assistance should be obtained not only from the laboratories in the conference area, but also from other countries; the information presented by WHO that the US Public Health Service had indicated its willingness to co-operate and assist in the organization of the conference was welcomed by the subcommittee.

(4) The subcommittee felt that at the conference complement-fixation, tube-flocculation, and slide-flocculation tests for blood or spinal fluid should be examined, besides tests designated for verification of specificity of seroreactions. The treponema immobilization test (Nelson) was considered an essential activity of the conference.

During the discussion on evaluation of serological methods by means of a conference or other type of WHO-sponsored comparison, the following further points were recorded:

(1) Reliability and technical simplicity could be evaluated and recorded at a conference; at the same time tests most useful and adaptable to different conditions in various parts of the world could be selected.

(2) It would be possible at a conference to have each specimen distributed to all serologists in the same condition and at the same time.

(3) Evaluation of methods at previous conferences had been a useful basis for selection of tests for nationwide application in several countries.

The view was expressed that a central conference would record only the ability of some technicians to perform certain techniques during 10 to 15 working days, and that even extremely good results would not necessarily be obtained by all, or even many, other technicians elsewhere and under other circumstances. Consideration was, therefore, given to the possibility of studying in more than one laboratory the sensitivity and specificity of each method to be evaluated.

The subcommittee considered extracts of reports on earlier laboratory conferences, particularly the 1941 conference held in Washington, D.C., USA, and received additional information on techniques for blood and spinal-fluid taking. A statistical survey of some of the results was presented to the subcommittee, and the wish was expressed that material from the 1941 conference and later evaluations be used for statistical analysis of variations in results from duplicate testing.

### 3.3 *Selection of participants*

Various methods for the selection of conference participants were considered. The subcommittee felt strongly that no logical and scientifically founded selection could be made of the various techniques available, on the basis of the written texts in the preliminary applications. From a laboratory standpoint, the actual techniques were in many cases unknown to the members of the subcommittee. Many tests were being used only nationally and some had been modified so recently that written descriptions were not available. The subcommittee agreed that evaluation of tests in previous national or international laboratory conferences was not a sound basis for selection, since several tests had since been modified and could not be judged on the basis of earlier performance.

The subcommittee discussed the usefulness of regional conferences either as a preliminary or as an alternative to a central conference. It was felt that preliminary regional conferences would involve heavy budgetary outlays and would give rise to new selection problems, as several widely used tests would be included and non-author serologists would have to perform the tests thus adding further to the problems.

The subcommittee considered the possibility of selecting participants for a conference by a preliminary survey, utilizing suitable serum samples in dried form, and was in favour of performing such a selection when and if such standard sera had been established through the pilot study (see section 3.1).

### 3.4 *Recommendations*

The subcommittee adopted the following resolution :

The Subcommittee on Serology and Laboratory Aspects of the Expert Committee on Venereal Infections and Treponematoses,

Having studied the documentation on the progress made in the organization of the International Serodiagnostic Laboratory Conference ;

Having considered the various technical and other aspects of the problem of standardization and evaluation of serological tests for syphilis ; and

Having considered the advice of the representative of the Expert Committee on Biological Standardization,

RECOMMENDS

1. that a limited pilot study be carried out, as suggested in the plan drawn up in co-operation with the Expert Committee on Biological Standardization,[18] with a view to establishing the practicability of applying freeze-dried

[18] See Annex 1, page 23.

syphilitic sera and false-positive sera as references at different levels of reactivity;

2. that the results of the pilot study—should they be satisfactory—be used as a basis for the establishment of a large collection of dehydrated sera to serve as a testing grid for selecting tests to be included in the projected international serodiagnostic laboratory conference;

3. that the final arrangements for the International Serodiagnostic Laboratory Conference to be held in 1951 be postponed until the results of the above-mentioned pilot study have become available;

4. that the value of the pilot study in the long-term policy of WHO for the standardization of the serology of syphilis be further studied at the next meeting of the subcommittee, and that close liaison be maintained with the Expert Committee on Biological Standardization in this regard.

## 4. National and International Serodiagnostic Laboratory Activities

### 4.1 *Inter-laboratory test evaluation*

Having considered the report on the exchange of blood samples between the Statens Seruminstitut, Copenhagen, Denmark, the Institut Pasteur, Paris, France, the Venereal Diseases Reference Laboratory (Public Health Laboratory Service), St. Peter's Hospital, London, United Kingdom, and the Venereal Disease Research Laboratory (US Public Health Service), Communicable Diseases Center, Chamblee, Ga., USA (formerly Staten Island, N.Y.), the subcommittee was in favour of continuing this exchange and extending it to include other areas. It was felt that this exchange between national laboratories would furnish information on:

(*a*) the relative sensitivity of a single test as between laboratories;

(*b*) the relative sensitivity levels of different testing procedures routinely used in the various laboratories;

(*c*) the influence of transportation on such samples;

(*d*) specimens from donor categories not locally available to the different laboratories.

The subcommittee agreed that clinical information on samples exchanged between laboratories was necessary if the possible differences in sensitivity were to be evaluated.

It was considered important that in the exchange there should be at least one test common to the participating laboratories. If these laboratories were, by mutual agreement, to use the same procedures and antigens

from the same lots, for tests on exchange samples, statistical evaluation of the results obtained would be facilitated.

During the initial stages of the inter-laboratory test evaluations certain customs difficulties had arisen in the transmission of some serum samples between countries. These were resolved by the combined action of the ministry of health concerned, the receiving laboratory, and WHO. In order that such difficulties might not arise in the extended exchange programme, the subcommittee suggested that WHO study the best way in which sample parcels could be described and distinctively marked to allow rapid passage through post and customs.

### 4.2 *Stability of blood samples in postal transmission*

Before convening an international laboratory conference further data should be obtained on possible deterioration in seroreactivity when blood samples are sent by post from areas not covered by the previous exchange experience. After a discussion of the various methods that might be employed, it was suggested that the laboratories represented by members of the subcommittee, and possibly also certain laboratories of members of the expert advisory panel, should collect further data by mailing blood samples and having them returned to their own laboratories for re-examination.[19]

As special categories of donor groups in tropical areas will be included in the conference, the Pan American Sanitary Bureau-supported laboratory in Guatemala was considered to have valuable background data which should be obtained as soon as possible.

The pilot study of freeze-dried sera referred to in Annex I (see page 23) is another form of exchange of samples and will be expanded whenever practical to include further laboratories.

### 4.3 *Conservation of sera*

The subcommittee considered reports on conservation experiments on *natural* sera during 1949-50. Good keeping quality was reported when sera were stored at —10°C and +4°C. Seven sera giving false-positive reactions had also remained sero-active after 30 days at —10°C. Several laboratories are using pooled sera (dried according to relatively simple

[19] Four samples should be taken from each case included in this test; one sample to be examined the same day, two samples to be sent by post to some other place from which they are to be returned immediately, the fourth sample to be kept at + 4°C in the original laboratory. The returned samples and the stored sample are to be examined at the same time. Duplicate examination should be performed on each sample tested, using quantitative methods in order to secure statistical material for evaluation of change in titre caused by transportation.

freeze-drying techniques) as controls of daily sensitivity. The planned activity of WHO in this sphere, using more refined drying methods as a basis for the projected pilot experiment, is outlined in Annex I (see page 23).

Consideration was given to the preservation of serological reactivity of different types of samples—sera, sera plus clot, citrated blood, heparinized blood, as well as separated sera and spinal fluids with or without a preservative (merthiolate). The subcommittee was informed that merthiolated serum had been used successfully in certain laboratories in the USA.

### 4.4 *Information on serodiagnostic methods used in different parts of the world*

The subcommittee studied the reports on serological methods used in laboratories throughout the world which had been received in reply to a WHO circular. Sufficient information was not yet available from a number of countries and the subcommittee wished to study this matter further at a later meeting. Specific information, by published reference or written text, should be obtained from each source as requested in the questionnaire. References to modified testing procedures should be accompanied by a detailed description of the modification.

## 5. Cardiolipin

Having reconsidered the recommendations on cardiolipin and purified lecithin (Pangborn) from its first session,[20] the subcommittee studied the development in the production and use of these reagents in different parts of the world during the last year. The subcommittee had the privilege of hearing a statement by Dr. Mary Pangborn on present methods of production and control of cardiolipin and lecithin. Advice was also given during the general discussion on proposed methods of control of these products.

### 5.1 *Considerations*

Production of cardiolipin and lecithin has increased so that these products are now commercially available, but unfortunately they have to be procured mostly from hard currency areas. Production has been started in a limited number of countries outside the USA but is sufficient only in a few instances to cover national needs. Limited laboratory production on an experimental scale has been started in several countries and more laboratories are planning such work. The information presented to the subcommittee indicated that production in small quantities is the rule

[20] *World Hlth Org. techn. Rep. Ser.* 1950, **14**, 14

rather than the exception, and that sufficient quantities are not available to meet the needs of all laboratories which will want this antigen for routine use. The subcommittee felt that large-scale production in soft currency areas should be encouraged, as this would increase the possibility of countries without any national production procuring cardiolipin antigens.

The subcommittee welcomed in this connexion the WHO/UNICEF-sponsored proposal for a cardiolipin-producing laboratory centre in India.

Many serological methods have been modified by the introduction of cardiolipin-lecithin antigens over the last year. Some of these methods are known to the subcommittee only by personal communications from the laboratories. Some laboratories are now using one or more cardiolipin antigens in addition to or as replacement for other antigens. It is generally felt that currency difficulties and technical problems in establishing national production are delaying both experimental work and routine use of cardiolipin antigens.

The use of antigens containing cardiolipin has offered a means for the simultaneous increase of sensitivity and specificity of serological tests for syphilis. A widespread use of cardiolipin antigen will offer new possibilities for standardization of the serological reactions to adequate levels of reactivity, and later for selection of one or more reactions as a universal standard.

The adoption of new reagents must be preceded by adequate experimental evidence that the product performs satisfactorily under local conditions affecting both the laboratory and the types of sera tested. The subcommittee felt that, in view of the anticipated developments in the use of cardiolipin, all precautions should be taken to ensure general control of the purity of cardiolipin antigens and that sufficient definition of all components of these antigens should be obtained. The advantages in using cardiolipin antigens would be lost if unsatisfactory products were placed on the market or produced in small lots in laboratories for local use without appropriate controls.

In the USA all production is covered by US Pat. No. 2,456,836 (21 December 1948), taken out by Dr. Pangborn and assigned to the New York State Department of Health on a non-commercial non-profit basis to secure the control of purity of cardiolipin. All commercial producers in the USA have signed an agreement stipulating that every lot of cardiolipin produced be controlled in the Division of Laboratories and Research, New York State Department of Health. Most of these producers are also requesting analysis of samples of the purified lecithin to be used, but this product is not covered by patent or by any agreement. The control analyses of both cardiolipin and lecithin have been performed free of charge. Since

production of cardiolipin has started on an experimental basis outside the USA, many samples have been received and tested in the Division of Laboratories and Research, New York State Department of Health. The subcommittee was informed by Dr. Pangborn that such work could not be continued indefinitely, as this burden was already heavy and likely to increase.

The subcommittee reiterated its views on the international nature of the problems relating to standardization of cardiolipin antigens and agreed on the following suggestions regarding the ways in which WHO might contribute to solving this question:

(1) a description of standard cardiolipin and purified lecithin [21] should be included in the *Pharmacopoea Internationalis*;

(2) the same description should be brought to the attention of the Expert Committee on Biological Standardization with a view to obtaining further advice from that committee as to the possibility of establishing international standards for cardiolipin and purified lecithin. The subcommittee welcomed the invitation from the Expert Committee on Biological Standardization for two members of the Subcommittee on Serology and Laboratory Aspects to take part in the coming meeting of that committee to discuss this question further;

(3) a limited number of laboratories should be selected to undertake control of new lots of cardiolipin and purified lecithin. The subcommittee suggested that WHO study the possibility of supporting such activities with grants, and of strengthening the technical staff of such laboratories by the allocation of WHO fellowships. One or more of these laboratories should be in a tropical or subtropical area, making sera of different local types available for testing purposes.

The question of control centres for cardiolipin and lecithin should be considered in connexion with the subcommittee's recommendation in the report on its first session:

"that a plan be drawn up ... for a worldwide system for standardization of serореactions and antigens in syphilis, aiming at the establishment of one or a few centres, to be designated later, delivering control sera and standard antigens to regional and national serodiagnostic laboratory centres." [22]

The subcommittee realizes that the laboratories selected to perform cardiolipin-control work will have to receive samples from the Division of Laboratories and Research, New York State Department of Health,

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[21] To be prepared by the Division of Laboratories and Research, New York State Department of Health, Albany, N.Y., USA.

[22] *World Hlth Org. techn. Rep. Ser.* 1950, **14**, 14

USA, for reference and for the necessary training in standardization work. The subcommittee also realizes that the selected laboratories must be kept in the closest possible contact with each other by means of the exchange of samples and information. Research work should also be carried out in these control centres on serological methods, other than those now employed, which might be useful in determining the purity or non-purity of cardiolipin and lecithin.

The present control of cardiolipin and lecithin requires the application of both chemical and serological methods.

The chemical control, which could be performed in well-equipped chemical laboratories, is a necessary preliminary procedure; only those products which are satisfactory by chemical tests should be submitted for serological assay. The serological control of cardiolipin and lecithin can be performed in a serological laboratory with a staff well-trained in comparing antigens, and having access to blood-donor groups comprising syphilitics, " normal " individuals, and, if possible, individuals with sera giving false-positive reactions. From each new lot of cardiolipin and lecithin, antigens for flocculation and complement-fixation tests should be prepared from a predetermined formula, using for a new cardiolipin the standard lecithin and for a new lecithin the standard cardiolipin. Such antigens should then be compared with antigens containing standard cardiolipin and standard lecithin in the same proportions. Parallel tests using the two antigens, which should be conducted on several hundred sera during the course of several days, should reveal no significant discrepancy; the testing should include quantitative procedures.

Statistical methods for determining the reproducibility of the test employed should be applied in order to avoid the rejection of samples on the basis of " differences " which actually fall within the errors of reading. Information on these methods of control should be published by WHO.

Purity control would be highly complicated if small-scale production in a great number of places were the rule, since the control work is the same for a lot containing 1 g as for one containing 20 g.[23]

The purity of cardiolipin and lecithin antigens depends not only on an adequate control of these components, but also requires that the alcohol and cholesterol or other additional substances used be of adequate purity. If some cardiolipin antigen should include other components these should be defined chemically. If this is impossible, a serological control similar to that indicated for cardiolipin should be performed, and products deviating in reactivity from earlier products should not be used until a full re-evaluation of sensitivity and specificity has been undertaken.

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[23] See *Bull. World Hlth Org.* 1951, **4**, in press.

The subcommittee agreed that the name cardiolipin should be used for the complex phosphatidic acid itself, while cardiolipin antigen was to be used for antigens prepared from cardiolipin by adding other substances, such as, for instance, lecithin, cholesterol, etc., for serological use. It should always be stated for what test a cardiolipin antigen was prepared.

The subcommittee considered recent information on improvements in methods for producing cardiolipin and lecithin (purification by means of acetic acid, the use of egg lecithin instead of heart lecithin, the possibility of controlling purity by means of chromatography). Experimental work in progress at present will probably make it possible to simplify the production of cardiolipin to some extent.

The preparation of uniformly pure lecithins could be greatly facilitated if egg lecithin were substituted for that made from heart muscle. It was recorded that unpublished extensive studies of egg lecithin and heart lecithin during the last year had shown that the optimal reacting zone for egg lecithin was somewhat broader than for heart lecithin, and that these products had been interchanged without any effect on sensitivity and specificity having been recorded on samples so far tested. The subcommittee expressed the wish that the description of both heart and egg lecithin might be considered in the monograph to be prepared for the Expert Committee on the Unification of Pharmacopoeias, and that the effect of the use of egg lecithin in the production of cardiolipin antigens should be evaluated on those types of sera which had so far not been tested.

Synthesis of cardiolipin has not yet been attempted as far as the subcommittee knows, and the subcommittee was advised that such a synthesis would most likely be very difficult.

Substances resembling cardiolipin, and belonging to complex phosphatidic acids, have been prepared from the tubercle bacillus, certain vegetables, and wheat. A complex phosphatidic acid has recently been isolated from wheat embryo in Finland.[24] This substance, sitolipin, is at present in the experimental stage, and initial information indicates that it might be applied in serological tests for syphilis. The subcommittee noted that sitolipin is probably cheap and easy to produce. Sitolipin developments should be followed in detail, and it is believed that, in order to avoid further confusion, the technique of preparation of sitolipin should be published in full as soon as possible, that pure samples of sitolipin should be distributed to laboratories with experience in work on cardiolipin for comparative purposes, and that extensive experimental work should be performed to ascertain the serological value of this new compound ; this work should be performed and controlled in a similar way to that on cardiolipin.

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[24] *Ann. Med. exp. Biol. fenn.* 1950, **1**, 72

5.2 *Recommendations*

The subcommittee adopted the following resolutions:

I. The Subcommittee on Serology and Laboratory Aspects of the Expert Committee on Venereal Infections and Treponematoses,

Having studied the report on production and control of cardiolipin and lecithin; and

Having noted the advice of Dr. Mary Pangborn, of the Division of Laboratories and Research, New York State Department of Health, USA,

RECOMMENDS

1. that cardiolipin production be encouraged in a few laboratory centres particularly in soft currency areas;

2. that the World Health Organization collect and make available to health authorities and major laboratories technical information on isolation, purification, and standardization methods for cardiolipin and lecithin, and on control of the products;

3. that the Expert Committee on the Unification of Pharmacopoeias be requested to consider the inclusion of monographs on cardiolipin and purified lecithin in the *Pharmocopoea Internationalis;*

4. that technical data on cardiolipin and lecithin production and control be transmitted to the Expert Committee on Biological Standardization with a view to further advice being obtained from that committee on the establishment of international standards of cardiolipin and lecithin;

5. 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 cardiolipin and lecithin.

II. The Subcommittee on Serology and Laboratory Aspects of the Expert Committee on Venereal Infections and Treponematoses,

In order to differentiate the many types of existing antigens for the serodiagnosis of syphilis, including antigens composed of cardiolipin and purified lecithin,

RECOMMENDS that all samples of antigens produced for serological tests for syphilis be accompanied by or labelled with special information regarding composition and characteristics.

### 6. Treponema Studies

The subcommittee noted with great interest the developments in WHO treponemal studies and the value of the co-operation planned between several WHO teams, located in areas where the different treponematoses are endemic, and the International Treponematosis Laboratory Centre in Baltimore, Md., USA.

The subcommittee welcomed the indication that treponema immobilization tests (Nelson) may be performed in laboratories in different countries. It would be particularly valuable if such activity were carried out in countries with a high prevalence of treponemal infections other than syphilis, such as Indonesia, Iraq, and Thailand, where WHO/UNICEF-supported programmes are now getting under way.

The subcommittee was in favour of including the treponema immobilizing test among those to be applied when freeze-dried serum specimens were evaluated. Further research work should be carried out with the Nelson test to verify its specificity and value in cases without clinical evidence of syphilis but with persistent seropositivity as demonstrated by one or more serological tests utilizing cardiolipin antigen.

The recent claims regarding the cultivation of virulent *T. pallidum* and their subsequent use in serological and skin tests for syphilis were discussed.[25] Information and data resulting from attempts to duplicate this work would be brought to the attention of the subcommittee and panel members. The subcommittee would also maintain interest in current efforts to cultivate treponema on artificial media, since success in this endeavour might contribute to improvement in serodiagnostic methods.

The subcommittee realizes that certain practical problems arise as a result of transport of infected material across national borders. Specific negotiations should be carried out with the appropriate customs and veterinarian authorities well in advance of such shipments, and WHO should assist in the arrangements when necessary. The methods by which WHO could assist in this matter should be specifically studied.

### 7. Mass Serological Examinations

The subcommittee studied the methods used for mass examination with special reference to the information collected by WHO.

The subcommittee was of the opinion that no *single* test was capable of producing positive results in *all* cases of syphilis or with *all* specimens

[25] US Pat. No. 2,513,327. 4 July 1950. *Treponema pallidum* culture and products thereof

reacting positively with other tests, and that therefore no single test could be exclusively recommended for mass serological testing. The subcommittee felt that the use of the term " screen test " might give rise to the idea that such a test had the ability to indicate all non-syphilitic cases by a negative reaction. It was agreed that a preferable term would be " screening procedure " provided it was understood that this meant a practical procedure which included a certain risk of false-negative and positive reactions which must be taken into account.

Whenever a laboratory must compromise between :

(*a*) the scientifically sound procedure of the use of a battery of several tests, chosen to supplement one another, and

(*b*) the practical problem of examining a great number of samples in a less efficient way because full serological examination with a number of testing procedures is not possible,

the subcommittee appreciates that it is sometimes necessary, because of local conditions, to use a single test in such mass examinations. The test selected under such circumstances should, however, have a high degree of sensitivity, consistent with adequate specificity. Whenever such a method is used in examining large population groups, certain cases in various stages of syphilis may be found to be negative. Such false-negative reactions will be observed not only in the very early stages of syphilitic infections, but also in some stages where strong positive reactions might be obtained by other tests. Whenever possible, physical examination of the population group thus surveyed should, therefore, be carried out in addition.

The subcommittee wished to draw attention to the following : technical conditions in both laboratory and the field, the prevalence of certain types of false-positive cases, and the incidence of treponematoses in the area should be considered before a specific test for mass examination is selected ; in some areas it may be preferable to carry out preliminary investigations to determine the most suitable testing procedure.

The subcommittee discussed practical methods for obtaining blood samples during mass testing programmes including collection in ordinary and in vacutainer tubes and the use of finger blood in capillary tubes.

### 8. System of Notation for Serological Tests

The system of notation for serological tests had been discussed at the first session of the subcommittee, and the following recommendations had been made :

(1) non-reactive sera (to be designated as —) ;

(2) doubtful reactive sera (to be designated as ±, indicating necessity of re-examination in cases without history or clinical signs of syphilis);

(3) reactive sera (to be designated as +);

(4) quantitative tests to be reported in terms of titre, the titre being defined as the highest reactive dilution of serum, for example, reactive up to 1/160.[26]

In view of the reference in an authoritative text[27] to the term "doubtful" as indicating "that the laboratory was not certain of the technical accuracy of its own performance", and of the connotation of laboratory inadequacy that so often accompanies this term, it is the opinion of the subcommittee that efforts should be made to delete this term from serological test reporting. The term "partially reactive" would designate the zone of test reactivity lying between the "reactive" and "non-reactive" (referred to as positive and negative) phases.

Recognizing the capacity of strongly reactive sera to produce sometimes only weak reactions in qualitative tests, specimens yielding weakly postive reactions should be subjected to quantitative testing before a report is submitted. All quantitative test reports such as "reactive to 1/60 or 1/120" should be made only when the next dilution fails to be *completely* reactive. The dilution schedule used should be made known to the recipient of the report.

Sufficient experience is not yet available to justify at present the selection of any one single serological standard procedure that could be recommended for general comparative use in and between laboratories in the world.

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[26] *World Hlth Org. techn. Rep. Ser.* 1950, **14**, 20

[27] Moore, J. E. (1949) *The diagnosis of syphilis by the general practitioner*, Washington, D.C. (*J. vener. Dis. Inform. Suppl.* No. 23), p. 48

## Annex 1

## PLAN FOR A PILOT STUDY OF FREEZE-DRIED SERA OF DIFFERENT LEVELS OF SENSITIVITY[1]

These proposals are put forward on the assumption that they will be implemented by the Statens Seruminstitut, Copenhagen, Denmark.

### 1. Donors

It is suggested that large blood samples be obtained from 12 donors whose serum reactions are as follows:

2 syphilitic patients strongly reactive
2 syphilitic patients moderately reactive
2 untreated patients in the early stages of syphilis weakly reactive
2 treated patients weakly reactive
2 non-syphilitic patients with false-positive sera
2 non-syphilitic persons non-reactive

The eight donors in the syphilitic category are to be proved syphilitic by clinical and bacteriological examination.

The false-positive patients will be perhaps sufferers from pneumonia.

### 2. Preparation of the Freeze-dried Samples

Each donor is to be bled so as to yield about 160 ml of serum.

The serum in each case will be distributed in exact 2-ml amounts into ampoules and freeze-dried in a thin layer.

When the main drying is complete, the ampoules will be further dried in vacuo over $P_2O_5$ in desiccators, filled with dry nitrogen and sealed, and marked by waterproof ink, if possible.

For the purposes of the test, it is important that the reactivity of the serum be determined, not by tests in the liquid state, but by tests on the dried sera reconstituted with the appropriate amount of distilled water. This applies particularly to the weakly reactive and false-positive sera.

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[1] Proposed by Dr. A. A. Miles, Director, Department of Biological Standards, National Institute for Medical Research (Medical Research Council), London, United Kingdom, and member of WHO Expert Committee on Biological Standardization.

### 3. Tests for Preliminary Characterization of the Sera

The various designations strongly reactive, moderately reactive, weakly reactive, and false-positive will be determined by the complement-fixation and Kahn tests in routine use in Copenhagen. Sera to be checked for particles microscopically visible (magnified 100 times).

The results of the tests in Copenhagen will constitute a basis for the measurement of gross deterioration when the samples are sent to distant participating laboratories.

### 4. Design of the Test

The participating laboratories which the members of the subcommittee represent are six in number.

Each will receive 12 ampoules of dried serum from each of the twelve donors.

On receipt of the material, the laboratory will immediately test 3 ampoules; 3 more ampoules will be stored in the dark at below 0°C (preferably at —10°C); 3 will be left in the dark at room temperature (daily temperature to be noted), as near as possible to 20°C; and 3 will be kept in the dark at 37°C.

After approximately three months, these 9 ampoules from each of the twelve donors will be given parallel tests on one day, using single batches of all the necessary reagents required.

It is not necessary for the 108 ampoules to be tested on the same day, but all the ampoules from a given donor must be so tested. If possible, at least one complement-fixation and two flocculation tests should be used, quantitative techniques being performed on all samples.

Bacteriological control should be performed, if possible.

The object in testing 3 ampoules at each temperature is to determine whether there is any inter-ampoule variation. For this reason, it is necessary to submit each ampoule to all the tests which the participating laboratory is adopting for the purpose of the experiment.

The test of the 3 ampoules made immediately upon receipt of the specimens is intended to give the participating laboratory some idea of the reactivity of the sera in their hands, and, by comparison with the declared reactivity determined by Copenhagen, to give a crude notion of how the dried sera have stood up to the hazards of transport.

The important and crucial test is the comparison of the three differently treated sets of ampoules after storage. In this test, the ampoules

stored at —10°C will, in a sense, serve as the standard serum for the day's test (since past experience indicates that storage at low temperature, —10°C, is the best means of preserving antibody potensive).

It is realized that the weakly reactive sera selected by Copenhagen may give negative results with certain techniques, so that persons using these techniques will not be able to determine deterioration in this particular sera. This is unavoidable in a restricted pilot test of the kind proposed. Ampoules should be checked for leakage, which causes increased deterioration, before distribution, when received, and after three months.

### 5. Methods to be Used by the Participating Laboratories

The prime object of the pilot test is to enable each of the participating laboratories to satisfy themselves that no deterioration takes place in the samples. The reactivity is measured by sensitive methods with which the laboratory is thoroughly familiar. There is, therefore, no need for each participant to use a wide variety of methods, but the methods chosen should be such that most of the usual complement-fixation and flocculation methods are represented in one or more of the six laboratories.

### 6. Reporting of Results

The collecting group is to report to WHO on their observations from the collecting and drying work combined with information on the check of re-dissolved samples. It is essential to report the number of rejections during the selection work of donors.

Each testing laboratory will send observations and results in detail to the Secretary of the Subcommittee on Serology and Laboratory Aspects, World Health Organization, Palais des Nations, Geneva, Switzerland, including an opinion on the behaviour of the reconstituted sera in serology tests for syphilis in the light of their experience with these samples.

These results and opinions will be combined in a general report which, together with the original data, will be sent to the participants and the Copenhagen group for their comments. WHO will then submit a final report to the appropriate committees (Subcommittee on Serology and Laboratory Aspects of the Expert Committee on Venereal Infections and Treponematoses, and the Expert Committee on Biological Standardization) in the light of these comments.

## 7. List of Participating Laboratories and of the Techniques Employed

| *Participating laboratories* | *Techniques* |
|---|---|
| Statens Seruminstitut<br>Copenhagen<br>Denmark | Complement fixation with crude and cardiolipin antigens<br>Kahn<br>Meinicke<br>VDRL (Venereal Disease Research Laboratory) slide |
| Institut Pasteur<br>Paris<br>France | Kolmer<br>Roulier<br>Kahn<br>Kline<br>Meinicke<br>VDRL |
| School of Tropical Medicine<br>Calcutta<br>India | Complement fixation (Medical Research Council) |
| Municipal and Beilinson Hospitals<br>Tel Aviv<br>Israel | Kahn<br>Meinicke<br>Rapid flocculation |
| Gade's Institute<br>University of Bergen<br>Norway | Complement fixation with crude and cardiolipin antigens<br>VDRL tube<br>Meinicke |
| Venereal Diseases Reference Laboratory<br>St. Peter's Hospital<br>London<br>United Kingdom | Complement fixation with crude and cardiolipin antigens<br>PPR (Price Precipitation Reaction) |
| Venereal Disease Research Laboratory<br>Communicable Diseases Center<br>Chamblee, Ga.<br>USA | Kolmer<br>VDRL tube<br>VDRL slide<br>Mazzini<br>Rein-Bossak |

## Annex 2

# EXPERT COMMITTEE ON VENEREAL INFECTIONS AND TREPONEMATOSES: CONSIDERATION OF SUBCOMMITTEE REPORT

### Resolutions

The committee adopted the following resolutions:

I. The Expert Committee on Venereal Infections and Treponematoses,

Having noted the considerable progress made by the Subcommittee on Serology and Laboratory Aspects in collecting information on cardiolipin antigens and on the developments relating to the holding of the International Serodiagnostic Laboratory Conference in 1951,

APPROVES the report of the subcommittee.

In so doing, the committee considers it highly desirable that the laboratory exchange of serum samples be extended to include a larger number of laboratories than at present.

II. The Expert Committee on Venereal Infections and Treponematoses

RECOMMENDS

1. that the establishment as soon as possible of international standards for cardiolipin and purified lecithin be explored in co-operation with the Expert Committee on Biological Standardization, that a limited number of laboratories be selected by WHO to determine that the purity of such products is in conformity with these standards, and that the Expert Committee on the Unification of Pharmacopoeias be requested to include a description of cardiolipin and purified lecithin in the *Pharmacopoea Internationalis*;

2. that pilot studies be carried out to ascertain the possibility of establishing freeze-dried sera as preliminary standards for different levels of serological reactivity in syphilis in preparation for the International Serodiagnostic Laboratory Conference;

3. that detailed preparations for the conference be temporarily postponed so that the possibility of using freeze-dried sera as a technical basis for the selection of participants can be considered in order to reduce the size

of the conference and the organizational burden of WHO and the host laboratory ;

4. that the possibility of establishing dried reference sera at various levels of reactivity be carefully considered when the results of the pilot studies mentioned in paragraph 2 are available.

**Members Circularized**

| | *Replies received* |
|---|---|
| Dr. W. E. Coutts, Professor of Venereology : Chief, Department of Social Hygiene, Public Health Administration, Santiago, Chile . . . . . | 26 October 1950 |
| Dr. R. Degos, Professeur agrégé à la Faculté de Médecine de l'Université de Paris, France . . | 17 November 1950 |
| Dr. S. Hellerstrøm, Professor of Dermato-syphilology, University of Stockholm, Sweden. . . . | 18 November 1950 |
| Dr. E. H. Hermans, Medical Director, Anti-Venereal-Disease Association, Rotterdam, Netherlands . . . . . | 28 November 1950 |
| Dr. G. L. M. McElligott, Director, Venereal-Disease Department, St. Mary's Hospital ; Adviser in Venereal Diseases, Ministry of Health, London, United Kingdom . . . . . | 30 October 1950 |
| Dr. J. F. Mahoney, Commissioner of Health, City of New York, N.Y., USA . . . . . | 27 October 1950 |
| Dr. I. H. Nagi, Director, Venereal Diseases Section, Ministry of Public Health, Cairo, Egypt . . . | 19 November 1950 |
| Dr. R. V. Rajam, Professor of Venereology, Government General Hospital, Madras, India . . . . | 31 October 1950 |