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EXPERT COMMITTEE ON
VENEREAL INFECTIONS

Report on the First Session
of the Subcommittee on
Serology and Laboratory Aspects

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WORLD HEALTH ORGANIZATION
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GENEVA
MAY 1950

EXPERT COMMITTEE ON VENEREAL INFECTIONS

First Session of the Subcommittee on Serology and Laboratory Aspects

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- Dr P. Krag, Assistant Director, Serodiagnostic Department, State Serum Institute, Copenhagen, Denmark (*Chairman*)
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- Dr I. N. Orpwood Price, Director, Venereal Diseases Reference Laboratory (Public Health Laboratory Service), St. Peter's Hospital, London, United Kingdom (*Vice-Chairman*)

Consultants and Advisers :

- Dr P. V. Marcussen, Physician in charge, Venereal Disease Clinic, Municipal Hospital, Copenhagen, Denmark (Consultant on clinical problems of syphilis)
- Dr T. B. Turner, Professor of Bacteriology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md., USA (Consultant on the bejel project)
- Dr M. V. Veldee, Medical Director, Hyland Laboratories, Los Angeles, Calif., USA (Member of WHO Expert Committee on Biological Standardization)

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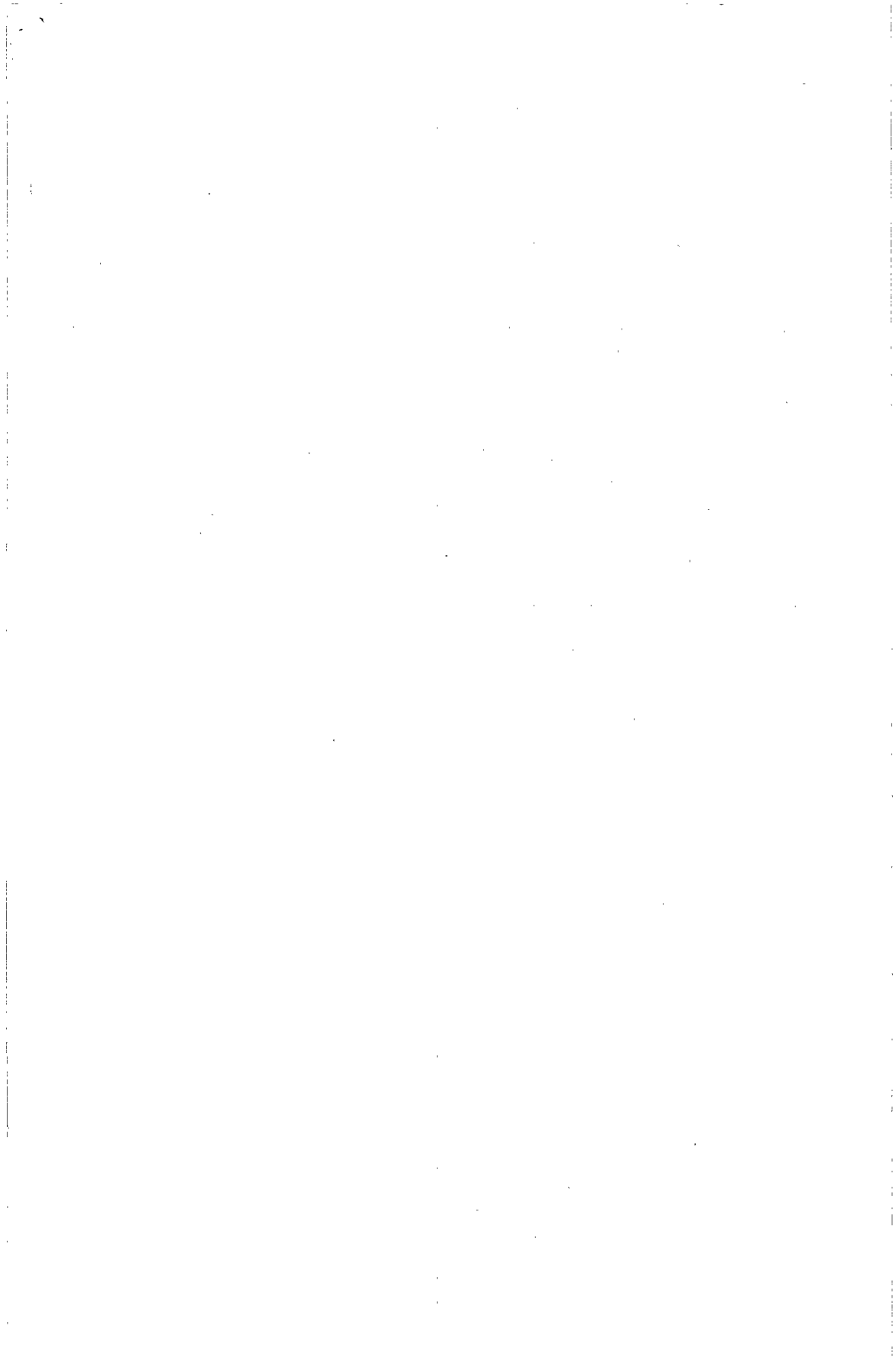
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- Dr A. Spillmann, Regional Adviser on Venereal Diseases for Europe, WHO

The report on the first session of this subcommittee was originally issued in mimeographed form as document WHO/VD/38, 10 November 1949.

COMMENTS BY THE EXECUTIVE BOARD

The Executive Board examined at its fifth session the report of the Subcommittee on Serology and Laboratory Aspects, which had been accepted by the Expert Committee on Venereal Infections at its third session. Attention was drawn to the difficulties which had often resulted from mass screening carried out in the field of tuberculosis control, when treatment facilities had not been available, but it was noted that, in the programme of venereal-disease control, hospitalization in the great majority of cases would not be necessary, since ambulatory treatment would be possible following mass examinations.

The Board agreed that, in view of the new serodiagnostic methods based on cardiolipin antigens, the holding of the international serodiagnostic laboratory conference, already approved by the Health Assembly, would be an important undertaking of the Organization in 1951 or 1952 as a basis for the wide standardization of serodiagnostic procedure on which any antisyphilis programme is dependent.



EXPERT COMMITTEE ON VENEREAL INFECTIONS

Report on the First Session of the Subcommittee on Serology and Laboratory Aspects¹

1. Introduction

The ad hoc Expert Committee on Venereal Diseases recommended in October 1948 that a subcommittee on serology and laboratory aspects be established as soon as possible, that this subcommittee be composed of not more than four members, and that a preliminary programme for the conduct of the next international serodiagnostic laboratory conference be drawn up by the subcommittee as soon as possible for consideration by the Expert Committee on Venereal Infections, to which the subcommittee would report.²

During the early part of 1949, members were appointed to the Subcommittee on Serology and Laboratory Aspects.

Dr P. Krag was elected Chairman and Dr I. N. Orpwood Price Vice-Chairman of the subcommittee.

The agenda was accepted, including items referred to the subcommittee by the parent committee, and items proposed by a corresponding member, Dr T. Vogelsang.

During the session 11 meetings were held and the report was approved by all members.

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

The Executive Board

- (1) APPROVES the publication of the report of the Subcommittee on Serology and Laboratory Aspects accepted by the Expert Committee on Venereal Infections ; and
- (2) REQUESTS the Director-General to draw the attention of Member Governments to :

(a) the desirability of national laboratories guiding standardization work in serology, and

(b) the holding of the international serology conference, approved by the Health Assembly,

and to facilitate the arrangements for this conference in every way possible.

² *Off. Rec. World Hlth Org.* 15, 25

2. Prospectus

A venereal-disease activity is dependent to a major degree upon the efficient conduct of serological tests for syphilis and other laboratory procedures. It has been rightly stated by the ad hoc Expert Committee on Venereal Diseases that : (a) there is great lack of uniformity of procedure technique; (b) the manner of reporting results has had the effect of producing confusion and rendering many studies in serology of syphilis in the past valueless ; and (c) it is possible for an individual under the present lack of uniformity to be judged as being syphilitic in one country and considered to be free from the disease in another.³

The subcommittee is in agreement with the outlook and philosophy stated in the reports on the two sessions of the Expert Committee on Venereal Diseases⁴ and has noted the objectives set forth by the parent committee and the preparatory work carried out by WHO up to the present in serology and laboratory aspects with the advice of the parent committee. Among the visualized activities of WHO on this question, the international serodiagnostic laboratory conference approved by the World Health Assembly is undoubtedly the major undertaking, and the subcommittee feels that a major proportion of its work at its first session and at sessions during the next two years should be devoted to developing sound plans for the conference of serodiagnostic laboratory workers.

However, consideration should also be given by the subcommittee in due time to the standardization on purity control of serodiagnostic reagents (e.g., cardiolipin), the application of seroreactions for special purposes (mass serological screening techniques) and the laboratory work relating to experimental syphilis and other treponematoses.

3. International Serodiagnostic Laboratory Conference

3.1 General

International serological laboratory conferences were held in Copenhagen in 1923 and 1928, and in Montevideo in 1930. A fourth international serological laboratory conference was scheduled to take place in Copenhagen in 1939 but was cancelled owing to the outbreak of war. Two years later it was found necessary for evaluating the seroreactions in the Western Hemisphere to hold a serological laboratory conference in Washington, 1941. Results from this conference have been useful in guiding scientific developments in the USA during the years which followed.

³ *Off. Rec. World Hlth Org.* 8, 62

⁴ *Off. Rec. World Hlth Org.* 8, 60; 15, 18

During the past five or six years the need for another international serodiagnostic laboratory conference has become increasingly evident due to the following developments : (a) mass testing of large sections of populations has placed more importance on test specificity ; (b) many laboratories have adopted the use of a multiple "battery" of serological test procedures in order to offer more clues to the diagnostician regarding possible "false-positive" reactions. This development has multiplied the work of the testing laboratory but has been only partially successful in solving the problem.

Intense laboratory investigations in the USA and elsewhere during these years have resulted in several new seroreactions, many of which are based on antigen components of more purified types than were available for the older seroreactions. Antigens composed of cardiolipin and purified lecithin have been shown to be capable of a higher degree of specificity and sensitivity than was obtained with cruder extract antigens. This offers the possibility that the chemical composition of serological antigens may now be more closely studied, and reference standards may possibly be developed.

Evaluation studies between many laboratories during the past few years have shown that discrepancies continue to exist between the same tests in different laboratories and different tests in the same laboratories to such a degree that additional efforts toward standardization of testing procedures are called for in each country and internationally.

During the last two years there has been developed the treponemal antibody technique (Nelson's treponema immobilization test), the details of which are referred to in section 6 of this report. Although the chief value of this technique would appear to be in the immunology of syphilis and other treponematoses, it may also play an important role in evaluating biologically false-positive tests for syphilis. This additional armament is of significant value since ordinary verification tests in serology of syphilis have not yet been evaluated in an international serodiagnostic laboratory conference. Experience has shown that the treponemal antibodies detected by this new technique are independent of and can be separated from the reagins detected by our ordinary serodiagnostic tests, even those employing cardiolipin-lecithin antigens.

The finding of surprisingly large numbers of positive seroreactions in some geographical areas (tropical) and the prevalence of seropositivity depending to some extent on the reactions used, indicates that the evaluation of relative test-efficiency and the selection of reactions which may be most useful in the various areas are world problems. Donor-groups from several geographical areas will therefore be necessary for the contemplated international serodiagnostic laboratory conference. The avail-

ability of source material from venereal-disease field units of WHO in various parts of the world would be of importance in this regard.

3.2 *Organization*

The subcommittee has carefully studied the preliminary data for the conference collected by WHO and the ad hoc Expert Committee on Venereal Diseases including objectives, organization, and operation of the conference. The principal features of the parent committee's statements in this regard meet with the approval of the subcommittee. The subcommittee desires to commend particularly the Chairman of the parent committee for the thorough manner in which the features of the conference have been outlined.

The subcommittee also studied the views of its members as expressed in the memoranda exchanged over the last few months in preparation for the first session. This has permitted detailed discussions of the actual preparations, organization, and operation of the conference during the session. The outline for the conference forming the basis for the subcommittee's discussions and the memoranda expressing the views of each member of the subcommittee are given in annexes. Additional details will be resolved at subsequent sessions of the subcommittee. It is, however, desired to record the following observations at this time :

3.3 *Time, place, and participation*

The time of year for the conference should be, if possible, chosen with due consideration to meteorological conditions. The conference should be held in a city with a large medical centre, having access to significant reservoirs of clinical syphilis, and with an airport on one or several of the main world air-routes. The laboratory space should be selected with a view to obtaining the best working conditions for a maximum number of participants.

With this in mind, WHO should explore the possibilities of holding the conference in Europe, especially Paris, London, or Copenhagen. These cities would be convenient from the point of view of easy access by air from areas of South-East Asia, Middle East, and tropical America—a few days each way—permitting minimum transportation time for serum samples.

The proposed conference should be announced as soon as possible by WHO through health administrations, large laboratories, and WHO publications. Preliminary applications for participation in the conference should be received as soon as possible.

It is estimated that approximately 40 procedures will be available for the conference. Each author-serologist should be allowed to enter more than one reaction.

3.4 *Criteria for participation and selection*

(a) Determination of eligibility for admittance of a testing procedure to the conference to be made solely on technical grounds ;

(b) Test-author or workers designated by him to be allowed to perform the author's procedure ;

(c) Applications for admittance to the conference to include statements regarding number of laboratories at present employing the procedure ; test-author's opinion of the contribution offered by the distinctive method and detailed technique description to accompany preliminary applications ;

(d) When two or more tests are entered by a single author, only one may be accepted if the total number of applicants is greater than the prescribed limit ;

(e) Conference director to pass on applications for admittance to the conference in consultation with the subcommittee and a limited number of ad hoc consultants, selected for the purpose.

3.5 *Test material*

The subcommittee is of the opinion that the following should apply to the test material :

(a) Not fewer than 3,000 sera should be tested ; approximately 200 sera would be tested each day ;

(b) 250 spinal fluids should be tested ; approximately 16 would be tested each day.

3.6 *Categories of sera*

3.6.1 *Normal*. No evidence of disease, but not a serologically preselected group ; to be obtained from three or more geographical areas.
Total number approximately 1,500.

3.6.2 *Abnormal non-syphilitic*

(a) Malaria : to be accompanied by information about temperature reading and estimated parasites in the blood at time blood-sample is obtained ;

(b) Leprosy : to include different forms of the disease, e.g., cutaneous, nodular ;

(c) Infectious mononucleosis ;

(d) Upper respiratory infections : febrile and afebrile state at time blood is taken ;

(e) Persons under treatment for various diseases or persons who have undergone surgical repair who are febrile at the time blood is obtained ;

(f) Herpes genitalis ;

(g) Pregnancies ;

(h) Cases recently vaccinated against smallpox, not pre-selected serologically.

Total number for (a) to (h) to be approximately 500.

(i) False-positive reactions :

If possible no more than 300 sera ⁵ should be obtained from preselected donors ; the following conditions of presumptive false-positive reactions should be met :

(a) evident false-positive reactions

(b) absence of evidence of syphilis with discordant seroreactions.

Sera from patients who have no history or clinical evidence of syphilis in which a positive reaction disappeared spontaneously without specific therapy, during a short period of serological observation, would be accepted as falling within the false-positive reactor category.

3.6.3 *Syphilitics*

(a) Primary syphilis, darkfield positive, untreated ;

(b) Secondary syphilis, untreated ;

(c) Late syphilis (of more than 4 years' duration) with clinical manifestations.

Total number approximately 1,000.

The special categories should include donor-groups who have received adequate treatment and also those that have not received sufficient treatment.

The number of fresh, untreated cases should be not less than 100.

3.6.4 *Categories of spinal fluids*

(a) Donors without any evidence of syphilis ;

(b) Donors with syphilis but without central nervous involvements ;

(c) Donors with syphilis having central nervous system involvement.

3.6.5 *Reports.* Reports on each donor should include record of physical examination and clinical history. Reports on syphilitic donors should also include all treatment-schedules and serological findings.

⁵ If suitable preservation methods are found, sera samples will be preserved for distribution at the conference.

3.7 *Further planning of the conference*

The administrative organization of the conference and the procedures to be advised for analysis of findings, conclusions and report — although preliminarily outlined in the annexes, and discussed in some detail by the subcommittee — should be considered at the next session, when further definite views have been formulated on these points. It is necessary, for such further consideration, to obtain the record of the preparations for the proposed international serological laboratory Conference in Copenhagen in 1939, as well as the Washington Conference in 1941.

Among details to be discussed at subsequent meetings are the following :

- (1) Size of blood samples to be drawn from donors.
- (2) Size of samples of spinal fluid.
- (3) Category of donor-groups.
- (4) Necessity for quantitative reporting of results of test without regard to type of antisyphilis treatment.
- (5) Sensitivity of seroreactions for control of treatment results (reactions exhibiting early seronegativity to be allowed time for comparative study with tests exhibiting gradual decrease of sero-activity).

The subcommittee would finally point out the opportunity arising during a conference of this type to develop international understanding generally, and to exchange technical information on serological matters and laboratory aspects. At least one day should be set aside during the conference for presentation of technical papers, demonstration of techniques, and discussion of possible exchange of technical personnel between laboratories.

The subcommittee adopted the following resolution :

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

Having noted

- (a) the recommendations of the ad hoc Expert Committee on Venereal Diseases at its two sessions, and
- (b) the approval by the World Health Assembly of the holding not earlier than 1950 of the first international serological laboratory conference to be organized by WHO,

Having studied and discussed in considerable detail the outline and supporting documentation for the preparation, operation, and organization of the conference, and

Being of the opinion that the holding of this conference is a necessary and worthwhile undertaking which can only be organized internationally by the World Health Organization,

RECOMMENDS

- (1) that the conference be held late in 1951 or early in 1952, depending on the availability of suitable laboratory accommodation and other factors ; that it be held in Copenhagen, London, or Paris for reasons outlined, that the final selection of a site be made by WHO in 1950, and that possibilities be explored in detail with the health administrations concerned ;
- (2) that WHO draw the attention of health administrations and main serological laboratories to the conference, requesting that preliminary applications be registered with WHO not later than 1 May 1950 for participation in the conference ;
- (3) that the final outline of proceedings for the conference be made available to health administrations and participants before the end of 1950 ;
- (4) that WHO take the necessary steps for obtaining adequate test-material from different parts of the world, including specimens from WHO venereal-disease fields units, laboratories, and other institutions co-operating with WHO ;
- (5) that plans for the conference be completed in detail by WHO as soon as possible, for further study by the subcommittee at its subsequent session.

4. National and International Serodiagnostic Laboratory Activities

4.1 *Inter-laboratory test evaluation*

The subcommittee feels that the preliminary pilot test evaluation of serum specimens and antigens carried out by the national laboratories of Bulgaria, Denmark, Ethiopia, Finland, and Italy — the US Public Health Service Venereal Disease Research Laboratory, New York, acting as reference centre — has provided valuable experience in the testing phase of the WHO venereal-disease programme as a preliminary to the international serodiagnostic laboratory conference.⁶

A more systematic procedure for such inter-laboratory test performance evaluations should be established, and a plan developed for further interchange of specimens and antigens. It is particularly desirable to initiate an exchange of sera between central laboratories in Denmark, France, the United Kingdom, and the USA, as a preliminary to drawing up a broader plan to include national laboratories in all WHO regions. Initial experience among a limited number of laboratories is required, although pilot

⁶ Unpublished working document WHO/VD/51

undertakings of the kind referred to above should go forward simultaneously.

4.2 *Conservation of sera*

The present knowledge of methods for conservation of sera is limited, especially in the case of false-positive sera. Keeping this in mind, it is felt that the use of preserved sera will be of the greatest importance for operation of the international serodiagnostic laboratory conference. Furthermore, it seems that a future expanded exchange system among laboratories, and ultimately the creation of a worldwide organization for standardization of seroreactions in syphilis, is desirable, one or a few world centres being responsible for delivering control sera and antigens to regional and national laboratory centres. The experience of the international conference and the initial exchange programmes would provide a basis for further planning. A project of this kind would be extensive and could hardly be completed by WHO within less than five years.

4.3 *Establishment of national laboratory centres*

The subcommittee suggests that WHO should draw the attention of national health-administrations to the necessity for establishing in each country a national laboratory centre for serodiagnostic procedures in syphilis which would participate in the envisaged international system. This would enable the national centres to guide other laboratories in the country towards the desired serodiagnostic standardization of antigens and test methods.

4.4 *Collection of information on laboratory performance*

Emphasis is placed on the necessity for obtaining at the earliest possible date information on the present techniques and use of seroreactions in syphilis in the various laboratories of different countries. Such information would be used to obtain the widest possible basis for developing a plan for the proposed international serodiagnostic centres for standardization of seroreactions and antigens in syphilis.

The following information would appear to be essential and should be obtained from health administrations :

(1) For laboratories performing more than 10,000-20,000 tests a year : Name, address, director, number of sera tested during the last three years, reactions employed (with specific reference to written text), and extent of use of screening methods.

(2) For smaller laboratories performing less than 10,000 reactions a year : number of laboratories falling in this category, names and number

of techniques employed, and approximate total number of tests carried out by these laboratories altogether.

Should an original test-author work in a laboratory performing less than 10,000 tests per annum, similar information to that requested under (1) above should be included.

The subcommittee adopted the following resolution :

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

RECOMMENDS

- (1) that WHO draw the attention of health administrations to the necessity of maintaining a national reference laboratory for serodiagnosis of syphilis to control and guide serodiagnostic performances of local laboratories ;
- (2) that a study of preserved sera be initiated as soon as possible on the basis of a preliminary exchange system between the serology laboratories of the US Public Health Service Venereal Disease Research Laboratory, New York, the Institut Pasteur, Paris, the State Serum Institute, Copenhagen, and the Venereal Diseases Reference Laboratory, London ;
- (3) that a plan be drawn up by subcommittee members, based on the above experience, for a worldwide system for standardization of seroreactions 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 ;
- (4) that a study be made by WHO of location and activities of the main laboratory centres in various countries where serology in syphilis is carried out, including reactions employed.

5. Cardioliipin

The second session of the ad hoc Expert Committee on Venereal Diseases pointed out the importance of WHO's encouraging a wider use of cardioliipin-lecithin antigens in an effort towards further standardization of serology in syphilis.⁷

The subcommittee observes that there would appear to be restrictive factors for a truly wide availability of cardioliipin, in view of the technical difficulties encountered in large-scale production. There is further the patent protection of its production. In this connexion, the subcommittee studied a communication from the New York State Department of Health,

⁷ *Off. Rec. World Hlth Org.* 15, 24

the patent holder, stating that the patent had been established for non-profit purposes to secure the purity of cardioliipin products. The subcommittee considers that the suggestion made by the New York State Department of Health to WHO to secure and administer this patent internationally should receive further consideration. The subcommittee feels that WHO may not be prepared to accept administration of international patents; on the other hand, it would, under the constitution of WHO, be desirable to standardize diagnostic procedures and substances, and the possibility might be explored with the WHO Expert Committee on Biological Standardization of the future establishment of an international cardioliipin reference standard, should developments indicate this to be desirable. The possibility of applying WHO regulations to test procedures and purity control of diagnostic substances might also be explored, and information on the exact terms of the patent should be collected. A high standard of purity of cardioliipin is essential for the successful application of all types of serodiagnostic antigens containing cardioliipin and purified lecithin; current purity control tests should be studied by the subcommittee.

Finally, as a basis for an evaluation of world requirements of cardioliipin, WHO should study the actual and projected availability of the substance in various countries.

As soon as further information on the above subject has been acquired, the subcommittee would wish to consider the matter further with a view to more definite technical recommendations. At this time the subcommittee recommends that:

(1) ways and means be studied by WHO aiming at the stimulation of cardioliipin-lecithin antigens production;

(2) liaison be maintained with the Expert Committee on Biological Standardization with a view to the possible future establishment of an international cardioliipin standard.

6. Bejel, Syphilis, and Other Treponematoses

It is understood that under the designation of "treponematoses" are to be grouped the diseases caused by similar micro-organisms, possibly identical with, but certainly closely allied to, the *Treponema pallidum* of syphilis. The diseases are considered to be yaws, pinta, and bejel as well as syphilis. It is recognized that these diseases are responsible for a great amount of human suffering, and that some of the group assume the proportion of major public-health problems in certain geographical areas.

By courtesy of the Department of Bacteriology, Johns Hopkins School of Hygiene and Public Health, Baltimore, Md., arrangements were made

for subcommittee members to study investigations into treponemal antibodies and recently established immobilization techniques.

The possibility of obtaining at this time tissue-free suspensions of sufficiently concentrated quantities of treponemata for study of the immunological aspects in syphilis, biologically false-positive serological reactions, as well as the biological and immunological relationship between strains of the treponematoses group, including bejel, is a significant technical contribution developed over the last two years. The subcommittee had an opportunity of seeing the various techniques demonstrated, including the equipment required. In syphilis, in general, the titre of immobilizing antibody is found to be high and is stated to be at times clearly different from the reagin titres obtained through the use of lipoid antigens. After specific treatment, the Nelson antibody persists longer than the reagin. In cases of recognizedly biological false-positive reactions, only negative findings with the Nelson technique were recorded. Strains of *T. pallidum* and *T. pertenue* could be differentiated by the Nelson technique.

The subcommittee is of the opinion that the Nelson technique now in development is a different approach to the problem of false-positive reactions, and that the results are extremely promising not only for the possible clarification of the nature of such reactions but also for the study of strains of treponemata with reference to their biological and immunological inter-relationship.

In the documentation available to the subcommittee, it is noted that the First and Second World Health Assemblies have recognized the urgency of the venereal-disease control programme and have stressed the importance of promoting similar measures also in regard to the control of yaws, pinta, and bejel. As a means of implementing the latter programme, the Second Health Assembly authorized the Executive Board to create an expert group on treponematoses to consist of nine members, three of whom are to be drawn from the roster of the Expert Committee on Venereal Infections.⁸ The subcommittee is of the opinion that this arrangement, or a modification thereof, could have the effect of providing close coordination between the groups engaged in the combating of infections which are closely related from the serological standpoint.

Bejel appears to be of sufficient public-health importance in the Eastern Mediterranean area to warrant consideration of steps toward the establishment of a control mechanism. The subcommittee is in entire agreement with the WHO bejel project.⁹ From the laboratory viewpoint mention

⁸ *Off. Rec. World Hlth Org.* 21, 28

⁹ Unpublished working document WHO/VD/23

is desirable of the following lines of investigation, the results of which might be helpful in formulating the definite programme, in adding valuable information to the present knowledge of this disease, and, by inference, in assisting in a better understanding of other treponematoses :

- (1) studies designed to portray the serological relationship between bejel and syphilis ;
- (2) clear presentation of the serology of bejel ;
- (3) comprehensive study of the bejel treponema in the experimental animal (rabbit) ;
- (4) an experimental re-survey of immunity and cross-immunity in experimental treponematoses (syphilis, pinta, yaws, and bejel).

The subcommittee adopted the following resolution :

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

Having studied the technical developments relating to treponemal antibodies, and the fundamental importance of acquiring further knowledge on the biological and immunological inter-relationship between the causative agents of members of the treponematoses group, and

Wishing to emphasize the unique opportunity offered through WHO venereal-disease field units and co-operating laboratories and institutions in many regions of the world for the organization of the proposed comparative investigation outlined in the WHO bejel project,

RECOMMENDS

- (1) that this co-operative study be carried forward along the lines suggested ; and
- (2) that an outstanding investigative guiding laboratory centre be selected for this purpose by WHO early in 1950.

7. Mass Serological Examinations

With the increasing general application of serodiagnostic tests for syphilis during and after the war, and the mass application of various tests to population groups, the finding of positive seroreactions due to causes other than syphilis has gained an increasing interest. The importance of mass-screening procedures is recognized by the subcommittee particularly in such areas where laboratory facilities are available on a limited scale. The problem exists also, however, in highly-developed areas where mass screening is performed from time to time, or where physicians carry out preliminary office tests in industrial groups. The problem may also arise where large laboratories receiving thousands of samples daily

cannot carry out routine flocculation and complement-fixation tests without increasing staff beyond reasonable cost limits. With various modifications, the usual procedure for mass testing demands that blood be obtained by venopuncture. In addition there are the tests performed on a single dried drop of blood collected on slides or filter paper (Chediak and Ko-Da-Guo techniques).

While the subcommittee recognizes the limitations of the use of a single test in mass screening, it would appear that this is a practical procedure under certain circumstances. The subcommittee further recognizes that not only false-positive but also false-negative reactions are obtained, and suggests that whenever possible two or more tests be employed.

First-hand statements on experiments carried out by investigators in the USA on methods that are applicable to mass testing were obtained by the subcommittee. In a statement to the subcommittee, Dr R. Hogan, US Public Health Service, described a sensitive slide test, based on cardiolipin-lecithin antigen, utilizing dried blood specimens collected on filter paper. This procedure has possibilities inasmuch as samples collected can be mailed to a central laboratory. Preliminary experience in the application of this method is encouraging, results indicating only approximately 1% negative reactions in seropositive syphilis. Recognized limitations of this test are now being studied by the US Public Health Service.

A second avenue of approach has been to experiment with rapid procedures in ordinary slide tests. Preliminary observations indicate that with unimportant technical modifications tests can be carried out in 30 minutes.

The subcommittee further had the opportunity of hearing a statement by Dr R. V. Rajam of the Government General Hospital, Madras, India. The problem of mass-serological testing for syphilis in less-developed tropical areas embraces the following considerations :

- (1) limited availability of laboratory facilities ;
- (2) lack of equipment, trained assistance, and electricity supply ;
- (3) need for rapid tests for mass-screening purposes in such areas ;
- (4) choice of method should include consideration of economy, technical simplicity (no water-bath), and the high degree of reliable positivity in cases of syphilis (and other treponematoses) ;
- (5) it would be preferable that all doubtful specimens should be sent to central laboratories ;
- (6) positive cases should be treated as a result of the findings of the original examinations ;
- (7) a limited proportion of biologically false-positive reactions would be acceptable ;

(8) mobile laboratory field units may prove useful in less-developed tropical areas ;

(9) low humidity combined with high temperature, known to interfere with slide reactions, should be kept in mind ;

(10) in some areas in India a prevalence rate of positive seroreactions of approximately 10% is estimated.

At the present time sufficient information is not at hand to justify a recommendation by the subcommittee for the worldwide use of a single reaction or technique in view of the varying circumstances and the varying purposes for which tests are employed. In selecting techniques for mass purposes it will be necessary to evaluate the merits of the test from the points of view of economy, the availability of technical personnel, and pre-existing technical knowledge among the groups launching the venereal-disease-control programme.

The subcommittee notes with considerable interest that the WHO field unit in the Simla area in Northern India appears to be obtaining useful results applying a mass-screening technique based on a modified Meinicke test. This technique is employed simultaneously with slide tests based on cardiolipin-lecithin antigen (Venereal Disease Research Laboratory, Staten Island, N.Y.). In view of expected further results and analysis of these findings, the subcommittee proposes to discuss this item at a subsequent meeting when such information is available.

Recommendation

Following a preliminary discussion at its first session, the subcommittee recommends that techniques suitable for mass-serological examination be further studied and that information collected, including results of mass-serological surveys and statistical material from field units, be entered on the agenda for analysis at the next meeting of the subcommittee.

8. Memoranda by Corresponding Members of the Subcommittee

The subcommittee expresses its appreciation of the interest shown by corresponding members and their valuable contribution to the meeting. The following questions thus submitted and supported with documentation were considered by the subcommittee :

- (1) Necessity of both flocculation and complement-fixation reactions
- (2) Standardization of the complement-fixation test
- (3) Standardization of flocculation reactions
- (4) Procedures for mass-serological examinations

The subcommittee feels that until the WHO international serodiagnostic laboratory conference has been held and results have been evaluated, laboratories in various countries may choose to follow the recommendations of the third International Serological Laboratory Conference (Montevideo, 1930) concerning the use of one complement-fixation test and at least one flocculation test. Standardization of tests, of performance, and types of seroreactions to be determined at the national level have been recommended by the ad hoc Expert Committee on Venereal Diseases, and the subcommittee will consider further the possibility of recommending the establishment of standardized serological techniques and antigens.

Problems relating to mass examinations have been referred to in section 7 of the report.

9. Revision of the System of Notation for Serological Tests Introduced by the League of Nations Health Organization

The subcommittee considered the introduction of a uniform but simple method of recording results of serological tests in syphilis. The system introduced by the Health Organization of the League of Nations and applied in the individual treatment booklet issued under the Brussels Agreement would appear to be subject to revision. The subcommittee intends to study this question on a broad basis and is of the opinion that, pending the establishment of a special international treatment booklet under the projected health regulations for venereal diseases, the revised Brussels Agreement booklet, to be issued in 1950, might be based on the following system of notation :

- (1) non-reactive sera \div
- (2) doubtful reactive sera $+\div$ (indicating necessity of re-examination in cases without history or clinical signs of syphilis)
- (3) reactive sera $+$
- (4) quantitative tests should be reported in terms of titre, the titre being defined as the highest reactive dilution of serum, for example, reactive up to 1/160.

10. Further Meetings and Agenda

After consideration of the various items placed on its agenda at the first session, and of the activities approved or projected by WHO in the serology and laboratory aspects of syphilis, and on the basis of the technical discussions during the meetings, and the statements of members and specially invited experts, the committee adopted the following resolution :

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

RECOMMENDS

- (1) that at least two meetings be held in 1950 ;
 - (2) that a limited number of experts be co-opted for these meetings to consider special items referred to the subcommittee, particularly those relating to treponematoses other than syphilis ;
 - (3) that the following items be included on the agenda for the next meeting :
 - (a) further details for the planning and organization of the international serology conference ;
 - (b) evaluation of results in serum exchange, and preservation of sera and cerebrospinal fluids ;
 - (c) studies on collected information on cardiolipin-lecithin antigens ;
 - (d) studies of mass-serological screening procedures ;
 - (e) studies of the laboratory aspects of the treponematoses survey ;
 - (f) revision of the League of Nations system of notation for sero-diagnostic tests.
-

Annex 1

AN OUTLINE FOR AN INTERNATIONAL SEROLOGY
CONFERENCE ¹

As stated in the report of the ad hoc Expert Committee on Venereal Diseases of the Interim Commission, concern is felt over the inability of the present serology structure to contribute effectively to the international control of syphilis. A wide variety of test methods are in use in various parts of the world. In regard to many, no valid estimate is available as to the efficiency of performance or reliability of findings. Differences exist in such basic respects as preparation of antigen, concentration of components, duration of inactivation and incubation, and reading and recording of reactions. Results obtained by the various methods are not comparable and, not infrequently, are widely discrepant.

Moreover, the serology of syphilis is passing into a transitional stage as regards the value of test findings in the recognition of the disease and in the management of treatment. The future will probably demand the use of multiple tests, in place of a single method, and the quantitative determination of the concentration of reacting substance. The latter service is considered to be essential to effective use of penicillin therapy.

To the increased demands mentioned above may be added the numerical increase in specimen load which will result from the broadening scope of the control programme. It would appear that the broad programme is feasible from an administrative standpoint, but that some rationalization of serological methods and practices will be needed if unity of action and effective international interchange of information is to be attained.

The most rapidly effective means of focusing attention upon the relative merits of test methods is considered to be the serology conference. Through the medium of this type of assembly results may be produced which illuminate the shortcomings of methods as well as indicate the procedures which may be expected consistently to produce a high standard of findings. A critical analysis of findings may well serve to indicate methods whose elimination, or replacement by a more consistent method, would be of value to the control effort.

Serology conferences have been held in Copenhagen, 1923 and 1928, in Montevideo, 1930, and in Washington, D.C., 1941. Each is considered

¹ Submitted by Dr J. F. Mahoney, Medical Director, Venereal Disease Research Laboratory (US Public Health Service), Staten Island, N.Y., USA ; as from 1 January 1950, Commissioner, State Department of Health, New York City, N.Y., USA.

to have produced information which was helpful in guiding the development of serological methods during the periods immediately following each conference. In the event of the inclusion of an international conference in the programme of the World Health Organization for the year 1950 or later, an early consideration of the following general topics will be essential.

1. Rationale

The rationale of incorporating an international serodiagnostic laboratory conference into the general plan of the World Health Organization is outlined below.

Undoubtedly forces are being set in motion which may have a far-reaching effect upon the future of syphilis as a public-health factor. Antibiotic therapy is capable of curtailing the infectious period of the disease. As an increasing number of early infections are brought under treatment, a spiral of decline in incidence may be set in motion. A dramatic decline in incidence would be accompanied by an equal decline in the public-health importance of the infection. Such an occurrence would argue against the advisability of conducting the proposed conference. At present the newer therapy has not been in use for a sufficient period for evidence of a favourable epidemiologic influence to become visible.

2. Cost of Conference

A practical estimate of cost will require policy declarations as to the site of the conference, number of participants, payment of travel and per diem of participants, and compensation of blood and spinal fluid donors.

3. Selection of Site of Conference

In the selection of the site for an international gathering of the type contemplated, the following general considerations are pertinent:

(1) Geographical location: a large city conveniently located with regard to relative ease of access from all parts of the world.

(2) Accessibility of a large medical centre: the selection of donors and the establishing of the presence or absence of syphilis and the presence of other disease entities will necessitate the collaboration of a large medical staff.

(3) Availability of laboratory space and equipment: Floor space approximately 200 square feet per participant will be required in premises equipped with the usual laboratory services (gas, water, air, etc.), furniture, and equipment.

(4) Availability of large reservoir of clinical syphilis : A great number of syphilitic donors will be required for contribution of serum and spinal fluid specimen material.

(5) Availability of scientific personnel capable of being trained in details of conduct.

(6) Availability of secretariat, including interpreters and translators.

(7) Availability of adequate mail service, both air and rail.

4. Administrative Organization

(1) Creation, within the structure of the Venereal Diseases Section of WHO, of the position of conference director, and the appointment of a consultant body of serologists and public-health administrators.

(2) Preparation of a preliminary draft of qualifications for participation, and of rules and regulations for conduct.

(3) Determination of the time of the conference ; an allowance of at least two years, and possibly three years, for preparation is essential.

5. Conduct of Conference

(1) Assembly of all qualified participants.

(2) Assignment of adequate floor space and equipment to each participant.

(3) Collection, preparation, and distribution to the participants of blood and spinal-fluid specimens having the following origins :

(a) Blood-serum specimens from individuals free of history and/or evidence of syphilis and of other diseases or pathologic conditions (normal group).

(b) Blood-serum specimens from individuals in whom the presence of syphilis has been excluded, in so far as is possible, but in whom other infectious diseases can be demonstrated.

(c) Blood-serum specimens from individuals in whom syphilis in any of its stages, treated or untreated, is known to exist.

(d) A representative group of spinal-fluid specimens from (i) normal individuals, (ii) patients with central nervous system syphilis, and (iii) patients displaying evidence of other central nervous system conditions.

(e) A large number of especially prepared merthiolated serum specimens of graded degrees of positivity ranging from small to large amounts of reacting substance. The purpose of this section would be to test the capacity of the test procedures to quantitate the concentration of reacting substance.

6. Duration of Conference

Each participant to be presented with 100 individual specimens, under code, on each of 20 days.

7. Analysis of Findings, Conclusions, and Report

(1) A numerical rating based upon the ability of the test method to identify syphilis, to escape non-specific reactions, and to detect relative concentrations of reacting substance.

(2) Adequate publication of all findings and conclusions.

Annex 2

**MEMORANDUM ON THE WHO INTERNATIONAL
SERODIAGNOSTIC LABORATORY CONFERENCE¹**

In the report on the first session of the ad hoc Expert Committee on Venereal Diseases, mention was made of the possibility of the syphilis control problem being altered in magnitude or complexion in the years immediately ahead.² This possibility was based upon the impact (immeasurable at present) which the general adoption of antibiotic therapy might have upon the incidence of the disease and upon the seriousness of its late manifestations. Mention was also made in the report of a spiral of decline in incidence which might result from the shortening of the infectious stages of the disease and consequent curtailment of opportunities for transmission.

In the interval since the publication of the report, no major changes of the above-mentioned character have taken place. Although there are some indications of a decline in the number of new syphilitic infections in some areas of the world, the character of the trend cannot be determined at present and probably will not be clearly discernible for another five years.

In planning a gathering of the type contemplated — an international serodiagnostic laboratory conference — it must be assumed that when the project has been completed there will still be a need for the information thus obtained. On this basis it would appear advisable to proceed with the organization of the conference without further immediate contemplation of the probable public-health status of syphilis at the time of distribution of the final report.

A period of at least three years will be required for the organizational work. Should changes in the basic nature of syphilis become evident during the planning phase, the effort could be halted or the project altered to meet the demands of the changing situation.

The initial step in planning appears to be the creation of a preliminary administrative organization for the collection of information upon which subsequent decisions will be based.

¹ Submitted by Dr J. F. Mahoney, Medical Director, Venereal Disease Research Laboratory (US Public Health Service), Staten Island, N.Y., USA; as from 1 January 1950, Commissioner, State Department of Health, New York City, N.Y., USA.

² *Off. Rec. World Hlth Org.* 8, 62

Preliminary points to be decided are the selection of a site for the conference, qualifications for participation, probable number of participants, estimate of costs, and designation of dates for the actual conduct of the conference. Much of this information may be assembled under the guidance of an administrative assistant to the conference director and presented to the administrative group charged with the formulation of early policy.

The selection of the conference director may well be deferred until the real dimensions of the undertaking can be visualized. Because of the medical nature of many of the problems to be decided, it would appear essential that the position of conference director be occupied by a medical man with experience in many geographical areas and with special training or interest in the field of syphilis. The selection must be made from a very limited group of serologists of acceptable standing and broad experience. A staff of serologists, interpreters, statisticians, clerical assistants, and sub-professional technicians may be recruited in an orderly manner as the need arises.

A very important matter of policy to be determined concerns requirements and qualifications for participation in the conference. Previous conferences of the same general nature have restricted participation to individuals who have contributed to the development of distinctive test methods. On this basis, the total number of participants in the conference would be approximately fifty. This figure includes an estimate of the number of workers having methods which, although now in the investigating stage, will probably be ready for evaluation when the conference convenes.

Participants may be approached by individual invitation or by solicitation of applications through suitable notices in the medical press of the world. An application would require the support of a brief statement setting forth the technical differences justifying the classing of the method in question as individual.

An important decision to be made is in regard to the willingness of WHO to defray the expenses of the participants, both as regards travel to and from the point of meeting and maintenance during the period of the conference. These items of expense will be considerable, and unless the serologists and their assistants have access to other sources of reimbursement, attendance by many desirable participants will probably be found impractical.

The collection of blood specimens from individual donors in many parts of the world and transportation of these specimens to the meeting place will represent a major organizational undertaking. Whole blood specimens may suffice in instances in which samples can be flown to their

destination under refrigeration. In other circumstances, the use of preserved specimen material will be required. The size of the blood specimen from each donor will be calculated in accordance with the number of participants.

In each collecting area the services of at least one consultant of recognized standing will be required. The consultant will (1) select individuals in whom syphilis is known to exist, (2) select patients in whom disease conditions other than syphilis can be demonstrated, and (3) select individual donors who are free from evidence of syphilis and other diseases and pathologic conditions (normal group).

The total number of collecting centres, the number of each kind of specimen to be collected, the amount of blood to be withdrawn, and the manner of reimbursing the medical personnel as well as the donors are points which will require consideration.

Of great importance to the world health picture is the problem posed by the failure of some test procedures to portray the syphilis status of a population group in one geographical area while being capable of producing reliable results in another area. There is some evidence to support the belief that the prevalence of the disease in some world areas has been greatly exaggerated, being based on a high proportion of positive serological findings. It is very possible that this picture is the result of an environmental condition quite distinct from syphilis. The findings of the conference should be of a nature adequate for the resolution of this problem.

An advisory group of syphilologists, serologists, and health administrators will give counsel in matters pertaining to the clinical and technical aspects of the conference. It would be helpful if two members of this group were to act with the conference director as a board of referees authorized to arbitrate in any matters of controversy which may arise during the course of the conference.

Annex 3

COMMENTS ON THE PROPOSED SEROLOGY CONFERENCE¹

A thorough examination of the serodiagnostic methods for syphilis will be of great importance ; first, because the second World War prevented direct contact among European laboratories and between them and laboratories in other parts of the world, and, secondly, because a wide variety of techniques have come into use, based on new principles, e.g., the purified cardiolipin, lecithin, and cholesterol antigens.

If the incidence of fresh syphilis should decline in many areas during the next two years, there would still be a need for a serology conference inasmuch as the efficiency of the new penicillin preparations is sufficiently great to provide an impetus for mass serological examinations. The newer penicillin preparations permit treatment and ensure a cure of the infectious patients revealed by mass surveys in a relatively short time as compared with the prolonged and unpleasant treatment of patients in the past with salvarsan and bismuth.

Consequently, it would appear essential that the conference indicate a test which combines technical simplicity with minimum of cost, at the same time affording sufficient security to those patients who may benefit from treatment. It is assumed, of course, that seropositive cases would be verified by more than one test.

In addition to the study of actual seroreactions, it will be of great importance to take the opportunity of making an official evaluation of the various verification tests, regardless of whether the test is a verification method for a single ordinary test, a special test, or a technique based on the isolation and addition of certain serum fractions followed by comparative evaluation of the seroreactions by means of several of the ordinary tests.

I must support Dr Mahoney's statement that two or three years will be necessary for the preparation of a profitable serology conference. In my opinion 1952 will prove the best time. An essential reason for postponing the conference until 1952 is the fact that in several European countries, owing to shortage of personnel, laboratory work has been limited to necessary routine details, leaving no possibility for research. If the conference is planned for 1952 and participants are informed now of its

¹ Submitted by Dr P. Krag, Assistant Director, Serodiagnostic Department, State Serum Institute, Copenhagen, Denmark.

impending schedule, an excellent possibility exists for participation by several European laboratories.

The number of participants will to some extent be limited by the volume of blood obtainable from each donor. Dividing the participants into groups or teams would complicate the analysis of the results, since overall failure of a test performed by one group might weight the results unfavourably too much in one direction.

<i>Participants</i>	<i>Sera</i>	<i>Sera</i>	<i>Sera</i>
1 - 15	1 - 50	51 - 100	
16 - 30	1 - 50		101 - 150
31 - 45		51 - 100	101 - 150

Even if the participants were divided into teams, a large number of donors would be required to contribute a serviceable quantity of material.

As a great many new and valuable techniques have come into use in US laboratories since the 1941 Washington conference, and as it will prove difficult to have more than twenty participants at a single conference, I suggest that :

(1) A serology conference, composed of USA participants only, be held in 1951 in the USA in a convenient city. It would be possible to select specially recommendable techniques, e.g., one to two complement-fixation tests, one to two tube-precipitation tests, one to two slide tests, and one to two verification tests ; total : four tests.

(2) A conference sponsored by WHO be held in 1952 in Europe ; the above-mentioned USA reactions would be compared with those employed elsewhere in the world.

(3) The two conferences should be planned by the same subcommittee on serology, and the same body of experts should evaluate the 1951 and 1952 results.

(4) Such a scheme would result in a clear survey of both conferences, and the non-American laboratories would have another year in which to complete reorganization and engage in scientific research. The travelling expenses of participants would also be somewhat reduced.

A serology conference sponsored by WHO should not only provide information regarding individual reactions, but also offer a possibility of evaluating the merits of several combinations of reactions, i.e., sets of two to three reactions with different sensitivity and specificity—several laboratories in their routine results are now interested in the relation between two to three well-known reactions—and of informing the physicians of the various chances of specificity in the different combinations of readings of these reactions.

In order to facilitate the practical use of quantitative methods, it will be of value to determine the titration series with which the lowest number of glasses offers the most exact description of the titre of a serum.

In Denmark, and in other laboratories in certain Scandinavian countries, it has been demonstrated that a dilution series, each glass of which contains one-third of the quantity of serum in the previous glass, offers a good basis for estimation of serum titre when readings are made in haemolysis percentages and the latter are used for estimation of two interpolation values between neighbouring glasses.

Instead of titre or dilutions a special logarithmic term called "the degree of strength" is used to indicate the titre.

Sera and blood for the conference must be contributed from many different sources; first, to ensure representative specimens for various stages of the disease and to allow for conditions under which they were obtained which might be responsible for provoking false reactions; and secondly to avoid an exaggeration of the number of nonspecific reactions in the country in which the conference takes place. If the country in question contributes many sera with suspected nonspecific reactions, it might deliver a hundred specimens of preserved sera all of which have been positive when examined locally, and which by clinical observation and serological examination have appeared to be nonspecific.

Since any test has its own tendency to be false under certain circumstances, reactions which have the same percentage of nonspecificity in heterologous material may not be positive in homologous patient material. Consequently, the foreign reactions may be negative in cases where the local tests may be positive. However, the opposite result will not be encountered in such a collection of sera.

Dr Mahoney proposes about 2,000 specimens of sera, a number which I consider the minimum. 3,000 specimens of sera distributed in working weeks of five days, 200 a day, appear reasonable and could be dealt with in three weeks.

The fact that the stability of specific and nonspecific sera under storage conditions may create essential differences should be kept in mind, and care should be taken to find a method which allows preservation of nonspecific sera in such a way that they are not altered.

The technique for forwarding of specimens should be elaborated before the actual conference.

At the conference it will hardly be possible to have the individual tests carried out by persons other than the authors or their authorized representatives, but after the conference it will be of the utmost importance to promote close co-operation between neighbouring countries in order to make yearly regional exchanges of sera a common rule.

A similar exchange of sera among a limited number of specially selected laboratories might be effected for the purpose of verifying the serological level in the various parts of the world. An exchange of antigens will not suffice to solve this problem, as the reading of reactions in most precipitation tests is based on individual judgment and consequently requires an evaluation of sera of various strengths. A consideration to be emphasized in this connexion is that, as these sera will be used in control tests, the laboratories may feel inclined to treat them with special care and entrust their examination to a specially skilled assistant. It is also possible that the laboratory will perform more than one test on a specimen, and in doubtful cases it may test a specimen by different techniques on the basis of which it may decide whether a specimen originally positive and later doubtful is to be considered positive or doubtful.

No risk is involved in judging the sensitivity of mixed pooled sera by quantitative reactions, as the quantitative result in another verification test will be of no assistance in indicating whether the reaction should be one degree higher or lower.

Annex 4**MEMORANDUM ON THE WHO INTERNATIONAL
SERODIAGNOSTIC LABORATORY CONFERENCE¹**

The various documents circulated for consideration and comment concerning preparations for an international serodiagnostic laboratory conference deal with a question of indisputable interest.

The suggestions for the organization of the technical work at the conference and its administrative aspects as well as considerations for selection of the conference site appear to be satisfactorily set forth and to be given in sufficient detail to arrange a programme for the conference. However, in my opinion, certain points have not been covered adequately and others have not yet been taken into account.

The chief problems to be confronted in the organization of the conference are the considerable extension of the use of serological methods, and the development of systematic procedures for the diagnosis of syphilis. When the last international serological conference met, the case-finding of syphilis by mass serological surveys had not been contemplated.

In order to deal with these circumstances, the first task of the conference should be, as in the case of preceding ones, the selection of standard serological techniques to be carried out with standard antigens. This necessitates the judicious choice of a testing method as a basis for proposing the selection of technique. These points have been considered in the proposals for the organization of the conference. I recommend that the techniques chosen should be qualitative and quantitative and as reliable in routine work as in the hands of experts; thus they should combine, as far as possible, technical simplicity with maximum accuracy.

The serodiagnosis of syphilis on a large scale demands the perfecting of a special serological test comprising one or, better, a number of reactions. It should be simplified as far as possible in order to facilitate a preliminary mass survey. Standard plans for serological investigations applicable to groups, e.g., factories, towns, regions, countries, should also be developed.

In addition to the selection of serological methods and reagents, it appears necessary, in order to ensure lasting co-ordination of research and standardization of serodiagnostic methods, to establish a permanent centre for serological reference and control which, to be effective, should be carried out on a world scale and on a regional and national scale.

¹ Submitted by Dr R. Laporte, Chef du Service de la Sérologie, Institut Pasteur, Paris, France.

Permanent Structure for Serological Reference and Control

1. *World centre*

On the world scale, the centre to be set up should possess a technical and administrative organization capable of ensuring the direction, co-ordination, and control of the regional and national centres, whose essential task should be to ensure liaison between the world centre and routine serological laboratories.

The world centre should ensure the diffusion and permanent control of standard serological methods, and supply the regional centres with standard reference reagents (antigens, sera, etc.); it should centralize, classify, and ensure the distribution of statistical information sent in by the regional and national centres; and it should centralize information concerning serological research, carry out its own test experiments on improvements in old techniques and on new techniques, and ensure, if necessary by instituting training courses, the additional technical instruction which may be necessary for the members of the regional centres.

2. *Regional or national centres*

On the regional or national scale, the centres to be set up should be in direct contact with heads of laboratories and laboratory workers. The serological laboratories, which, in a country like France, carry out on an average 90% of the total serological examinations, are small laboratories which experience considerable difficulty in checking the value of their own work despite the amount of work carried out daily. It is clear that, under these conditions, no improvement in serological results can be expected if the difficulties which these laboratories experience in working under correct control conditions are not overcome.

Of course, the establishment of control bodies for these laboratories lies entirely with the countries concerned, but, in my opinion, the international serological conference should draw up a detailed plan for the standard organization of such control, and should discuss in what way the centres made responsible for it can be ensured adequate working facilities.

Each national centre should ensure the control of the antigens used in the country in question by reference to the standard antigens supplied by the world centre; it should supply standard control sera, of controlled clinical origin, to the routine laboratories; and it should make a serological examination of samples of sera or of cerebrospinal fluid from the routine laboratories so as to check the accuracy of the results obtained by the latter.

For this purpose, a laboratory inspection service, set up as part of the national health-organization, should work in collaboration with the national centre. The drawing-up of a standard plan of organization seems advisable.

The national centre should be made responsible for the inspection, and, if necessary, approval of the technical instruction given on the national level to approved laboratory personnel (executive scientific staff, and assistant technicians). This instruction should include a compulsory period of probation, followed by a qualifying examination, for laboratory staff found to be insufficiently trained.

The national centre should examine new methods and techniques, and forward the results of inquiries carried out in this connexion to the world centre; conversely, it should ensure the circulation of tested technical improvements brought to its notice by the world centre.

It should collect and classify statistical documents on the serological organization and functioning of the country in question. This information would be transmitted to the world centre and would be included in a general publication.

It is felt that each of these points should be studied in detail by the international conference so that, after public discussion, the world serological centre can definitely be established.

One question in particular deserves attention, namely, the creation of collecting centres for standard sera and cerebrospinal fluids of known serological activity (need for a standard quantitative method) and of varying serological quality.

A detailed plan for such centres should be drawn up, especially as regards:

(a) the collection of sera in sufficient quantity, after rigorous clinical examination of the donors; these sera should be homogeneous, i.e., mixtures of different sera should be prohibited;

(b) the study of means for storage and transport, as well as initial and periodical serological control, of these standard sera.

It would be logical for questions concerning the collection, storage, and transport of standard sera to be settled before the opening of the conference by an expert committee designated for this purpose.

To summarize, the subjects which should be thoroughly examined and publicly discussed by the conference, after careful preparation by the experts, are:

- (1) reference tests and standard reagents;

- (2) the plan of organization for mass-serological case-finding surveys and the selection of simple techniques suitable for this purpose ;
 - (3) the selection, storage, and distribution of standard sera with variable and measured positivity ;
 - (4) the setting-up of a permanent world reference and control centre for the serology of syphilis.
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Annex 5**MEMORANDUM ON THE WHO INTERNATIONAL
SERODIAGNOSTIC LABORATORY CONFERENCE¹**

It is assumed that the object of the conference is the comparative testing of sera by various technical methods under conditions as near as possible to those found in ordinary practice. Thus an attempt will be made to assess which technical methods yield the most useful information to the clinician. Any tendency to run the tests under "streamline" conditions or any unhealthy spirit of competition should be eliminated, because it is reasonable to suppose that all the participants will be seeking information as to the value of their methods rather than attempting to show off the excellence of their techniques. It is therefore suggested that at the meeting of the Subcommittee on Serology and Laboratory Aspects the following points might be discussed with advantage :

1. Blood Specimens

Specimens should come from sources both far and near, and be sent not only as sera separated from the clots, but also as whole blood from which the sera should be separated in the routine manner on arrival at the designated laboratory. The question as to whether preservatives, such as merthiolate, should be added to the specimens before testing should be discussed in some detail, because, while this can be easily adopted in routine hospital or clinic practice, some method must be arranged for a similar procedure in the case of the ordinary general practitioner.

The total number of specimens to be tested at the conference should also be defined, and their choice should be representative of all stages of syphilis, both treated and untreated. Furthermore, some specimens should be included from patients who do not present, and never have presented, any signs, symptoms, or history of syphilis, and also from patients suffering from conditions such as malaria and leprosy which are thought to be frequent causes of false-positive serological tests for syphilis.

2. Complement

The source of complement to be used in the complement-fixation tests should also be discussed. For some years many laboratories in this country

¹ Submitted by Dr I. N. Orpwood Price, Director, Venereal Diseases Reference Laboratory (Public Health Laboratory Service), St. Peter's Hospital, London, United Kingdom.

have been using a preserved complement (not dried) with satisfactory results, and I personally would prefer to use this preserved complement at the conference.

3. Antigen

It would appear that many people throughout the world are now trying out cardiolipin antigen, but have adapted it to their own technique rather than use the technique advocated by those responsible for the discovery of this type of antigen. It is, therefore, suggested that those among the participants in the conference who have had experience with cardiolipin antigen as adapted to their own technique should test the sera not only with the Wassermann antigen they usually use but also with cardiolipin antigen.

4. Reporting

It is suggested that the results of the serum tests be in the first instance reported as "positive", "doubtful", or "negative", depending on which term is applicable. In view of the admitted importance of quantitative reactions, all positive serum tests should be reported in a quantitative manner. I suggest that this should be done in terms of serum dilutions because it seems to me that this is the most practical method.

5. Number of Specimens to be Tested Daily

This should be clearly defined with the time factor necessary for the various techniques to be employed clearly in mind. It is suggested that not more than 50 sera be tested daily.

6. Technicians

Each participant in the conference should be allowed one technician to help him carry out the work. The technician should be chosen from his own laboratory.

Annex 6**COMMENTS ON THE WHO INTERNATIONAL
SERODIAGNOSTIC LABORATORY CONFERENCE¹**

Copies of memoranda by Drs Krag, Laporte, Mahoney, and Orpwood Price on the proposed international serodiagnostic laboratory conference have been reviewed. The organizational concept of this study is well expressed in these memoranda.

An early definition of the aims of the serological congress will be helpful in formulating plans. The objectives may be several and each may wholly or in part be met by only certain donor categories. So the number of donor divisions, the number of specimens in each category, and the maximum number of tests that can be performed daily by each participant will determine the number of testing days that must be allowed.

International serological congresses have in the past produced evidence regarding the relative specificity, sensitivity, and reproducibility of serological tests. Attention has not been given to the adaptability of these tests to population groups in several parts of the world or to the testing of individual specimens containing preservatives.

The use of chemical preservatives may cause difficulties in some testing procedures and would create an additional division for statistical evaluation of results. Whole, untreated blood and spinal fluid would be preferred testing samples. Selection of the site for this congress may be influenced by its proximity to main lines of world air-routes so that specimens may be obtained from the desired geographical areas within a limited time, thereby eliminating the need for preservative.

This conference will offer an opportunity to replace the often misunderstood terms, "positive", "doubtful", and "negative", that carry diagnostic connotations, with more suitable words or phrases. The designations "reactive", "partially reactive", and "nonreactive" are suggested with the endpoint titre being attached to all reports of "reactive".

It is also suggested that applications to the serology conference be closed six to nine months before the starting date so that final arrangements will not be interfered with by late applicants. The application period could be open for one year. Applications should be accompanied by a detailed outline of test technique, which may be amended before the starting date.

¹ Submitted by A. Harris, Senior Serologist, Venereal Disease Research Laboratory (US Public Health Service), Staten Island, N.Y., USA.

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