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EXPERT COMMITTEE ON VENEREAL INFECTIONS AND TREPONEMATOSES

SUBCOMMITTEE ON SEROLOGY AND LABORATORY ASPECTS

Third Report

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EXPERT COMMITTEE ON VENEREAL INFECTIONS AND TREPONEMATOSES

SUBCOMMITTEE ON SEROLOGY AND LABORATORY ASPECTS

Third Session

Copenhagen, 31 August–5 September 1953

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Secretariat :

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

* Attended two meetings.

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EXPERT COMMITTEE ON VENEREAL INFECTIONS AND TREPONEMATOSES

SUBCOMMITTEE ON SEROLOGY AND LABORATORY ASPECTS

Third Report *

1. Introduction

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

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

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

2. Developments and Perspectives

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

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

1. NOTES the third report of the Subcommittee on Serology and Laboratory Aspects of Venereal Infections and Treponematoses;
2. THANKS the members of the subcommittee for their work; and
3. AUTHORIZES publication of the report.

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

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

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

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[1] *Wld Hlth Org. techn. Rep. Ser.* 1950, **13**; 1953, **63**

[2] *Wld Hlth Org. techn. Rep. Ser.* 1950, **14**; 1951, **33**

[3] Resolution EB7. R66, *Off. Rec. Wld Hlth Org.* **32**, 28

[4] *Wld Hlth Org. techn. Rep. Ser.* 1953, **63**, 25

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

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

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

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

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

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

[6] See *Wld Hlth Org. techn. Rep. Ser.* 1952, **56**, 8 (section 10.2).

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

3. Cardiolipin Antigen

3.1 *Observations*

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

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

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

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

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[7] Unpublished working document WHO/VD/SERO/39

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

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

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

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

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

[9] *Wld Hlth Org. techn. Rep. Ser.* 1952, **56**, 8 (section 10.2)
[10] *Wld Hlth Org. techn. Rep. Ser.* 1954, **86**, 11 (section 11)
[11] *Wld Hlth Org. techn. Rep. Ser.* 1950, **33**, 19 (section 5.2, I.5)
[12] Unpublished working document WHO/VD/SERO/40