WORLD HEALTH ORGANIZATION

REGIONAL OFFICE FOR THE WESTERN PACIFIC

REPORT

MEETING OF THE SCIENTIFIC WORKING GROUP ON THE DEVELOPMENT OF RAPID DIAGNOSTIC METHODS FOR *M. LEPRAE* INFECTION

Tokyo, Japan

18-21 June 1985

Manila, Philippines

March 1986

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MEETING OF THE SCIENTIFIC WORKING GROUP ON THE DEVELOPMENT OF RAPID DIAGNOSTIC METHODS FOR *M. LEPRAE* INFECTION

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NOTE

The views expressed in this report are those of the members of the Working Group on the Development of Rapid Diagnostic Methods for M. leprae Infection and do not necessarily reflect the policies of the World Health Organization.

This report has been prepared by the Regional Office for the Western Pacific of the World Health Organization for the governments of Member States in the Region and for the members of the Working Group on the Development of Rapid Diagnostic Methods for M. leprae Infection, which was held in Tokyo, Japan, from 18 to 21 June 1985.

CONTENTS

1. INTRODUCTION

Leprosy is a public health problem of increasing concern in many countries of the Western Pacific Region. The establishment of simple, sensitive and rapid diagnostic methods for the detection of inapparent forms of Mycobacterium leprae infection and for monitoring the effects of chemotherapy application is very important and should be urgently explored.

The successful isolation of phenolic glycolipids antigen from M. leprae-infected armadillo tissues specific to M. leprae has opened the door to the development of rapid diagnostic methods for M. leprae infection. Research on the development of rapid diagnostic methods for M. leprae infection using natural phenolic glycolipids antigen and the synthetic oligo saccharide antigen is currently ongoing in Japan and the United States of America.

In the light of this recent development, a meeting of the Working Group on the development of the Rapid Diagnostic Methods for M. leprae infections was held in Tokyo from 18 to 21 June 1985 to strengthen cooperation in the development of rapid diagnostic methods for early M. leprae infection and to discuss and formulate a strategy for application of the methods for the control of leprosy in the Region.

The meeting, the first of its kind to examine the progress of the studies in the Region, was attended by fifteen experts/researchers from China, Japan, Samoa and the United States of America.

2. SUMMARY AND COMMENTS OF DISCUSSIONS

The objectives of the Working Group were:

(1) to review research and development of rapid diagnostic methods of M. leprae infection;
(2) to develop collaborative activities in the development of rapid diagnostic methods for M. leprae infection in the Western Pacific Region; and
(3) to recommend plans for future development and application in the Region.

The meeting started with a brief review by Dr Noordeen of the applicability of the various available Cell Mediation Immunity (CMI) assays to leprosy. He noted that lymphocyte transformation tests and skin testing with various forms of lepromin and soluble antigens had inherent problems, but were still very useful as shown by the recent Venezuelan studies, in detecting sub-clinical infection.

Dr Noordeen also reviewed the status of research on the protein antigens of the leprosy bacillus and their potential in the context of rapid diagnostic methods. Much of this new information had emerged from the work of the recent very successful Scientific Working Group on Immunology of Leprosy (IMMLEP) on monoclonal antibodies.

During the open discussions, the members defined the major requirements of any successful rapid immunodiagnostic tool for leprosy:

- Sensitivity and specificity in detecting sub-clinical infection in leprosy endemic areas;
- Ability to predict clinical leprosy;
- Simplicity, practicability and cost-effectiveness;
- Value in gauging the efficacy of vaccination. An important requirement of an ideal test was that vaccination/skin testing should not of themselves produce a positive serological response and in this respect, the phenolic glycolipid appeared ideal in that vaccination did not induce antibodies against the glycolipid.

It was observed that the topic of the phenolic glycolipid and its relatives had been a major theme of the Working Group because it was specific to M. leprae; it was highly immunoreactive; it was widely available through IMMLEP and the U.S. programmes in a very pure form; and it was the molecule on which many investigators in the Western Pacific Region were working.

The above information set the scene for the discussions on the narrower topic of phenolic glycolipids, by describing the broad outcome of the second IMMLEP Workshop on Enzyme-Linked Immunosorbent Assay (ELISA) and the glycolipid studies recently conducted by Dr Howard Engers of the WHO Immunological Research and Training Centre. The outcome of this endeavour had been highly successful. There was confirmation of the specificity of the glycolipid/ELISA assay (of the order of 90% specificity). The assay was also highly sensitive at the lepromatous end. There was a very good accord between the various laboratories involved in the study of the analysis of some 129 sera specimens.

An important outcome of this workshop was the new emphasis placed on the new semi-synthetic antigens related to phenolic glycolipid. The results with the disaccharide-based semi-synthetic antigens were highly reproducible; such antigens were ultimately inexpensive and could be produced in vast quantities. The water-soluble synthetic antigens, unlike the lipid-soluble glycolipid, were amenable to simple and varied serological tests.

Another recommendation from the IMMLEP Workshop was the need for a standard ELISA protocol; already, there was good conformity between the participating laboratories. A standardized positive serum should be made available, the continued monitoring of anti-glycolipid IgM and IgT should proceed, and the effects of vaccination on anti-glycolipid antibodies should be determined.

Dr Brennan (United States of America) described the chemistry of the several generations of semi-synthetic glycoprotein antigens based on the glycolipid, particularly those used in the IMMLEP Workshop, i.e. those containing an octyl-8-methoxycarbonyl. All showed very good concordance with the native glycolipid in ELISA and about the same specificity. They also corresponded to the native glycolipid in showing a high seropositivity at the multibacillary end and about 30% at the pauci-bacillary tuberculoid end. In addition, a strong case was presented, based on the use of a variety of semi-synthetic antigens, that the more the natural sugar structure introduced into the semi-synthetic compounds, the greater the specificity and activity. Accordingly, future diagnosis conducted in the context of IMMLEP or the Western Pacific Region programmes would use the so-called natural disaccharide-octyl-8-methoxycarronyl-BSA antigen (ND-O-BSA) in detecting tuberculoid leprosy and this would emphasize the need for second assays, perhaps based on IgG-inducing protein specific antigens.

Dr Brennan also raised the issue of specific, rapid and sensitive sero-diagnosis based on glycolipid antigenemia detection rather than antibody assay, particularly by immunological methods backed up by chemical means. The so-called DOT-ELISA provided the requisite sensitivity. It was inexpensive, highly diagnostic and could be applied to urine and serum. Glycolipid analysis in lepromatous serum showed an immediate and sensitive response to chemotherapy. In limited studies, serum glycolipid levels showed good concordance with the clinical and pathological classification, the description of skin lesions, and the number of *M. leprae* in punch biopsy.

In general discussions after the presentation, there appeared to be general enthusiasm for the concept of glycolipid detection, particularly in the context of tissue specimens. This could be accompanied by extraction or *in situ* immunofluorescence with anti-glycolipid monoclonal antibodies and used as a gauge of, perhaps, infectivity, successful chemotherapy and the state and composition of immune complexes such as ENL.

Dr Izumi (Japan) presented other novel chemical strategies for the preparation of synthetic antigens. Initially, a variety of fully synthetic glycoconjugates were prepared containing a lipophilic aglycone. Most of these were active in an ELISA inhibition assay and some of the conventional ELISA; that containing a steriled alcohol was the most promising. More importantly, they described the synthesis of a natural disaccharide-phenyl propionate-BSA (ND-P-BSA) containing 20-30 moles of disaccharide per mole of carrier polypeptide. This compound had been tested by Dr Izumi and appeared highly active; it had also been tested in Dr Brennan's laboratory and showed identical serological activity to the ND-O-BSA used in the IMMLEP Workshop. The ND-P-BSA would be used immediately in "field" serodiagnosis trials.

In addition, Dr Fujiwara was on the verge of synthesizing the entire trisaccharide-phenylpropionate unit, and conjugated to a protein carrier. This would be the most complete synthetic structure synthesized to date and would be the closest to the natural compound.

Japan had reported very significant progress in the application of ELISA testing to Dr Fujiwara's compound. Dr Izumi reported striking concordant rates and correlation/co-efficients when comparing the synthetic compounds against the glycolipid.

Dr Izumi reported certain anomalies in their inhibition assay. However, now that they were testing the multiple epitope glycoconjugates, there appeared to be less need for an inhibition assay. In addition, some of the coating methods in use had been questioned, particularly the case of ethanol for the native glycolipid and stearyl glycoconjugate. In view of the apparent success of the IMMLEP Workshop on phenolic glycolipid, the fact that all participating laboratories in Seattle, Colorado, Hawaii and London used aqueous buffers or simple water for coating purposes, and the recommendation to standardize ELISA, Dr Izumi suggested the use of aqueous buffers for coating phenolic glycolipid and glycoconjugates. In addition, the practice of pre-treating plates with ethanol should, in the interests of conformity, be discontinued. Again, in the interests of conformity, Dr Izumi stressed the need for data on a truly non-exposed population.

Dr Douglas (United States of America) had apparently no major problem with ELISA, perhaps because he had adhered fairly and rigorously to the early recommendations. He described the use of biotin/avidin amplification, which merely meant that individual sera could be diluted further. For coating purposes, he advocated a volatile buffer. This was a recommended ploy, particularly suitable for pre-coating and shipping of plates to endemic areas. The discussion on Dr Douglas' paper emphasized the need for further statistical evaluation of results.

It was noted that the fascinating feature of Dr Douglas' study was the composition of the population in Micronesia, specifically Ponape, and the excellence of the facility in Cebu (Leonard Wood Memorial Foundation), Philippines, for monitoring in terms of specific antibody and the outcome of the joint chemotherapy trial. In Cebu, there was a great opportunity to test the synthetic antigens generated. In addition, there were all sorts of caveats to the Cebu study: use of paper disc and glycoconjugates, varied chemotherapy, ELISA amplification, opportunity for applying glycolipid detection and a wealth of records. With a good ELISA and antigens, Dr Douglas and Dr Steenbergen were in a position to answer many of the questions raised by Dr Noordeen. Ponape presented the opportunity for pre-clinical diagnosis, retroactive studies, longitudinal studies, etc. The preliminary studies in the context of multidrug therapy and glycolipid-based ELISA looked promising.

Presentations from other participants in the Working Group addressed topics other than ELISA and glycolipid-based antigens. Dr Abe (Japan), in particular, described the progress with his FLA-ABS test. There was no doubt that there was a very strong element of specificity in this test.

Dr Abe presented evidence that much of the specificity was due to the specific glycolipid and specific epitopes within the 55-65 polypeptide. He also reported progress in defining the immunoglobulin class of the antibodies in leprosy sera with the FLA-ABS test and the application of the test to sub-clinical infection among contacts.

Dr Abe also presented some observations on the development of rapid, specific serodiagnostic field tests for leprosy, and the desirability of confirming results by referral of specimens to a central fully developed laboratory.

Dr Izumi described a complement fixation test for detecting anti-glycolipid antibodies. A good degree of concordance among sera from LL, TT, occupational contacts and tuberculosis patients, and non-contacts using complement fixation and ELISA was reported. Complement fixation was an inexpensive assay, even if unwieldy, and had the potential for improvement with the new neo-glycoconjugates. It was closer to the field situation and obviated the need for an ELISA reader.

Dr Brennan described the so-called DIG-ELISA using the new semi-synthetic antigens. It was a combination of GBL diffusion and ELISA terminology. It was inexpensive, specific, sensitive and showed good correlation with the "glycolipid-ELISA". The disadvantage was that one needed enzyme conjugated anti-human IgM, although not an ELISA reader.

3. SUMMARY OF RECOMMENDATIONS

(1) WHO should collaborate in implementing and disseminating within the Region a standardized ELISA protocol, similar to the protocol being recommended by the Scientific Working Group on Immunology of Leprosy (IMMLEP). It should provide for purposes of field application the newly synthesized disaccharide-containing antigens. A comparison should be carried out within the collaborative laboratories of the serological activity of such antigens and information on this should be provided to the ongoing IMMLEP Workshop to permit further evaluation.

(2) WHO should collaborate in the training of manpower to be utilized in conducting and promoting the development of ELISA capability among laboratories in the Region.

(3) WHO should promote the technology, at least in some laboratories, for glycolipid antigen detection and quantitation and evaluate it as a sensitive parameter of clinical progress in the patient, in collaboration with the Scientific Working Group on Immunology of Leprosy and the Scientific Working Group on Chemotherapy of Leprosy (IMMLEP and THELEP).

(4) Research in the development of simplified field tests for sub-clinical infection should be encouraged.

(5) Further research in the Region leading to novel antigens which may be more useful in the investigation of paucibacillary disease and in the evaluation of cellular immunity in leprosy should be promoted in collaboration with IMMLEP.

(6) The serological tests should be evaluated through adequately designed longitudinal studies so that they can be developed as epidemiological tools.

(7) Multicentric seroepidemiological studies should be promoted using standardized protocols and reagents.

(8) WHO should promote the development of central reference and referral laboratories at the regional and national level which will play a pivotal role in these collaborative studies.

ANNEX 1

OPENING REMARKS OF DR H. NAKAJIMA,
WHO WESTERN PACIFIC REGION, TO THE WORKING GROUP ON
DEVELOPMENT OF RAPID DIAGNOSTIC METHODS FOR M. LEPRAE INFECTION
TOKYO, JAPAN
18-21 June 1985

Distinguished Members, Ladies and Gentlemen,

It is my capacity as Regional Director of the World Health Organization Regional Office for the Western Pacific that I have the pleasure of welcoming you to this Working Group on Development of Rapid Diagnostic Methods for Mycobacterium leprae infection.

I wish to express my gratitude to the Government of Japan for agreeing to hold this meeting in Tokyo and to Professor M. Ishidate, Chairman of the Board, Sasakawa Memorial Health Foundation, and their staff for their valuable assistance in organizing this meeting. On this occasion, I am happy to see Dr Noordeen from IMMLEP, who in spite of his busy schedule, has found the time to attend this meeting.

Leprosy is a public health problem of increasing concern in many countries of the Western Pacific Region. The establishment of simple, sensitive and rapid diagnostic methods for the detection of inapparent forms of Mycobacterium leprae and for monitoring the effects of the chemotherapy applied is very important and should be explored urgently, particularly in view of the introduction of WHO-recommended multidrug therapy.

The successful isolation of phenolic glycolipid antigen from Mycobacterium leprae-infected armadillo tissues specific to Mycobacterium leprae has opened the door to the development of rapid diagnostic methods for Mycrobacterium leprae infection. It has also been demonstrated that the phenolic glycolipid has a unique sugar arrangement and that crucial part of the phenolic glycolipid antigen is composed of this oligosaccharide.

The oligosaccharide of the phenolic glycolipid was recently synthesized in the United States and Japan. It was also demonstrated that the synthesized antigen has antigenicity similar to the natural phenolic glycolipid antigen. Research on the development of rapid diagnostic methods for mycobacterium infection using the natural phenolic glycolipid antigen and the synthetic oligosaccharide antigen is currently going on in the United States and Japan.

In the global context, IMMLEP has initiated research programmes on the development of rapid diagnostic methods for Mycobacterium leprae infection. Available data show that the results of these studies are encouraging.

Annex 1

In the light of the above considerations, this is therefore, an opportune time to hold this meeting of the Working Group in order to strengthen cooperation in the development of rapid diagnostic methods for Mycobacterium leprae infection and to discuss and formulate a regional plan for the application of these methods for the control of leprosy in the Region.

This meeting is the first of its kind to examine the progress of studies in the Region. This will enable the participants in the group meeting to discuss their achievements in a regional context and it is hoped to develop strategies for the application of these diagnostic methods.

Finally, I wish to express hope that the final outcome of this meeting will contribute and complement global effort to the development of rapid diagnostic methods for Mycobacterium leprae infection especially in the Western Pacific Region.

ANNEX 2

LIST OF MEMBERS AND SECRETARIAT

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ANNEX 3

AGENDA

Tuesday, 18 June

0830 hrs Registration

0900 Opening ceremony

- Introduction
- Opening remarks
- Self-introduction
- Designation of Chairman, Vice-Chairman and Rapporteur
- Administrative announcements
- Group picture taking

1030 COFFEE BREAK

1100 Adoption of the agenda

"IMMLEP strategy on the development of rapid diagnostic methods for M. leprae infection" by Dr S.K. Noordeen, Chief, LEP/HQ

1200 LUNCH BREAK

1400 Discussion on the progress of development of diagnostic methods

(1) "Immunofluorescent diagnostic methods" by Dr M. Abe

1530 COFFEE BREAK

1600 Discussion on the progress of development of diagnostic methods (continuation)

1830 BANQUET DINNER, Kiku Restaurant, 5th Floor, Sasakawa Memorial Health Foundation Building

Annex 3

Wednesday, 19 June

Discussion on the progress of development of diagnostic methods

0900 hrs (2) ELISA - by Dr P. Brennan, Dr J. Douglas, Dr S. Izumi and Dr T. Fujiwara

1030 COFFEE BREAK

1100 Discussion on the progress of development of diagnostic methods (continuation)

1200 LUNCH BREAK

1400 Discussion on the progress of development of diagnostic methods (continuation)

1530 COFFEE BREAK

1600 Discussion on the progress of development of diagnostic methods (continuation)

(3) "Other methods" by Dr S. Izumi

Thursday, 20 June

0900 hrs Group discussion - Future collaboration on the development of the methodology

1030 COFFEE BREAK

1100 Group discussion - Future collaboration on the development of the methodology (continuation)

1200 LUNCH BREAK

Annex 3

1400 Group discussion - Field application of the rapid diagnostic methods

1530 COFFEE BREAK

1600 Group discussion - Field application of the rapid diagnostic methods (continuation)

Friday, 21 June

0900 hrs Recommendations

1030 COFFEE BREAK

1100 Recommendations (continuation)

1130 Closing ceremony

ANNEX 4

DELIBERATIONS OF SUB-GROUP I

The deliberation of this sub-group resulted in a number of specific, highly focused recommendations and some which had more long-term implications.

It was agreed that a major priority is the standardization of the various ELISA protocols now being used in the Western Pacific Region. The new standardized procedure should conform as closely as possible to the ELISA protocol as used in the context of the recent WHO/IMMLEP Workshop. Details of this protocol are contained in Appendix I. A minor digression is the use of an ammonium acetate-based buffer, which, being volatile, allows for reproductible coating and easier shipping of pre-coated ELISA plates to endemic areas. It is recommended that ELISA plates pre-coated with antigens be made freely available, at least initially, in order to get field application of the technology under way. The Honolulu laboratory is willing to provide this service, at least on an interim basis.

The emergence of the two new semi-synthetic antigens, both natural disaccharide containing glycoconjugates, the "natural disaccharide-octyl-8-methoxy-BSA" (ND-O-BSA) and the "natural disaccharide-phenylpropionate-BSA" (ND-P-BSA), is regarded as a key and exciting development. These are the recommended antigens for field testing in the Western Pacific Region in 1985. Hopefully, by 1986, the "natural trisaccharide-octyl-8-methoxy-BSA" (NT-O-BSA) and the "natural trisaccharide-phenylpropionate-BSA" (NT-P-BSA) will be available. It is also recommended, as a matter of urgency, that comparative testing of the two disaccharide-containing semi-synthetic antigens be conducted in contributing laboratories in the Region (Kyoto, Cebu, Hawaii, Shanghai, Tokyo and Colorado) and that the results be transmitted through Dr Noordeen to Dr Howard Engers of the WHO Immunological Research and Training Centre in Geneva for consideration in the context of the broader IMMLEP programme. Any duplication of the IMMLEP Workshop programme should be avoided; the activities in the Western Pacific should be regarded as complementing the IMMLEP/ELISA Workshop. For purposes of comparing the activities of the newly synthesized disaccharide-containing glycoconjugates and future products, such as those based on trisaccharide, a set of 30 reference sera should be generated representing different spectral forms of leprosy, also tuberculosis and normal persons. These reference sera will be collected by the Tokyo Laboratory (Dr Abe) and aliquots maintained in each of the contributing laboratories.

To facilitate immediate "field" serodiagnosis using the disaccharide-containing neo-glycoconjugates, every effort should be made to provide not only pre-coated ELISA plates but anti-human IgG and IgM conjugates. In general, in field testing, both conjugates should be used in order to probe the clinical implications of anti-glycolipid IgM and IgG. The Hawaii (Dr Douglas) Laboratory, in conjunction with the Colorado Laboratory (Dr Brennan), will undertake to provide such materials at least on an interim basis.

Annex 4

Recommended procedures for storing and shipping of sera, the application of blood samples to paper discs and subsequent elution of antibodies are contained within Annex 2.

The second major recommendation concerns the need to provide facilities for the training of personnel in the standard ELISA protocol. In general, it is recommended that provisions be made available to allow those people competent in the art of the standard ELISA. In fact, those people responsible for the formulation of the standard assay used in the IMMLEP Workshop, should visit relevant laboratories in the Western Pacific Region in order to transmit the ELISA skills. Alternatively, personnel should be encouraged to spend some time in training in the appropriate laboratories, perhaps through a WHO-sponsored fellowship. In addition, a specific recommendation is made that personnel in the Kyoto Laboratory responsible for testing the newly synthesized antigens emerging from the Nara Laboratory, should travel to one of these laboratories, or to the new WHO Immunological Research and Training Centre in Geneva to become familiar with the standard ELISA protocol and engage in the comparative testing of the semi-synthetic antigens against the set of stock of sera.

A third major recommendation is the implementation of glycolipid detection, as distinct from anti-glycolipid antibody assays, in the context of the planned longitudinal studies. Glycolipid may be a more accurate and specific gauge of bacterial index and a truer yardstick of infection, and apparently provides a good reflection of the success of chemotherapy. In the absence of even simpler tests, the DOT-ELISA may present the requisite sensitivity and simplicity and when combined with limiting dilution of the trapping antibody may provide some degree of quantitation. It is recommended that in the major Regional Central Laboratories, high pressure liquid chromatography with a fluorescent detector and possibly automated be available to quantitate glycolipid. Fluorescence may provide the ultimate in the sensitive quantitation of glycolipid in serum, urine and tissue. In situ methods for glycolipid detection in tissue, such as the use of fluorescence-conjugated anti-glycolipid monoclonal antibody, should be developed as a sensitive and specific qualitative diagnostic tool and a means of gauging the status of glycolipid-containing immunocomplexes in leprosy states.

A fourth major recommendation concerns the need for a simple field test for initial screening for leprosy, combined with the existence of a major central reference laboratory (ies) with the capability of providing more sophisticated confirmatory data. The ideal situation is the use of one highly sensitive test in the field. What this should be is not yet clear. Perhaps the outcome of the extensive comparative evaluation now under way, of some 400 serum specimens involving the FLA-ABS test and glycolipid-based ELISA will throw some light on this issue. Candidate simple field assays are passive hemagglutination, or latex agglutination, or "dip-stick" ELISA or DIG-ELISA or "card" tests all based on the inherent specificity within the newly synthesized glycoconjugates. Such simple assays have yet to be fully developed. It is a recommendation that this type of developmental research be encouraged. The concept of a central

Annex 4

regional laboratory met with considerable enthusiasm. The basic necessities within such a central leprosy diagnostic laboratory are: deep-freeze storage facilities (-40° to -85°C) for large number of sera, stable vacuum and electrical sources, automatic diluting devices, micropipetting devices, automatic plate washer, ELISA reader, air conditioning, constant temperature (37°C) water baths, microtiter plate shaker, etc.

A further recommendation concerns the ongoing search for new M. leprae antigen. A categorical statement is being made that serodiagnosis based on glycolipid-related glycoconjugates is not the full answer to leprosy serodiagnosis; glycolipid-based antigens have limited applicability in paucibacillary leprosy. Pursuit of other antigens, peptide based or carbohydrate based, should be encouraged. It is recommended that "skin test" antigens be explored in order to derive greater specificity from them, since they should provide the most sensible approach to gauging specific delayed type hypersensitivity. On a practical note, the need to remove residual glycolipid, due to its potential humoral immunogenicity and CMI immunosuppressive activity, from skin test preparation is important. The greater availability of M. leprae as a source of other putative specific antigen is regarded as a major priority. Finally, the issue was addressed to more thorough immunological exploration of the so-called "common" antigens of the leprosy bacillus. In theory, at least such antigens could be endowed with complete cross reactivity in terms of humoral response but could contain within them the wherewithal of specific T-cell responses, and could thereby hold the secret of the selective immunological energy and consequently be of potential use in diagnosis.

A final recommendation concerns the continuation of this Working Group interaction. It has proved very beneficial, useful and promising. Our goal is that hand-in-hand with the overall authority of the WHO/IMMLEP body, leprosy will be eradicated from the Region, if not in our time, at least in the time of our children.

PROTOTYPE FOR STANDARD ELISA

A. ELISA Procedure for ND-BSA Coated Microtiter Plates

1. Wash wells four times (2x by suction, 2x by dump) with PBST.
2. Add 100 ul of blocking solution, PBST containing 1% (w/v) BSA.
3. Incubate at 37°C for one hour.
4. Empty blocking solution by suction.
5. Add 50 ul of serum diluted (1:300) in PBST containing 10% (v/v) normal goat serum (NGS).
6. Incubate serum at 37°C for one hour
7. Wash wells five times (3x by suction, 2x by dump) with PBST.
8. Add 50 ul of peroxidase-conjugated anti-human IgM conjugate diluted (1:1000 to 1:6000)* in PBST-10% NGS.
9. Incubate conjugate at 37°C for 45 min. - one hour.*
10. Wash wells five times with pbst.

Annex 4

11. Add 50 ul of substrate solution.
12. Incubate substrate at room temperature for 12-30 min.* in dark. Read using 414nm filter and stop at OD of 0.42 for positive control. (414nm reading of OD of 0.42 approximates 492nm reading of OD of 1.1)
13. Add 50 ul of 2.5N H_2SO_4 to stop reaction.
14. Read absorbance at 488-492nm.

Note:

- All the volumes used in this assay are for the round bottom microtiter plate; make double the volume for the flat bottom plate.
- PBST-10% NGS can be replaced with PBST-1% BSA.

* Conditions must be adjusted with standard positive sera.

B. Composition of Materials Used in D-BSA ELISA

Phosphate-buffered solution (PBS), pH 7.4

Na_2HPO_4 12.8 g, NaH_2PO_4 2.62 g, NaCL 0.58 g, D.W. 1000 ml (or phosphate-buffered Saline)

PBST: PBS containing 0.1% (v/v) Tween 80 or Tween 20.

Citrate-phosphate buffer, pH 5.0

Citric acid 4.67 g, Na_2HPO_4 7.3 g, D.W. 1000 ml

Substrate solution:

Citrate-phosphate buffer, pH 5.0	50 ml
O-phenylenediamine	20 ml
30% H_2O_2	

Sulfuric acid, 2.5 N

Sulfuric acid (H_2SO_4) 70 ml, D.W. 930 ml

Conjugate: Peroxidase-conjugated goat anti-human IgM

BSA: Bovine serum albumin

NGS: normal goat serum

ANNEX 5

DELIBERATIONS OF SUB-GROUP II

Dr Li Huang Ying reviewed the preceding presentation in relevance to the objectives and outcome of this sub-committee meeting.

She reminded members of the demonstrated specificity and sensitivity of the techniques and the limitation that it is poorly sensitive in paucibacillary leprosy. The outcome of the meeting were recommendations as to:

1. Need of which type of serodiagnostic tests;
2. How to apply the technology in field situations keeping the logistical realities of the field in mind.

It was observed that PGL ELISA is highly specific and sensitive for multibacillary leprosy though its sensitivity for paucibacillary leprosy is weak. The sensitivity and specificity of subclinical leprosy (all types) needs to be explored further.

It was recommended to collect as much sample material, under excellent and comparable documentation as possible for future analysis. It was strongly indicated that the PGL ELISA and PGL antigen determination can become an extremely useful tool in the monitoring of treatment efficacy of MDT in multibacillary patients. Such a tool is a valuable addition to the skin smear examination as a treatment monitoring tool.

PGL ELISA or any other valid serodiagnostic test can become a useful monitoring tool of the epidemiological transmission potential in the population.

It was suggested that once the technology has proved its validity as an indication for M. leprae infection, it could be used to measure the impact of MDT on a population through sequential cross-sectional examination of the risk age group. This would become a measure of trend of leprosy as it applies to the subclinical reservoir in the population. Such outcome can give important correlations with the incidence trend. The declining incidence trend in some countries of the Region can also be related with this technology.

In order to realize the above-mentioned possibilities of application, the technology needs to be tested in pilot areas to determine the sensitivity, specificity and resulting predictive value as a function of the prevalence.

In such test, a good demographic data base, good case ascertainment and a stable cooperative population are an essential.

Annex 5

If references about the epidemiological impact of MDT are expected, a high treatment coverage is needed.

The Republic of Korea has some leprosy settlement villages with different epidemiological condition that could present interesting results in this respect. A comparative study of different intervention strategies seems feasible.

Saba (Malaysia) with a small stable population is currently being investigated longitudinally for other diseases and this collection could become useful to leprosy as well.

Philippines (Cebu), China and Micronesia are other areas of feasible implementation. The technology can address important epidemiological issues and can enhance the understanding of the natural history of the disease. In addition, it can be useful as an epidemiological tool in the MDT evaluation and a clinical tool as a treatment monitor and relapse predictor in multibacillary leprosy, especially if the AG detection methods can be of great value in the latter when the sensitivity is adequate.

In order to determine the strategy of the application, it can be said that:

1. It can answer scientific epidemiological questions; and
2. It can be applied to the leprosy control strategy in the future.

It was felt that the technology as a diagnostic test would be of less importance since other methods, clinical, bacteriological and histological, are available to detect clinical diseases far more easily.

The AG detection can, however, be of great importance in clinical management.

As an epidemiological tool, the technology may stimulate us to design strategies on how to deal with an identified subclinical (and potentially infectious) reservoir in the population.

In good epidemiological hands, the technology may identify high risk groups in the population. Operational constraints may, however, restrain us from addressing this issue in terms of control work.

Annex 5

CONCLUSION

This technology is a good epidemiological research tool that needs to be tested through adequately designed longitudinal studies.

These should be carried out as multi-country studies using standardized protocols and reagents. Such collaborative effort would call for a central reference laboratory. This can become a valuable training centre, and it may, in addition develop an appropriate technology for the field and supply the needed reagents as a field supportive service. The implementation of the findings may, however, be too premature to include as a tool in the strategic management of leprosy control.