REPORT

WORKING GROUP ON RAPID DIAGNOSTIC TEST FOR LEPROSY

Manila, Philippines
29-31 March 1989
WORKING GROUP ON RAPID DIAGNOSTIC TEST FOR LEPROSY

Convened by the
REGIONAL OFFICE FOR THE WESTERN PACIFIC
OF THE
WORLD HEALTH ORGANIZATION

Manila, Philippines
29-31 March 1989
The views expressed in this report are those of the members of the Working Group on Rapid Diagnostic Test for Leprosy 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 Rapid Diagnostic Test for Leprosy, which was held in Manila, Philippines, from 29-31 March 1989.
CONTENTS

1. INTRODUCTION ....................................................... 1

2. SUMMARY OF COMMENTS ON DISCUSSION ............................. 1
   2.1 General review ...................................................... 2
   2.2 Discussion of participants' report in which the particle agglutination test was evaluated .............. 2
   2.3 Development in antigen (PGL-I) detection and other reports .................................................. 3
   2.4 Discussions on the usefulness of the gelatin particle agglutination assay in a leprosy control programme ................................................................. 4

3. RECOMMENDATIONS ...................................................... 5

ANNEXES:

ANNEX 1 - OPENING REMARKS OF THE REGIONAL DIRECTOR, WHO WESTERN PACIFIC REGION ..................... 7

ANNEX 2 - LIST OF MEMBERS, OBSERVER AND SECRETARIAT ........... 9

ANNEX 3 - PROGRAMME ..................................................... 13

ANNEX 4 - METHOD FOR GELATIN PARTICLE AGGLUTINATION TEST .... 15

APPENDIX 1 - MICROΤITER PARTICLE AGGLUTINATION TEST KIT FOR DETECTION OF ANTI-PGL ANTIBODY ............................................. 17
1. INTRODUCTION

The fourth meeting of the working group on the development of rapid diagnostic methods for Mycobacterium leprae was convened in the WHO Regional Office in Manila on 29-31 March 1989 to review progress in these areas since its third meeting in July 1987, especially in the development and assessment of facile and sensitive agglutination assay.

In particular, the group discussed the preliminary results arising from the application of a gelatin particle agglutination test developed in Japan, and the ongoing use of conventional ELISA employing a variety of neoglycoprotein antigens. In addition, assays directed towards the detection of antigen were discussed and further coordinated research in these topics was recommended.

The objectives of the working group were:

(1) to review progress and identify the problems and constraints in the implementation of ELISA assay in serodiagnosis of leprosy;

(2) to review the regional protocol and results of gelatin particle agglutination assay;

(3) to review the status of the development of antigen assay to monitor multidrug therapy and as an early diagnostic test for M. leprae infection; and

(4) to make recommendations on the role of these diagnostic methods available, and their importance and relevance as a tool for epidemiological study and monitoring of multidrug therapy in leprosy control programmes.

The meeting was opened by Dr Liu Guo-bin on behalf of Dr S.T. Han, Regional Director. Dr Liu traced the history of the three previous meetings indicating that previously the immunological parameters of the various assays had been primarily examined and none of them were being implemented in leprosy control. He invited the Group to examine the comparative results on the gelatin particle agglutination assay with a view to its implementation in the leprosy control programme and perhaps its application towards eliminating disease from at least some selected areas in the Western Pacific region. Dr Y. Yuasa and Dr P. Brennan were selected as Chairman and Vice-Chairman, respectively, and Dr J.T. Douglas and Dr Chan as Rapporteurs.
2. SUMMARY OF COMMENTS ON DISCUSSION

The agenda was as follows: First, Dr Brennan summarized past achievements; second, the presentations addressing individual experiences with the gelatin particle agglutination test were considered, followed by a description of new results on antigen detection in leprosy; at this point, Dr Noordeen discussed the epidemiological perspectives of serodiagnostic tests; and finally, Dr Yuasa initiated an open discussion on the usefulness of the gelatin particle agglutination assay in leprosy control.

2.1 General review

Dr Brennan commended the WHO personnel for their foresight in realizing the worth of PGL-I and associated assays in leprosy control programmes, from the time of its early inception. He reviewed the major accomplishments from the earlier meetings in Tokyo (1985), Manila (1986) and Manila (1987). These meetings examined: the various strategies for synthesis of neoantigens, their comparative testing, the matter of whether an IgG response versus IgM was important, and finally, the introduction by the Japanese investigators of the gelatin particle agglutination assay. In addition, Dr Brennan emphasized some fundamental points, such as the fact that the other antigens of M. leprae (both protein and carbohydrate based) should be pursued as serodiagnostic tools to develop greater sensitivity especially for the paucibacillary leprosy.

2.2 Discussion of participants' report in which the particle agglutination test was evaluated

Dr Wu Q. X., Dr E.C. Deulako, Dr S. Chanteau, Dr S.N. Cho, Dr K.D. Sukumaran, Dr G. Chan, Dr R. Cellona, Dr P. Brennan, Dr J.T. Douglas and Dr L. K. Due each reported on their experiences with the gelatin particle agglutination assay (GPAT). Some investigators had conducted extensive comparison with the existing conventional ELISA (regarded as the "gold standard" assay) with semi-synthetic neoglycoprotein antigens, whereas others had used GPAT in its own right against sera from patients with well documented clinical symptoms. When GPAT and ELISA were compared, the agreement rate for determining positive and negative sera was generally found to be 86%. The correlation between ELISA and GPAT titre as analysed by regression analysis of paired values resulted in \( r = 0.68, p < 0.001 \). GPAT was compared to ELISA for sensitivity and specificity. The sensitivity was found to be from 0% to 5% lower than the ELISA, while the specificity was 10% lower than the ELISA at 89%. Some ambiguity was experienced in reading marginal results and some people adopted a system of + 1, + 2, + 3 and flexibility in adopting a cut-off titre was recommended. The use of a modified diluent and higher incubation temperatures which will be part of a new standard protocol (see Annex 4) are expected to correct some of the ambiguities noted above.

However, as a simple assay, the GPAT system is more amenable than ELISA in terms of field applications.
2.3 Development in antigen (PGL-I) detection and other reports

Dr Cho described his antigen detection method and indicated that when applied to serum it demonstrated a rapid clearance of PGL-I levels 60-90 days after the start of chemotherapy. This was in contrast to findings when biopsy tissue blocks were used where PGL-I antigen persisted for several years. Dr Cho suggested that PGL-I antigen detection in serum is useful in monitoring clinical trials of new antimicrobials for treatment of leprosy. An advantage of the test is that drug trials can be accomplished in a short time (90-120 days), since successfully treated individual become antigen negative in 60-90 days. However, the relative insensitivity of the test (requiring 25 ng/ml of serum) requires that multibacillary patients with a BI index of 3 or greater must be used in these trials. Nevertheless, the sensitivity rate represents a fourfold increase over figures reported at the third Working Group.

Dr Chanteau reported on results of PGL-I antigen detection obtained in French Polynesia. Circulating PGL-I antigen could be detected in 100% of multibacillary patients at a level ranging from 50 to 8000 ng/ml, and in 20% of the paucibacillary patients at a low level from 12 to 50 ng/ml. Multibacillary patients put on MDT showed a PGL-I decrease of 89 ± 11% within one month. In a short-term trial comparing the effectiveness of Pefloxacain and Ofloxacain for the treatment of multibacillary patients, a significant correlation was found between PGL-I decrease and the morphological index decrease of bacilli recovered from serial biopsies. Dr Chanteau intends to compare PGL-I decrease and killing rate of M. leprae in order to further evaluate the usefulness of this test to assess the effectiveness of chemotherapy. PGL-I was detected at a low level among anti-PGL-I positive contacts who had no evidence of clinical disease.

Dr Chanteau regarded the existing PGL-I detection assays as a good research tool, but added that they were not yet applicable as a field test. She felt that there was a need to improve the methodology in terms of ease and sensitivity of antigen detection in order to render this test accessible to a broader range of studies.

Dr Izumi described a simple specific immunohistopathological technique for the detection of PGL-I in biopsy specimens involving the use of anti-PGL-I murine monoclonal antibodies and readily available commercial reagents.

In addition to the topics discussed above, Dr Izumi presented information on use of prevalence, specificity and sensitivity in evaluating appropriate cut-off points. He suggested the use of receiver operating characteristic curve ("ROC test") as a means of evaluating diagnostic tests.

Dr Douglas presented data on early detection of leprosy from his work with Leonard Wood Research Centre in Cebu. He indicated that only one out of five multibacillary index cases had ELISA positive contacts. Of these serologically positive contacts, 9/53 had developed a disease. One per cent of the ELISA negative (4/397) had developed disease. It was interesting that the ELISA negative individuals developed
paucibacillary disease, whereas most of the ELISA positive contacts developed multibacillary disease. This represented approximately a two-fold increase in previously reported data. The interesting aspect of the study is that there was an accumulation of new cases emerging from the ELISA positive cohort. In other words, the longer the ELISA positive cohort was monitored, the more new cases continued to emerge. The distribution of types of new cases that were developed from the ELISA positive group mimicked the ELISA positive distribution found in existing cases: greater numbers of MB in relation to PB.

Dr Douglas also gave a short report indicating that a high rate of relapse was found in PB cases who were ELISA positive at the start of treatment: 26% (6/23) relapsed compared to 1.5% (1/67) in the ELISA negative PB patients. These compliant patients had been monitored for 18 to 24 months post-PB treatment and had developed increased BI at the time of relapse.

2.4 Discussions on the usefulness of the gelatin particle agglutination assay in a leprosy control programme

Dr Noordeen discussed progress in the development of serodiagnostic tests for leprosy and its indications at a global level. Dr Noordeen also outlined the requirements of an ideal immunological test, stressing the critical need for specificity and sensitivity in view of the low incidence of leprosy in most population. He demonstrated the effects of selective serological examination of the contacts within a population group and how this will deal with less than 10% of the disease problem. He challenged the Working Group to examine GPAT or any serodiagnostic test in terms of programme needs, such as cost effectiveness for identification of high-risk groups where intervention may be required; detection of early leprosy when clinical signs are equivocal; detection of early relapse; and monitoring of patients for progress under chemotherapy.

Dr Yuasa and Dr Brennan further exhorted the Working Group to begin to consider how the GPAT test could address these practical problems and how the technology could be used as a further tool towards the elimination of leprosy in some parts of the Western Pacific region. Dr Cellona, in response, considered the assay excellent for randomized surveys of prevalence in a given area and where MDT had been broadly implemented. The assay may be used to monitor compliance and as a screen for new cases. Dr Douglas emphasized the role of the test as a "flagging" tool to draw attention to high-risk individuals. Dr Chan regarded the test as being highly applicable in a clinical setting, such as the situation in Manila where about 5000 patients are being considered for removal from the patient registry. These patients could be screened by GPAT to provide a gauge of the possibility of relapse.

In summary, throughout the discussions, the possibility of applying the test as an indicator of relapse in patients after the completion of therapy was stressed. In addition, it was also suggested that this test would be highly suitable in certain communities (e.g., island populations) where MDT is winding down as a possible gauge for the measurement of extent of disease control. Where resources are
available, it was felt that the test would also be useful in monitoring contacts. The participants were particularly pleased with the applicability and simplicity of the gelatin particle agglutination test.

3. RECOMMENDATIONS

The Working Group endorsed the previous recommendations arising from the meeting held in Tokyo in 1985 and Manila in 1986 and 1987, and, further noting the usefulness of GPAT, as well as recognizing ELISA as a possible back-up, recommended the following:

(1) In leprosy control programmes where coverage of existing patients by MDT is completed, GPAT could be applied in mass survey to monitor the effectiveness of the control measures. This application should be particularly useful in certain areas, such as on islands with stable populations.

(2) GPAT is recommended as a simple additional tool for field use in monitoring the occurrence of relapse after the completion of therapeutic regimens on multibacillary patients.

(3) Where resources exist, GPAT is recommended for the purpose of monitoring contacts of patients for the early detection of disease.

(4) It is recommended that appropriate expert groups within the WHO framework should study the feasibility of the prophylactic regimens for the high-risk persons identified by the serological tests.

(5) It is recommended that due attention should be given to the continued research efforts for the development of additional serological tools, such as detection of PCL and antibodies against other M. leprae antigens for possible future application in the leprosy control programme.
OPENING REMARKS OF THE REGIONAL DIRECTOR
OF THE WORLD HEALTH ORGANIZATION
REGIONAL OFFICE FOR THE WESTERN PACIFIC

Distinguished Members, Ladies and Gentlemen:

On behalf of Dr S.T. Han, our Regional Director, I have pleasure in welcoming you to this meeting of the Working Group on Rapid Diagnostic Test for Leprosy.

You may recall that the first meeting on this topic was held in Tokyo in 1985, the Working Group met again both in 1986 and in 1987. Since then Members of the group have conducted many trials to develop serodiagnostic tests which are sensitive, specific and practical.

There are at present four more widely recognized serological tests for the detection of leprosy infection: Flourescent Antibody Serology (FLA-ABS), Gelatin Particle Agglutination Test, Enzyme Linked Immunosorbent Assay, and antigen detection tests. However, for technical and operational reasons, none of them have been used in a routine leprosy control programme. In the past working group meetings, the emphasis was on the defining of the immunological characteristics and experimental procedures of the various antigens and tests. At this meeting, may I request you to focus your discussion on the applicability of these tests to the leprosy control programmes. With your profound knowledge and experience, I am sure you'll be able to identify and recommend the most suitable ones.

Your recommendations will help us in formulating our plans to eliminate leprosy in a few selected countries in the South Pacific.

We will work closely with the Member States to implement your recommendations for their leprosy control programmes.

Allow me in conclusion to give you my best wishes for the successful outcome of this meeting. I will look forward to your recommendations with great interest.

Thank you for your attendance. I wish you an enjoyable stay in Manila.
LIST OF MEMBERS, OBSERVER AND SECRETARIAT

1. MEMBERS

CHINA
Dr Wu Qin Xue
Nanjing Institute of Dermatology
Nanjing

FIJI
Dr E.C. Daulako
Medical Superintendent
P.J. Twomey Memorial Hospital
Tamavua Heights
Suva

FRENCH POLYNESIA
Dr S. Chanteau
Chief
Department of Laboratory and Immunology
Institut Territorial de Recherches
Medicales Louis Malarde
B.P. 30 Papeete
Tahiti

JAPAN
Dr Masahide Abe
c/o Sasakawa Memorial Health Foundation
The Sasakawa Hall
3-12-12, Mita, Minato-ku
Tokyo 108

Dr Tsuyoshi Fujiwara
Associate Professor
Institute for Natural Science
Nara University
Misasagi-cho 1500
Nara 631

Mr Mikio Ikeda
General Manager
Immunoserology Department
Fujirebio, Inc.
51 Komiya-cho, Hachioji
Tokyo 192
Annex 2

Dr Shinzo Izumi  
National Institute for Leprosy Research  
4-2-1 Aoba-cho, Higashimurayama-shi  
Tokyo 189

Dr Yo Yuasa  
Executive and Medical Director  
Sasakawa Memorial Health Foundation  
The Sasakawa Hall  
3-12-12, Mita, Minato-ku  
Tokyo 108

REPUBLIC OF KOREA  
Dr Sang Nae Cho  
Assistant Professor  
Department of Microbiology  
Yonsei University  
College of Medicine  
C.P.O. Box 8044  
Seoul

MALAYSIA  
Dr K.D. Sukumaran  
Institute for Medical Research  
Serology and Immunology Division  
Jalan Pahang  
Kuala Lumpur

PHILIPPINES  
Dr Roland Cellona  
Chief  
Epidemiology Branch  
Leonard Wood Memorial Centre for Leprosy Research  
Cebu City

Dr Gertrude Chan  
Research Institute for Tropical Medicine  
Alabang, Muntinlupa  
Metro Manila
2. OBSERVER

Ms Menilou Parrilla
Leonard Wood Memorial Centre
for Leprosy Research
Cebu City
Philippines

3. SECRETARIAT

Dr S.K. Noordeen
Chief, Medical Officer, Leprosy
Division of Communicable Diseases
World Health Organization
1211 Geneva 27
Switzerland
Annex 2

WHO REGIONAL OFFICE FOR THE WESTERN PACIFIC

Dr T. Umenai
Director, Disease Prevention and Control
WHO Regional Office for the Western Pacific
P.O. Box 2932
Manila
Philippines

Dr A.A. Galvez
Regional Adviser in Chronic Diseases
WHO Regional Office for the Western Pacific
P.O. Box 2932
Manila
Philippines

Dr Jong-Wook Lee (Operational Officer)
Medical Officer
Regional Leprosy Control
WHO Regional Office for the Western Pacific
P.O. Box 2932
Manila
Philippines
PROGRAMME

29 March 1989
(Wednesday)

8:00 - 9:00 - Registration
9:00 - 9:30 - Opening Ceremony
9:30 - 9:45 - Coffee Break
9:45 - 12:00 - Presentation of results of gelatin particle agglutination test (GPAT)
1. China
   - Dr Li Futian
   - Dr Wu Qin Xue
2. Fiji
   - Dr E.C. Daulako
3. French Polynesia
   - Dr S. Chanteau
12:00 - 1:30 - Noon break
1:30 - 2:30 - Presentation of result of GPAT (continuation)
4. Republic of Korea
   - Dr Sang Nae Cho
5. Malaysia
   - Dr K.D. Sukumaran
6. Philippines
   - Dr Gertrude Chan
   - Dr R. Cellona
2:30 - 2:45 - Coffee Break
2:45 - 3:45 - Presentation of result of GPAT (continuation)
7. United States of America
   - Dr Patrick Brennan
   - Dr James T. Douglas
8. Viet Nam
   - Dr Le Kinh Due

30 March 1989
(Thursday)

8:00 - 9:30 - Discussion on the usefulness of GPAT in a leprosy control programme
9:30 - 9:45 - Coffee break
9:45 - 12:00 - Discussion on the usefulness of GPAT in a leprosy control programme (continuation)
12:00 - 1:30 - Noon break
1:30 - 2:30 - Discussion on the usefulness of GPAT in a leprosy control programme (continuation)
2:30 - 2:45 - Coffee break
2:45 - 3:30 - (1) Discussion on the usefulness of ELISA as epidemiological and monitoring tool in a leprosy control programme.
(2) Status of development of antigen detection test
Annex 3

31 March 1989  
(Friday)

8:30 - 9:30 - Discussion of the previous recommendations on the following:
(1) The serodiagnostic method applicable to field condition
(2) Identification of institute/country where the field test will be applicable in national leprosy control programme
(3) Formulation/revision of protocol for field situation

9:30 - 9:45 - Coffee break
9:45 - 12:00 - Finalization of recommendations
12:00 - 2:00 - Noon break
2:00 - 2:30 - Presentation of recommendations
2:30 - 3:00 - Closing ceremony
METHOD FOR GELATIN PARTICLE AGGLUTINATION TEST
(Please see instructions for MLPA* Kit, Appendix 1)

Recommendations for adjustments to the GPAT

For standardization of the assay it is suggested that the test be incubated at 37°C for one hour. In areas where incubators are not available, the test may be performed at room temperature for two hours. It is also recommended that incubation be carried out in an environment free of excessive vibration.

---

*Mycobacterium Leprae Particle Agglutination. It was developed by Fujirebio, Inc., Tokyo, Japan.
MLPA

Microtiter Particle Agglutination Test Kit for Detection of Anti-PGL Antibody.
### 1. KIT COMPONENTS

MLPA consists of the following reagents and supplies.

#### Reagents:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reconstituting Solution</td>
<td>23ml × 1 vial</td>
</tr>
<tr>
<td>Serum Diluent</td>
<td>34ml × 1 vial</td>
</tr>
<tr>
<td>Sensitized Particles (Lyophilized)</td>
<td>3.0ml × 5 vials</td>
</tr>
<tr>
<td>Control Particles (Lyophilized)</td>
<td>3.0ml × 2 vials</td>
</tr>
<tr>
<td>Positive Control (Lyophilized)</td>
<td>0.5ml × 1 vial</td>
</tr>
</tbody>
</table>

* After reconstitution

#### Supplies:

1. Dropper .......................................................................................... 25μl × 2 pcs.
   
   Use exclusively for dispensing reconstituted Sensitized Particles and Control particles.
Please Prepare

1. U-shaped rigid microplates*
2. U-shaped rigid microplate cover**
3. Micropipette with tips, 25μl and 50μl
4. Multipipette with tips, 25μl
5. Volmetrip pipettes, 1.0ml, 5.0ml, and 10.0ml
6. Tray mixer, or automatic vibrator**
7. Microplate viewer**
8. Droppers (supplied in a kit)

* Microplates are sold separately by Fujirebio Inc.
** Preferred but not required equipment

2. METHOD FOR MICROPLATE WASHING

I. New Microplate
   When washing is needed, it should be done as follows:
   1. Rinse with warm water thoroughly, then rinse with running water, and if possible, again with purified water.
   2. Dry in an incubator, not exceeding 37°C, or in open air.

II. Used Microplate
   1. Immerse a used plate in a sodium hypochlorite solution to disinfect (overnight for a 1% solution, or 30 to 60 minutes with a 2.5% solution).
   2. Clean off the plate thoroughly in running water, and follow the instructions for cleaning a new plate.

Notice:
Insufficient washing may produce distorted pattern results.
3. TEST PROCEDURE

Preparation of Reagents

Reconstitute sensitized and control particles with 3.0 ml of reconstituting solution and positive control with 0.5 ml respectively. Let it stand at room temperature for 30 min. prior to testing.

Qualitative Test (Screening)

1. Dropping of Serum Diluent

Drop 75 µl of serum diluent into well no. 1 and 25 µl each into wells no. 2 and no. 3 with a multipipette.
2. **Adding of Test Serum**

Add 25μl of the serum into well no.1 with a micro pipette. Mix content fluid by filling and discharging the micropipette repeatedly for 3 or 4 times in well no.1.

Before sampling a serum specimen, a serum collection tube should be well mixed by a Vortex Mixer or shaking to get a homogeneous specimen. When precipitate is found in serum, centrifuge it at 2,000 rpm for 5 min. or filtrate.

![Graph](well no.) 1 2 3

3. **Serum Dilution**

Dispense 25μl of diluted solution in well no.1 into no.2 with a multipipette. Mix content of well no.2 in the same way in as step 2, and then transfer 25μl of diluted mixture into well no.3. Repeat this step again in well no.3 except discarding 25μl of diluted mixture.

![Graph](well no.) 1 2 3

(serum dilution rate) 1:4 1:8 1:16
4. Dropping of Particles

Add one drop (25μl) of control particles in well no. 2, and one drop (25μl) of sensitized particles in well no. 3 using the droppers supplied in the kit.

5. Mixing

Mix the content of each well thoroughly using a tray mixer (approx. 15 sec.) twice. In case an automatic vibratory shaker is not available, tap the edge of the microplate several times by hand.
6. Incubation

Cover the plate and allow to stand for 2 hrs. at room temperature.
In order to avoid any distorted pattern formations, do not move the plate after it is set on the bench.
Appendix 1

7. Interpretation

Rate as −, ±, or + according to the criteria shown in the photograph.

Interpretation Criteria

<table>
<thead>
<tr>
<th>Negative</th>
<th>Inconclusive</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>(−)</td>
<td>(±)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

![Interpretation Criteria Diagram]
Quantitative Test

Quantitative test is performed in the case that the antibody titer is necessary.

Test procedure is same as that of qualitative test except that the test is carried out in the wells from no.3 to no.12.

Absorption procedure

If a test shows agglutination with both control and sensitized particles, it should be retested after the following absorption procedure.

1. Place 0.35 ml of reconstituted control particles in a small test tube.
2. Add 0.05 ml of test serum, mix thoroughly and incubate at room temperature for 20 min. (mix well by shaking 1 or 2 times).
3. Centrifuge for 5 min. at 2,000 rpm.
4. Place 50μl of absorbed serum (serum dilution 1:8) in well no. 3, then repeat the test.
Appendix 1

Agglutination Pattern

※1 Control particles added
※2 Sensitized particles added
※3 Interpretation
※4 Final dilution
Appendix 1

*1 Control particles added
*2 Sensitized particles added
*3 Antibody titer
*4 Final dilution