WORLD HEALTH ORGANIZATION
REGIONAL OFFICE FOR THE WESTERN PACIFIC

REPORT

REGIONAL WORKSHOP ON COURSE ON LABORATORY DIAGNOSIS OF POLIOMYELITIS AND POTENCY TESTING FOR VIRUS VACCINES

Beijing, People's Republic of China
11-27 June 1990

Manila, Philippines
August 1990

(WP)LAB/ICP/EPI/001-E ENGLISH ONLY

REPORT

REGIONAL TRAINING COURSE ON LABORATORY DIAGNOSIS OF POLIOMYELITIS AND POTENCY TESTING FOR VIRUS VACCINES

Convened by the

REGIONAL OFFICE FOR THE WESTERN PACIFIC
OF THE
WORLD HEALTH ORGANIZATION

Beijing, People's Republic of China, 11-27 June 1990

Not for sale

Printed and distributed
by the

Regional Office for the Western Pacific of the
World Health Organization
Manila, Philippines
August 1990

NOTE

The views expressed in this report are those of the participants in the Regional Training Course on Laboratory Diagnosis of Poliomyelitis and Potency Testing for Virus Vaccines 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 governments of Member States in the Region for the participants in the Regional Training Course on Laboratory Diagnosis of Poliomyelitis and Potency Testing for Virus Vaccines held in Beijing, People's Republic of China from 11 to 27 June 1990.

CONTENTS

1. INTRODUCTION

The Regional Training Course on Laboratory Diagnosis of Poliomyelitis and Potency Testing for Virus Vaccines, organized by the World Health Organization Regional Office for the Western Pacific was conducted from 11 to 27 June 1990 in Beijing, China.

The specific objectives of the training course were:

(1) to review the current status of the laboratory diagnosis of poliomyelitis and potency testing of virus vaccines (poliomyelitis and measles) in the Region;

(2) to review the WHO manuals on the isolation and identification of poliovirus and on vaccine potency testing;

(3) to review specimen collection during epidemiological surveillance (from suspected poliomyelitis cases and apparently healthy contacts);

(4) to introduce standard methods and practice in laboratory techniques for isolation and identification of poliovirus (types 1-3) wild and vaccine strains; and

(5) to standardize and practise the technique for potency testing of virus vaccines (poliomyelitis and measles).

There were 14 participants from China, two each from the Philippines, Korea and Viet Nam, and one from New Zealand, making a total of 21 participants from five countries.

The course was directed by a team from the National Institute of Public Health and Environmental Protection, Bilthoven, the Netherlands, including Dr Henk Bruning, Dr Anton van Loon, Dr Fred van Nimwegen, and Dr Paul Oostvogel.

Dr Akio Hagiwara of National Institute of Health, Tokyo, Japan and Mrs Margery Kennett, Fairfield Hospital, Victoria, Australia were temporary advisers.

Dr Sima Huilan, Regional Adviser, Health Laboratory Services, WHO/WPRO Manila was the operational officer.

The WHO secretariat in Geneva was represented by Dr Takatoshi Kobayakawa, Medical Officer, Expanded Programme on Immunization, and Dr Julie Milstien, Scientist, Biologicals Unit.

The course was opened by Dr Bernard P. Kean, WHO Representative in China, on behalf of Dr Sang Tae Han, Regional Director for the Western Pacific. Dr Kean expressed his gratitude to the Government of the People's Republic of China for agreeing to hold the training course at the Sino-Danish Biomedical Post Graduate Training Centre in Beijing.

The World Health Organization's Regional Committee for the Western Pacific has resolved to make every effort to eradicate poliomyelitis in the Region by 1995. Dr Kean described the present incidence of poliomyelitis in the Region and outlined the steps required to further reduce its incidence and prevalence.

This involves increasing immunization coverage using a potent vaccine and strengthening the surveillance of potential cases. Special efforts must be made to upgrade the laboratory techniques for isolating and identifying the poliovirus and for testing vaccine potency.

The training course was planned to enable participants to exchange information and ideas about establishing a regional laboratory network for poliovirus confirmation and vaccine potency testing and to practise in their own countries the techniques learned on the course.

Dr Wang Chao, Deputy Director, Department of Epidemiologic Prevention, Beijing, welcomed the participants.

Also present at the opening ceremony were Professor Zhang Yi Hao, former Director of the National Vaccine and Serum Institute, Beijing; Dr Yang Baoping, Chief, EPI Division, Department of Epidemiologic Prevention, Beijing, Mrs Xue Puying, Department of Foreign Affairs, Beijing; and Professor Guo Cunsan, Director, Sino-Danish Biomedical Postgraduate Training Centre, Beijing.

The Sino-Danish Biomedical Postgraduate Training Centre (PTC) was an excellent location for the course as the dormitory was in the grounds of the Centre, which meant that participants had easy access to the laboratory and classrooms.

The list of participants is given in Annex I.

2. PLANNING OF THE COURSE

In preparing the timetable for the training course, due attention was given to:

(a) the stated objectives of the course;

(b) the contents of similar courses on potency testing for viral vaccines in Cairo, Egypt in November/December 1989 and Coonoor, India in March/April 1990;

(c) the content of the global workshop on the laboratory diagnosis of poliomyelitis at Rijksinstituut voor Volksgezondheid en Milieuhygiene (RIVM) (National Institute of Public Health and Environmental Protection), Bilthoven, the Netherlands in November 1989;

(d) the availability of resources in laboratories in countries of the Region;

(e) the application of several nonstandard techniques used in the Region.

Each day's programme was designed to cover presentation of principles and practice. A generous portion of time was spent on bench work and group discussions, and several relevant field visits were made.

The agenda is given in Annex 2.

3. CONCLUSION AND EVALUATION OF THE COURSE

3.1 Selection and qualification of participants

The course included 21 participants: 14 participants from China, two participants each from Korea, Viet Nam, the Philippines, and one from New Zealand. The course was intended for those responsible for performing vaccine potency testing, serology, and poliomyelitis

diagnostic techniques in production, national quality control, and national diagnostic laboratories. The mix of participants was as follows: six from production laboratories; two from quality control laboratories; and 12 from EPI/diagnostic laboratories. One participant, from Viet Nam, works in a hospital microbiology laboratory, but was sent because her director felt it would be useful to convert this laboratory to a poliomyelitis diagnostic laboratory.

Of the 14 Chinese participants, three were from production laboratories, one was from the National Quality Control Laboratory and one was from the designated National Reference Laboratory for Poliomyelitis Diagnosis. The remaining nine were from nine different provinces; three of these were epidemiologists with good laboratory background, and the other six were full-time laboratory professionals.

In general, the selection of participants was excellent; most of them used good laboratory techniques and had a good understanding of the techniques being used. The participant from New Zealand were exceptionally good, functioned as a co-facilitator.

The two participants from Viet Nam were the weakest in understanding the theory and practice used. One had no virology experience at all.

The participants from the Philippines were both chosen from the in-process production control laboratory. It would have been useful if the proposed diagnostic laboratory Research Institute for Tropical Medicine (RITM) had been represented.

In addition, of those countries who may be considered for certification of national diagnostic laboratories in the near future, Malaysia was conspicuous by its absence from the course.

The course was conducted in English, with all training materials in English. This posed quite a problem for class participation and understanding of the materials covered. Some of the communication difficulties were obviated by the use of small working groups (three or four participants) with at least one person in each group fluent in both Chinese and English. The non-Chinese participants all had an acceptable working knowledge of English, except for the Vietnamese, who had some difficulty. At least half the Chinese participants also had problems working in English.

3.2 Selection and qualification of facilitators

The course facilitators included four scientists from the RIVM, Netherlands, who were charged with developing and carrying out the course curriculum, two scientists from proposed regional laboratories for poliomyelitis diagnosis in the Region, who had already participated in one global level training course; and two representatives from the WHO secretariat in Geneva.

The RIVM staff had developed an excellent course curriculum and the course was outstanding. A few problems arising in the diagnostic segment, which will be dealt with later, probably stem from two major factors: (1) this was the first course of this type, including curriculum topics in both control and diagnostic areas; and (2) in addition to the planned curriculum, the course covered evaluation of two alternative diagnostic methods suggested by scientists within the Region.

The temporary adviser from Australia was essential to coordinate the diagnostic portion of the course, as well as the part on laboratory safety.

3.3 Arrangements on site

The PTC is without doubt the best training site ever used for one of these courses by WHO. The fact that the participants and facilitators were in constant close contact and with ready access to the laboratory even on evenings and weekends contributed to an esprit de corps

and to an efficient, positive working environment. The physical layout of the PTC was perfect for this course, and the facilities for photocopying, videotaping, and secretarial work were excellent.

Arrangements for field trips as part of the course curriculum were excellent, and all support received by the PTC, from accommodations and transport to social activities was efficient and pleasant. The staff were always willing to do whatever was necessary to meet needs and requests. The participants would have preferred to work six-day weeks. This is important for a course involving cell culture, and it is hoped that future course agendas will take this into account.

3.4 Evaluation of course content

As this was the first course of this type to combine vaccine potency testing and serology with diagnostic methods, it was an experiment. The experiment was successful, and the course will serve as a model for future courses.

The diagnostic part of the course, which was intended to be interwoven throughout the two-and-a-half-week period, but to comprise only two days of actual experimental work, was extended to four days. This was because of the evaluation of two experimental techniques was added to the curriculum: the IgM method and the microplate assay. These will be described below. The result of this extension was that the facilitators felt the need to rush through parts of the curriculum, without the leisure to put the various components into context, and to discuss them fully with the participants. It is hoped that future courses will not meet these problems. In principle, however, the facilitators felt that the combined curriculum was useful and necessary. In fact, many of the participants are doing both types of work, and need to be exposed to standard methodologies from both curricula.

The one major problem in the practical work was that the preferred method for specimen processing for poliomyelitis diagnostic work, according to the WHO manual for poliomyelitis diagnostic laboratories (in press), did not work for the faecal specimens used in the course. Although this method is recommended in the manual, it has not yet been tested in a course of this type. The previous course, in the Netherlands, used chloroform extraction of faeces, a suggested alternative method. It had been intended to use the chloroform extraction procedure here, but it was discovered the night before the actual laboratory work was to be carried out that the only centrifuge tubes available were not resistant to chloroform. Thus the WHO method was substituted, without trial, in this laboratory. It did not work.

3.5 Evaluation of training materials

Three types of training materials were used in the course: the BLG/EPI restricted document, Laboratory Methods for the Titration of Live Virus Vaccines Using Cell Culture Techniques (BLG/EPI/89.1), which is being tested in the regions for comments prior to finalization; the EPI draft Manual for the Virological Investigation of Poliomyelitis; and handouts for the practical work (Annex 3) and lectures prepared by the course facilitators.

The document BLG/EPI/89.1 has been tested in two previous training courses in two other WHO regions, and, with the relevant handouts, which will be included in a subsequent revision, has been found useful and comprehensive.

The Manual for the Virological Investigation of Poliomyelitis was tested in a global training course held in November in 1989 at the RIVM, and comments received from participants were included in its revision. However, the specimen treatment method noted in the manual as the preferred method has not actually been subjected to field laboratory testing; it proved unsatisfactory under the conditions of the course. The facilitators thus recommend that the description of this method be modified, either by changing the centrifugal force needed for treatment of faecal specimens prior to inoculation, or by recommending inclusion of

chloroform extraction. The recommended method should be satisfactory for all samples given the equipment found in most laboratories.

A second comment on the manual was that for courses of this type, a modular format, similar to that used in BLG/EPI/89.1, would be more useful. For courses of this type, which cover a curriculum suitable for participants with mixed backgrounds (which seems to be more the rule than the exception), parts of it could then be extracted as required for the course curriculum. In its present format, it is not readily adaptable for teaching purposes.

3.6 Commentary on non-standard methods included in the course

Two non-standard methods were evaluated in the course at the suggestion of regional scientists. The first was the IgM technique for poliomyelitis diagnosis, developed by Professor Zhang Li-Bi, of the Institute of Virology, Chinese Academy of Preventive Medicine in Beijing (Annex 4). This is the laboratory designated by the Chinese authorities as the National Reference Laboratory for Poliomyelitis Diagnosis. Training in the use of this technique has been carried out by Professor Zhang throughout China, and many provincial laboratories are using it. Professor Zhang was present at the course for two days, and provided protocols, samples, and demonstrations on using the test. The entire procedure was recorded on video for future reference.

Three sera were tested by this technique, and each of the six laboratory groups performed the test. Correct results were obtained by four of the six groups, and misleading results from the other two. The source of the incorrect results was thought to be the addition of sera to the wrong wells in one case and improper washing in a second case. However, the controls necessary for pinpointing the source of problems had not been set up.

The course facilitators felt that the method shows promise, but that it should be routinely performed with more controls. In addition, crucial data on the sensitivity and specificity of the test have not yet been made available. Further questions to be answered include those relating to the interference from immunization and/or concurrent enterovirus infection, and the ability to use the test to distinguish between infection by wild or vaccine-like poliomyelitis viruses. Although the test does show promise, it is felt that it could not be used without some concurrent isolation, necessary for the characterization of circulating polioviruses. A detailed summary of comments by course facilitators is annexed (Annex 4).

The second technique, the microplate method, was suggested by Japanese researchers as a means of saving on reagent costs as well as time. The test was evaluated during the course. The general concensus was that the method needs further work before it can be recommended for routine use.

3.7 Planned follow-up activities

All participants will receive a panel of samples to test and the necessary reagents to test them, to ascertain whether they can satisfactorily identify poliovirus isolates and test for poliovaccine potency after participation in this course. In addition, a kit of standard reagents will be supplied to each of the participants on request to enable them to standardize their own laboratory techniques.

It is planned to contact each participant by letter at least once in the next 12-month period to determine what problems or successes they may be having in applying the course procedures, and to solicit their comments on the course and training materials. If any of the laboratories are selected to take part in the global network for laboratory support for the eradication of poliomyelitis, they will be contacted regularly after that period. WHO and the facilitators can provide technical support to the participants on request.

ANNEX 1

LIST OF PARTICIPANTS

1) CHINA

DR CANG YAO-QUING
Assistant Researcher
Shanghai Institute of Biological Products
Ministry of Public Health
1262 Yan-An Road (W), Shanghai
People's Republic of China
Post Code: 200052

2)

DR HU HAN-XIAN
EPI Section
Hubei Provincial Anti-Epidemic Station
Wu Chang, Wuhan, Hubei Province
People's Republic of China
Post Code: 430070

3)

DR JIANG YI-DE
Assistant Research Fellow
National Institute for the Control of Pharmaceutical
and Biological Products, Ministry of Public Health
Temple of Heaven, Beijing
People's Republic of China
Zip Code: 100050

4)

DR JIANG YONG-ZHEN
Research Associate, Institute of Virology
Chinese Academy of Preventive Medicine
100 Ying Xing Jie, Xuan Wu Qu
Beijing, People's Republic of China
Zip Code: 100052

5)

MS LI PING
In-charge of Laboratory, Diagnosis of Poliomyelitis
EPI Section, Health and Epidemic Prevention Station
Jandong Street, Xian, Shanxi Province
People's Republic of China
Post Code: 710054

6)

DR LIU CHUN-MEI
Health and Anti-Epidemic Station of Liao-Ning Province
3 Nanjin Street, Hepingqu, Shenyang
Liao-Ning Province
People's Republic of China
Post Office Box: 110005

7)

DR LIU QU
Inspector, Health and Anti-Epidemic Prevention Station
Huaisu Street 50, Chengdu, Sichuan Province
People's Republic of China
Post Code: 610031

Annex I

8) DR TAN SHUN-GE
Assistant Researcher, Institute of Medical Biology
Chinese Academy of Medical Sciences
Hua Hong, Dong Xi San Qu, Kunming
People's Republic of China
Post Code: 650107

9) DR TIAN XIAOEN
Chief of Section of Planning on Immunization
Henan Provincial Anti-Epidemic Station
Zhengzhou City, Henan Province
People's Republic of China
Post Code: 450003

10) MR WANG YAN
Assistant Engineer, National Vaccine and Serum Institute
Sanjianfang, Chaoyangqu, Beijing
People's Republic of China
Post Code: 100024

11) DR WU CHENG-MIN
Technician in charge, EPI Section
Health and Epidemic Prevention Station
176 Xingang West Road, Guangzhou
People's Republic of China
Post Code: 510300

12) DR XU AIQIANG
Doctor on Epidemiology
Shandong Provincial Hygiene and Anti-Epidemic Station
20 Jingshi Road, Jinan, Shandong Province
People's Republic of China
Post Code: 250014

13) DR ZHENG KUI-CHENG
Physician of Epidemiology
Fujian Provincial Center of Sanitary and Epidemic Prevention
Fuzhou, Fujian Province
People's Republic of China
Post Code: 350001

14) DR ZHOU YING-PING
Vice Director, EPI Laboratory Prevention Station
80 Taoyuan Road, Nanning, Guangxi Province
People's Republic of China
Post Code: 530013

NEW ZEALAND

15) MR DAVID FEATHERSTONE
Scientist, Virology Laboratory
New Zealand Communicable Disease Centre
Kenepuru Drive Porirua, Wellington, New Zealand
P.O. Box: 50-348 Porirua

Annex 1

PHILIPPINES

16) MRS ILUMINADA V. CRUZ
Bacteriologist III
Biologicals Production Service, Department of Health
DOH Compound, Alabang 1702, Muntinlupa
Metro Manila, Philippines

17) MISS MARILOU S. PADUGA
Bacteriologist I
Biologicals Production Service, Department of Health
DOH Compound, Alabang 1702, Muntinlupa
Metro Manila, Philippines

REPUBLIC OF KOREA

18) DR YOUN-HO CHUNG
Public Health Doctor
Division of Enterovirus, Department of Virology
National Institute of Health
5 Nokbun-dong, Eunpyung-ku, 122-020
Seoul, Korea

19) MR MOON-BOO KIM
Researcher, Division of Enterovirus
Department of Virology, National Institute of Health
5 Nokbun-dong, Eunpyung-ku, 122-020,
Seoul, Korea

SOCIALIST REPUBLIC OF VIET NAM

20) DR DANG THU DUNG
Microbiologist, Institute for Clinical Research of
Tropical Diseases, Bach Mai University Hospital
Hanoi, Viet Nam

21) MRS NGUYEN THI KIM TAM
Chief Laboratory for Quantity
Control of Poliomyelitis National Vaccine
National Institute of Hygiene and Epidemiology
National Centre for Control of Vaccine
1 Yersine Street, Hanoi, Viet Nam

TEMPORARY ADVISERS

22) DR H. BRUNING
National Institute of Public Health
and Environmental Protection (RIVM)
Antonie Van Leeuwenhoeklaan 9
P.O. Box 1, 3270 BA Bilthoven
The Netherlands

Annex 1

23) DR AKIO HAGIWARA
Department of Enteroviruses
National Institute of Health
Gakuen 4-7-1, Musashimurayama
Tokyo 190-12, Japan

24) DR MARGERY KENNETT
Virologist, Virology Department
Fairfield Hospital, Yara Bend Road
Fairfield 3078, Victoria, Australia
P.O. Box: 65

25) DR ANTON VAN LOON
Virology Laboratory
National Institute of Public Health
and Environmental Protection (RIVM)
Antonie Van Leeuwenhoeklaan 9
P.O. Box 1, 3270 BA Bilthoven
The Netherlands

26) DR F. VAN NIMWEGEN
National Institute of Public Health
and Environmental Protection (RIVM)
Antonie Van Leeuwenhoeklaan 9
P.O. Box 1, 3270 BA Bilthoven
The Netherlands

27) DR PAULUS OOSTVOGEL
National Institute of Public Health
and Environmental Protection (RIVM)
Antonie Van Leeuwenhoeklaan 9
P.O. Box 1, 3270 BA Bilthoven
The Netherlands

SECRETARIAT

27) DR SIMA HUILAN
Regional Adviser
Health Laboratory Services
WHO Regional Office for the
Western Pacific
Manila

28) DR T. KOBAYAKAWA
Expanded Programme on Immunization
Word Health Organization
1211 Geneva 27, Switzerland

29) DR J. MILSTIEN
Scientist, Biologicals Unit
World Health Organization
1211 Geneva 27, Switzerland

ANNEX 2

AGENDA

Monday, 11 June 1990

Opening Session

Introduction of participants

Tea Break

Lecture: Regional overview on polio eradication
(Dr Sima Huilan, WHO/WPRO)

Lunch

Lecture: Laboratory safety (Mrs Margery Kennett)

Practice: Preparation for the next day
Cell counting
Pretreatment filter discs

Tuesday, 12 June 1990

Film: EPI Documentary - Recognize the Disease

Lecture: The EPI vaccine (Dr Julie Milstien)

Tea Break

Lecture: Introduction measles haemagglutination (HA) and HA-inhibition (HI)

Practice: Pretreatment of sera
Performing HI-assay
Performing HA-assay

Lunch

Tuesday, 12 June 1990 (afternoon)

Practice: Reading and reporting results
Preparation for the next day

Wednesday, 13 June 1990

Presentation by a participant

Lecture: Introduction polio serum neutralization test

Tea Break

Practice: Polio serum neutralization assay (Micro)

Annex 2

Lunch

Practice: Preparation for the next day

Thursday, 14 June 1990

Introduction OPV-potency testing (cpe)

Tea Break

Practice: Add Hep2 cells to polio SN plates
Trivalent oral polio vaccine potency testing CCID50 Micro

Lunch

Lecture: Preparing and testing anti-sera
(Dr Henk Bruning)

Practice: First reading and reporting - polio
SN assay

Preparation for the next day

Lecture: Difference between plaque assay
and cpe test (Dr Henk Bruning)

Friday, 15 June 1990

Lecture: Introduction measles-cpe test

Practice: Measles cpe test (CCID50)

Tea Break

Lecture: Adverse Reactions (Dr Julie Milstien)

Lunch

Practice: Reading and reporting results - first week

Monday, 18 June 1990

Lecture: Collection, transportation and registration of faecal specimens
(Dr Paul Oostvogel)

Preparation of faecal specimens and inoculation of cell cultures
(Mrs Margery Kennett)

Tea Break

Preparation of specimens

Lunch

Practice: Inoculation of cell cultures with faecal suspensions

Annex 2

Lecture: Introduction yellow fever plaque assay

Practice: Yellow fever potency testing (plaque assay)

Tuesday, 19 June 1990

Lecture: Isolation and identification from polio and other enteroviruses from faeces
Rapid simultaneous isolation and identification of polio viruses
(Mrs Margery Kennett)

Film: The Polio Virus

Tea Break

Practice: Isolation and identification from polio and other enteroviruses from faeces

Lunch

Practice: Addition of cells
Examination and registation of previous experiments

Tea Break

Lecture: Staining (Dr Henk Bruning)

Wednesday, 20 June 1990

Lecture: Introduction single IgM for the diagnosis of poliomyelitis

Practice: Single IGM for the diagnosis of poliomyelitis (Dr Zhang Li Bi)

Tea Break

Practice: Single IGM for the diagnosis of poliomyelitis

Examination and registration of previous experiments

Lunch

Wednesday, 20 June 1990 (afternoon)

Field Visit: National Laboratory for the Control of Pharmaceuticals and Biologicals, Beijing

Tea Break

Lecture: Preparing and testing of anti-sera (continued) (Dr Henk Bruning)

Annex 2

Thursday, 21 June 1990

Lecture: Use of filter discs for blood collection
(Dr Henk Bruning)

The EPI laboratory network (Dr Paul Oostvogel)

The WPRO laboratory networkk (Dr Julie Milstien)

Tea Break

Practice Single IGM for the diagnosis of poliomyelitis

Examination and registration of previous experiments

Lunch

Practice: Single IGM for the diagnosis of poliomyelitis

Lecture: Reading and calculation of microtitre and plaque assays (Dr Henk Bruning)

Tea Break

Lecture: Interpretation of microtitre tests for polio and enterovirus typings.

Friday, 22 June 1990

Field Visit:A routine immunization session -Tong County

Lunch

Practice: Examination and registration of previous experiments

Yellow fever plaque assay

Tea Break

Lecture: Calculation of experimental results

Monday, 25 June

Field visit: National Vaccine and Serum Institute, Beijing

Lunch

Lecture: Intratypic differentation:
State of the art at a reference laboratory
(Dr A.M. Van Loon)

Cell banking (Dr H. Bruning)

Acceptance of results (Dr J. Milstien)

Annex 2

Tea Break

Practice: Examination and registration of previous experiments

Tuesday, 26 June

Return of course examination
Evaluation of practical work
Discussions on the examination results

Tea Break

Evaluation - WHO Manual

Lunch

Closing Ceremony

ANNEX 3

LIST OF HANDOUTS

1.	EPI Update May 1989 Poliomyelitis Global Eradication by the year 2000	
2.	Regional Overview of Poliomyelitis eradication in the Western Pacific Region	- Dr Sima Huilan
3.	Report on WHO/RIVM Workshop on Laboratory Diagnosis of Poliomyelitis	- Mrs M. Kennett
4.	Cell Counting	- Dr H. Bruning
5.	Requirements for EPI vaccines	- Dr J.Milstien
6.	Adverse Reactions	- Dr J. Milstien
7.	Preparation of specimens for isolation of polio and other entero virus	- Mrs M. Kennett
8.	Identification of Polio and other enterovirus	- Mrs M. Kennett
9.	How to test suitability of antipolio sera	- Dr H. Bruning
10.	Cpe versus plaque tests	- Dr H. Bruning
11.	Optional test circumstances	- Dr H. Bruning
12.	Timetable for polio laboratory development	- Dr P. Oostvogel - Dr J. Milstien
13.	Intratype differentiation of polio virus isolates	- Dr A. van Loon

ANNEX 4

DETECTION OF POLIOVIRUS-SPECIFIC IgM ANTIBODIES BY AN IgM ANTIBODY-CAPTURE ELISA

(Prof Dr Zhang-Li bi)

Principle:

anti-Hu-IgM --IgM --Poliovirus antigen --Anti-polio virus IgG --Anti-rabbit IgG-HRP

(serum) (rabbit) (goat)

Reagents used:

Anti-Hu-IgM: Affinity-purified polyclonal antisera (Sigma).
Monoclonal (home-made).

Poliovirus antigen: Sonicated tissue culture supernatant from VERO cells infected with Sabin strains.

Control antigen: Idem from uninfected cells

Anti-poliovirus IgG (rabbit): Antibodies against poliovirus type 1, 2 or 3 raised in rabbits by immunization with purified poliovirus.
Antibodies are said to be type specific.

Anti rabbit IgG (HRP) (goat): Home-made; HRP conjugation method not given.

Other reagents: See annex 1

Note: Concentration and buffer competition of the substrate (OPD) solution were not given; instead data were given for preparation of the substrate for alkaline phosphatase conjugates.

Procedure: See annex 2.

Results:

Results are measured as optical densities and are expressed as the ratio between the reaction with the poliovirus antigen (P) and the control antigen (N). A P/N ratio > 2.1 is considered to indicate the presence of poliovirus (type) specific IgM.

Kinetics of the assay system:

Details on the kinetics of the assay system were not given and may well be not known very well (personal communication Prof. Zhang Li-bi). This is particularly regrettable with the respect to the correlation between serumdilution and optical density in infants vs. children and adults.

Annex 4

Detection rate:

In suspected patients poliovirus-specific IgM antibody was detected in between 13% (6/46) and 92.3% (48/52) of patients. It is remarkable that IgM antibody to more than one type was found in only one out of 367 positive patients (annex 3, table 5). The relation to the onset of illness (paralytic?) was studied in more than 200 suspected cases. Results are given in table 1. The numbers of patients in each group are not known.

Table 1. Correlation between days after onset of illness in patients suspected of poliomyelitis and the detection of poliovirus specific IgM antibodies

Days after onset	Percentage IgM positive
0-3	61%
4-21	76%
22-25	40%
25-55	no data
>55	0%

Sensitivity: comparison with virus isolation.

So far, data regarding sensitivity as related to virus isolation are available from two laboratories. Data from Beijing and Shan dong show that IgM antibodies to poliovirus were detected in 76.9% and 80%, respectively, of suspected patients. The figures for virusisolation are 42.2% and 45%, respectively (table 2). Comparison of the isolated virustype and the type specificity of the IgM response showed infection by the same virus in 39 out of 42 patients (annex 3, table 4)

Table 2. Correlation between virus isolation and detection of poliovirus-specific IgM in suspected patients.

	IgM detection			
Virus isolation	Beijing (n=52)		Shan dong (n=20)	
	+	-	+	-
+	18	5	7	2
-	22	7	9	2

Specificity:

A P/N ratio > 2.1 is considered to indicate a positive IgM reaction. However, no data regarding the frequency distribution of P/N ratio's in a sufficiently large number (>100) of negative patients are available. A blocking assay with different enterovirus antigens was carried out to confirm the specificity of a positive IgM reaction in the poliovirus type 1 IgM test. Reactivity was neutralized by the homologous antigen only (annex 3 table 2). Additional data on specificity were obtained by examining a few sera from patients with other enterovirus infections as demonstrated by detection of a specific IgM

Annex 4

response. No cross-reactivity was observed in the assay for poliovirus type 1 IgM antibody. (annex 3, table 1)

Conclusion :
Detection of type-specific IgM antibodies to polioviruses may facilitate and complement the diagnosis of poliomyelitis in suspected patients. The test is relatively simple and easy to perform and to standardize, obviates the need for tissue culture facilities and results may be obtained within 24 hours after receiving specimens. However, it can not completely replace virus isolation which is absolutely needed for genetic and antigenetic analysis of virus strains. In addition, the IgM assay cannot (yet) discriminate between antibody induced by wild or vaccine type viruses. This last problem may be of limited importance as one may expect the IgM response after wild type virus infection to be restricted to one type.

The presently available data on sensitivity and specificity appear promising but are clearly insufficient for recommendation of the method to diagnose poliomyelitis. In addition, data on technical aspects of the assay are nearly completely lacking. Therefor further studies are needed to determine the value of the technique for diagnosis of poliomyelitis. These studies should at least address the following points:

Technical aspects:

- Dose response relationships, notably serumdilution vs. optical density in sera from infants.
- Effect of using wild type viral antigens endemic to the region
- Frequency distribution of sera from patients with and without poliovirus infection
- Development of a confirmatory assay (blocking test, immunoblotting)

Sensitivity and specificity:

- Performance of the test in specimens from well characterized patients with poliovirus as well as other infections (not only by enteroviruses), preferably in a prospective study.
- Follow-up studies in patients and vaccinees to determine the type specific IgM response in relation to onset of disease and to study persistence of IgM antibody.

In addition, it is recommended to develop a similar assay for detection of IgA antibodies to poliovirus. This may be useful for serological diagnosis of poliomyelitis in partially immunized patients.

Beijing, 24/06/90
A.M. van Loon.

ANNEX 5

EVALUATION QUESTIONNAIRE

1. Educational gains

1.1 Were the following objectives met?		Yes	No	No reply
(a)	To review the current status of laboratory diagnosis of poliomyelitis and potency testing of virus vaccines (polio and measles) in the Region	100%		
(b)	To review the WHO manuals on isolation and identification of polio virus and on vaccine potency testing;	100%		
(c)	To review the specimen collection during epidemiological surveillance (from suspected poliomyelitis cases and apparently healthy contacts);	100%		
(d)	To introduce standard methods and practice on laboratory techniques for isolation and identification of polio virus (by types 1-3 and wild, vaccine-strain);	90%	10%	
	Two people would have liked to learn more of identification techniques of polio wild strains and vaccine strains. One of them wants to learn more of polymerised chain reaction in detail.			
(e)	To standardize and practice the technique for potency testing of virus vaccines (OPV and measles)	100%		

If no, please describe:

1.2	Have new skills or concepts been learned at the course?	100%		
1.3	Can these skills and concepts be applied in your country?	100%		

2. Process and outcome

2.1	Were you able to express your ideas or poblems at the course?	86%		14%

Annex 5

2.2	Was there enough opportunity to exchange knowledge and experience with other participants?	86%	14%	
	If response to any of the above is no, give comments as appropriate:			
2.3	Were you satisfied with all training materials provided? 76%	5%	9%	
	If no, please explain for specific paper(s):			
2.4	Specify which of the training materials distributed for the course are suitable for wider distribution:			
	a) Laboratory methods for the titration of live virus vaccines	52%		
	b) Laboratory techniques for isolation and identification of polio virus	9.5%		
	c) Polio and measles handouts	9.5%		
	d) Examination items	9.5%		
	e) Reference antisera for Polio I, II and III	9.5%		
	f) Reference OPV and measles vaccines	9.5%		
	g) Polio virus video	9.5%		
2.5	Did you have enough time to study the training materials?	62%	33%	5%
	One person replied: "Sometimes yes but sometimes no because of our heavy schedule during the weekdays, even weekend but I think I would rather review this manual when I come back to my country."			
2.6	Were methods of introduction and presentation of different topics satisfactory?	66%	34%	
2.7	Were you fully satisfied with the			
	a) formal discussions?	100%		
	b) laboratory discussions?	86%	14%	
	If no, please explain:			

Annex 5

2.8 Field Visits

2.8.1	If there were field visits as part of the course, were they useful to meet the objectives?	90%	10%
	If no, explain your response:		

3. Organization of the course

Were the duration and scheduling of different activities - lectures, benchwork practice, disicussions, etc. - satisfactory?	90%	10%

4. Administrative aspect

Are organization or administrative arrangements for travel, accommodation, per diem, meeting room and secretarial support satisfactory?	81%	5%	14%

5. Is there any better way to achieve the course objectives? 5% 43%

One participant said: "With the help of video and film." Another one said: "Yes", but did not specify how.

6. What follow-up activities, if any would you recommend?

a) National government

2 people - not specified	10%
1 person - to set up the National Control Authority	5%
1 person - EPI meetings about methods with laboratory and epidemiology staff	5%
1 person - training for the staff who are involved in laboratory diagnosis of poliomyelitis and those in the production and control of vaccines	5%

b) WHO

8 people - not specified	38%
1 person - continue training of methods expanding DPT	5%

c) By other agencies

1 person	5%