WORLD HEALTH ORGANIZATION
REGIONAL OFFICE FOR THE WESTERN PACIFIC

REPORT
SIXTH MEETING ON LABORATORY SURVEILLANCE FOR
POLIOMYELITIS ERADICATION IN THE WESTERN PACIFIC REGION

Manila, Philippines
6 November 2002
REPORT

SIXTH MEETING ON LABORATORY SURVEILLANCE FOR POLIOMYELITIS ERADICATION IN THE WESTERN PACIFIC REGION

Convened by:

WORLD HEALTH ORGANIZATION

REGIONAL OFFICE FOR THE WESTERN PACIFIC

Manila, Philippines
6 November 2002

Not for sale

Printed and distributed by:

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

April 2003
NOTE

The views expressed in this report are those of the participants of the Sixth Meeting on Laboratory Surveillance for Poliomyelitis Eradication in the Western Pacific Region and do not necessarily reflect the policies of the World Health Organization.

This report has been printed by the Regional Office for the Western Pacific of the World Health Organization for the participants in the Sixth Meeting on Laboratory Surveillance for Poliomyelitis Eradication in the Western Pacific Region, which was held in Manila, Philippines, 6 November 2002.
SUMMARY

Representatives of the Polio Laboratory Network in the Western Pacific Region met in Manila on 6 November 2002. The meeting was attended by: representatives of the Regional reference laboratories (RRLs) and national poliomyelitis laboratories (NPLs); temporary advisers from the Centers for Disease Control and Prevention (CDC), Atlanta, United States of America; and representatives from the WHO Vaccine Assessment and Monitoring Unit (WHO/VAM) in Geneva and the WHO South-East Asia Regional Office in New Delhi.

The Polio Laboratory Network continues to be well established in this Region, and is being used to provide essential information for action in responding to the eventual importation of wild poliovirus and detection of vaccine-derived polioviruses. Performance levels have been maintained as required for certification of poliomyelitis eradication since the Region was declared poliomyelitis-free. The formal system for annual accreditation of network laboratories is well established, and almost all laboratories in the Western Pacific Region’s Polio Laboratory Network are performing at WHO accreditation standard.

The workload of the laboratory network has constantly grown over the past years and is expected to further increase. This increase is mainly due to the new requirements for intratypic differentiation of all poliovirus isolates requiring also larger sequencing capacity as all poliovirus isolates with discordant results need to undergo immediate further sequence analysis.

Thus, even more funding support is required to ensure that the laboratory network is maintained at least until global certification. Technical Advisory Groups recommended that countries should aim to reduce the time period between onset of paralysis and availability of intratypic differentiation results from 90 to 60 days. Although laboratories can only contribute to a certain extent as delays often occur during investigation, steps prior to receipt of specimens or isolates at the laboratories, aiming at shorter turnaround times will increase workload as well.

Enzyme-linked immunoassay (ELISA) intratypic differentiation (ITD) testing has been successfully established at all three RRLs, and reporting of results by two methods has commenced. In order to increase ITD capacity in the Region, selected NPLs were reviewed and found to be operating at very high-quality standards. Accreditation is still pending, however, as new P3 ELISA reagents were only recently received. Once testing methods are fully established and final proficiency test results are available, then the decision can immediately be made.

Internal quality control is an important performance indicator for laboratory accreditation. As there is still a wide range of in-house quality control, a move towards standardization of quality control is still required. The introduction of the revised WHO Polio Laboratory Manual, which contains a comprehensive section on internal quality control, was a great step forward. It was noted that another updated version of the laboratory manual is expected to be available at the end of first quarter 2003 and will contain new sections related to internal quality control (i.e. mycoplasma testing, cell line sensitivity for virus isolation, guidelines for suspected viral contamination, and containment requirements).

As virological surveillance for poliovirus will continue as a requirement for the post-certification era of poliovirus eradication, it is essential that the Western Pacific Region’s Polio Laboratory Network be sustained as a functioning component of the WHO Expanded Programme on Immunization, requiring continuous support from national authorities and partner agencies.
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ANNEX 1 TIMETABLE OF THE MEETING
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1. INTRODUCTION

The Polio Laboratory Network continues to be well established in this Region, and is being used to provide essential information for action in responding to eventual importation of wild poliovirus and detection of vaccine derived polioviruses (VDPV). Performance levels have been maintained at those levels required for certification of poliomyelitis eradication since the Region was declared as poliomyelitis-free. The formal system for annual accreditation of Polio Network Laboratories is well established, and almost all laboratories in the Regional Laboratory Network are performing at WHO accreditation standard.

Concerns, however, have been expressed about maintaining certification standards for reporting and investigating acute flaccid paralysis (AFP) cases and collecting adequate stool specimens in the future as after certification priorities may be moved to other public health activities and certain complacency develop under the assumption that poliovirus transmission has stopped in the Region.

The workload of the laboratory network is expected to further increase, mainly due to the new requirements for intratypic differentiation (ITD) of all poliovirus isolates requiring also larger sequencing capacity as all poliovirus isolates with discordant ITD results need to undergo immediate further sequence analysis.

Thus, even more funding support is required to ensure that the laboratory network is maintained at least until global certification. The Technical Advisory Groups (TAGs) also recommended that countries should aim to reduce the time period between onset of paralysis and availability of ITD results from 90 to 60 days. Although laboratories can only contribute to a certain extent as delays often occur during investigation steps prior to receipt of specimens or isolates at the laboratories, aiming at shorter turn-around times will increase workload as well.

To maintain excellent performance levels it is crucial to give special attention to further standardizing the laboratory data management system, establishing a support system for equipment maintenance, distribution of selected supplies and developing standardized approaches on in-house quality control. Additional support and assessment is also required to meet the continuing demand for training in basic laboratory techniques.

1.1 Objectives

The objectives of the meeting are:

(1) to review the current status of the laboratory network with regard to levels of performance, and to identify and document outstanding problems related to routine laboratory responsibilities and to propose solutions; and

(2) to identify available resources to address specific operational issues e.g., the management of vaccine derived polioviruses and non-polio enterovirus isolates.

1.2 Organization

The meeting was attended by representatives of the regional reference laboratories (RRLs) and national poliomyelitis laboratories (NPLs), temporary advisers from the Centers for Disease Control and Prevention (CDC), Atlanta, United States of America, and a secretariat.

Annex 1 shows the timetable of the meeting, and Annex 2 contains the list of participants.
1.3 Opening ceremony

Dr Shigeru Omi, Regional Director of the WHO Western Pacific Region, welcomed all participants and expressed appreciation that despite significant workload increases due to new requirements for ITD, sequencing and timeliness of results, performance levels are being kept at high standards.

Dr Omi summarized that enzyme-linked immunoassay (ELISA) ITD capacity has been established at all RRLs and selected NPLs, sequencing capacity been strengthened and the majority of laboratory results are available within 60 days of onset of paralysis. Dr Omi noted that these achievements are particularly remarkable in a Region that has been certified poliomyelitis-free for two years and that now faces a risk that ongoing priorities may shift to other public health activities or complacency may develop.

Dr Omi noted that the process of annual accreditation continues to provide documentation that laboratories have the capability and capacity to detect and report wild polioviruses and VDPVs in a timely and accurate fashion.

Dr Omi encouraged the group to address several issues including: stricter shipment requirements for virus isolates as infectious substances resulting in significantly higher costs; expanding standardized internal quality control; and ensuring proper equipment maintenance to address bio-safety concerns.

Dr Bruce Thorley was requested to serve as chairperson, Dr Phan Van Tu as vice-chairperson and Dr Youngmee Jee as rapporteur.

2. PROCEEDINGS

2.1 Overview of the Polio Laboratory Network

2.1.1 Global update

Progress in the poliomyelitis eradication initiative has continued to be very encouraging with the number of poliomyelitis endemic countries decreasing from 10 to six, the number of infected districts within those countries decreasing, and the quality of surveillance continuing to show impressive improvement. On the other hand, the number of confirmed cases has shown an increase from 483 in 2001 to 706 as of 1 October 2002. It is expected that the total number of confirmed cases for 2002 will exceed 1000. Ninety-eight percent of all confirmed cases until October 2002 have occurred in three countries: India (76%), Nigeria (16%) and Pakistan (6%), representing the three remaining high-transmission foci of Northern India, Northern Nigeria/Niger and Pakistan/Afghanistan.

The increase in confirmed cases has occurred against a background of continually improving surveillance. The rates for AFP detection and investigation continue to rise, and stool collection rates have shown significant improvements in the areas where they were previously most lacking. The most dramatic improvements have been seen in Africa, particularly in Nigeria. The number of AFP cases reported in the Eastern Mediterranean Region has increased by 20% over those reported in the same time period in 2001, and an increase of 14% has been achieved in the South East Asian Region.
In Afghanistan only seven confirmed cases have been detected this year. Surveillance quality remains high and these cases have been widely dispersed, suggesting there are few remaining foci in Afghanistan. Despite the recent security problems, AFP reporting and stool collection rates have continued to be of high quality for most of Afghanistan. In Pakistan the number of AFP cases reported in 2002 is approximately equal to that reported in 2002, with high quality surveillance and improved stool collection rates. The foci of transmission of wild poliovirus seen in 2001 have been maintained, but the number of confirmed cases has continued to show a decline.

Poliovirus types 1 and 3 continue to circulate in the northern states of Nigeria, and across the northern border in Niger, but no wild viruses have been detected in the southern and central states of Nigeria up to September 2002. Routine immunization rates in the northern states remain low, and approximately 50% of the non-polio AFP cases are either non-immunized or under-immunized. Immunization rates have shown an improvement with time, but progress has been slow. Surveillance quality in the country has greatly improved since 2000, and every state now has an AFP reporting rate of greater than two cases per 100,000 population less than 15 years of age. Stool collection rates have also shown significant improvement over the past two years.

India reported 535 cases to September 2002, most of these from the state of Uttar Pradesh, and mostly poliovirus type 1. An outbreak of poliovirus type 1 was detected in eastern Uttar Pradesh in the early part of the year and has continued through the high transmission season. Transmission in the neighbouring state of Bihar appears to be dispersed, while an outbreak in the eastern state of West Bengal is largely restricted to a single district. Earlier this year virus reintroduced from Uttar Pradesh initiated an outbreak in the western state of Gujarat. Most other cases detected in India appear to be sporadic introductions from Uttar Pradesh and Bihar. The quality of immunization activities has been improving throughout India over recent years, particularly in Uttar Pradesh, but these improvements need to be maintained for at least another four rounds of supplementary immunization if wild virus transmission is to be halted in this state.

Critical activities for the initiative over the coming year are: to maintain access to all children in poliomyelitis-susceptible areas; continue to push for improvements in the quality of oral poliovirus vaccine (OPV) immunization and AFP surveillance, particularly at sub-national level; ensure the continued availability of funds by filling the US$ 275 million funding gap; and ensure that requirements for laboratory containment of wild poliovirus are achieved.

African Region

Of 117 wild poliovirus confirmed cases detected in the African Region up to September 2002, 113 have come from Nigeria, two from the bordering country of Niger, and two from the southern part of Zambia. The two cases detected in Zambia are believed to be importations from neighbouring Angola. No recent cases have been detected, however, in Angola itself.

In 2000 there were three genotypes of poliovirus type 1 detected in the African Region: WEAF-A, WEAF-B and WEAF-C. In 2002 WEAF-A has been restricted to the imported cases in Zambia. WEAF-C, despite being widespread in previous years, was last detected in sporadic cases in Mauritania and Algeria in 2001. Viruses belonging to the WEAF-B genotype are now only found in northern Nigeria and Niger. Poliovirus type 3 viruses continue to be isolated in northern Nigeria and Niger, these viruses belonging mainly to the P3 WEAF-B genotype.

In addition to the detection of wild polioviruses, an outbreak associated with VDPV has been detected on the island of Madagascar. The first case was detected in 2001 and a further four cases
have been detected in 2002. The outbreak virus is derived from the Sabin type 2 vaccine strain, but appears to have been circulating in the under-immunized population on the island for two to three years. Investigation of the outbreak found many unreported AFP cases around the VDVP-associated cases, suggesting that under-reporting has been a problem in the country for some time.

**Eastern Mediterranean Region**

The number of countries in the Region reporting wild poliovirus transmission has decreased to four at the end September 2002. Confirmed cases have been reported from Pakistan, Afghanistan and Somalia, and wild poliovirus isolates have been detected in environmental surveillance samples from Egypt, in the absence of poliomyelitis cases.

Poliovirus types 1 and 3 continue to be detected in Afghanistan and Pakistan. The number of cases has decreased, and transmission of both serotypes appears to be increasingly restricted to specific locations. The major foci of transmission in Pakistan; Sindh, Baluchistan and North West Frontier province, have continued from previous years. Genomic heterogeneity has decreased significantly in recent years, but at least six distinct lineages of poliovirus type 1 continue to circulate in their traditional foci. Relative dominance of these lineages has, however, shown some change in the past year, with the lineage previously associated with transmission in North West Frontier province becoming more significant in other parts of the country.

In Egypt, three distinct lineages of poliovirus type 1 have been detected in environmental surveillance samples. In 2001 wild poliovirus was detected in sites in eight provinces of the country, but this has reduced considerably in 2002 with sites in Upper Egypt (provinces south of the Nile delta) being free of virus since April. The wild poliovirus type 3 isolates from Somalia originate from the area around Mogadishu and represent the same lineage as detected previously. The last wild poliovirus detected in Sudan was a case with onset in April 2001, and it is hoped that this country is now free of poliomyelitis. The last wild virus detected in Iran was in 2001, which represented an importation from Afghanistan/Pakistan. With improved vaccine coverage and surveillance in the border areas of Iran it is hoped that outbreaks due to further importations can be avoided.

**2.1.2 Western Pacific Region**

The performance of the Polio Laboratory Network remains at good quality levels. For the Region as a whole in 2001, 87% of laboratory results were available within 28 days of specimen receipt at the laboratory. As of 31 October 2002, 91% of results have been available within 28 days of receipt at the national laboratory, and 100% within 42 days. Reporting timeliness lower than 80% was noted in the NPL in New Zealand and four sub-national laboratories in China.

Ninety-five percent of (at least preliminary) ITD results were available within 14 days after receipt at the RRL, and 99% ITD results were available during the current standard of 28 days after receipt.
For the Region as a whole in 2001, non-polio enteroviruses (NPEV) were isolated from 11% of all AFP case specimens processed. The rate was 9% as of October 2002. Although no longer a criterion for accreditation, the NPEV isolation rate is still used as a direct measure of laboratory sensitivity. Relatively low rates are noted for the NPLs of Malaysia (5%), the Philippines (8%) and Viet Nam (Ho Chi Minh City) (6%) and have been discussed with proposed solutions including a more detailed protocol (and documentation) for cell sensitivity checks, adjustments in cell storage and incubator functions and eventual temporary re-introduction of Hep2C cell lines.
In 2001, all RRLs were visited and fully accredited. All NPLs were also reviewed in 2001; seven were fully accredited [Hong Kong, Malaysia, New Zealand, Papua New Guinea, the Philippines, Singapore and Viet Nam (Ho Chi Minh City)], one was provisionally accredited [Viet Nam (Ha Noi)], and for two laboratories (Mongolia, Republic of Korea), accreditation was kept pending until the first quarter of 2002. The NPL in Republic of Korea was subsequently fully accredited, and the NPL in Mongolia was provisionally accredited. All but one (Tibet) of the 31 sub-national laboratories in China have shown to operate at WHO accreditation standard in 2001.

In 2002 to date, two RRLs [China Centre for Disease Control and Prevention (CDC, China), Beijing and National Institute of Infectious Diseases (NIID), Japan] were already visited and reviewed and fully accredited. The review of the third RRL [Victorian Infectious Disease Reference Laboratory (VIDRL), Australia] was planned in December 2002. Six NPLs were visited and reviewed [Hong Kong, Malaysia, Mongolia, Philippines, Singapore, Viet Nam (Ha Noi)] and fully accredited. The review of the NPL in New Zealand was scheduled in December 2002. The NPL in Viet Nam (Ho Chi Minh City) was also visited, but accreditation was kept pending as no passing score was achieved in the most recent isolation and identification proficiency test (PT). A detailed action plan was developed with specimens tested in parallel at the RRL in Australia. Accreditation indicators of all laboratories are detailed in Table 1.
Table 1: Results of accreditation reviews, 2002 (as of 5 November 2002).

<table>
<thead>
<tr>
<th>Country</th>
<th>Accred level</th>
<th>Current status</th>
<th>Date of latest accreditation</th>
<th>AFP test results reported 28 days</th>
<th>ITD results on poliovirus isolates reported 28 days</th>
<th>Number of specimens tested</th>
<th>NPEV isolation rate</th>
<th>Isolates confirmed by RRL</th>
<th>Isolates forwarded within 14 days</th>
<th>Internal QC procedures</th>
<th>Most recent ITD PT result</th>
<th>Most recent isolation PT result</th>
<th>Operating procedures and work practices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>RRL</td>
<td>Accredited</td>
<td>Aug-01</td>
<td>94</td>
<td>NA</td>
<td>100</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Yes</td>
<td>100</td>
<td>100</td>
<td>PCR, Probe, ELISA 100</td>
</tr>
<tr>
<td>China</td>
<td>RRL</td>
<td>Accredited</td>
<td>Jun-02</td>
<td>NA</td>
<td>99.9</td>
<td>100</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Yes</td>
<td>100</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>Japan</td>
<td>RRL</td>
<td>Accredited</td>
<td>Aug-02</td>
<td>100</td>
<td>100</td>
<td>NA</td>
<td>23.6</td>
<td>NA</td>
<td>NA</td>
<td>Yes</td>
<td>100</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>NPL</td>
<td>Accredited</td>
<td>Apr-02</td>
<td>100</td>
<td>NA</td>
<td>620</td>
<td>11.3</td>
<td>none isolated</td>
<td>none from AFP</td>
<td>Yes</td>
<td>100</td>
<td>100</td>
<td>PCR, Probe 100</td>
</tr>
<tr>
<td>Republic of Korea</td>
<td>NPL</td>
<td>Accredited</td>
<td>Mar-02</td>
<td>84</td>
<td>NA</td>
<td>676</td>
<td>6</td>
<td>none isolated</td>
<td>none from AFP</td>
<td>Yes</td>
<td>80</td>
<td>NA</td>
<td>84</td>
</tr>
<tr>
<td>Malaysia</td>
<td>NPL</td>
<td>Accredited</td>
<td>Jul-02</td>
<td>96</td>
<td>NA</td>
<td>201</td>
<td>8.5</td>
<td>100</td>
<td>100</td>
<td>Yes</td>
<td>100</td>
<td>NA</td>
<td>97</td>
</tr>
<tr>
<td>Mongolia</td>
<td>NPL</td>
<td>Accredited</td>
<td>Sep-02</td>
<td>89</td>
<td>NA</td>
<td>474</td>
<td>5.5</td>
<td>100</td>
<td>0*</td>
<td>Yes</td>
<td>100</td>
<td>NA</td>
<td>94</td>
</tr>
<tr>
<td>New Zealand</td>
<td>NPL</td>
<td>Accredited</td>
<td>Aug-01</td>
<td>100</td>
<td>NA</td>
<td>150</td>
<td>13.6</td>
<td>100</td>
<td>100</td>
<td>Yes</td>
<td>100</td>
<td>PCR 100</td>
<td>100</td>
</tr>
<tr>
<td>Philippines</td>
<td>NPL</td>
<td>Accredited</td>
<td>Apr-02</td>
<td>100</td>
<td>NA</td>
<td>992</td>
<td>6.9</td>
<td>90</td>
<td>100</td>
<td>Yes</td>
<td>100</td>
<td>NA</td>
<td>89</td>
</tr>
<tr>
<td>PNG</td>
<td>NPL</td>
<td>Accredited</td>
<td>Nov-01</td>
<td>100</td>
<td>NA</td>
<td>139</td>
<td>10.5</td>
<td>100</td>
<td>0+</td>
<td>Yes</td>
<td>60</td>
<td>NA</td>
<td>94</td>
</tr>
<tr>
<td>Singapore</td>
<td>NPL</td>
<td>Accredited</td>
<td>Apr-02</td>
<td>98</td>
<td>NA</td>
<td>476</td>
<td>34.2</td>
<td>96.4</td>
<td>100</td>
<td>Yes</td>
<td>100</td>
<td>PCR 100</td>
<td>97</td>
</tr>
<tr>
<td>Vietnam, Ha Noi</td>
<td>NPL</td>
<td>Accredited</td>
<td>Jul-02</td>
<td>98</td>
<td>NA</td>
<td>441</td>
<td>23</td>
<td>100</td>
<td>100</td>
<td>Yes</td>
<td>100</td>
<td>NA</td>
<td>94</td>
</tr>
<tr>
<td>Vietnam, Ho Chi Minh City</td>
<td>NPL</td>
<td>Pending</td>
<td>Jul-02</td>
<td>97</td>
<td>NA</td>
<td>267</td>
<td>7.5</td>
<td>100</td>
<td>67</td>
<td>Yes</td>
<td>70</td>
<td>NA</td>
<td>87</td>
</tr>
</tbody>
</table>

* delay caused by difficulties to identify courier service in Mongolia that would accept infectious substances
+ required procedures were explained and timely shipment assured in the future
As the NPL in Papua New Guinea did not achieve a passing score in the most recent PT, arrangements continue for parallel testing at the RRL in Australia and have been made for performance review and recommendations towards repeat PT and accreditation in November 2002 with the review planned either at the end of the year or in early 2003. All other laboratories passed the isolation and identification PT during the first distribution (Table 2).

**Table 2: Poliovirus isolation and identification proficiency test results by country, 1999-2002.**

<table>
<thead>
<tr>
<th>Country</th>
<th>1999**</th>
<th>2000</th>
<th>2001</th>
<th>2002***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hong Kong</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Malaysia</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Mongolia</td>
<td>100</td>
<td>100</td>
<td>100*  (69)</td>
<td>100</td>
</tr>
<tr>
<td>New Zealand</td>
<td>84</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Papua New Guinea</td>
<td>96</td>
<td>100</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>Philippines</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Rep. of Korea</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>Singapore</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Viet Nam (Ha Noi)</td>
<td>84</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Viet Nam (Ho Chi Minh City)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>70</td>
</tr>
</tbody>
</table>

* repeat PT; ** new scoring system; *** reduced reporting time of results of 28 days (before 42)

Technical support was provided in close collaboration with the CDC in Atlanta and NIID in Tokyo to the RRL in Beijing to conduct genetic sequencing procedures; phylogenetic analysis of sequence data; and facilitate introduction and use of the screening enzyme-linked immunoassay (EIA) developed by National Institute of Public Health and the Environment (RIVM), the Netherlands.

In order to increase ITD capacity in the Region, the NPLs in Hong Kong and Singapore were reviewed and found to be operating at very high quality standards; accreditation is still pending, however, as new P3 ELISA reagents were only recently received and the PT still has to be passed once testing methods are fully established.

Training has been provided at NIID in Japan for staff from the NPLs in Mongolia and Viet Nam (Ha Noi). Review of work practices and on-the-job training was also provided to both laboratories.

An important milestone has been reached in the organization of the poliomyelitis laboratory data. Before 2002, all information for the laboratory investigation of AFP cases did not have a single proper database. Specimen test results, both from the national polio laboratories and RRLs are stored per country in separate Excel spreadsheets.

However, starting in 2002, a new Microsoft (MS) Access-based system has been set up to store poliomyelitis laboratory, as well as AFP surveillance data. The information received from countries, varying in formats, can be imported into the new database through several methods: (1) manual data entry for paper reports; (2) a pre-formatted Excel file for spreadsheet reports; or (3) a click and upload interface for EPI Info-based REC file datasets. Since the data is now stored in MS Access, limitless reports can be generated from it as necessary, currently one of which is the weekly poliomyelitis bulletin. The sections in the bulletin for the laboratory investigation, which used to be computed semi-manually, are now fully automated.

The database for the China poliomyelitis laboratory network was also reviewed and modified and expanded to harmonize data reporting from sub-national laboratories and the RRL, completely merge laboratory and AFP surveillance data and additionally manage data from individual ITD method reporting and sequence data.
All poliovirus isolates are supposed to be subjected to two ITD methods, one antigenic and one molecular. ELISA testing capacity has been established at all RRLs, and reporting to the Western Pacific Regional Office by individual ITD method has commenced. Poliovirus isolates with non-conclusive ITD results are subjected to sequencing of the VP1 Region. Searches for potential VDPV included retrospective virological analysis.

The results from NIID and VIDRL are listed in Table 3.

**Table 3: Results of prospective and retrospective laboratory testing for VDPV**

<table>
<thead>
<tr>
<th>Year/Country</th>
<th>Poliovirus isolation</th>
<th>ITD method not conclusive</th>
<th>Results</th>
<th>Sequence result</th>
<th>Similarity to Sabin VP1</th>
<th>Specimen origin</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>Hong Kong</td>
<td>4 polio type 1 ELISA</td>
<td>non Sabin-like</td>
<td>Sabin-like</td>
<td>&gt; 99.5%</td>
<td>non-AFP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>Malaysia</td>
<td>1 polio type 1 ELISA</td>
<td>double reactive</td>
<td>VDPV?</td>
<td>9/903= 99%</td>
<td>non-AFP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Singapore</td>
<td>2 polio type 1 ELISA</td>
<td>non Sabin-like</td>
<td>Sabin-like</td>
<td>99.7%</td>
<td>non-AFP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 polio type 1 ELISA</td>
<td>double reactive</td>
<td>Sabin-like</td>
<td>99.7%</td>
<td>non-AFP</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>Hong Kong</td>
<td>1 polio type 1 ELISA</td>
<td>non Sabin-like</td>
<td>Sabin-like</td>
<td>99.7%</td>
<td>non-AFP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 polio type 1 ELISA</td>
<td>non Sabin-like</td>
<td>VDPV</td>
<td>98.6%</td>
<td>non-AFP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 polio type 1 ELISA</td>
<td>double reactive</td>
<td>Sabin-like</td>
<td>99.7-99.9%</td>
<td>non-AFP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 polio type 2 ELISA</td>
<td>double reactive</td>
<td>Sabin-like</td>
<td>99.7%</td>
<td>non-AFP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malaysia</td>
<td>1 polio type 1 ELISA</td>
<td>double reactive</td>
<td>Sabin-like</td>
<td>99.3%</td>
<td>AFP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>New Zealand</td>
<td>1 polio type 1 ELISA</td>
<td>non Sabin-like</td>
<td>VDPV</td>
<td>98.9%</td>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 polio type 1 ELISA</td>
<td>non Sabin-like</td>
<td>VDPV</td>
<td>98.8%</td>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 polio type 2 ELISA</td>
<td>non Sabin-like</td>
<td></td>
<td></td>
<td></td>
<td>M EF-1 reference strain</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 polio type 3 ELISA</td>
<td>non Sabin-like</td>
<td></td>
<td></td>
<td>Saukett COP reference strain</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 polio type 1 ELISA</td>
<td>double reactive</td>
<td>Sabin-like</td>
<td>99.6%</td>
<td>non-AFP</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 polio type 3 ELISA</td>
<td>double reactive</td>
<td>Sabin-like</td>
<td>99.8%</td>
<td>non-AFP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>Australia</td>
<td>1 polio type 1 ELISA</td>
<td>double reactive</td>
<td>Sabin-like</td>
<td>99.6%</td>
<td>non-AFP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 polio type 2 ELISA</td>
<td>double reactive</td>
<td>Sabin-like</td>
<td>99.8%</td>
<td>non-AFP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cambodia</td>
<td>1 polio type 3 MoAb IT</td>
<td>Sabin-like?</td>
<td>99.4%</td>
<td>AFP</td>
<td>3D 78% to Sabin 1, 75% to Sabin 2, 77% to Sabin 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hong Kong</td>
<td>2 polio type 1 ELISA</td>
<td>non Sabin-like</td>
<td>Sabin-like</td>
<td>99.7%</td>
<td>non-AFP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 polio type 1 ELISA</td>
<td>double reactive</td>
<td>Sabin-like</td>
<td>99.8%</td>
<td>non-AFP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 polio type 3 ELISA</td>
<td>double reactive</td>
<td>Sabin-like</td>
<td>99.8%</td>
<td>non-AFP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 polio type 1 ELISA</td>
<td>double reactive</td>
<td>pending</td>
<td>non-AFP</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Korea</td>
<td>1 polio type 3 PCR</td>
<td>non Sabin-like</td>
<td></td>
<td></td>
<td>reference strain Lederle III</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Philippines</td>
<td>5 polio type 1 ELISA</td>
<td>double reactive</td>
<td>Sabin-like</td>
<td>&gt; 99.5%</td>
<td>AFP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Viet Nam</td>
<td>1 polio type 1 ELISA</td>
<td>non Sabin-like</td>
<td>Sabin-like</td>
<td>99.2%</td>
<td>AFP</td>
<td></td>
</tr>
</tbody>
</table>
The isolates identified in New Zealand were submitted to the NPL for testing while establishing a national inventory for wild poliovirus infectious materials for laboratory containment. The VDPV in Hong Kong was isolated from a routine stool sample of an immunocompromised infant. The potential VDPV in Malaysia was isolated from a routine stool sample of a non-AFP case, but no further clinical information is currently available. AFP surveillance quality in the respective area was assumed to be good. The isolate from Cambodia may require further sequence analysis. Surveillance for potential VDPV in China was presented during the meeting.

Following a poliomyelitis outbreak in 2001 associated with VDPV, the quality of AFP surveillance in the Philippines significantly improved in 2002 with all regions except one achieving an annualized non-polio AFP rate above 1 per 100,000 under age 15 years. Adequate stool collection exceeds 80% in all but two regions. No new VDPVs were detected supporting the assumption that the nationwide immunization response was able to successfully interrupt virus circulation. Two rounds of National Immunization Days (NIDs) were conducted in February and March 2002, targeting all children under-five years of age and delivering OPV to approximately 70% of the target group in a house-to-house approach.

2.1.3 South-East Asia Region

The third quarter of 2001 saw an increase in the number of both type 1 and type 3 poliovirus isolations, with the total for the year at 268 cases. Of these, 209 were type 1, 56 were type 3, and three cases were mixed infections. Wild virus was found in cases from 63 districts. The number of wild virus cases did not decline significantly through 2002, and July saw the largest monthly total of confirmed cases for the past three years. This rise in cases was associated with a large outbreak of type 1 poliovirus in eastern Uttar Pradesh and a smaller focus of transmission in West Bengal. In 2002 to 23 September, a total of 480 confirmed cases were reported; 435 type 1 and 45 type 3 polioviruses detected in 86 districts. There has been a steady decline in the level of type 3 poliovirus detected since 1999. Of the cases detected in 2001 and 2002, 77% were less than 24 months of age at onset of paralysis and 41% were under-immunized.

In 2001, 210 of 212 poliovirus isolates were subjected to genomic sequence analysis. Up to the end of September 2002, 187 isolates were sequenced. Three major type 1 lineages have been detected in 2001 and 2002, each with several sub-lineages. Representatives of all three lineages have been detected in Uttar Pradesh. Representative of a lineage classified as “C” was the most prevalent in 2001, but has declined significantly in 2002, although it has remained prevalent in western Uttar Pradesh. Lineage “B” increased to be the most prevalent group in 2002, particularly the sub-lineage B2. The outbreak in West Bengal in 2002 is associated with the B1 sub-lineage. An outbreak in Gujarat in 2002 is associated with reintroduction of virus belonging to the “A” lineage from Uttar Pradesh.

It is now possible to follow in great detail the transmission chains of different viruses around India as they are eradicated from one area only to be reintroduced later from remaining foci. It appears that wild poliovirus transmission was almost halted in central and eastern Uttar Pradesh in the low season between 1999 and 2000, but these areas were re-seeded with virus from western Uttar Pradesh in 2001.

2.2 Accreditation and proficiency issues

The accreditation checklists were last revised in February 2000 and in light of current conditions, a number of amendments were proposed during the last global network meeting. These include the requirements to adopt laboratory timeliness proposals made in 2001 by the Technical Consultative Group (TCG) to accommodate the detection of VDPVs and to take into account the processing of samples from sources other than AFP surveillance.
The following general changes were also proposed:

1. To remove statements on the possibility of providing interim reports;
2. To include questions related to containment under a combined bio-safety/containment section;
3. To include a question about report submission to the WHO regional offices with agreed frequency and format; and
4. To change all references to wild polioviruses to "wild polioviruses and suspected VDPV isolates."

Review of accreditation reports has revealed that although the network has now been in place for over 10 years, there are still laboratories experiencing cell culture problems.

2.2.1 National poliomyelitis laboratories

The following changes to the accreditation criteria for the national laboratory checklist were proposed:

<table>
<thead>
<tr>
<th>Old criterion</th>
<th>New Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>= 80% of reports within 28 days</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Tests on = 150 samples per year</td>
<td>Unchanged</td>
</tr>
<tr>
<td>= 90% poliovirus accuracy of identification of polioviruses</td>
<td>Unchanged</td>
</tr>
<tr>
<td>= 80% poliovirus isolates sent for ITD within 14 days of detection</td>
<td>= 80% poliovirus isolates sent for ITD within 7 days of detection</td>
</tr>
<tr>
<td>Internal quality control implemented</td>
<td>Unchanged</td>
</tr>
<tr>
<td>= 80% score on PT test</td>
<td>Unchanged – except report within 28 days</td>
</tr>
<tr>
<td>= 80% on on-site review</td>
<td>Unchanged</td>
</tr>
</tbody>
</table>

2.2.2 Regional reference laboratories

The following changes to the accreditation criteria for the regional reference laboratory checklist were proposed:

<table>
<thead>
<tr>
<th>Old criterion</th>
<th>New Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITD results on = 80% of polioviruses reported within 28 days</td>
<td>ITD results on = 80% of polioviruses reported within 14 days</td>
</tr>
<tr>
<td>= 90% score on ITD PT test</td>
<td>= 90% score on ITD PT test – score to be recorded for each ITD PT test</td>
</tr>
<tr>
<td>= 90% score on isolation and typing PT test</td>
<td>Unchanged – except report within 28 days</td>
</tr>
<tr>
<td>= 90% on on-site review</td>
<td>Unchanged</td>
</tr>
<tr>
<td>= 80% of isolation reports within 28 days</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Internal quality control implemented</td>
<td>Unchanged</td>
</tr>
</tbody>
</table>
2.2.3 Intratypic differentiation functions at selected NPLs

The following changes to the accreditation criteria for the supplementary national laboratory checklist were proposed:

<table>
<thead>
<tr>
<th>Old criterion</th>
<th>New Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITD results on = 80% of polioviruses reported within 28 days</td>
<td>ITD results on = 80% of polioviruses reported within 14 days</td>
</tr>
<tr>
<td>= 80% wild polioviruses and suspected VDPV referred for sequencing within 7 days of detection</td>
<td>= 90% score on ITD PT test - score to be recorded for each ITD PT test</td>
</tr>
<tr>
<td>= 90% score on ITD PT test</td>
<td>= 90% score on ITD PT test</td>
</tr>
<tr>
<td>= 90% poliovirus accuracy of identification of polioviruses</td>
<td>To be removed</td>
</tr>
<tr>
<td>= 90% on on-site review</td>
<td>Unchanged</td>
</tr>
</tbody>
</table>

2.3 Implications from search for vaccine derived polioviruses

The Regional network has started to implement the requirement of all poliovirus isolates regardless of source being subjected to one antigenic and one molecular ITD method. A workshop conducted with the support of the Global Specialized Laboratory, RIVM in the Netherlands, held at VIDRL, Melbourne in November 2001, facilitated the introduction of ELISA ITD testing at all RRLs and the NPLs in Hong Kong, New Zealand and Singapore.

Problems were encountered with the serotype 3 components of the ELISA; a deficient batch of type 3 antiserum was identified and it became apparent that the inactivated non-Sabin-like controls are only stable when stored undiluted at 4ºC. Revisions will be made accordingly in the polio laboratory manual. All sera should continue to be stored at -20ºC.

Vaccine-related virus strains that are clearly not normal are those that show significant sequence variation from the parent Sabin strains, by common agreement greater than 1% in the VP1 region. Sources of these strains are the outbreak associated VDPVs (cVDPV), strains isolated from long term virus excretors, who are usually immunodeficient in some way (iVDPV), and other strains of ambiguous origin. A further distinction can be made in that those believed to have circulated in human populations always show signs of recombination. A common characteristic of all polioviruses, of whatever origin, appears to be that circulation results, sooner or later, in recombination either with other polioviruses or with non-polio enteroviruses.

Three further characteristics have been noted for VDPVs known to have circulated in human populations: all have occurred in areas or sub-populations with low vaccine coverage, all have occurred in areas with no homologous wild poliovirus present, and all have been detected using the surveillance strategy recommended for use by the Western Pacific Region Polio Laboratory Network.

2.4 Constraints to producing timely laboratory results

With the current quality requirements for individual steps of AFP case investigation, 86 days can be reached without jeopardizing individual quality indicators. It has been proposed during the recent meeting of the Global Polio Laboratory Network to reduce the turnaround time
for ITD testing by 50% as well as timely shipment requirements for poliovirus isolates. But even with these reductions, 66 days can be reached without jeopardizing individual quality indicators as indicated in Table 4.

**Table 4. Quality indicators in AFP case investigation.**

<table>
<thead>
<tr>
<th>Steps in AFP case investigation</th>
<th>Max. # days allowed</th>
<th>Required standard</th>
<th>WPR 2002</th>
<th>Starting January 2003</th>
<th>Max. # days allowed</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP cases with adequate stool samples</td>
<td>14</td>
<td>80%</td>
<td>87%</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Transport of stools to NPL within 3 days</td>
<td>3</td>
<td>41%</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Transport of stools to NPL within 7 days</td>
<td>28</td>
<td>80%</td>
<td>91%</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>NPL results within 28 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shipment of isolates to RRL within 14 days</td>
<td>14</td>
<td>80%</td>
<td></td>
<td>reduced to 7 days</td>
<td>7</td>
</tr>
<tr>
<td>ITD results within 28 days</td>
<td>28</td>
<td>80%</td>
<td>99%</td>
<td>reduced to 14 days</td>
<td>14</td>
</tr>
<tr>
<td>ITD results within 60 days of onset</td>
<td></td>
<td>80%</td>
<td>79%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As per dataset of 1 November 2002, 41% of stool samples in the Region reached the NPL within three days of collection, 85% were received within one week and 15% reached the NPL later than one week after collection. These figures were similar in 2001. Higher than average rates for stool samples taking longer than a week to reach the NPLs are observed in Australia, Malaysia, Mongolia, the Philippines and Viet Nam. Cambodia and Lao People's Democratic Republic do also face problems in timely shipment of stool specimens from the point of collection to the capital, but as NIID in Tokyo functions also as the RRL, time savings can be made as there is one shipment less.

Detailed analysis of AFP cases with ITD results available after 60 days of onset reveals: (a) late stool specimen collection in Cambodia and Malaysia; (b) late stool sample collection in Mongolia and the Republic of Korea and shipment delays of the isolates to the RRL; (c) delays in transporting stool specimens to the NPL in Viet Nam and shipment delays to the RRL; and (d) late stool collection, delays in transporting stool specimens to the NPL in the Philippines and shipment delays to the RRL. Delays in international shipment are mainly caused by decreased acceptance and/or stricter shipping requirements for infectious substances.

### 2.5 Quality control

#### 2.5.1 Virus titration and evaluating cell line sensitivity using authenticated Sabin strains

The requirement for standardized preparations of Sabin viruses to be used as test controls and for a titration standard has been long appreciated. During the eighth meeting of the Global Laboratory Network in October 2002, it was announced that standards for each serotype have now been prepared by National Institute for Biological Standards and Control (NIBSC), United Kingdom. The initial aim is to supply each of 250 laboratories (all Polio Laboratory Network members and some additional laboratories) with 10 ampoules of standard preparations each year. Two thousand five hundred (2500) ampoules of each type have been prepared for the first batch of standards.
Standard protocols for the use of these standards, based on the WHO standard procedure for vaccine potency assays, are now being prepared and will be distributed with the standards. It is recommended that laboratories use the standards to establish their own banks of authenticated Sabin strain preparations for use in cell sensitivity assays.

Distribution to laboratories in the Western Pacific is anticipated in the second quarter of 2003.

2.5.2 Testing cell lines for the presence of Mycoplasma

The effects of mycoplasma contamination on cell cultures include suppression of cell division, depression of the general health of the cells, and diminution of the ability of cells to support virus replication, leading to loss of cell sensitivity. Several methods are available for detection of mycoplasma infection, and all have relative advantages and disadvantages. The ideal assay would be rapid, sensitive, use existing resources available in the poliomyelitis laboratory, easy to perform and inexpensive. The ideal assay, however, does not exist. The reality is that most assays take at least one day of laboratory staff time to perform, require additional resources, are not easy to perform, and the least expensive (Hoechst 33258 stain) is the least sensitive.

During the eighth meeting of the Global Laboratory Network, it was summarized that descriptions of three methods, the Hoechst stain, a commercial EIA fluorescence assay and a commercial PCR assay, have been proposed for inclusion in the WHO Polio Laboratory Manual. If it is agreed that these three methods are the most appropriate for inclusion, then the next step will be to determine which laboratories should be carrying out the assays. Appropriate training in mycoplasma detection should be provided in conjunction with a general cell culture-training course at the first opportunity.

2.5.3 Other quality issues

In order to further discuss technical issues within the Western Pacific Regional Polio Laboratory Network, four groups were formed with Dr Stuart Blacksell, Mr David Featherstone, Dr Bruce Thorley and Dr Nalini Withana acting as facilitators within the groups. Each group was allowed 45 minutes to formulate issues and a further 45 minutes was used to report the issues to the group. The following areas of concern were discussed and recommendations formulated:

I. Virus Isolation and identification (Facilitator: Dr Bruce Thorley)

A. RIVM antisera pools

The use of RIVM monospecific antisera was noted in the detection of NPEV.

B. Low titre polioviruses in a mixture with another poliovirus

It was noted that there are the possibilities of mixed infections with poliovirus masking NPEV growth and visa versa. It was suggested that titration of virus isolates from $10^1$ to $10^8$ may reveal differences in cytopathic effect (CPE) suggestive of multiple infection. It was also suggested to pick out well and repeat poliovirus neutralization to see if the neutralization titres are concordant.
C. Growth of RD-A cells

Some laboratories reported that they were unable to maintain RD-A cells beyond five days. The following solutions were suggested:

- Check water and media;
- Check water conductivity and water QC (have there been recent resin changes);
- Poor quality water storage container can contaminate the water; and
- Cells should be changed onto maintenance medium at four days.

When all else fails new cells should be requested from the RRL. It was noted that RD-A cells are more fastidious than other lines and require a bit of extra care.

D. Unusual CPE

Unusual morphology of RD-A cells was noted in some laboratories. It was reported that the cells reflected a rounded morphology but without lysis. Discussion centred on the fact that it could be a number of factors that may include old age of RD-A cells. It was suggested that the cells be passed into Hep-2c cells, as these cells are more useful for the isolation of adenoviruses. It was noted that adenovirus is more focal CPE than the generalized polio/enterovirus CPE.

II. Tissue culture (Facilitator: Mr David Featherstone)

A. Standardized Standard Operating Procedures (SOPs) for tissue culture (TC)

The issue of a standardized SOP for cell susceptibility was raised. It was noted that a complete method will be provided in the updated WHO Polio Laboratory Manual. It was suggested that cells be assessed at passage 3, 8 and 15.

B. Ensure the quality of the cells

It was noted that there is currently no recommended method for mycoplasma testing of cell lines. Staff needed to know (1) What method to use; (2) the frequency of testing and this should be included in the updated WHO Polio Laboratory Manual.

C. Fetal Calf Serum (FCS) quality

FCS batch variation was noted as a general problem that was difficult to resolve. It was suggested that each laboratory attempt to select a gold standard serum and make aliquots of this serum for future comparison of growth characteristics with new FCS.

D. NPEV growth in L20B

The growth of NPEVs should be noted, assessed and recorded. Dr Walter Dowdle volunteered to compile the Regional database.

E. Role of TC laboratory facility

Each laboratory has its own system for cell cultures. Some laboratories have centralized cell culture facilities, and others are maintained with the poliovirus laboratories. These differences should be noted as centralized cell culture facilities may have difficulties following the poliovirus laboratory standard operating procedures (SOPs).
F. Count cells rather than use split ratios

It was recommended that cell counts (using a counting chamber with trypan blue) be used when trypsinizing cells rather than using split ratios. This will maintain standardized cell numbers.

G. Liquid nitrogen (LN) and CO₂: recognize the risk

The danger of working with liquid nitrogen and CO₂ was discussed. Provision for adequate ventilation at all times was recognized. Furthermore, it was recognized that more than one person should be responsible for checking and filling of the LN tanks to prevent them running dry.

III. Standard Operating Procedures (Facilitator: Dr Nalini Withana)

A. Write individual SOPs

SOPs are important documents for each laboratory and maintenance of these documents should also be reflected in the laboratory accreditation process. They should be based on the WHO Polio Laboratory Manual but should reflect local deviations and situations (i.e., freezer numbers and locations where viruses are stored).

B. Simplification of language

As appropriate (given the level of English language comprehension in the individual country), it is important that SOPs are translated into local languages and are understandable to laboratory staff at all levels.

C. Continuous training

SOPs should reflect a culture of continuous training and revision in the laboratory and should be a “living document”.

D. Implementation of SOPs

SOPs must be retained in an appropriate area of the laboratory where they are accessible to all staff. It may be necessary to have multiple copies of the SOPs.

E. Importance of continual review of SOPs

Revisions should be incorporated as required, and SOPs should be reviewed (at least) on an annual basis.

2.6 Bio-safety issues including certification and monitoring (facilitator Dr Stuart Blacksell)

A. Biological Safety Cabinets (BSCs)

BSCs should be accredited annually. In a worse case scenario where there is no available accreditation mechanism, efforts should be made to accommodate local in-house accreditation using the following principles:

- Regular decontamination of BSC using portable formaldehyde gas generator;
- Airflow check at various points on the work surface using an anemometer (minimum 0.45 meters/secs); and
• Check the integrity of the down flow HEPA filter using blood agar plates strategically located on the work surface and checking for growth at 18 hrs incubation.

BSCs should be fully cleaned on the working surface after use and the sump should be cleaned at least once a week.

B. Bio-safety officers

There should be at least one trained microbiological safety officer who can deal with staff induction and microbiological safety issues.

C. Virus spills

Contingency plans should be developed for different size contaminated material spills. Examples are given below. SOPs should be developed and recommended procedures should be incorporated into future updates of the WHO Polio Laboratory Manual. General concepts for spills clean up are suggested and detailed below.

Spill Clean-up Protocol

Any potentially contaminated clothing must be removed and placed in a biohazard waste bag for autoclaving. If the spill is outside of a BSC, then the laboratory must be evacuated immediately. It is the responsibility of the last person out to ensure that all doors have been closed. The room must remain vacant with the door shut for least 30 minutes to allow time for the laboratory ventilation system to clear any aerosolized material from the room, and thus decrease the risk of inhaling biohazardous materials.

If this spill is outside of the laboratory, then immediate cleanup is essential. If outdoors, then personnel should remain upwind from the spill, if at all possible.

If the spill is inside a centrifuge, then the centrifuge should be closed as soon as the spill is noticed. It should be wiped with 1% hypochlorine. Wipe dry after 20 minutes and if buckets are available, decontaminate with running water and dry. If possible, the rotor and its content should be moved to a BSC.

If the spill is contained inside a BSC, then the room need not be evacuated, however, the BSC must remain running for at least 10 minutes before resuming use. The spill tray underneath the work area and the trough below the air intake grill must be cleaned as well. These are likely to be contaminated when the spill is large.

Hands and any other contaminated skin must be washed thoroughly with soap and water.

Everyone not needed for spill cleanup must be cautioned to stay away from the spill area. Signs may be posted if necessary.

Appropriate personnel protection must be worn. At a minimum, disposable gloves, eye protection and a laboratory coat should be worn. An N95 dust mask respirator is advised for spills greater than ~ 10mL outside a BSC, or any spill inside a centrifuge, because of the likelihood of splashing and/or aerosolization of the biohazardous material.

Any sharp contaminated objects must be removed from the spill area using mechanical means, never with hands. After all sharps are removed, disinfectant must be poured carefully around the edges of the spill with care taken to avoid splashing. Paper towels can be used to absorb as much of the spilled material as possible. Working from the outside of the spill toward the center avoids spreading contamination. Alcohol is not recommended as a disinfectant.
After initial cleanup, the spill area must be flooded with disinfectant and left to soak for at least 20 minutes (adequate contact time is important to ensure complete decontamination). A final wipe-down should be done with clean paper towels soaked with disinfectant. Laboratory personnel should be sure to disinfect any equipment, walls or other areas likely to have been splashed by the spill.

All contaminated waste must be disposed of properly.

D. Waste disposal

It is important that secondary containers (polypropylene plastic or stainless steel) are used for the storage and transport of autoclave bags. This is to contain any liquid spills caused by bag puncture or split. Furthermore, it is important that appropriate autoclave bags are used for sterilization of waste.

E. Surface disinfectants

There is currently no standard for surface disinfectant for the WPR Polio Laboratory Network. At CDC, a 1% v/v chlorine solution is used although this is considered to be corrosive (especially in autoclaves and BSCs). Another disinfectant is PHORAID, which is currently in use at VIDRL. It is PVP iodine based non-corrosive with a long shelf life. Another alternative is VIRKON made by Antec International uses a stabilized peroxidizing agent, surfactant, organic acids and inorganic buffers that is non-corrosive but results in a "cloudy build up" after prolonged use on stainless steel surfaces. A list of suitable disinfectants should be prepared and distributed/or included in the updated WHO Polio Laboratory Manual.

F. Autoclaves

Elements required for effective autoclave use. A autoclaves must be used properly to effectively decontaminate potentially biohazardous materials. The following elements all contribute to autoclave effectiveness.

Temperature. Adequate chamber temperature is at least 121°C.

Time. Adequate autoclaving time is a minimum of 30 minutes, measured after the temperature of the material being sterilized reaches 121°C and 15 psi pressure. The tighter the autoclave is packed, the longer it will take to reach 121°C in the center of the load.

Contact. Steam saturation of the load is essential for effective decontamination. Air pockets or insufficient steam supply will prevent adequate contact. To ensure adequate steam contact, leave autoclave bags partially open during autoclaving to allow steam to penetrate into the bag. Add a small amount of water inside the bag to help ensure heat transfer to the items being decontaminated (do not add water if it will cause bio-hazardous materials to splash out of the bag).

Container. Use leak-proof containers for items to be autoclaved. Place plastic bags inside a secondary container in the autoclave in case liquids leak out. Plastic or stainless steel containers are appropriate secondary containers. Make sure plastic bags and pans are autoclavable, to avoid having to clean up melted plastic.

Indicators. Tape indicators can only verify that the autoclave has reached normal operating temperatures for decontamination. Most chemical indicators change color after being exposed to 121°C, but cannot measure the length of time spent at 121°C. Biological indicators (such as Bacillus stearothermophilus spore strips) and certain chemical indicators (such as
Sterigage) verify that the autoclave reached adequate temperature for a long enough time to kill micro-organisms.

A chemical indicator should be used in every load to monitor the effectiveness of individual autoclave runs (temperature only).

Once a month, either a biological indicator (such as Bacillus stearothermophilus spore strips) or a chemical indicator that measures both time and temperature (such as Sterigage) should be used. Bury the indicator in the center of the load to validate adequate steam penetration. Keep a logbook to record the results.

3. CONCLUSIONS AND RECOMMENDATIONS

3.1 Conclusions

The performance of the Western Pacific Regional Polio Laboratory Network remains at good quality levels. For the Region as a whole to date (31 October 2002), 91% of results have been available within 28 days of receipt at the national laboratories. Ninety-five per cent of ITD results are available within 14 days after receipt at the RRL and 99% ITD results are available during the current standard of 28 days after receipt.

For the Region as a whole in 2002 to date, NPEVs were isolated from 9% of all stool specimens processed from AFP cases. Although no longer a criterion for accreditation, the NPEV isolation rate is still used as a direct measure of laboratory sensitivity. Relatively low rates are noted in several NPLs and have been discussed with proposed solutions including a more detailed protocol (and documentation) for cell sensitivity checks, adjustments in cell storage and incubator functions and consideration for the temporary re-introduction of Hep2 cell lines.

In 2002 to date, two RRLs had already been visited and fully accredited. The review of the third RRL was planned in December 2002. Eight NPLs passed the isolation and identification PT during the first distribution. Seven NPLs were visited and fully accredited, the review of one NPL is scheduled in December 2002. Two NPLs still have to perform a repeat PT and achieve a passing score before the accreditation review can be scheduled. Arrangement for parallel testing of stool specimens at a RRL has been made for both laboratories. All but one of the 31 sub-national laboratories in China have shown to operate at WHO accreditation standard in 2001.

ELISA ITD testing has been successfully established at all three RRLs. VIDRL (Melbourne) is currently using PCR and NAPH as their second ITD method, CCDC (Beijing) is implementing PCR-RFLP and NIID (Tokyo) is using monoclonal antibodies plus random partial sequencing. Reporting of ITD results by two methods has been fully implemented by VIDRL since mid-2001. Reporting from CCDC and NIID has commenced.

VIDRL is also reporting ITD results of all poliovirus isolates from non-AFP cases by two methods to the Western Pacific Regional Office which covers most of the poliovirus isolates from non-AFP cases (Australia, Hong Kong, Malaysia, New Zealand, Papua New Guinea, Singapore) in the Region. The network in China does not test stool specimens from non-AFP cases, but lists contacts of AFP cases. Poliovirus isolates from non-AFP cases can furthermore be expected from Japan, Mongolia and the Republic of Korea with all except the ones from Japan reported to the Western Pacific Regional Office.
In order to increase ITD capacity in the Region, the NPLs in Hong Kong and Singapore were reviewed and found to be operating at very high quality standards; accreditation is still pending, however, as new P3 ELISA reagents were only recently received. Once testing methods are fully established and final PT results will be available, the decision can immediately be made.

The required 80% of ITD results available within 60 days of onset is not yet reached although significant improvements to 79% in 2002 to date (compared to 47% in 2001) have been made, particularly in China. Detailed analysis of AFP cases with ITD results not available after 60 days of onset reveals the main reasons as late stool specimen collection and delays in sending stool samples from the point of collection to the NPL. Forty-two percent of stool samples in the Region reach the NPL within three days of collection, 42% takes longer than three days but less than eight days to reach the NPL and 16% reach the NPL later than one week after collection. Delays in international shipment are mainly caused by decreased acceptance and/or stricter shipping requirements for infectious substances.

The database for the Region and that for the China poliomyelitis laboratory network were modified and expanded to manage data from individual ITD method reporting and sequence data; additionally in China to harmonize data reporting from sub-national laboratories and the RRL, completely merge laboratory and AFP surveillance data.

A poliomyelitis outbreak caused by VDPV type 2 occurred in Madagascar in 2002 and VDPV type 1 was identified in an AFP case in Romania in July 2002 as well as in contact persons. A new imported wild poliovirus type 1 with closest relatives being viruses from Northern India was identified in a routinely collected stool samples in late 2001 in Georgia with delays in virological investigation as not related to an AFP case. All incidents highlight the need to continue high quality AFP and virological surveillance systems for all polioviruses.

The Western Pacific Regional Office provided support in supply distribution, mainly poliovirus and enterovirus antisera but stricter global shipment and quarantine requirements for infectious substances/dangerous goods have caused problems i.e. sending ELISA reagents and PTs became increasingly difficult as items perceived as originating in the Philippines have been rejected several times.

Problems were also encountered in shipping isolates from NPLs to RRLs with courier companies accepting infectious substances in the country of origin but not at the destination. New shipment companies were finally identified but at much higher costs.

The Western Pacific Regional Office provided support for equipment maintenance, mainly filter changes in bio-safety cabinets, in several network laboratories but capacity to commission and certify their function is not yet established in several countries.

Internal quality control is an important performance indicator for laboratory accreditation. As there is still a wide-range diversity in in-house quality control (QC), a move toward standardization of quality control is still required, although the introduction of the revised laboratory manual, which contains a comprehensive section on internal QC was already a great step forward. However, depending on the needs of individual network members it might still be required to develop regional guidelines incorporating outlines of member laboratory QC methods. It was noted that another updated version of the laboratory manual is expected to be available at the end of first quarter 2003 and will contain new sections with several related to internal QC (i.e. mycoplasma testing, cell line sensitivity for virus isolation, guidelines for suspected viral contamination, containment requirements).
3.2 Recommendations

(1) As virological surveillance for poliovirus will continue as a requirement for the post-certification era of poliovirus eradication, it is recommended that the Western Pacific Region’s Polio Laboratory Network be sustained as a functioning component of the WHO EPI, requiring continuous support from national authorities and partner agencies.

(2) With the Region being declared free of indigenous wild poliovirus, the Polio Laboratory Network has the opportunity for increased vigilance to detect VDPVs. The VDPV observations are important and need to be fully documented as part of the records of the poliovirus eradication process; for example, the documentation and reporting of ELISA ITD results that, while meeting the specified test criteria, have unusual OD values.

(3) It is noted that the changes to the accreditation criteria for the Western Pacific Region Polio Laboratory Network will be implemented from early 2003. The laboratory staff will endeavour to fully comply with these changes. For example, for AFP cases the time period for the reporting of poliovirus ITD results has been reduced from 28 to 14 days, and poliovirus isolates should be shipped for ITD within seven days after results became available.

(4) It is recognized that shipping of infectious materials has become problematic in the previous 12-month period. Laboratory staff are recommended to strictly follow International Air Transport Association (IATA) dangerous goods shipping regulations at all times. It is also recommended that staff receive appropriate training to achieve IATA dangerous goods shipping qualifications.

(5) Laboratories are recommended to embrace the concept of a quality systems approach to provide a mechanism of continual monitoring and improvement. This should include increased awareness of high quality cell culture procedures during a training course for WPR poliovirus network laboratory staff. This should include revised recommendations for determining cell line virus susceptibility using authenticated Sabin strains, which are anticipated to be distributed in 2003.

(6) It is recommended that the data collected at individual WPR poliovirus network laboratories regarding the isolation of NPEVs in the L20B cell line be collated for further analysis and evaluation and will be forwarded to the WHO head office.

(7) A major revision of the WHO Poliovirus Laboratory Manual will be completed by March 2003. It is recommended that these revisions are noted and incorporated into revised standard operating procedures at all WPR poliovirus network laboratories.

(8) Appreciating the second edition of the Global Action Plan for Laboratory Containment of Wild Poliovirus Infectious/Potentially Infectious Materials, laboratories should strictly implement separation of infectious and non-infectious materials and ensure complete inventories of all such materials to continue serving as models of excellence in laboratory containment.
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LIST OF PARTICIPANTS

1. REGIONAL REFERENCE LABORATORY STAFF

Dr Hiroyuki Shimizu, Department of Virology II, National Institute of Infectious Diseases
Gakuen 4-7-1, Musashimurayama-shi, Tokyo 208-0011, Japan,
Tel: +81-42-561-0771, Fax: +81-42-561-4729, E-mail: hshimizu@nih.go.jp

Dr Bruce Thorley, Regional Poliovirus Reference Laboratory, Epidemiology & Public Health Division, Victorian Infectious Diseases Reference Laboratory, 10 Wreckyn Street, North Melbourne 3051, Victoria, Australia,
Tel: 61-3 9342-2607/2600, Fax: 61-3 9342-2665, E-mail: bruce.thorley@mh.org.au

Dr Zhang Libi, Director, National Reference Laboratory for Poliomyelitis, China Center for Disease Control and Prevention (CCDC), 27 Nanwei Lu, Beijing 100050, China,
Tel: (8610) 6317-1710, Fax: (8610) 6317-1724, E-mail: chenli0102@hotmail.com

2. NATIONAL POLIOMYELITIS LABORATORY STAFF

Dr Wilina Lim Wei-ling, Consultant - Medical Microbiologist, Department of Health, Hong Kong Government, 9/F Public Health Laboratory Centre, 382 Nam Cheong Street, Shek Kip Mei, Kowloon, Hong Kong, Tel: (852) 2319 8252; Fax: (852) 2319 5989, E-mail: wllim@pacific.net.hk

Dr Nor Shahidah Khairullah, Head, Division of Virology, Institute of Medical Research Jalan Pahang, 50588 Kuala Lumpur,
Tel: +603-440-2345, Fax: +603-2693-6323, E-mail: norshahidah@imr.gov.my

Dr Sarankhuu Amarzaya, Head of National Polio Laboratory, Department of Virology Public Health Institute, Enkhtaivan Street-17, Ulaanbaatar 49,
Tel: (976) 11 452 664, Fax: (976) 11 452 664, E-mail: amarzs@yahoo.com

Dr Qiu Sue Huang, Science Leader-Virology, Communicable Disease Group, Institute of Environmental Science and Research, Kenepuru Drive, Porirua
Tel: +64-4-914-0764, Fax: +64-4-914-0770, E-mail: sue.huang@esr.cri.nz

Dr Peter Max Siba, Assistant Director and Head, Microbiology and Virology Unit
Papua New Guinea Institute of Medical Research, P.O. Box 60, Goroka, EHP 441,
Tel: +675-732-2800, Fax: +675-732-1998, E-mail: pmsiba@pngimr.org.pg
Annex 2

Ms Judy B. Bocacao, Research Analyst, National Poliomyelitis Laboratory, Research Institute for Tropical Medicine, Department of Health, Filinvest Corporate City Compound, Alabang, Muntinlupa City, Tel: +632-809-7599; 807-2628 (Loc. 605), Fax: +632-842-2245/2828, E-mail: jbocacao@ritm.gov.ph

Ms Eichelle J. Lintag, Research Assistant, Laboratory Containment for Wild Poliovirus Research Institute for Tropical Medicine, Department of Health, Filinvest Corporate City Compound, Alabang, Muntinlupa City, Tel: +632-809-7599; 807-2628 (Loc. 605), Fax: +632-842-2245/2828, E-mail: eichelle@edsamail.com.ph

Dr Youngmee Jee, Senior Researcher, Laboratory of Enteroviruses, Department of Virology, National Institute of Health, 5 Nokbun-dong, Eunpyung-gu, Seoul 122-701, Tel: +82-2-380-1493, Fax: +82-2-382-6542, E-mail: ymeejee@nih.go.kr

Dr Chan Kwai Peng, Consultant, Department of Pathology, Singapore General Hospital, Outram Road, Singapore 169608, Tel: +65-326-5435, Fax: +65-323-4921, E-mail: gptckp@sgh.com.sg

Dr Nguyen Thi Hien Thanh, Chief of Enterovirus Laboratory, National Institute of Hygiene and Epidemiology, Ministry of Health, No. 1 Yersin Street, Hanoi 10 000, Tel: +84-8-211-534, Fax: +84-8-213-782, E-mail: nththanh@fpt.vn

Dr Phan Van Tu, Director, Laboratory of Enteroviruses, Pasteur Institute, Ministry of Health, 167, Duong Pasteur, Q3, Ho Chi Minh City, Tel: +84-8-230-352 / 820-2878 or 820-2835, Fax: +84-8-8231-419, E-mail: phantu@hcmc.netnam.vn

3. TEMPORARY ADVISER

Dr Walter Dowdle, Director of Programmes, The Task Force for Child Survival and Development, 750 Commerce Drive, Suite-400, Decatur, Georgia 30030, United States of America, Tel: 1 404 687 5608, Fax: 1 404 371 1087, E-mail: wdowdle@taskforce.org

Dr Mark Pallansch, Chief, Enterovirus Section, Respiratory and Enteric Viruses Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road E-05, Atlanta, Georgia 30333, United States of America, Tel: 404-639-2749, Fax: 404-639-4011, E-mail: map1@cdc.gov

4. SECRETARIAT

Dr E. B. Doberstyn, Director, Combating Communicable Diseases, Regional Office for the Western Pacific, United Nations Avenue, 1000 Manila, Philippines, Tel: +632 528-8001, Fax: +632 521-1036, E-mail: doberstyn@wpro.who.int

Dr Yang Baoping, Regional Adviser, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue, 1000 Manila, Philippines, Tel: +632-528-9747, Fax: +632-521-1036, E-mail: yangb@wpro.who.int

Dr Yoshikuni Sato, Medical Officer, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue, 1000 Manila, Philippines, Tel: (632) 528-9742, Fax: (632) 521-1036, E-mail: satoy@wpro.who.int

1 Observer as representative of Dr Julia Fem Paladin, Head Virology Department, RITM, Muntinlupa City
Annex 2

Dr Sigrun Roesel, Medical Officer, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue, 1000 Manila, Philippines, Tel: +632-528-9741, Fax: +632-521-1036, E-mail: roesels@wpro.who.int

Dr Osman Mansoor, Medical Officer, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue, 1000 Manila, Philippines, Tel: +632-528-9748, Fax: +632-521-1036, E-mail: mansooro@wpro.who.int

Dr Jeffrey McFarland, Medical Officer, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue, 1000 Manila, Philippines, Tel: +632-528-9746, Fax: +632-521-1036, E-mail: mcfarlandj@wpro.who.int

Mr Wayne Antkowiak, Technical Officer, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue 1000 Manila, Philippines, Tel: +632-528-9751, Fax: +632-521-1036, E-mail: antkowiak@wpro.who.int

Dr Hiroko Tanaka, Technical Officer, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue, 1000 Manila, Philippines, Tel: +632-528-9744, Fax: +632-521-1036, E-mail: tanakah@wpro.who.int

Dr Stuart Blacksell, WHO Consultant, Wellcome Unit, Faculty of Tropical Medicine Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand, Tel: +66 2 2460832, Fax: +66 2 2467795, E-mail: sdb_thai@hotmail.com

Dr Lisa Lee, Medical Officer, Expanded Programme on Immunization, WHO Representative's Office – China, 401, Dongwai, Diplomatic Office Building, Chaoyang District, Beijing 100600 China, Tel: +8610 6532 7189 to 92, Fax: +8610 6532-2359, E-mail: leel@chn.who.int

Dr J. Mendsaikhan, Immunization Officer, Expanded Programme on Immunization, WHO Representative's Office, Ministry of Public Health, Ulaanbaatar-13, Mongolia Tel: +976-11-32 7870, Fax: +976-11-32 4683, E-mail: mendsaikhan@mog.wpro.who.int

Dr Rikke Schultz, Technical Officer, Expanded Programme on Immunization, WHO Representative's Office, 63 Tran Hung Dao Street, Hoan Kiem District, Ha Noi, Viet Nam Tel: +844 943-3734, Fax: +844 943-3740, E-mail: schultzr@vtn.wpro.who.int

Mr David Featherstone, Coordinator, Vaccine-Preventable Disease Laboratory Network Vaccines Assessment and Monitoring, Health Technology and Pharmaceuticals World Health Organization, CH-1211 Geneva 27, Switzerland Tel: +41 22-791-4422, Fax: +41 22-791-4210, E-mail: featherstoned@who.int

Dr Nalini Withana, Virologist/Consultant, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Mahatma Gandhi Road, New Delhi 110002, India Tel: +91-11 331 7804, Fax: +91-11 331 8607, E-mail: withanan@whosea.org