REPORT

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

Manila, Philippines
8-9 July 1999

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December 2000
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NOTE

The views expressed in this report are those of the participants of the third 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 third meeting on Laboratory Surveillance for Poliomyelitis Eradication in the Western Pacific Region, which was held in Manila, Philippines, 8-9 July 1999.
SUMMARY

Representatives of the Regional Poliomyelitis Laboratory Network met in Manila on 8 and 9 July 1999. The meeting was attended by representatives from the laboratories in the Regional poliomyelitis laboratory network, with the exception of China, Hong Kong and Malaysia together with representatives from three provincial poliomyelitis laboratories in China, and representatives from the WHO South East Asia Regional Office (SEARO) and WHO Headquarters in Geneva. Discussed topics included the current status of the Regional poliomyelitis laboratory network, recent changes to the WHO laboratory accreditation process, proposed changes to recommended laboratory methodologies, and the role of network laboratories in implementing the plan of action on wild poliovirus containment.
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1. INTRODUCTION

The first meeting on Laboratory Surveillance for Poliomyelitis Eradication in the Western Pacific Region was held in Manila from 16 to 18 October 1995, attended by 24 participants, including representatives from each of the national poliomyelitis laboratories in the Region, representatives from the Western Pacific Regional Reference Laboratories, a representative from the South-East Asia Regional Reference Laboratory, Thailand, and WHO staff from Geneva. The second meeting was held on 23 June 1997 also in Manila to discuss the accreditation of network laboratories, laboratory results reporting and information flow, the containment of virus stocks, and international packaging and shipping requirements for diagnostic specimens and virus isolates.

The two meetings were very successful in improving coordination and communications within the laboratory network. Standard procedures for laboratory processing of stool specimens, laboratory data management and results reporting have been developed and the poliomyelitis laboratory network in this Region is now fully established. Two years have now passed since the last wild poliovirus was isolated, and it is believed that wild poliovirus transmission has ceased. Laboratory workload for the past three years has remained stable with approximately 10,000 stool specimens of acute flaccid paralysis (AFP) cases being received and processed each year. Despite this high workload, however, laboratory performance standards have been consistently improving, thanks to the WHO laboratory accreditation scheme. The major tasks remaining for the poliomyelitis laboratory network are to maintain the high standard of work until global certification of poliomyelitis is attained, and to contribute to ensuring that all remaining stocks of wild poliovirus infectious materials are either destroyed or safely stored under maximum containment conditions.

Although most of the poliomyelitis network laboratories in the Region have attained accreditation status, and can be expected to retain that status over the coming years, some laboratories are still experiencing difficulties in achieving the required performance levels. During the third meeting, it was necessary to review the performance status of the Regional laboratory network as a whole, identify the remaining difficulties, and propose technical solutions. The process should be viewed as part of an overall review of the poliomyelitis surveillance system leading to development of a strategic plan for continued development of the poliomyelitis laboratory network, through the period of certification of poliomyelitis eradication and on to cessation of poliomyelitis immunization.

A list of meeting participants is provided in Annex 1.

2. OBJECTIVES

The objectives of the meeting were:

(1) to review the current status of the laboratory network with regard to required performance levels;

(2) to identify and document remaining problems and deficiencies in the network;
(3) to propose solutions to the remaining problems, or propose mechanisms by which solutions can be sought; and

(4) to review the plan of action and implementation guidelines for containment of laboratory stocks of wild poliovirus

3. PROCEEDINGS

3.1 Regional overview

As no new wild-poliovirus-associated case of poliomyelitis has been detected since March 1997, under conditions of high quality surveillance, there is good reason to believe that indigenous wild poliovirus transmission in the Western Pacific Region has finally ceased.

As of 13 July 1999, 6404 AFP cases with onset in 1998 were reported in the Region; 86% had two stool samples taken within 14 days of onset. Surveillance and supplementary immunization activities were intensified throughout 1997 and 1998. As a result, even though over 13 000 AFP cases have been investigated throughout the Region since the onset of the last poliomyelitis case on 19 March 1997, no new cases of poliomyelitis have been detected. Therefore, the Regional total for poliomyelitis is zero cases for 1998, under conditions of high quality surveillance. It has been the experience in the Western Pacific Region that the efforts to eradicate the last few remaining wild polioviruses are far more intense than they were at the beginning of the poliomyelitis eradication initiative.

The process for certifying the Region as poliomyelitis-free by the end of 2000 is well on its way. However, supplementary immunization will continue in high-risk areas, particularly in the waterways of Cambodia and Viet Nam where the last wild polioviruses were detected. High routine immunization coverage and high quality AFP surveillance will need to continue even after Regional certification until global certification is achieved.

At the third meeting of the Regional Certification Commission in August 1998 in Brunei Darussalam, the national plans of action of recently-endemic countries and the progress reports of non-endemic countries and the Pacific island countries and areas were reviewed and approved. The Commission endorsed the guidelines for investigation and response to wild poliovirus importation and the Regional plan of action for the safe handling and containment of polioviruses. Finally, the Commission endorsed the revised Manual of Operations for use by countries while submitting documentation for certification of poliomyelitis eradication in the year 2000.

3.2 South-East Asia Regional laboratory overview

The number of reported poliomyelitis cases in the Region decreased by 82% from 25 711 cases in 1998 to 4662 cases in 1999. Emerging poliomyelitis-free countries in SEAR now include Bhutan, Maldives, Sri Lanka (1993), Indonesia (1995), Myanmar (1996), and Thailand (1997). The remaining poliomyelitis-endemic countries - Bangladesh, Democratic People’s Republic of Korea, India, and Nepal - need to add extra rounds of high quality national immunization (NIDs) and implement expansive house-to-house, door-to-door, boat-to-boat poliomyelitis immunization campaigns.

The interruption of major chains of transmission of poliovirus through yearly high quality NIDs continues to receive priority in all SEAR member countries, and for the third consecutive year, NIDs were synchronized in South Asia in Bangladesh, Bhutan, India, Maldives, Myanmar, Nepal and Thailand.
During the past year there has been a dramatic improvement in the quality of AFP surveillance in many of the countries of the Region. India reached certification standards in AFP surveillance by reporting 9457 cases of AFP, thus achieving a non-poliomyelitis AFP rate of 1.45 per 100,000 children under 15 years of age. Similarly, Thailand was able to achieve a non-poliomyelitis rate exceeding 1 per 100,000 and Bangladesh doubled its rate to 0.31 per 100,000.

The South-East Asia Region (SEAR) laboratory network consists of four Regional Reference Laboratories: The National Institute of Communicable Diseases (NICD) in New Delhi, India; the Enterovirus Research Centre (ERC) in Mumbai, India; the Medical Research Institute (MRI) in Colombo, Sri Lanka; and the Virus Research Institute (VRI) in Bangkok, Thailand. There are seven National Laboratories in India, three in Indonesia, and Bangladesh and Myanmar each has a national laboratory. A poliomyelitis laboratory is currently being established in the Democratic People’s Republic of Korea.

In 1996, the network investigated 1418 stool specimens from AFP cases and 1044 specimens from contacts. In 1997, poliomyelitis network laboratories investigated 6376 specimens from AFP cases. In 1998 this total rose to 19,789 specimens. To date in 1999, poliomyelitis network laboratories have received 6263 specimens. The dramatic increase in stool specimens tested over the past two years has been due to improvements in AFP surveillance, particularly in India. In 1998 wild poliovirus was obtained from 2010 cases from two countries. Wild poliovirus types 1 and 3 were isolated from cases in India and Bangladesh. Wild poliovirus type 2 was isolated from 83 cases from northern India. Details of wild poliovirus isolations and interpretation of the situation in 1998 are given in the Table.

<table>
<thead>
<tr>
<th>Country</th>
<th>P 1/w</th>
<th>P 2/w</th>
<th>P 3/w</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangladesh</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>Ongoing p1/w and p3/w</td>
</tr>
<tr>
<td>DPR Korea</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Unconfirmed p1/w from 4 cases in 1996</td>
</tr>
<tr>
<td>India</td>
<td>731</td>
<td>8</td>
<td>90</td>
<td>Ongoing p1/w, p2/w and p3/w</td>
</tr>
<tr>
<td>Indonesia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>P1/w and p3/w isolated in 1995</td>
</tr>
<tr>
<td>Myanmar</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>P1/w and p3/w isolated in China in 1995/1996</td>
</tr>
<tr>
<td>Nepal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>P1/w isolated in 1996</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Last isolate in 1993</td>
</tr>
<tr>
<td>Thailand</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>P1/w isolated in 1996</td>
</tr>
<tr>
<td>Regional total</td>
<td>734</td>
<td>8</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>

Together with the increase in workload, the quality of laboratory work has been steadily improving. Poliomyelitis laboratories in Ahmedabad, Bangalore, and Mumbai and the
laboratories in Myanmar, Sri Lanka and Thailand, isolated non-polio enteroviruses in 10% or more of the stool specimens. However, more than half of the laboratories in the SEAR polio laboratory network need to improve isolation performance of the non-polio enteroviruses.

In 1998, the laboratories in Indonesia and Sri Lanka processed at least 80% of the stool specimens received in the 28-day turn around time recommended by WHO. In India, the high volume of specimens generated by expansion of field surveillance teams in 1998 produced large backlogs in some laboratories.

Priorities for 1999 include achieving full accreditation for all regional network laboratories and strengthening communication between the laboratories and the national programmes. To improve the quality of laboratory work, a protocol for in-house quality assurance will be developed and distributed to each laboratory for implementation. In anticipation of the requirements for containment of wild poliovirus stocks, each laboratory has been requested to maintain an inventory of wild poliovirus isolates and materials that may contain wild poliovirus, in accordance with the accreditation requirements.

3.3 Global overview

Good progress continues to be made towards the goal of global poliomyelitis eradication. Mass immunization campaigns intended to provide two doses of oral poliovirus vaccine to all children under five years of age are fundamental to the strategy of interrupting the transmission of poliovirus. At the end of 1997, only five countries where poliomyelitis was endemic had not conducted full NIDs. There are now only two countries in this category, and in 1998 both conducted subnational campaigns. NIDs were conducted in 84 countries in 1998, reaching over 450 million children.

Many countries in Africa have conducted NIDs for three years and most of the others have done so for two years. In South Central Asia, three NIDs have been organized in Afghanistan and Myanmar; Bangladesh, India and Nepal have conducted four NIDs, and Pakistan has conducted five. During one NID in India, 134 million children were reached. In 1998 the MECACAR countries of the Eastern Mediterranean and European Regions completed a fourth year of NIDs. Operation MECACAR Plus was initiated in 1999 with NIDs in selected high-risk countries and mopping-up campaigns in high-risk areas of other countries.

During the past year there was a marked improvement in AFP surveillance activities. A total of 21,508 AFP cases were reported in 1998, whereas in 1996 and 1997 the corresponding numbers were 13,857 and 17,365. These figures represent rates of 1.09, 0.57 and 0.72 cases per 100,000 children respectively in countries with AFP surveillance. Globally, 68% of AFP cases had two adequate stool specimens. AFP surveillance has been initiated in all countries where poliomyelitis is endemic. In some countries, however, surveillance does not extend beyond the major urban centres.

The reported incidence of poliomyelitis increased from 5,186 confirmed cases in 1997 to 5,298 in 1998. However, this figure should be interpreted with caution since reporting from a number of important countries was incomplete. Despite the slight increase in the number of confirmed cases, the geographical distribution of poliovirus continues to shrink. It is approaching two years since the last poliomyelitis case was identified in the Western Pacific Region, which is preparing to be certified free of the disease in 2000. In the European Region, wild poliovirus was identified during 1998 only in south-eastern Turkey, where there were 23 virologically confirmed cases; mopping-up immunization was conducted during the autumn of 1998 in this area and in bordering areas of Iran, Iraq, and Syria. Turkey was to conduct a full NID and mopping-up in 1999 and 2000.
To support surveillance and the detection of poliovirus, the global poliomyelitis laboratory network has continued to expand in size. The network now consists of 119 national and sub-national laboratories, 15 Regional reference laboratories and six global specialized laboratories. Development of network laboratory facilities has also continued, with essential supplies and equipment being provided to more than 40 laboratories. A new tissue culture cell line with increased specificity and sensitivity for the detection of polioviruses, the L20B cell line, has now been introduced in laboratories in all Regions. The annual poliomyelitis laboratory accreditation scheme has been continued and extended. In all, 80% of the laboratories have now been reviewed for accreditation, and 80% of these have passed the review.

Wild poliovirus is now largely confined to South Central Asia, West Africa, Central Africa and the Horn of Africa. In Asia, a major poliovirus reservoir exists in Afghanistan, Bangladesh, India, Nepal, and Pakistan; in 1998 these countries reported more than 90% of all poliomyelitis cases. Wild poliovirus type 2, the poliovirus type most susceptible to breaking its chain of transmission, was still circulating in at least three areas (Afghanistan, northern India and West Africa) in 1997 and 1998. A large outbreak of poliomyelitis associated with wild poliovirus type 2 has been reported in northern India in the first quarter of 1999. Wild poliovirus type 1 and type 3 transmission occurred in many countries in a wide belt running from West Africa to India and Bangladesh. There is an increasing amount of genomic sequence information available on the wild polioviruses that continue to circulate. The findings demonstrate the rapidly declining genomic diversity of all three poliovirus serotypes, and strongly suggests that there are less than ten remaining major foci for transmission of wild poliovirus.

The national laboratory reports are attached as Annexes 3 to 15 to the report.

3.4 Update on L20B distribution and recommendations for use

L20B cells have now been distributed from the global cell bank at National Institute for Biological Standards and Control (NIBSC) to 18 Regional distribution centres, and secondary cell banks have been established and validated at each centre. The Regional distribution centres have now either distributed, or are in the process of distributing the cells to all national and sub-national laboratories in the network. Many network laboratories now have more than 12 months’ experience in growing and using these cells.

There were concerns that some early batches of L20B cells were contaminated with mycoplasma, as one of the national laboratories found evidence of mycoplasma by PCR. After extensive investigation by culture, DNA staining, electron microscopy and PCR, the global cell bank can be certified as mycoplasma-free. It is clear that each Regional distribution centre must check its cell banks for mycoplasma, so that the cells distributed to national and sub-national laboratories can also be certified as mycoplasma-free.

A proposal for the use of L20B cells within the poliomyelitis eradication programme has now been developed and has been widely distributed. It is recommended that L20B cells replace Hep-2 cells in the primary isolation of poliovirus from stool specimens, and a flowchart describing the recommended protocol has been developed (Figure). It is now known that a small percentage of poliovirus isolates does not grow well in L20B cells on first passage, and may not produce recognizable CPE. They do, however, grow in RD cells, and on re-passage in L20B cells, these isolates produce recognizable CPE. It is important, therefore, that in order not to miss any Sabin virus, all cultures positive in RD cells but negative in L20B cells on first passage should be re-passaged in L20B cells.

Correct use of L20B cells within the programme should make it unnecessary for laboratories to carry out routine serotyping of non-poliomyelitis enteroviruses, as growth in L20B cells provides an effective screen for polioviruses present in poliomyelitis/non-poliomyelitis
mixtures. Many laboratories, however, will decide to continue to serotype non-poliomyelitis enteroviruses for their own reasons. It should also be borne in mind that the ability to detect non-poliomyelitis enteroviruses will continue to be monitored as one of the laboratory performance criteria.
It is now recognized that a small number of viruses other than polioviruses are also capable of growth or of producing CPE in L20B cells. These include adenoviruses, reoviruses and some non-poliomyelitis enteroviruses, particularly members of the Coxsackie A group. National laboratories that isolate non-polioviruses in L20B cells should inform their Regional reference laboratory. After consultation with the reference laboratory, non-polioymyelitis enteroviruses found to grow on L20B cells should be forwarded to the reference laboratories for identification and characterization. All data on non-polioviruses found to grow in L20B cells should be collated in the WHO Regional Office and forwarded to the NIBSC, Potters Bar, England, for analysis. Any viruses that remain unidentified by the Regional Reference Laboratories should also be forwarded to NIBSC.

In order to maintain confidence in sensitivity of the cells to poliovirus infection, NIBSC recommended that sub-cultures should not be used beyond 15 passages, but cells be replaced from stocks held in liquid nitrogen. For the same reason, it is important that regular tests of virus sensitivity should be standard procedure for tissue culture use.

3.5 Changes to the laboratory accreditation process and proficiency test panels

3.5.1 Laboratory accreditation

All of the national and regional reference laboratories in the Western Pacific Region, with one exception, have now been reviewed for WHO laboratory accreditation. In addition to providing documentation on the proficiency of the laboratories, the accreditation process itself has been a factor in the improvement of laboratory performance. Accreditation has encouraged laboratories to evaluate and document the procedures they are using and to identify areas of weakness. It has also identified resource requirements, such as equipment, supplies and laboratory staff. The most significant areas of weakness that have been exposed by the accreditation process include lack of sensitivity in isolating viruses (both poliomyelitis and non-poliomyelitis enteroviruses), delays in reporting of results, poor laboratory management structure and poor data management. Technical and procedural problems have also been disclosed, particularly related to the use and maintenance of laboratory equipment. For those laboratories that fail to be accredited, visits by laboratory experts have been organized and work plans and timetables have been developed to address their areas of weakness. Their resource requirements have been reassessed and training programmes have been arranged for the laboratory staff.

The accreditation process has provided a strong stimulus to improve the quality of laboratory performance, but it is now apparent that some refinement of the process is required. The first is the formal acceptance of the category of provisionally accredited. This category is applied to laboratories that have passed the most recent proficiency test, but failed to reach one of the other performance criteria (reporting time, number of specimens tested, non-poliomyelitis enterovirus isolation rate, confirmation of isolates, review of operating procedures and work practices) required for full accreditation. The Regional laboratory coordinator makes the decision to award provisional accreditation status. A clear description of why a laboratory failed to achieve full accreditation must be given, and a detailed plan of action developed to improve laboratory performance within the next 12 months. The laboratory must receive another accreditation review visit by the end of that year. A laboratory that again fails to meet all six accreditation criteria cannot be given provisional accreditation status for a second year in succession. Such laboratories must be given the status of “not accredited”, and an accredited laboratory must confirm the test results on all specimens from AFP cases that are reported by the non-accredited laboratory.

The respective roles and responsibilities of the regional and global laboratory coordinators have been ambiguous and must be made clear. Accreditation of national and recognized sub-national laboratories is the responsibility of the WHO Regional Offices. Ideally, all national and recognized sub-national laboratories should be visited by the Regional poliomyelitis laboratory.
coordinator at least once every year. Where this is not possible, laboratory consultants or other experts should visit the laboratories on behalf of WHO. These visits should be combined with the annual accreditation review visit. On completion of an accreditation review, the WHO Regional Office should be informed of the result. It is the responsibility of the Regional Office to make official announcement of the accreditation review result. Accreditation of Regional reference laboratories is the responsibility of WHO Headquarters. Ideally, the WHO global laboratory coordinator accompanied by the Regional laboratory coordinator will make accreditation review visits to all Regional reference laboratories. Where this is not possible, the visit should be made by the Regional laboratory coordinator accompanied by a representative acting on behalf of WHO/HQ.

For laboratories that serve as both national and Regional reference laboratories, it has been agreed that laboratories should be accredited only at their highest designated level. Therefore, Regional reference laboratories that also act as national laboratories should be accredited only as Regional laboratories, not as National laboratories.

It is now apparent that several laboratories in the network are capable of carrying out intratypic differentiation, although they are not regarded as Regional reference laboratories. In many instances it would be of benefit to the network to have these laboratories carry out intratypic differentiation, reducing the workload on the Regional reference laboratories. It is not appropriate, however, that these laboratories themselves become Regional reference laboratories. A mechanism is being developed to recognize the proficiency of these laboratories in carrying out intratypic differentiation while retaining their status of on network of national laboratories. The current accreditation checklists for both national and Regional reference laboratories are being reviewed and will be revised appropriately. The proposed revisions were presented for discussion at the Global Poliomyelitis Laboratory meeting held in Geneva from 29 September to 1 October 1999.

Replacement of HEp2 cells with L20B cells is expected to result in a general decrease in the non-polio enterovirus (NPEV) isolation rate for many laboratories. Although this decrease is expected to be small, it again brings into question the relevance of the long-standing performance-monitoring criterion of a minimum NPEV isolation rate of 10%. It has been proposed that the accreditation requirement of a minimum annual 10% NPEV isolation rate be dropped and replaced by documentation of regular in-house quality control checks.

3.5.2 Proficiency test panels

Based on the recommended use of L20B cells for the isolation of polioviruses, a revised scoring system for proficiency testing has been devised. The following rules will be applied to all proficiency tests carried out in Regional network laboratories:

1. Reporting the correct polioviruses in a sample scores 30 points, reporting the correct result of poliovirus negative samples scores 5 points.

2. Contamination with poliovirus or missing the isolation of poliovirus in a sample results in a 20-point deduction.

3. Reporting any enterovirus in an enterovirus-negative sample results in a 10-point deduction.

4. Typing of enteroviruses and identification of enteroviruses in mixtures with polioviruses results in no extra score.
The score is calculated as the fraction of the achieved points per maximum number of points that can be obtained (for example: \(\frac{100}{130} = 76\%\)).

Reports have to be sent within 42 days after confirmation of receipt of panel. For every week or part of a week that the results are reported later than the agreed date, 5\% of the total maximum score will be subtracted.

It is now irresponsible of the poliomyelitis eradication initiative to be distributing infectious wild poliovirus isolates in areas of the world that are free of circulating wild poliovirus. Furthermore, to comply with requirements of Phase I of the Plan of Action on wild poliovirus containment, laboratories in the Americas, Western Pacific and European Regions of WHO should no longer receive proficiency test panels containing infectious wild polioviruses. There remains, however, a requirement for regional reference laboratories in these regions to be able to carry out intratypic differentiation (ITD) assays for wild poliovirus and to be able to demonstrate proficiency in detecting and correctly identifying wild polioviruses. To this end, inactivated virus controls for use in the ELISA ITD and appropriate genomic sequence material for use in nucleic acid probe hybridisation and diagnostic PCR ITD have been developed. Proficiency test panels using the proposed non-infectious wild poliovirus controls were developed and distributed to a small number of selected Regional reference laboratories in July 1999. Recipient laboratories tested the panels and reported the results in September 1999, so that results could be available for discussion during the Global Poliomyelitis Laboratory meeting.

Correct use of L20B cells should make it unnecessary for laboratories to carry out routine serotyping of NPEVs, as growth in L20B cells provides an effective screen for polioviruses present in poliomyelitis/non-poliomyelitis mixtures. The ability to detect non-poliomyelitis enteroviruses will continue to be monitored as one of the laboratory performance criteria; however, the requirement to confirm the ability to type NPEVs in the proficiency tests becomes questionable. It has, therefore, been proposed that the proficiency test scoring system be adjusted so that laboratories correctly isolate non-poliomyelitis enteroviruses, but reporting them simply as untyped NPEV should not be penalized. Furthermore, laboratories reporting only poliovirus in poliovirus/NPEV mixtures should not be penalized for failing to report the NPEV. A new scoring system has been developed and has been in use since July 1999.

3.6 Containment of wild polioviruses

Once poliomyelitis is eradicated, the laboratories of the world will be the only remaining source of the virus. Safe handling and, ultimately, maximum containment of poliovirus and potentially infectious materials in the laboratory is crucial. Until now, poliovirus biosafety concerns have been minimal. Universal immunization with inactivated poliovirus vaccine (IPV) or oral poliovirus vaccine (OPV) has reduced the risk of disease for laboratory workers and the general public. Current technologies and biosafety practices have further reduced the risks of poliovirus contamination of the environment.

The probability of a laboratory-associated poliovirus infection is small, but the consequences of an infection grow greater with time. A chance reintroduction of wild poliovirus from the laboratory into the community after cessation of transmission presents a threat to poliomyelitis eradication. A chance reintroduction of wild poliovirus after cessation of immunization presents a threat to public health of global proportions. The world now faces the formidable, but not insurmountable, challenge of locating the many laboratories that have wild poliovirus infectious, or potentially infectious, materials and ensuring that they are adequately contained in the laboratory, rendered non-infectious, or destroyed. The required action and timetable for implementation are described in the WHO Global Action Plan for Laboratory Containment of Wild Polioviruses.
Guidelines for implementing Phase I of the Global Action Plan for Laboratory Containment of Wild Polioviruses have now been developed and are being field-tested in the Western Pacific Region. The guidelines describe how to carry out two critical steps in the implementation of the initial phase of containment: surveying all medical/biological laboratories that might possess wild poliovirus infectious and/or potentially infectious materials and establishing a global inventory system for laboratories that retain such materials.

The purposes of the global survey are:

- to acquaint all biomedical laboratories with the Global Action Plan;
- to effect disposal of all wild poliovirus infectious and/or potentially infectious materials no longer needed by the laboratory;
- to ensure safe handling of all such materials; and
- to establish a global inventory of laboratories that retain such materials.

The survey is hierarchical, beginning with notification by WHO and proceeding through ministries of health, agencies and institutions, to the laboratories. Because many laboratories that might possess such materials are outside the health sector, completion of the survey will require ministries of health to enlist the co-operation of other ministries, including education, defense, and the environment.

The purposes of the inventory are:

- to document location and type of wild poliovirus infectious and/or potentially infectious materials being retained;
- to meet the country requirements for Regions to be certified as poliomyelitis free; and
- to maintain a current list of laboratories for notification to initiate containment procedures one year after detection of the last wild poliovirus.

The data for the inventory system are obtained from the global survey, beginning with a thorough search by each laboratory for any materials in its possession that meet the definition of wild poliovirus infectious or potentially infectious materials. The laboratory submits a complete list of all such materials it intends to retain, to its parent agency/institutional inventory. Data from all laboratories listed on the latter are included in the national inventory, maintained by each country. Summary data from the national inventory are submitted to the appropriate WHO Regional office to be included in the Regional inventory, which, in turn, compiles the data for the WHO global inventory.

The Regional survey of all biomedical laboratories and agency/institutional and national inventories should be complete before the end of year 2000, in keeping with the current progress on poliomyelitis eradication and certification of the Region as poliomyelitis-free. The data from the national inventory should be submitted to the national committee for the certification of the eradication of poliomyelitis as well as to WHO. Completion of a national inventory from all member states in a WHO Region is a prerequisite for certification of the Region as poliomyelitis-free.

3.7 Technical issues
3.7.1 Non-typable non-poliomyelitis enteroviruses

Throughout the poliomyelitis laboratory network, non-poliomyelitis enteroviruses that cannot be typed with conventional enterovirus typing panels are increasing. These viruses are strongly associated with aseptic meningitis, neonatal sepsis-like disease, acute conjunctivitis, and some, such as enterovirus 71, can be strongly associated with AFP. In all, there are almost 70 recognized serotypes of human enteroviruses, and the standardized WHO typing pools contain antisera to only a subset of these. Generating and distributing panels containing antisera to all known enteroviruses is not a practical proposition, partly because of the antigenic heterogeneity within serotypes and the rapid progress of antigenic drift between serotypes. A possible solution may be provided by the use of molecular methods for typing enteroviruses using PCR. Specialized laboratories within the network are currently investigating these methods.

3.7.2 Tissue culture growth media formulations

With the introduction of L20B cells it has become apparent that recommendations on tissue culture growth medium composition are not clear and need to be reassessed. Investigations on the media used in different network laboratories have revealed that there are large variations in media composition and that these variations have largely unknown comparable effects on cells: their growth, maintenance and susceptibility to virus infection. The key media components are the salts (Earle’s or Hank’s), the bovine serum, L-glutamine, sodium bicarbonate, HEPES buffer, and antibiotics. Common variations in media formulations have been reviewed and preliminary evaluations of effects of variation in key components have been made. Revised recommendations were presented at the Global Poliomyelitis Laboratory Network meeting in September 1999.

3.7.3 In-house quality control

All poliomyelitis network laboratories are subject to annual on-site review for accreditation, and carry out at least one proficiency testing exercise each year. However, the accreditation reviews and proficiency tests do not provide a full monitor of routine accuracy and sensitivity. Confidence in these aspects of laboratory activity can be provided by demonstration of good laboratory practice, an essential element of which is in-house quality control. Some aspects of quality control are already incorporated into the formal reporting system (condition of specimens on arrival, NPEV isolation rate, timeliness of reporting) or are assessed at review (laboratory layout, cell quality, media labelling and storage, record keeping, temperature monitoring, etc.) but many other aspects of quality control cannot be monitored in this way. One obvious example is routine testing of cell cultures for sensitivity to viral infection, or for mycoplasma contamination. Another example may be routine proficiency or virus isolation and characterization. Guidelines on proposed in-house quality control activities were prepared and presented at the Global Poliomyelitis Laboratory Network meeting held from 29 September to 1 October 1999.

3.8 Extending the laboratory network

While the eradication of poliovirus remains the main focus of the laboratory network, it is becoming increasingly appropriate to extend the activities of the poliomyelitis laboratories to include surveillance of other diseases. The laboratory has two main functions in control of measles: monitoring and verifying virus transmission through confirmation of outbreaks, confirmation of cases and identification of measles virus strains; and monitoring the susceptibility profiles of populations through serosurveys. Many network laboratories already participate in programmes for the control and elimination of measles. Other laboratories will be drawn into this activity over the coming years. As yet, the nature and extent of a measles laboratory network have to be fully determined, but it is clear that the five main priorities of the network will be:
to develop standards for laboratory diagnosis of measles;

• to establish mechanisms for confirming and referring specimens;

• to establish training resources;

• to provide reference materials; and

• to provide databases of epidemics and virus information.

It is clear that many poliomyelitis network laboratories will eventually be included in a network for measles and/or other rash and fever diseases.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Overview

It is now more than two years since the last wild poliovirus was isolated in the Western Pacific Region. The next milestone in the Regional poliomyelitis eradication initiative will be certification of poliomyelitis-free status, and the Regional poliomyelitis laboratory network will have an important part to play in gaining certification. Although wild poliovirus has been eliminated from this Region, all three serotypes of poliovirus continue to circulate in other parts of the world, particularly the Indian sub-continent and Africa. The risk of importation of wild poliovirus into this Region remains high, and surveillance, particularly in border and known high-risk areas, must be maintained at the highest level.

Recommendation

All network laboratories should endeavour to maintain the high standard of virological surveillance required to detect importation of wild poliovirus and to meet the requirements for Regional certification of poliomyelitis-free status.

4.2 National laboratory reports

Many laboratories continue to experience severe staffing problems due to high turnover of experienced staff. Equipment maintenance and replacement, obtaining high quality media and reagents, and securing support for laboratory activities are common problems encountered in network laboratories. The WHO Regional office plays a key role in collaborating with laboratories to overcome these problems, and the position of Regional Poliomyelitis Laboratory Coordinator is now essential to maintaining the Regional poliomyelitis laboratory network.

As a requirement for certification of poliomyelitis-free status, all poliovirus isolates, regardless of origin, must undergo intratypic differentiation to demonstrate that they are not wild poliovirus. Several national poliomyelitis laboratories in the Region are now capable of carrying out intratypic differentiation of poliovirus isolates, and these laboratories could be used for this task, decreasing the workload placed upon the Regional reference laboratories.

Recommendations
• WHO should endeavour to fill the vacant position of Regional Poliomyelitis Laboratory Coordinator as soon as possible.

• WHO should investigate the possibility of increasing the number of laboratories accredited to carry out intratypic differentiation to include selected national poliomyelitis laboratories. These laboratories could be expected to carry out ITD on poliovirus isolates from non-AFP cases.

• All member countries must ensure that every poliovirus isolate, regardless of source of isolation, must be subject to intratypic differentiation in an accredited laboratory to prove that it is not wild poliovirus.

4.3 Cell lines and isolation

L20B cells have now been distributed to all network laboratories in the Region. These cells are very easy to use and will allow laboratories to reach the required timeliness indicators more easily. Following the distribution of L20B cells, there is an urgent need for distribution of new, well-characterized RD cells. This will be coordinated at the global level, and new cells will be available for distribution to all laboratories shortly.

Recommendations

• All laboratories should use a minimum of two cell lines (L20B and RD) for isolation of polioviruses from stool samples, according to the WHO recommendations.

• All RD-positive, L20B-negative isolates must be passaged once in L20B cells before the presence of poliovirus can be excluded.

• Non-poliovirus isolates found to grow in L20B cells should be reported to the Regional reference laboratory for confirmation, identification and possible inclusion in the global data bank.

4.4 Accreditation and proficiency

With one exception, all laboratories in the Region have now been through the accreditation process at least once. All laboratories are operating at a high standard of performance. Recent changes to the accreditation process including the inclusion of the category of “provisional accreditation” have been implemented. Further modifications to improve the system were discussed at the Global Poliomyelitis Laboratory Network meeting in September 1999.

Since wild poliovirus has been eliminated from this Region, it is no longer acceptable to distribute proficiency test panels for intratypic differentiation that contain infectious wild poliovirus. Alternative panel components that contain non-infectious wild poliovirus markers have been developed and will be distributed to the Regional reference laboratories in 2000.

Recommendation

All laboratories must rapidly communicate confirmation of receipt (or non-receipt) of proficiency test panels.

4.5 Containment of wild polioviruses

The Region has a goal of certification of poliomyelitis-free status by the end of the year 2000. This leaves very little time to implement Phase I of the containment plan, and urgent action
is required to initiate this process in all Member States as soon as possible. In many Member States, there are many laboratories outside of the authority of ministries of health that may have wild poliovirus infectious or potentially infectious materials. Mechanisms must be developed in each country to obtain inventories of all laboratories so that the action plan on containment can be implemented.

**Recommendations**

- The WHO Regional Office should send a letter to the heads of government of all Member States explaining the requirements for poliovirus containment and requesting that they nominate an official body or individual to take responsibility for coordination of containment.

- WHO should investigate the possibility of providing technical consultants to countries that face particular problems in implementing the containment plan of action.

- All network laboratory staff should be familiar with the WHO Plan of Action and guidelines on containment so that they can act as a technical resource in their country.

4.6 **Technical issues**

Throughout the poliomyelitis laboratory network, non-poliomyelitis enteroviruses that cannot be typed with conventional enterovirus typing panels are increasing. Some of these enteroviruses, such as enterovirus 71, can be strongly associated with AFP. Specialized laboratories in the network are investigating these viruses and producing specific reagents for their characterization.

An essential element of good laboratory practice is in-house quality control, and all network laboratories should be carrying out routine in-house quality control activities to ensure that the high standards of laboratory performance are maintained. WHO guidelines on establishing in-house quality control activities were provided before the end of 1999.

**Recommendations**

- All laboratories should use WHO recommended methods for isolation and characterization of polioviruses.

- All network laboratories should be carrying out in-house quality control activities.

- WHO should provide an updated poliovirus laboratory manual, containing all recent changes to recommended methodologies, as soon as possible.

4.7 **Extending the laboratory network**

While the eradication of poliovirus remains the main focus of the laboratory network, it is becoming increasingly relevant to extend the activities of the poliomyelitis laboratories to include surveillance for other diseases. Many network laboratories already participate in programmes for the control and elimination of measles. Other laboratories will be drawn into this activity over the coming years. As yet, the nature and extent of a measles laboratory network have not been determined, but it is clear that many poliomyelitis network laboratories will be included in that network.

**Recommendation**
Poliomyelitis laboratories should participate in surveillance activities of other infectious diseases.
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Introduction

- The Division of Virology, in the Institute for Medical Research continues to function as the National WHO Poliomyelitis Laboratory for Malaysia.
- Our last indigenous wild poliovirus was isolated in year 1984.
- Imported cases of wild poliovirus isolated in 1992 (1 case) and in 1993 (1 case).

Source of Antisera Cells

<table>
<thead>
<tr>
<th>Source</th>
<th>Antisera/cells</th>
</tr>
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<tbody>
<tr>
<td>Manila</td>
<td>RIVM Polio pool Antisera</td>
</tr>
<tr>
<td></td>
<td>ECHO pool Antisera (A-G)</td>
</tr>
<tr>
<td></td>
<td>Coxsackie B pool Antisera</td>
</tr>
<tr>
<td>Japan</td>
<td>NIH EV 71 Antisera</td>
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<tr>
<td></td>
<td>Coxsackie B1-B6</td>
</tr>
<tr>
<td>Melbourne</td>
<td>VIDRL RD</td>
</tr>
<tr>
<td></td>
<td>L 20B</td>
</tr>
<tr>
<td></td>
<td>Hep-2C</td>
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Competency

<table>
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<tr>
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<th>1997</th>
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<th>1999</th>
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<tbody>
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<td>Results &lt; 28 days</td>
<td>68%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Results &gt; 28 days</td>
<td>32%</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td>NPEV Rate</td>
<td>13.9%</td>
<td>2.08%</td>
<td>2.04%</td>
</tr>
<tr>
<td>Proficiency Test</td>
<td>(i) 73.2%</td>
<td>93.4%</td>
<td>Pending</td>
</tr>
<tr>
<td></td>
<td>(ii) 84%</td>
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</tbody>
</table>
Facilities

Space
Equipment Adequate
Reagents

Last RD and L20 B supply

1 April 1999 from National Polio Laboratory, VIDRL
North Melbourne Victoria, Australia

Problems

(1) **High Staff Turnover**

1 Scientific Officer, 1 Part-time technical staff will remain.

(2) **New staff will require training**

Extramural training to be continued.

(3) **Facilities, funding, reagent – No major problems**