Fourth informal consultation on the polio laboratory network

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<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>AFP</td>
<td>acute flaccid paralysis</td>
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<tr>
<td>AFR</td>
<td>WHO African Region</td>
</tr>
<tr>
<td>AMR</td>
<td>WHO Region of the Americas</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention, United States</td>
</tr>
<tr>
<td>CPE</td>
<td>cytopathic effect</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EMR</td>
<td>WHO Eastern Mediterranean Region</td>
</tr>
<tr>
<td>EUR</td>
<td>WHO European Region</td>
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<tr>
<td>IPV</td>
<td>inactivated polio vaccine</td>
</tr>
<tr>
<td>MEM</td>
<td>minimum essential medium</td>
</tr>
<tr>
<td>OPV</td>
<td>oral polio vaccine</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
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<td>regional reference laboratory</td>
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<td>VDPV</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>WPR</td>
<td>WHO Western Pacific Region</td>
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Objectives

1. To accelerate progress towards achieving the availability of the services of an accredited Network Laboratory to every country.
2. To review the accreditation status of laboratories and make recommendations on further developing the accreditation process.
3. To review the status of distribution of L20B cells and develop guidelines on their use in the isolation of polioviruses.
4. To review current genomic sequencing information on circulation of wild polioviruses.
5. To review the standard network recommendations and practices of intratypic differentiation in the network laboratories.
6. To review the status of studies on the role of environmental surveillance for wild polioviruses.
7. To review the rational and status of data management and communication within the laboratory network.
8. To discuss the global action plan for containment of laboratory stocks of wild polioviruses.
9. To review the annual plans of action for the regional laboratory networks.

The meeting was opened by Dr B. Melgaard, Director GPV, on behalf of the Director-General Dr Gro Harlem Brundtland. The meeting agenda and list of participants is attached (Annexes 1 and 2). Dr Anton van Loon, University Hospital Utrecht, Netherlands was elected as Chairman and Dr Mark Pallansch, Centers for Disease Control and Prevention, Atlanta as Rapporteur.
1. Global overview of progress towards polio eradication

Reported global coverage with three doses of oral polio vaccine has been maintained at a high level for the past eight years, and almost all countries that need to conduct supplementary immunization activities have either done so or have plans to conduct these activities within the coming few months. In addition, there is an increasing tendency for the timing of supplementary immunization activities to be coordinated between adjacent countries and areas.

The quality of acute flaccid paralysis (AFP) surveillance has also improved dramatically over the past few years, particularly in India and some of the countries in Africa. Almost all countries known or suspected to have endemic polio are now conducting some form of AFP surveillance, and work is in hand to develop surveillance systems for those countries that still need them. There is no room for complacency however, as it is believed that at least 52 countries remain endemic for polio, with at least eight major foci of transmission. Seven of the remaining endemic countries are in areas of conflict, and these require special strategies to ensure the success of the polio eradication programme. 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 early 1998. Wild poliovirus type 1 and type 3 transmission occurred in many countries in a wide belt running from West Africa to India and Bangladesh.

Priorities for 1998 and 1999 are to interrupt wild poliovirus transmission in all remaining infected countries, to strengthen the global AFP surveillance system, to ensure that all countries have access to accredited polio laboratories, to begin the process of certifying the global eradication on polio, and to advocate for more funding support.
2. Accreditation of Global Polio Network Laboratories

2.1 Current status of laboratory network accreditation

Of the 138 laboratories listed as WHO polio laboratories, 87 have been accredited or provisionally accredited for 1998 (Table 1). In all, 80% of the laboratories have now been reviewed for accreditation, and 80% of these have passed the review. 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 polio and non-polio 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 from laboratory experts have been organized, work plans and timetables developed to address the areas of weakness, resource requirements reassessed and training arranged for laboratory staff.

Table 1. Accreditation status of network laboratories by WHO Region

<table>
<thead>
<tr>
<th>Region</th>
<th>Type of laboratory</th>
<th>Number of laboratories</th>
<th>Passed</th>
<th>Provisional pass</th>
<th>Non-accredited</th>
<th>Pending</th>
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<td>1</td>
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<tr>
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<td>138</td>
<td>74</td>
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2.2 Proposed changes to the accreditation process

The accreditation process has provided a strong stimulus to improving the quality of laboratory performance, but it is now apparent that some refinement of the process is required. The first is formal acceptance of the category of provisionally accredited. This category would apply to laboratories that have passed the most recent proficiency test, but fail to reach one of the other performance criteria (reporting time, number of specimens tested, non-polio enterovirus isolation rate, confirmation of isolates, review of operating procedures and work practices) required for full accreditation. The decision to award provisional accreditation status would be made by the Regional Laboratory Coordinator. A clear description of why the laboratory failed to achieve full accreditation would be given, and a detailed plan of action developed to improve laboratory performance within the next 12 months.

The respective roles and responsibilities of the Regional and Global Laboratory coordinators have to be made clear, and guidelines should be distributed as soon as possible. For the laboratories that serve as both National and Regional Reference Laboratories, a decision must be made as to whether these laboratories should be accredited twice, as both National and Regional Laboratories, or once at their highest designated level. It was agreed that Regional Reference Laboratories should be accredited only as Regional Laboratories, not as National Laboratories. It was also agreed that accredited laboratories that continued to meet the first five required performance criteria would not necessarily require review visits. The Regional Laboratory Coordinator should make the decision whether or not to carry out an accreditation review visit, but may consult with the Global Laboratory Coordinator if uncertain of the requirement.

As yet, no accreditation procedure has been proposed for the Global Specialized Laboratories.

2.3 Recommendations

2.3.1 The additional category of provisionally accredited should be utilized for laboratories that achieve a passing score in the proficiency panel test, and meet four of the five remaining criteria. Provisional accreditation is given at the discretion of the Regional Laboratory Coordinator. A detailed plan of action must be developed to resolve problems within one year.

2.3.2 Results of accreditation exercises, including comments, should be monitored by WHO both regionally and globally to assure consistent application of accreditation criteria throughout the Network.

2.3.3 Accreditation criteria should be refined to include guidelines on accreditation of Regional Reference Laboratories, responsibilities of Regional and Global Laboratory Coordinators, requirements for yearly accreditation reviews, and procedures for laboratories which repeatedly fail to achieve accreditation.

2.3.4 Procedures should be established for review of accredited laboratories that fail a proficiency panel test.

2.3.5 Criteria and a timetable for the accreditation of Global Specialized Laboratories should be developed and presented at the next informal consultation on the polio laboratory network.
3. Molecular epidemiology of wild poliovirus circulation

3.1 Global overview

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 suggest that there are only eight remaining major foci for transmission of wild poliovirus. This information is becoming of increasing importance in the final stages of the eradication programme, allowing the tracking of virus genotypes from one area to another, and the focusing of resources on the most appropriate areas.

3.2 Regional review - Africa

Molecular sequence information is now available on many of the recent African wild poliovirus isolates. Wild poliovirus type 2 was isolated in Benin in late 1997 and in Nigeria in early 1998. Isolates from the two countries belong to different genotypes and are not closely related, suggesting at least two independent foci of transmission in west Africa. Comparison of these isolates to other wild poliovirus type 2 isolates from elsewhere in the world suggests that the West African strains are indigenous to Africa. All available evidence demonstrates extensive wild poliovirus type 1 circulation in western and central Africa, with significant cross-border transmission in western Africa. Four indigenous African poliovirus type 1 genotypes have been in circulation in sub-Saharan Africa since 1995, the majority of recent virus isolates belonging to the genotype designated as West African. As of the beginning of 1998 at least five different lineages of the West African genotype had been identified, suggesting that even national immunization days (NIDs) the number of wild poliovirus type 1 strains in circulation in west and central Africa had not been significantly reduced.

At least four reservoirs of circulating wild poliovirus type 3 in sub-Saharan Africa (Cameroon, Central African Republic, Nigeria and Togo) exist, representing at least three distinct genotypes. Viruses isolated from Madagascar in 1995 and 1997 suggest the recent existence of at least two separate reservoirs of wild poliovirus type 3 of a genotype unique to Madagascar and unrelated to other African genotypes.
3.3 Country reviews

Egypt

Wild poliovirus type 2 isolates have not been detected in Egypt for many years. The last wild poliovirus type 3-associated case in Egypt was detected in December 1996. Sequence data from 12 type 3 isolates from 1996 suggest that all viruses belong to the same genotype, with two distinct lineages. There was no clear geographical separation of these two lineages however.

Wild poliovirus type 1 has continued to be isolated through to the end of 1997, with all recent isolates belonging to the same genotype. The amount of sequence divergence between the isolates has been shown to decrease with time, being 4 to 5% in 1995 to less than 1% in 1997. Despite the small amount of genomic diversity, the 1997 isolates could be grouped into two distinct genetic clusters. There was no clear geographic separation of these two clusters, however.

Pakistan

The last wild poliovirus type 2 isolate in Pakistan came from a case with onset of paralysis in April 1997. This virus was very similar to viruses isolated from cases in Afghanistan, but distinct from recent wild poliovirus type 2 isolates from India. Wild poliovirus type 3 continued to circulate in Pakistan at least to the end of 1997, but very little genomic sequence information is available on the isolates that have been obtained. Limited information indicates that all recent isolates are related, and suggests a relationship with wild poliovirus type 3 isolates from northwestern India.

All wild poliovirus type 1 isolates from the past two years belong to the same genotype, contrasting with the four genotypes reported in 1990. Viruses belonging to this genotype have also been detected in the Islamic Republic of Iran, but are distinct from those recently reported in India. The 1997 Pakistan isolates fall into three main genetic clusters, which are similar to those reported from The Islamic Republic of Iran. Very little information is available on wild poliovirus type 1 isolates from Afghanistan, but it is suspected that they are very similar to viruses circulating in Iran and Pakistan.

Turkey

Two wild poliovirus type 3 and 28 wild poliovirus type 1 isolates have been obtained from the southeastern part of Turkey in the past two years. Available molecular sequence information suggests that all wild poliovirus type 1 isolates belong to the same genotype, but form two clusters. There is clear evidence that this group of viruses has been in circulation in the area for at least the past eight years. The 1998 Turkish wild poliovirus type 1 isolates are similar to viruses detected in the adjacent border area of Iraq in 1997.

3.4 Recommendation

3.4.1 Greater use should be made of information obtained from virological surveillance in directing and targeting activities in the eradication of polioviruses and in planning the final stages of the eradication programme.
4. Review of the Manual for the virological investigation of poliomyelitis

4.1 Standardization of cell culture media and techniques

The Manual for the virological investigation of poliomyelitis\(^1\) was first produced in 1990, and although a revision has been distributed, clear recommendations on the composition of tissue culture media used for growth of cells and virus are needed. As a result, there is now a large variation in the media composition being used within the laboratory network. These variations include the type of basic salts solution used (Earle's or Hank's), type and amount of serum supplements used, antibiotics used, and concentrations of glutamine, bicarbonate, and HEPES buffer. These variations have largely unknown effects on cell growth, maintenance and sustainability. The effects on sensitivity to virus infection are also unknown.

There are clearly advantages to the programme in making recommendations on the standard media to be used. Recent studies carried out at the Centers for Disease Control and Prevention (CDC), Atlanta, have investigated the effect of various changes in media composition. For cell growth, they have found that the most consistent results were obtained using MEM with Earle's salts, with 2 mM glutamine, penicillin and streptomycin as antibiotics, and with 10% fetal bovine serum as growth medium supplement (2% in maintenance medium). Investigations on the effects of using MEM with Hank's salts, the HEPES and bicarbonate concentrations, and incubation with or without a 5% carbon dioxide atmosphere, are continuing. The team found that in both RD and L20B cells, the concentration of fetal bovine serum supplement in the growth medium could be varied between 5% and 10% without affecting sensitivity to virus infection. The effect of different concentrations of fetal bovine serum in virus titration assays, however, has not been investigated.

The sensitivity of a cell line can be described as the property to detect virus by growth in the cells resulting in recognized cytopathic effect (CPE). Studies were undertaken by CDC, Atlanta, to compare the sensitivity of four widely-available cell lines (RD (CDC), RD (RIVM), L20B, HEp-2(C)) to infection by various titres of Sabin poliovirus. Under the conditions used at CDC, RD (CDC) cells were found to be more sensitive to Sabin poliovirus infection than RD (RIVM), RD cells were more sensitive than L20B cells, and L20B cells were more sensitive than HEp-2(C) cells.

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\(^1\) Printed as an informal document available from the WHO V&I Document Centre, ordering code WHO/EPI/GEN/97.01.
4.2 Review of L20B distribution and planned introduction

L20B cells have now been distributed from the global cell bank at 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. L20B cells have been distributed currently to all anglophone laboratories in the WHO Regions for Africa, the Americas, the Eastern Mediterranean, South-East Asia and the Western, plus Bangui and the majority of laboratories in the European Region.

There have been concerns that some distributed 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, however, 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. This raised the question of standard methodologies for mycoplasma testing, and the urgent requirement for establishing guidelines for routine testing.

It was reported that Sabin polioviruses grown in L20B cells can acquire neurovirulence markers. However, this also happens when Sabin viruses are grown in other cell lines, such as primary monkey kidney, Vero and human fibroblasts, and also in vivo in the human gut. It is currently believed that this does not represent a significant biosafety problem, and that standard BSL-2/polio containment measures should be adequate. Laboratory staff should be aware, however, that reversion to neurovirulence could occur and ensure that the appropriate safety measures are enforced.

A proposal for the use of L20B cells within the polio eradication programme was presented. It was proposed that L20B cells replace HEp-2 cells in the primary isolation of poliovirus from stool specimens, and a flowchart describing the recommended protocol was discussed (Figure 1). A significant feature of the proposed protocol was that any culture positive in RD cells but negative in L20B cells on first passage should be re-passaged in L20B cells. A small percentage of Sabin poliovirus isolates have been observed not to 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.

Use of L20B cells within the programme should make it unnecessary for laboratories to carry out routine serotyping of non-polio enteroviruses, as growth in L20B cells provides an effective screen for polioviruses present in polio/non-polio mixtures. Many laboratories, however, will decide to continue to serotype non-polio enteroviruses for their own reasons. It should also be borne in mind that the ability to detect non-polio enteroviruses will continue to be monitored as one of the laboratory performance criteria, and the ability to type non-polio enteroviruses will continue to be evaluated in the proficiency tests.
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.
4.3 Comparative sensitivity of L20B and L α cells

A n investigation of the comparative sensitivity of two cells lines, L20B and L α, expressing the human cellular gene for the poliovirus receptor, has been carried out by the National Institute for Infectious Diseases (NIID), Tokyo, Japan. These studies have shown that the overall efficiencies of isolation for all three serotypes of poliovirus are the same in both cell lines, but that the rate of virus growth tends to be more rapid in L20B cells. However, the group observed a decline in sensitivity in high passage levels of L20B cells and stressed the need for frequent replacement of the cells.

4.4 Recommendations

4.4.1 The laboratory manual should be revised to include more specific information about recommended media composition, guidelines for the use of L20B cells, guidelines and recommended methods for routine mycoplasma testing, methods for virus sensitivity testing, example laboratory worksheets and recommended workflow by first quarter 1999.

4.4.2 Regular sensitivity assessments of cell lines should be included in the standard operating procedures of all laboratories.

4.4.3 Following the establishment of L20B cells in network laboratories, the two recommended cell culture lines for poliovirus isolation and typing will be RD and L20B cells.
5. Intratypic differentiation of polioviruses

5.1 Review of current recommendations

Five methods for intratypic differentiation of poliovirus isolates are used; the two most common being polyclonal absorbed ELISA and probe hybridization. The other tests in use are PCR-RFLP, PCR and monoclonal antibody neutralization. Available evidence suggests that all five methods are capable of giving acceptable levels of accuracy, as judged from the results of annual proficiency tests, but that a new comparative study would be required to provide a detailed analysis of relative accuracy. There is inadequate evidence to support the proposition that only one method of intratypic differentiation can be routinely applied, unless genomic sequencing of all isolates is used as an additional method.

5.2 Recommendations

5.2.1 All Network laboratories performing intratypic differentiation must subject all poliovirus isolates to two WHO-recommended intratypic differentiation methods (one antigenic one molecular) or one of the other accepted methods if used in conjunction with genomic sequencing.

5.2.2 All wild poliovirus isolates from polio-free or recently endemic countries should be analysed by genomic sequencing to determine the likely origin of the virus and extend results of intratypic differentiation.
6. Review of environmental surveillance for wild polioviruses

6.1 Role and operational realities of environmental surveillance in the polio laboratory network

The cornerstone of effective wild poliovirus surveillance remains AFP surveillance, but environmental surveillance could offer substantial supplementary information under some circumstances. This is particularly true where sewage networks serve large communities and AFP surveillance is inadequate. Important developmental work in this field has been carried out by the National Public Health Laboratory, Helsinki, and conclusions from their work were presented. Regular sampling of inlet material from sewage treatment plants, carried out biweekly or monthly, could provide evidence for continued circulation in the community despite the apparent absence of clinical cases. In the absence of sewage systems, environmental surveillance should be restricted to the most recent problem areas, and the sampling sites could comprise rivers, pools of wastewater and other potentially contaminated water in densely populated areas.

It has been estimated that in industrialized countries it would be possible to detect wild poliovirus in a single 1-ml sample of raw sewage, without concentration, if there were 100 people shedding virus out of a population of no more than 10 000. In substantially larger populations, however, the number of samples needed to detect virus would be prohibitive, and larger volumes of sample would have to be concentrated before testing. Several techniques have been developed for the concentration of sewage and waste water samples, the final choice being dependent upon available laboratory resources.

The techniques presently available for environmental monitoring are appropriate for countries using inactivated polio vaccine (IPV). In countries with active oral polio vaccine (OPV) immunization activities, however, the presence of large amounts of OPV-derived viruses in environmental samples many reduce the sensitivity of this type of surveillance. Further collaborative studies are required to determine if proposed methods of detecting wild poliovirus in wild virus/OPV mixtures can be used in countries with persisting wild poliovirus circulation.

6.2 Evaluation of environmental surveillance in Mumbai

Preliminary results of investigations being carried out by ERC, Mumbai, India, were presented. The primary objective of the investigation was to establish and evaluate a standard method of environmental sampling for the detection of wild polioviruses in
an area where these viruses continue to circulate widely. Two sites in Mumbai were chosen; in one site samples were collected from a sewage pumping station, in the other samples were collected from a large lagoon into which untreated domestic sewage regularly flows.

A PEG precipitation method has been used to concentrate samples by a factor of approximately 200:1 before inoculation onto L20B cells. For some samples a second concentration method, a two-phase separation technique was used in parallel with PEG precipitation. This method was found to give a concentration factor of less that that obtained with PEG precipitation, and was less reproducible. For those samples tested in parallel, however, the two methods gave comparable results.

The study began in August 1997 and is continuing. From samples collected between August 1997 and July 1998, poliovirus types 1 and 3 were the most frequently isolated. All of the polio type 2 isolates were vaccine-like on intratypic differentiation. Most of the polio type 1 isolates were wild type. At least 8 of the 40 poliovirus type 3 isolates were wild type. When compared with virus isolation results from AFP cases from Mumbai over the same time period, environmental sampling gave very similar results to AFP surveillance, except that polio type 3 was detected more frequently in environmental samples.

6.3 Recommendations

6.3.1 The first priority of Network laboratories is testing specimens from AFP surveillance, and this activity should not be compromised by laboratories carrying out evaluation studies or supplemental surveillance activities.

6.3.2 Results from studies on the evaluation and use of environmental surveillance techniques for the detection of poliovirus, as in Mumbai and Cuba, should be presented at the next Global Laboratory meeting.
7. Laboratory communications

7.1 Data management

The traditional laboratory results reporting system, of reporting directly back to the submitting physician or lower-level laboratory, works very well for reporting diagnostic results on patients (Figure 2). It is a system that everyone understands; it clearly defines the relationship and responsibilities of the submitter and the reporter, and it places a high priority on confidentiality of results. However, the system is inappropriate for epidemiological surveillance programmes in general, and for the polio eradication programme in particular. In an eradication programme decisions have to be made, and actions taken, in a coordinated manner at several levels at the same time. This is particularly relevant to the discovery of wild poliovirus from an area at a time when the virus has become rare. Actions must be taken at local level to carry out in-depth case investigation activities, at national level to plan immunization responses, and at international level to mobilize the support needed to mount the responses. Rapid notification at all levels is essential to providing timely action. A revised reporting system that calls for simultaneous reporting to several recipients was proposed to facilitate more efficient information flow (Figure 3). The proposed system does not take away the responsibility of the laboratory from reporting to the submitter, but does ensure that information flows rapidly throughout the system, so that all levels can take appropriate action.

Figure 2. Traditional investigation and laboratory report flow
7.2 Status of communication in the laboratory network

The laboratory accreditation process has demonstrated several areas of weakness in the global laboratory network that relate to reporting of results. Sometimes delays in reporting have been caused by excessive workloads, problems with methods or procedures, or by poor laboratory data management. Delays have also been caused by lack of the physical means to communicate. The laboratory network recognized that this was a significant problem for many laboratories and has established a programme to ensure that all laboratories have access to a reliable means of communicating results and receiving information. Activities are currently underway to ensure that 21 additional laboratories have access to electronic mail facilities by the end of 1998. An additional 10 are planned to have access by mid-1999, and a further 10 by the end of 1999. Several options are available for providing funding support for this activity; seed money can be supplied through WHO and all activities completed at a local level; WHO provides a consultant for installation, and for equipment and running costs; WHO provides running costs and channels funding from Rotary International for equipment and installation; or WHO establishes a “turn key” contract with an established communications provider, such as SITANET. In all cases, however, laboratory is held responsible for installation of a dedicated phone line needed to establish the e-mail connection. It is intended that by the end of 1999 all laboratories in the global network will have easy access to phone, fax or e-mail.

7.3 Review of polio virus database

The proposed global database on wild polioviruses was discussed as a mechanism for providing accurate information on the identity and probable origin of wild polioviruses isolated throughout the world. This information becomes essential in the final stages of the eradication in determining if wild poliovirus isolation is due to continued endemic transmission or to re-introduction. The information is also critical in assessing the scale and nature of the supplementary immunization activities that have to be mounted.
The global and regional Polio Laboratory Coordinators will be responsible for the regular updating of the global database. The data sources for the database will be all Specialised Reference Laboratories, Regional Reference Laboratories and those sub-regional or National Laboratories that do intratypic differentiation. The data will probably be entered at a single site (EPI surveillance unit/ Geneva). It is proposed that the Regional Polio Laboratory Coordinators would forward the relevant information to the surveillance team of the WHO Department of Vaccines and other Biologicals (V&B) for inputting.

New wild polioviruses would be entered immediately upon notification. Further considerations will be needed to establish the most appropriate mechanism for prioritising the collection of historical viruses and regularly updating the database with information from all levels of the network, particularly Regional and Specialised Reference Laboratories.

A method for classifying wild poliovirus genotypes was proposed. The serotype followed by a four-letter code identifies each genotype. The four-letter code indicates the geographic location where the genotype was first recognized or has a major reservoir. The first two letters indicate eight compass points from the geographical centre of the continent (e.g. NO, NE, EA, SE, SO, SW, WE, NW) or central (CE). The last two letters indicate continents (e.g. AF [Africa], AS [Asia], EU [Europe], NA [North America], SA [South America]). For a given serotype, if more than one genotype has been endemic to a region, a letter suffix (-A, -B etc.) specifies the individual genotypes. Vaccine-derived isolates have the genotype designation of Sab. Recombinants of vaccine-related viruses, if recognized, are designated SabR. For example:

- The type 1 genotype endemic to South Asia (e.g., IND) would be recorded as PV1/SOAS.
- One of the type 1 genotypes endemic to West Africa (e.g., NIE) would be recorded as PV1/WEAF-A.
- A second type 1 genotype endemic to West Africa (e.g., NIE) would be recorded as PV1/WEAF-B.
- A vaccine-related type 3 isolate would be recorded as PV3/Sab.

No standard method of designating individual isolate names has yet been developed. The laboratory number often assigned to an isolate is neither unique, nor of standard format between laboratories, therefore redundancy or ambiguity is possible. Furthermore, an isolate can acquire a new identity when it moves between laboratories and gets a new laboratory numbers. Further discussions are required before agreement can be achieved on the adoption of a standard nomenclature system for poliovirus isolates.
7.4 Recommendations

7.4.1 All Regional Laboratory coordinators should establish, and make explicit to network laboratories, standard data flow mechanisms for laboratory data in their Region.

7.4.2 Efforts to establish effective means of communication within the network should continue and be expanded to assure the rapid and reliable exchange of results, including establishment of e-mail in all network laboratories. Every network laboratory should have direct access to e-mail on a daily basis to facilitate rapid communication of laboratory results and receipt of laboratory-related information.

7.4.3 Results of intratypic differentiation testing in Regional Reference Laboratories should include reporting to the national EPI manager and the Regional Laboratory Coordinator, in addition to reports to the submitting laboratory. Results of additional testing in Specialized Laboratories should include reports to the Regional Laboratory Coordinator, in addition to reports to the submitting laboratory.

7.4.4 The proposed wild poliovirus database should be activated with the proposed fields and genotype designations. Submissions to the database should be through the Regional Laboratory Coordinators.
8. Global action plan for containment of laboratory stocks of wild polioviruses

8.1 Current status of the plan

The draft plan for containment of laboratory stocks of wild polioviruses has been widely circulated for comments and is now being amended in the light of comments received. Tasks remaining to be completed by WHO include finalizing the action plan and developing a World Health Assembly resolution on containment. There is also an urgent requirement to establish a focus of responsible individuals to coordinate, oversee and provide technical inputs for the containment process. Specific guidelines for nations on how to develop and maintain inventories of laboratories holding wild poliovirus-infectious materials must also be developed and tested as soon as possible. It is now an urgent requirement to develop criteria for identifying programmatically important wild polioviruses and to draw up guidelines on selection, handling and safe storage of these viruses. A proposed timetable for the polio eradication programme, including laboratory containment activities, was presented (Figure 4).

8.2 Recommendations

8.2.1 To facilitate the selection of viruses for shipment to interim wild poliovirus repositories, criteria for identification of programmatically important wild polioviruses should be developed and included in the revised containment plan.

8.2.2 Laboratories in the Global Poliovirus Network should immediately implement BSL-2/polio and be prepared to assist WHO Regions and individual countries in the implementation of the Phase I recommendations in the global containment plan.
Figure 4. Proposed polio eradication timetable

<table>
<thead>
<tr>
<th>Interrupting transmission</th>
<th>Certification</th>
<th>Laboratory containment of polio virus</th>
<th>Cessation of OPV immunization</th>
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<td>Wild polio virus containment</td>
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<tr>
<td>Last region certified</td>
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<td>Global certification</td>
<td>2005?</td>
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9. Meeting on the scientific basis for stopping immunization against poliomyelitis

9.1 Report from the meeting

The meeting was carried out in Geneva from 23 to 25 March 1998 with the objectives of reviewing available scientific information and studies in progress relevant to making a decision on stopping immunization against poliomyelitis, and defining priorities for research relevant for recommending a strategy for stopping immunization.

The single issue that has dominated debate on strategies for stopping immunization is whether vaccine-derived poliovirus (VDPV) strains will continue to circulate after the use of OPV is discontinued. Although persistent circulation of VDPV was actively debated prior to the introduction of OPV, a limited amount of relevant research has been carried out in recent years. Furthermore, the information that is available is not widely known.

If VDPV do not persist, the simplest and least costly strategy would be to simply stop immunizing with OPV. Ideally, cessation would be coordinated between countries, and a final series of mass campaigns may be necessary to boost population immunity. If VDPV do circulate persistently, then inactivated polio vaccine (IPV) could be used for an interim period. The effectiveness and feasibility of this strategy has, however, yet to be validated. A n alternative strategy would be to develop new, more stable attenuated polioviruses for OPV or genetically engineered viruses of lower virulence for IPV production. No such vaccines are currently licensed, but several candidate strains are under development.

9.2 Recommendation

9.2.1 Existing data should continue to be reviewed and studies conducted to assess issues related to stopping OPV vaccination, including the duration of poliovirus persistence in the environment.
10. Report of the meeting on the Global Commission for the Certification of Polio Eradication

10.1 Recommendations for the use of enterovirus surveillance as a certification strategy

The Global Commission for the Certification of Polio Eradication met in Geneva on 9 July 1998. One of the sessions included discussion on the use of enterovirus surveillance as a certification strategy, particularly for use in non-endemic countries. The Commission recognized that certain industrialized countries that had been polio-free for many years could not establish high quality routine AFP surveillance. An alternative strategy based on the combination of highly sensitive surveillance for suspected polio cases and data from enterovirus laboratories and laboratory networks could provide relevant information for certification. However, the technical basis and criteria for accepting enterovirus data should be established, and guidelines for countries wishing to use enterovirus laboratory information should be developed.

10.2 Requirements for an enterovirus surveillance system

Data collected on a monthly or quarterly basis from routine diagnostic investigations carried out by clinical virology laboratories could include the number of specimens, particularly faecal specimens, inoculated onto enterovirus-susceptible cell lines; the number and types of polioviruses isolated; and the number and types of non-polio enteroviruses isolated. Arrangements could then be established for the confirmation of all poliovirus isolates and a proportion of enterovirus isolates, and for intratypic differentiation and molecular characterization of the polioviruses.

Such a system may be useful in non-endemic countries with a high immunization coverage and highly developed healthcare system. It would be complementary to a clinical surveillance system for suspected poliomyelitis cases, and should be geographically and demographically representative. It would also be important to engage only qualified virus diagnostic laboratories with demonstrate proficiency.
11. Regional polio laboratory network status, priorities and 12-month plans of action

11.1 African Region

There has been a significant improvement in AFP surveillance in many African countries over the past 12 months, resulting in a greatly increased workload for the laboratories. By and large the Regional Polio Laboratory Network has managed to cope with this increased workload, although lack of communications facilities within the network continues to cause problems. Laboratory reporting time has continued to show improvement, with 66% of virus isolation results being reported within 28 days in 1997, improving to 77% in the first seven months of 1998.

Two additional laboratories (Addis Ababa in Ethiopia and Maiduguri in Northern Nigeria) were added to the existing network of 13 laboratories. Polio isolation activities have begun in Addis Ababa, but the Maiduguri laboratory, the second national-level laboratory to be assigned in Nigeria, is still awaiting delivery of essential equipment and supplies.

With only four fully accredited and three provisionally accredited laboratories in the region, improvement of laboratory performance leading to WHO accreditation is a major priority. Problems have been identified as lack of trained staff, inadequate preparation and maintenance of tissue culture, irregular supply of consumables and reagents, difficulties in shipping specimens and isolates, and inefficient communications systems.

Training issues will be addressed during two training programmes to be run in the Regional Reference laboratories in Ghana and the Central African Republic during the first half of 1999. These programmes will focus on cell culture preparation and maintenance as well as poliovirus isolation and characterization. Data management courses will also be held for laboratory staff.

Much of the equipment and supplies for the polio laboratories in Africa are ordered through WHO channels and this will continue for laboratories that are unable themselves to establish reliable supply mechanisms. Other laboratories, with well-established procurement systems, are encouraged to handle on their own much of the procurement of consumables and reagents. In conjunction with Rotary International, a desktop computer, together with telephone, fax and e-mail facilities will be established in five of the laboratories before the end of 1998. Similar facilities will be installed in another five laboratories before the end of 1999.
11.2 Eastern Mediterranean Region

Laboratory performance indicators have been maintained at high levels for the region as a whole, but poor or no AFP surveillance continues to be a problem in some countries. In 1997 only 53% of reported AFP cases had adequate stools collected, and there were delays in getting approximately 50% of specimens to the laboratory.

All 12 laboratories in the network have been visited in the past year, with 9 being accredited as WHO polio laboratories. Of the three laboratories that failed to achieve accreditation, one needs to improve on reporting performance, and should receive a follow-up visit in the near future. The other two laboratories require more substantial improvements in performance before they can be re-considered for accreditation.

Laboratory contamination has arisen as a major problem within the region, and the consequences to the programme can be very costly, with unnecessary field investigations, deterioration in relationships between laboratory and epidemiology staff, and loss of confidence of laboratory staff. Additional training, focusing on use of recommended techniques, optimal laboratory layout, correct use of laboratory equipment and better distribution of workload, is needed to solve the problem.

In the past year, 14 staff from 7 laboratories have received laboratory training. A workshop on electronic data management was held in May in Alexandria. Priorities for the coming year are to focus on data management, providing reliable computers and communications links to all laboratories, and encouraging laboratories to adopt a weekly reporting format. Training will be continued, but will focus on “in-country” rather than sending laboratory staff on training courses. Visits will be made to all poorly performing laboratories through the laboratory accreditation process.

11.3 South-East Asia Region

The significant improvement in AFP surveillance performance in the region in the past two years has resulted in a dramatic increase in laboratory workload. This is particularly true for India, where additional laboratories have been established to handle the increase in specimen numbers. New laboratories have also been established in Indonesia and Myanmar, but problems in handling the workload continue, with only approximately 40% of laboratory results in the region being reported within 28 days. There have also been long delays in reporting results of intratypic differentiation, although this has improved recently.

Five of the laboratories were accredited as WHO polio laboratories in 1997. To date in 1998 only two laboratories have been accredited. Accreditation reviews on a further three laboratories have been postponed until early 1999, and accreditation of the remaining nine laboratories remains pending. Problems have been detected in basic virological techniques in use, in receiving and maintaining stocks of essential laboratory reagents and consumables, and in data management and reporting.
The major priority for the region is to carry out training of laboratory staff, in basic virological techniques, in recommended intratypic differentiation methodologies and in data management. Laboratory consultants are being sent to selected laboratories to provide on-site training, and two training courses in basic virological techniques have been planned for 1999. A training workshop on data management for laboratory staff has also been planned.

Proficiency test panels were distributed to all Regional Reference laboratories in October, and panels for all national laboratories will be distributed in January 1999. Following analysis of the proficiency test results accreditation reviews for all laboratories in the region will be planned.

Distribution and supply of essential equipment and supplies has been centralized in the WHO Regional Office, allowing easier assessment of requirements and priority needs. Orders for procurement are currently sent every six months, which provides sufficient time for forward planning, but also allows the system to respond to urgent needs.

To improve laboratory reporting and feedback a new format for monthly reporting of laboratory results is being developed. It is intended that monthly reports will be issued starting January 1999.

11.4 European Region

Many of the laboratories in the European Polio Laboratory Network are well established, with many years of successfully isolating and characterizing enteroviruses, but the network itself is relatively new and continues to expand. At present there are 38 National Laboratories and 8 sub-national laboratories in the region, but several of these require essential equipment and supplies and many require reliable communications links before they can be considered as fully functional within the network.

Laboratory performance has continued to improve, but overall efficiency remains hampered by delays in transporting specimens to the laboratories, especially in the Newly Independent States and the Russian Federation, and in transporting virus isolates to the Regional Reference Laboratories. Although many laboratories are in urgent need of equipment and supplies, long delays for customs clearance are preventing laboratories from receiving the materials they need.

Accreditation of network laboratories remains a high priority for the region, with 28 out of 38 National Laboratories either provisionally or fully accredited. Several of the laboratories being considered for accreditation are in urgent need of upgrading, and for the staff to receive training in basic virological techniques. A accreditation review visits to four National Laboratories that have yet to be visited are planned for the end of 1998 and the first quarter of 1999. Repeat site visits have also been planned for many of the other national and sub-national laboratories during the first half of 1999.

To improve data management and communications within the network, computers with e-mail facilities are being supplied to 17 laboratories during the last quarter of 1998 and the first quarter of 1999.
A training workshop will be held in Moscow at the end of 1998 for staff from laboratories in the Russian Federation and the Newly Independent States. Arrangements are also being made for staff from other national laboratories to receive extra-mural training in the Regional Reference Laboratories.

11.5 Region of the Americas

The polio laboratory network was established in the American Region in 1986, and the last indigenous wild poliovirus isolated in 1991. Certification of regional eradication of wild poliovirus was announced in 1994 and since then there has been a slow but steady decline in AFP surveillance performance.

All laboratories take part in an annual proficiency test, and there is periodical follow up visits to the laboratories. Since the beginning of this year the consultants visiting the laboratories have been using the WHO checklist for accreditation to review the laboratories. This year visits to five of the seven laboratories (Argentina, Brazil, Guatemala, Mexico and Venezuela) were carried out; the other two National Laboratories (Colombia and CAREC) will be visited during the first quarter of 1999.

Since wild polioviruses have not circulated in the region for many years, it is appropriate to begin the process of containment of wild poliovirus stocks, and the laboratories will be participating in the implementation of Phase I activities according to the Global Plan of Action on containment.

During the next year the network will continue to implement the plan to introduce and evaluate PCR for poliovirus identification in network laboratories. Special studies on non-polio enterovirus will be carried out in three of the laboratories, and studies on developing a diagnostic test for enterovirus 71 will be continued.

11.6 Western Pacific Region

The last wild poliovirus-associated case in the Western Pacific Region had a date of onset of paralysis of 19 March 1997. Since then more than 15,000 stool specimens from AFP cases have been processed in network laboratories. Performance levels continue to improve, and are now approaching those required for certification of polio eradication.

Laboratory contamination remains a cause for concern, but strict adherence of WHO policy on limiting all laboratory work on wild poliovirus isolates to Regional Reference Laboratories has reduced the potential for cross-contamination. It is now appropriate to establish a system of maximum laboratory containment for wild polioviruses and wild poliovirus-infectious materials. To this end a Regional Action Plan for safe handling and maximum laboratory containment of wild polioviruses and potentially infectious materials, based on the Global Action Plan, has been developed.

Three National Laboratories in the region have yet to be accredited, and one was provisionally accredited for 1998. Accreditation of these four laboratories is a high priority activity for the coming year. Reported performance indicators of all other national polio laboratories will be reviewed to determine which require a review visit for 1999 accreditation.
L20B cells have now been distributed to all Network laboratories in the region, including provincial laboratories in the People’s Republic of China. It is expected that guidelines on the integration of these cells into the programme will be provided after the global laboratory meeting. Following distribution of the guidelines, close monitoring of laboratory performance indicators will be required to evaluate the impact of introducing these cells.

The Regional Action Plan for safe handling and maximum laboratory containment of wild polioviruses has been distributed and nations have been requested to ensure that all laboratories working with wild poliovirus-infectious and potentially infectious materials do so under strict BSL-2/polio conditions. They have also been requested to compile inventories of all laboratories retaining wild poliovirus-infectious and potentially infectious materials.
Annex 1: Agenda

10 October 1999, Day 1

8:15-8:30 Registration of participants
B. Melgaard

8:30-8:40 Introduction and objectives

8:40-9:00
1. Global overview of the progress towards polio eradication
M. Birmingham

2. Accreditation of global polio network laboratories

9:00-9:15 a) Current status of laboratory network accreditation
D. Featherstone

b) Recommended changes to accreditation process
R. Sanders

9:15-9:30 Discussion

10:00-10:15 a) Global overview
O. Kew

b) Regional review

10:15-10:25 Africa
N. Blackburn

10:20-10:30 Pakistan
H. Asghar

10:30-10:40 Turkey
H. van der Avoort

10:25-11:00 Coffee

c) Country reviews

11:00-11:10 India
J. Deshpande

11:10-11:20 Egypt
T. Naguib

10:20-10:30 Pakistan
H. Asghar

10:30-10:40 Turkey
H. van der Avoort

11:30-12:00 Discussion

4. Review of the Manual for the virological investigation of polio

12:00-12:15 a) Standardization of cell culture media and techniques
M. Pallansch

12:15-12:30 Discussion

12:30-14:00 Lunch
1 October 1999, Day 1 (continued)

14:00-14:15 b) Review of L20B distribution and planned introduction D. Wood
14:15-14:20 c) Comparative sensitivity of L20B and L alpha cells T. Miyamura

14:20-15:00 Discussion

5. Intratypic differentiation of polioviruses
15:00-15:15 a) Review of current recommendations H. van der Avoort
15:15-15:30 b) Role of PCR in intratypic differentiation M. Pallansch
15:30-16:00 Coffee

6:00-16:30 Discussion

6. Review of environmental surveillance for wild polioviruses
16:30-16:50 a) Current status
Mumbai environmental surveillance study J. Deshpande
b) Role and operational realities of environmental surveillance in the polio laboratory network T. Hovi

17:10-17:40 Discussion

17:40-18:00 Summary of Day 1

18:00 Official reception - WHO Restaurant - Main Building

2 October 1999, Day 2

7. Laboratory communications
9:00-9:10 a) Data management T. Burton
9:10- 9:20 b) Status of communication in the Lab network M. Birmingham
9:20-9:30 c) Review of wild polio virus database D. Featherstone

9:30-10:00 Discussion

8. Global action plan for containment of laboratory stocks of wild polioviruses
10:00-10:15 a) Proposed timetable and role of the network laboratories W. Dowdle

10:15-10:45 Discussion

10:45- 11:15 Coffee

11:15-11:30 9. Report on the scientific basis for stopping immunization against poliomyelitis meeting D. Wood
2 October 1999, Day 1 (continued)

11:30-12:00  Discussion

12:00-14:00  Lunch

10. Regional polio laboratory network status, priorities and 12- month plans of action

14:00-14:45  a) African Region  O. Tomori
             b) Eastern Mediterranean Region  E. de Gourville
             c) South-East Asia Region  N. Withana

14:45-15:30  Discussion

15:30-16:00  Coffee

16:00-16:45  d) European Region  G. Lipskaya
             e) Region of the Americas  G. Tambini
             f) Western Pacific Region  R. Sanders

16:45-17:15  Discussion

17:15-17:30  Conclusions and recommendations
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