New approaches to poliovirus diagnosis using laboratory techniques: Memorandum from a WHO meeting*

Laboratory diagnosis of poliomyelitis is an important part of the WHO initiative for global eradication of poliomyelitis. During the last year, new methods have been developed for the detection of poliovirus in clinical specimens, for intratypic differentiation, for the analysis of poliovirus neurovirulence, and for the detection of poliovirus antibodies. Progress in laboratory techniques for detection of poliovirus antibodies and for characterization of poliovirus isolates has suggested several new approaches to poliovirus diagnosis using laboratory techniques.

New methods for poliovirus detection

New methods for the detection of poliovirus in clinical specimens with and without cultivation of a virus are described below.

**Indirect immunofluorescence technique**

It has been demonstrated that an indirect immunofluorescence (IIF) technique can reliably be used under field conditions to rapidly identify and type poliovirus isolates in cell culture from stool specimens. Although it requires initial inoculation of the specimen(s) in cell culture, it can be used when the cytopathogenic effect (CPE) becomes apparent and results are obtained within hours. It is thus much quicker than the standard virus neutralization test. It has the added advantage that it is more readily and, in some cases, more sensitively detects the presence of polioviruses in poliovirus or poliovirus/enterovirus mixtures and is not influenced by the "breakthrough" phenomenon which may lead to misidentification of isolates when using the virus neutralization technique. With further development, it is likely to be possible to use the same technique to discriminate between wild and vaccine-related poliovirus isolates. The use of IIF for the direct detection of polioviruses in clinical specimens without a cell culture isolation step has not been thoroughly studied, but in some cases this may be possible. Although IIF currently requires the use of cell culture systems and an immunofluorescence microscope, the same instrument and similar techniques can be used for the identification of other viruses such as those associated with acute respiratory infections. In summary, the IIF technique appears to be a useful investigatory tool for the detection, typing and perhaps characterization of polioviruses from stool specimens and environmental samples, particularly in situations where there is a high prevalence of endemic non-polio enteroviruses.

**Polymerase chain reaction (PCR)**

Reverse transcription followed by the polymerase chain reaction (PCR) has been used for sensitive detection and identification of polioviruses based upon the nucleotide sequence characteristics of their RNA genomes. The sensitivity and selectivity of PCR will simplify the establishment of two parallel surveillance systems for poliovirus circulation. The first focuses on the characterization of viruses associated with cases of acute flaccid paralysis. The second would be designed to detect the silent transmission of wild polioviruses through community or environmental sampling.

Primer pairs have been designed which permit identification using PCR of each Sabin poliovirus vaccine strain based on the electrophoretic mobilities of the amplified cDNA products (Sabin 1, 97 base-pairs (bp); Sabin 2, 71 bp; Sabin 3, 53 bp). Samples containing mixtures of vaccine strains can be readily detected and their composition determined by PCR. Virus present in 1 μl of infected cell-culture supernatant fluid can be readily detected. Direct detection and specific identification of the Sabin vaccine strain viruses was possible in approximately 70% of stool

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samples analysed. Improvements in methodology may significantly increase sensitivities with crude clinical samples.

PCR primer pairs have also been developed which permit genotype-specific amplification of wild poliovirus sequences. Selective amplification of wild poliovirus sequences may occur in reactions containing large stoichiometric excesses (up to 10^6-fold) of Sabin-vaccine-strain-derived RNA.

Preliminary studies with sewage samples showed that the Sabin-derived strains which are typically present in waste water could be readily detected by PCR. Small quantities of wild polioviruses added to sewage specimens before processing could also be identified. Sewage samples obtained from areas of Brazil in which the last cases associated with wild polioviruses had occurred two years earlier were negative by PCR using primers specific for the previously indigenous wild polioviruses. The ability of PCR to detect silent transmission of wild polioviruses is currently being critically evaluated through sampling in areas of Colombia that recently (1991) had cases associated with wild polioviruses. Both sewage samples and pooled stool specimens will be tested.

PCR is now routinely used in the American region to identify cell culture isolates from suspected poliomyelitis cases. The classification of isolates by PCR has been highly consistent with parallel analysis using probe hybridization and, for wild viruses, partial genomic sequencing. The advantages of PCR (speed, specificity, and sensitivity) are well established with clinical isolates. The feasibility of PCR methods for detection of virus in original clinical or environmental specimens requires further evaluation.

Semi-nested PCR, using three different broadly reactive primers, has been used to search for enteroviral (polioviral) sequences directly in various crude specimens (stools, sewage, surface water). This approach permitted detection of very low amounts of the representative viruses in the original sample.

**Antigen-capture PCR (AC/JPPCR)**

The AC/PCR method couples a simple immunofinity procedure for purification of virus present in clinical samples with conventional PCR for detection of virus with a very high level of sensitivity. As developed for hepatitis A virus (HAV), AC/PCR is carried out in PCR tubes that are pre-coated with virus-specific monoclonal antibody. Dilute faecal suspensions are incubated in these tubes after which the tubes are washed extensively to remove unbound contaminating materials present in the suspension. RTaq (core) buffer is added to the tubes which are then heated briefly to 95 °C to denature the bound virus and release viral RNA. First strand cDNA synthesis and subsequent PCR amplification of viral nucleic acid is then carried out in the same reaction vessel. AC/PCR offers several advantages over conventional PCR. These include the technical simplicity of the procedure, which involves fewer manipulations of the sample and thus may pose fewer risks of cross-contamination. The antigen-capture step results in considerable purification of the sample prior to PCR. Substances present in faecal extracts or environmental samples which may have an inhibiting effect on enzymes used in PCR are thus effectively removed prior to the polymerase chain reaction. The purification of the sample prior to PCR also results in enrichment of the tested nucleic acid derived from virus, and this reduces the frequency and amount of stochastic PCR products arising from random priming of non-viral template nucleic acids. Finally, AC/PCR detects antigen-associated nucleic acid with a relatively high degree of specificity. In the context of poliovirus detection and characterization, the immunodeficiency-capture step included in AC/PCR may thus offer a second level of discrimination among virus serotypes, or even potentially differentiate between wild-type and vaccine-related virus strains. Although the sensitivity of AC/PCR appears comparable to more technically complicated methods involving the isolation of viral RNA by conventional methods, it is probable that the sensitivity of AC/PCR might be enhanced by the use of alternative materials for the solid-phase capture. Thus, it may prove useful to consider development of polystyrene PCR tubes or other solid-phase supports (such as beads) to replace the polypropylene tubes now in use.

AC/PCR has been used in a number of laboratories for detection of hepatitis A virus in human faecal samples, but very limited experience has been accumulated to date concerning the application of AC/PCR to detection of poliovirus. It is an attractive approach to consider in this context, however, because it may potentially allow a marriage of existing immunological and molecular biological tools available for the detection and characterization of polioviruses. In addition, the method is technically straightforward, which should facilitate its eventual transfer to other laboratories.

**Recommendations**

1. WHO should support collaborative studies aimed at the further development of PCR technology for detection of polioviruses in clinical samples and environmental specimens. These studies should include the application of AC/PCR for the detection of polioviruses. A useful strategy might involve a two-step procedure in which all samples would first be screened for the presence of poliovirus in polio-
virus type-specific assays using poliovirus type-specific capture antibodies and broadly-reactive PCR primers capable of detecting all polioviruses. Samples found to be positive in this screening assay would be retested by a PCR method utilizing genotype-specific oligonucleotide primers capable of differentiating vaccine and wild strains of polioviruses.

2. WHO should support collaborative studies designed to determine the optimal methods for the safe transport, collection and processing of samples from environmental (sewage) or community sources (pooled stool specimens from high-risk persons) to detect the silent transmission of wild polioviruses.

3. It is recommended that the IIF technique be further evaluated by several laboratories in a variety of circumstances to determine its general applicability.

**New methods for intratypic differentiation**

The objective of intratypic differentiation of poliovirus strains is to determine whether field isolates are vaccine-derived or wild virus. This is important for evaluation of paralytic patients and for epidemiological surveillance.

A number of methods for intratypic differentiation have been developed, including the following.

**Polyclonal antibody tests**

Neutralization assays or enzyme-linked immuno-sorbent assays (ELISA) that use cross-absorbed polyclonal antisera have been developed. Type-specific rabbit antisera raised against Sabin-vaccine-like viruses (SL) and wild viruses (NSL) have been made strain-specific by cross-absorption with the heterologous strain. These antisera have been used successfully for many years in a neutralization assay and an ELISA for intratypic differentiation of poliovirus strains. The indirect sandwich ELISA system uses poliovirus type-specific (bovine) antibodies for capture of virus and horseradish peroxidase (HRP)-labelled anti-rabbit sera for detection of bound anti-SL or anti-NSL antibodies. Clear-cut results are obtained in approximately 95% of isolates using either of the two methods. The use of well-established methodology is an advantage of the method; the assays are easy to perform and the results may be obtained within 24 hours when using the ELISA. However, production of the antisera is not without problems and absorption has to be carried out very carefully.

**Monoclonal antibody panels**

Monoclonal antibody panels have been developed for each of the three serotypes. The panel for each type includes six antibodies with different degrees of strain specificity. It is currently recommended that these panels be used in neutralization assays based on neutralization index measurements in which varying amounts of virus are exposed to a fixed amount of antibody. The identification of strains as Sabin-vaccine-like or wild-type is in broad agreement with that obtained by the use of absorbed polyclonal sera. The assay used well-established methodology. Difficulties with regard to stability, reconstitution, and specificity of these monoclonal antibodies have been experienced.

**Probe hybridization**

Plasmids have been constructed which generate riboprobes that permit the identification of Sabin-strain-derived polioviruses by blot hybridization. Two independent riboprobes have been developed for each Sabin strain. A broadly reacting riboprobe has been prepared that detects the presence of poliovirus (or enterovirus) RNA and provides a positive control reaction. A number of genotype-specific riboprobes have been prepared that provide positive identification of wild viruses for which the sequence is known. The use of these riboprobes requires prior amplification of the virus RNA either by cell culture propagation or by in vitro enzymatic amplification of cDNA before blot hybridization. However, the probe technology is easy to perform when adapted to sensitive non-isotopic detection systems.

An operational problem with conventional probe assays is the requirement for radioactive isotopes. Disadvantages of these radioactive probes are their short half-life, and the radiation hazards and disposal costs associated with radioactive waste. Non-radioactive labelling methods have been developed which provide sensitivities comparable to radioactive probes (that is, sub-picogram sensitivities without prior amplification of cDNA). For example, a system applying digoxigenin as a modification group in the probe sequence shows extremely low background in hybridization assays. Digoxigenin-labelled probes are stable and reusable, and may be detected by colorimetric, luminescence, or fluorescence indicator systems.

Non-radioactive labelled probes can be applied to blot hybridization (Southern, Northern, dot blot), solution hybridization, or in situ hybridization. Several enzymatic and chemical methods for the introduction of non-radioactive modification groups into nucleic acids (polynucleotides or oligonucleotides, RNA or DNA) have been established. Several detection systems are available. Non-radioactive probes can be prepared in central laboratories and shipped to other laboratories in the network. For
the blotting, hybridization and detection assays, only limited technical equipment is necessary. Application of non-radioactive labelling systems can easily be combined with amplification methods like PCR to improve sensitivity and specificity. Thus the use of non-radioactive methods in labelling probes should greatly facilitate the demonstration of probe technology in several laboratories.

**In vitro enzymatic gene amplification**

As described above, PCR has been utilized for the sensitive detection of Sabin-strain-derived polioviruses as well as selected wild poliovirus strains of well-known genotypes. Primer pairs have been designed which permit identification of each Sabin type by the electrophoretic mobilities of the amplified product. Primer pairs have also been prepared for specific wild poliovirus genotypes, so that their sequences may be selectively amplified in the presence of a large excess of vaccine virus genomes. The method is relatively easy and gives results within one day. A major problem with this approach, however, is the requirement that new primer pairs be developed and validated for each wild-type genotype.

**Restriction fragment length polymorphism (RFLP)**

Using viral RNA extracted from cell-culture supernatant fluid, the poliovirus genomic segment is reverse-transcribed and amplified by PCR, as described above. RFLP analysis of the resulting dsDNA is carried out by digestion with specific endonucleases followed by electrophoretic separa-

tion of DNA fragments of different lengths. The RFLP patterns derived from equivalent genomic segments of different virus strains can thus be compared rapidly and with a high level of specificity. The RFLP-1 assay examines a 480 nucleotide sequence between residues 2401 and 2880 (P1/Mahoney), a region coding for the N-terminal half of the capsid protein VP1 including the antigenic site 1. Generic primers capable of PCR amplification of all poliovirus strains are used. The endonucleases HaeIII, DdeI and HpaII are utilized for RFLP analysis because of their capacity to generate distinct fragment profiles for representative poliovirus strains with a known sequence in the genomic region: P1/Mahoney, P2/Lansing, P3/Finland/23127/84, and the three Sabin vaccine strains. The Sabin vaccine strains and related viruses can be identified by their Sabin-specific RFLP-1 profiles (one for each serotype), which are different from the profiles of the homotypic wild strains.

The technical requirements, advantages and disadvantages of the different methods are summarized in Table 1. It should be noted that, particularly in polio-free countries, methods biased towards detection of wild virus strains are preferred.

**Recommendations**

1. Although a number of laboratories have experience with one of the above-mentioned methods, few have experience with more than one. A collaborative study should be initiated involving well-experienced laboratories in order to determine the relative ability of each of the above-mentioned methods to correctly identify poliovirus strains.

### Table 1: Methods for intratypic differentiation of poliovirus

<table>
<thead>
<tr>
<th>Method</th>
<th>Technical requirements</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-adsorbed polyclonal antisera (neutralization, ELISA)</td>
<td>Tissue-culture facilities, ELISA equipment</td>
<td>Well-established methodology, easy, rapid (ELISA)</td>
<td>Poor standardization, indeterminate results in about 5% of strains</td>
</tr>
<tr>
<td>Monoclonal antibody panels (neutralization)</td>
<td>Tissue-culture facilities</td>
<td>Well-established methodology, clear-cut interpretation of results</td>
<td>Large number of monoclonal antibodies currently used</td>
</tr>
<tr>
<td>Probe hybridization</td>
<td>Labelled probes, hybridization equipment</td>
<td>Easy, rapid, easy to standardize, feasible with non-radioactive probes</td>
<td>Mixtures of types may not be readily resolved; requires virus isolation prior to hybridization</td>
</tr>
<tr>
<td><strong>In vitro enzymatic gene amplification (PCR)</strong></td>
<td>Primers, PCR equipment, electrophoresis</td>
<td>Strain-specific amplification possible from one sample, rapidity, easy interpretation, potential for application to clinical samples</td>
<td>Cross-contamination of samples; specific primers required for each genotype</td>
</tr>
<tr>
<td>Restriction fragment length polymorphism</td>
<td>Primers, PCR equipment, restriction enzymes, electrophoresis</td>
<td>Rapidity, clear-cut interpretation, easy detection of mixtures, identification of wild genotypes</td>
<td>Expensive, cross-contamination of samples</td>
</tr>
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</table>
2. At the level of Regional Reference Laboratories, a collaborative study should be carried out in order to determine whether it is possible to limit the number of monoclonal antibodies required for intratypic differentiation and to optimize this method.

3. It is recommended that the Manual for virological investigation of poliomyelitis be revised regarding the following procedures, depending on the results of the collaborative studies described above:
- the recommended typing procedure, as correct typing is crucial to correct intratypic differentiation;
- the recommended method for intratypic differentiation; a decision on the method to be used should be made after completion of the above-mentioned collaborative studies.

New methods for detecting poliovirus antibodies

The poliovirus neutralization assay is a sensitive and specific method for the determination of antibodies resulting from infection with either vaccine or wild virus. However, owing to significant difficulties in the interpretation of results as well as limitations on resources and time, serology is currently not a part of the poliovirus eradication effort. None the less, results presented at this meeting indicate that important progress has been made in the development of alternative methods for measuring poliovirus antibodies based on ELISA technology. These new methods may ultimately prove useful for the global eradication of poliomyelitis.

In three different laboratories, isotype-specific capture-ELISA tests have been developed for the detection of either IgM or IgA antibodies specific for each of the three types of poliovirus. Although the details of the three procedures vary slightly, the overall strategies are similar. Briefly, the antibody-class specificity is determined by an anti-mu or anti-alpha capturing antibody coating a solid-phase support. The sera to be tested is added, followed by the specific serotype of poliovirus as antigen. The detector antibodies are monoclonal antibodies specific for each of the three serotypes. The signal-generating system is then added to determine the extent to which the detector antibody has been bound. The methods are technically easy to perform, do not require extensive equipment, and can give results in less than two days.

The reported results have indicated a very good correlation in clinical cases between the presence of virus-specific IgM and virus isolated from the patient. A very low level of cross-reaction was observed with other enterovirus infections, and the frequency of multiple serotype responses was low. There was also a very good correlation between IgM and IgA response among vaccinees and seroconversion in the neutralizing antibody test. Data were also presented which suggested that IgM ELISA was more sensitive than virus isolation in the diagnosis of poliovirus-associated acute flaccid paralysis.

In contrast, the results of the capture ELISA for virus-specific IgG did not correlate well with the level of neutralizing antibodies. However, other assay configurations may provide a better measure of IgG. Results from a competitive binding ELISA indicate that this approach may be quite promising. In addition, by using adsorbed sera, the competitive binding assay could also specifically differentiate between OPV response and IPV response, although sensitivity was markedly reduced.

As a result of these reports and the subsequent discussion, it is clear that ELISA-based serology has a high potential for contributing to the eradication effort in three main areas: (1) the virus-specific diagnosis of acute flaccid paralysis due to poliovirus; (2) the measurement of immune response to vaccine; and (3) the determination of the prevalence of antibodies to poliovirus.

For virus-specific diagnosis, the capture IgM ELISA, and possibly IgA ELISA, have demonstrated promise for rapidly determining recent poliovirus infection. A collaborative study should be performed among at least three laboratories to assess the correlation between IgM and IgA response and poliovirus infection. This will require the identification of appropriate case and control sera, testing of the sera, and analysis of the results (see Annex). For discriminating between wild and vaccine infection, however, further work needs to be done to develop simple and rapid assays.

Among vaccinees, evaluation of class-specific antibody response and its correlation with neutralizing antibody is important. A collaborative study similar to that described above should be designed to evaluate the role of these assays in this setting. Since the sera are already identified and the methods will be identical, this evaluation should require only a small additional effort.

Although it is possible to assess the antibody response to recent virus infection, both in clinical cases or vaccinees, by measuring IgA and IgM antibody, the immune status of an entire population can probably only be measured by determining the IgG response. There was a consensus that the capture ELISA configuration was inappropriate for this purpose. Further assay development needs to be
continued for measuring IgG antibody levels which have a high quantitative correlation with neutralizing antibody.

Although not directly related to ELISA test development, the use of filter-paper for the collection of serum specimens will be an important part of the implementation of routine serology. The small volume of sera required by ELISAs makes this approach for sample collection very feasible. Although use of filter-paper collection has been described for other IgM assays, their use in these assays should be demonstrated.

**Recommendations**

1. A collaborative study should be conducted to assess the correlation between IgM or IgA antibodies detected by antibody-capture ELISA and recent infection with poliovirus in order to determine the utility of these assays in the virus-specific diagnosis of acute flaccid paralysis. Appropriate sera should be identified within three months and the study completed within one year.

2. A collaborative study should be conducted to assess the correlation between IgM or IgA antibodies detected by antibody-capture ELISA and the neutralizing antibody response to vaccines in order to determine whether these assays will be useful in evaluating the response to vaccines. Appropriate sera have been identified and the study should be completed within one year.

3. Results from the above studies should be evaluated with a view towards making specific recommendations for changes in or continuation of current programme policies concerning use of serology in the poliovirus eradication effort.

4. WHO should support the further development of assays to measure IgG antibody levels which correlate with the results of neutralizing antibody tests.

5. The use of filter-paper for collection of serum for testing in poliovirus ELISA tests should be evaluated.

6. WHO should support and coordinate a prospective clinical evaluation of the sensitivity and specificity of these newer serological procedures for virus-specific diagnosis in cases of acute flaccid paralysis. This study should include the collection of serial serum and faecal samples from well-characterized and confirmed clinical cases of poliomyelitis.

**New methods for analysing poliovirus neurovirulence**

During the past few years, several new methods have been developed for analysing the neurovirulence of polioviruses. These methods have distinct advantages over the monkey neurovirulence test: they are faster to perform, cost less, and are potentially more reliable.

**Transgenic mouse**

One of these new methods is a transgenic-mouse model for poliomyelitis. Transgenic mice have been developed which express the human-cell receptor for poliovirus (PVR). PVR transgenic mice are susceptible to poliovirus infection, and develop poliomyelitis after inoculation with any of the three serotypes of wild-type polioviruses. The mice are susceptible after inoculation intracerebrally, intramuscularly, intraperitoneally, intravenously, and (with a higher challenge dose) orally. PVR transgenic mice do not develop clinical disease after inoculation with any of the three serotypes of the Sabin vaccine strains. These mice therefore have the potential to be used in tests for the neurovirulence of polioviruses.

**Mutant assay by PCR restriction-enzyme cleavage (MAPREC)**

The second test, MAPREC, directly examines genomic loci known to be important for the attenuation phenotype. In this assay, PCR is used to amplify a region of the viral genome, and the relevant sequence differences are detected by restriction-enzyme cleavage of the amplified products. For example, MAPREC has been used to determine the percentage of C at nucleotide 472 in type 3 strains, one of the positions known to control the attenuation phenotype. Examination of over 70 vaccine lots and other type-3 virus strains has indicated that the proportion of virus with C at position 472 correlates well with the results of the monkey neurovirulence test. All vaccine lots which failed the monkey test contained >1% C at 472, while lots which passed contained <1% of C at 472. Mutations at other genomic positions failed to correlate with the results of the monkey neurovirulence test. MAPREC is therefore a rapid, inexpensive and reliable assay which shows great promise for determining the neurovirulence potential of polioviruses. However, specific MAPREC test procedures must be developed and evaluated for types 1 and 2 vaccine strains.

**In vitro translation assay**

The third method is an *in vitro* translation assay. Viral RNA is translated in cell-free systems, and the translation product is quantitated. In this assay, the translation efficiencies of Sabin-strain RNAs are significantly less than those observed for the wild-type counterparts. The differences in translation have been mapped to positions in the 5'-nontranslated
region that control the attenuation phenotype. This assay therefore employs a correlation between translational efficiency and neurovirulence to assess the attenuation of a particular strain.

All three assays have potential to supplement or perhaps even replace the monkey neurovirulence test. However, further studies are required in several laboratories before further decisions on the use of these assays can be made.

**Recommendations**

1. **PVR transgenic mice.** It is necessary to compare the sensitivity and variability of a transgenic-mouse neurovirulence assay with the monkey test. As a first step, it is recommended that the neurovirulence of type-3 strains be determined in one or more PVR transgenic-mouse lines. Three strains should be tested: an attenuated strain (P3/Sabin), a virulent strain (P3/Leon), and a strain of intermediate neurovirulence (e.g., ATCC VR-1004 or a similar strain). The mice should be inoculated intracerebrally, and clinical signs and lesions in the brain and spinal cord quantitated. Areas of the brain and spinal cord examined should be those pathognomonic for poliomyelitis. These studies should be carried out collaboratively in several different laboratories. The amount of virus inoculated, and the incubation time, should be determined by each laboratory, but as a starting point published values can be used (see Ren et al., *Cell*, 63: 353-362 (1990)). The assay should also be conducted with intramuscular inoculation, which appears to be as efficient as intracerebral inoculation and may be easier to perform. The goal of these studies should be to directly compare the results of the mouse neurovirulence assays with assays using the same viruses in monkeys. If the results indicate that the mouse assay is as sensitive and reproducible as the monkey test, then additional studies, first using vaccine-associated isolates of type 3 poliovirus and then using poliovirus serotypes 1 and 2, would be warranted.

2. **MAPREC.** Considerable data have been obtained which suggest an excellent correlation between the proportion of C at base 472 in the type-3 genome, and the monkey neurovirulence test. The participants therefore encourage similar studies using the poliovirus type 1 and 2 strains. For both serotypes, three viruses should initially be examined: a virulent strain (e.g., P2/MEF1 or P1/Mahoney), an attenuated strain (e.g., P1/Sabin or P2/Sabin), and strains of intermediate virulence. MAPREC should be used to determine the nucleotide at positions known to be involved in the attenuation phenotype of these strains. The results of MAPREC analysis should be compared with the results of WHO monkey neurovirulence tests, as was done with the type-3 strains.

3. **In vitro translation test.** The correlation between reduced translational efficiency in *vitro* and attenuation imparted by mutations in the 5′-nontranslated region should be further examined, and compared with the results of monkey neurovirulence tests. Initially, three strains of type-3 poliovirus should be examined, with high, low, and intermediate neurovirulence. The translational efficiency *in vitro* of the genomes of these viruses should be determined, and the accuracy and variability of the translation determined. Monkey neurovirulence tests should be conducted in parallel using the same viral strains. If preliminary results suggest the *in vitro* translation assay to be a good indication of attenuation, then examination of vaccine-associated type-3 isolates, as well as representatives of the other two serotypes, using *in vitro* translation and the monkey neurovirulence test, would be encouraged.

It would be most productive to conduct studies of these three new test procedures in parallel, using identical virus strains, so that the results of the different assays could be directly compared. This could most effectively be accomplished by one laboratory, but could also be achieved by an effective collaboration between different laboratories.

**Annex**

Information to be provided with serum samples to be tested in collaborative prospective and retrospective studies of new serologic procedures for diagnosis of poliovirus infections.

**Retrospective identification of cases (100 sera)**

1. Clinical diagnosis—acute flaccid paralysis (AFP).
2. Vaccination history by vaccine type and by date (indicate card or history).
3. Laboratory results of virus isolation and identification/characterization and serology (if done).
4. Date of onset.
5. Date of sera (0–4 weeks after date of onset).
6. Volume of serum sample (0.2 ml or more).
7. Age, sex, country.

**Controls (100 sera)**

1. Same information as cases.

Other acute flaccid paralysis with no poliovirus isolates.