



World Health
Organization

**The 17th Informal Consultation of the Global Polio Laboratory Network
22-23 September 2011, WHO Headquarters, Geneva, Switzerland**

Summary of discussions and recommendations

The World Health Organization convenes an annual informal consultation with participants representing six geographical regions and 20 percent of the 146 member laboratories of the Global Polio laboratory Network (GPLN). Focussed discussions are held on laboratory issues of relevance to the Polio Eradication Initiative (PEI) and policies and technical priorities are established to ensure that the network operates with accuracy and efficiency to confirm the locations in which Polio Viruses (PVs) are transmitted. Effective collaboration among surveillance, laboratory and immunization personnel is vital to target efforts to achieve the polio eradication goal.

Wild Polio Virus (WPV) detections

The GPLN tested 286,301 faecal samples and 26,188 non-Acute Flaccid Paralysis (AFP) samples between January 2010 and June 2011¹. Participants of the consultation reviewed the trends in the number and genetic relationships among wild PV (WPV) detected in 1750 AFP cases and 175 non-AFP samples between January 2010 and 21st September 2011.

- 22% percent of all reported WPV cases during the period reviewed were detected in the 4 **polio endemic countries** of Afghanistan, India, Nigeria and Pakistan. The situation in India is considered to be promising as no WPV has been detected in that country since a WPV serotype 1 (WPV1) case was found in West Bengal in January 2011, in a setting of high standards of performance for AFP surveillance. The reported decline in WPV3 cases in 3 polio endemic countries is also encouraging: no WPV3 was detected in Afghanistan and India in 2011, while the most recent WPV3 positive AFP case from Pakistan had paralysis onset in June 2011 and the most recent WPV3 positive sewage sample in the same country was collected in Sindh province in October 2010. The WPV1 situation in Pakistan is still a major concern to the PEI as WPV1 transmission is widespread in the insecure north western areas of the country and in Baluchistan, as well as in the highly populated Sindh province from which transmission spills over into other locations within Pakistan. WPV1 transmission continued mainly in southern provinces of Afghanistan in 2011.

¹ Workload between 01 July and 20 September 2011 was not summarized as all laboratory analyses had not been completed.

The number of WPV cases in Nigeria has declined substantially with reported total number of WPV cases of 388 in 2009, 21 in 2010, and 30 up to September 2011. However co-circulation of WPV1 and WPV3 and Vaccine Derived Polio Virus (VDPV) of serotype 2 (as discussed below) continues and there are occasional long gaps in genetic information that link related viruses, suggesting some weaknesses in AFP surveillance.

- 78% of cases and a single WPV1 in sewage reported globally since January 2011 represented transmission of imported viruses in 23 countries:

- **Countries with WPV cases or outbreaks following new importations** numbered eight in 2010² and four in 2011³. A WPV serotype 1 (WPV1) outbreak due to virus imported from Pakistan was newly detected in Xinjiang province China in 2011 with reported virus isolated from 10 cases, 13 contacts and 6 healthy children up to 21 September 2011. Control efforts were on-going at the time of the consultation. Large WPV1 outbreaks in Tajikistan and Republic of Congo (Congo) accounted for 26% and 25% of all reported cases globally, respectively, since January 2010. India was the source of the imported virus found in Tajikistan that spread and caused cases in three other European countries (Kazakhstan, Turkmenistan and Russian Federation) in 2010. Nepal had WPV1 cases in 2010 linked to transmission in Bihar, India. Angola was the source of imported WPV1 virus found in the Congo outbreak in 2010 and a single WPV1 case in Gabon in early 2011 represented importation of virus from Congo.

A new WPV1 outbreak in Senegal and cases in Liberia in 2010 were part of a larger outbreak that started with westward spread of virus from Nigeria in 2008, eventually affecting several countries in West Africa before being brought under control in 2010. In 2011, a new problem emerged in West Africa with the detection of a WPV serotype 3 (WPV3) outbreak in Ivory Coast with related cases also found in Guinea and Mali. Genetic data linked the first case in Côte d'Ivoire in 2011 to a WPV3 case previously found in Nigeria in 2009, suggesting sub-optimal surveillance in one or more West African countries that allowed transmission to go undetected.

- Four countries (Chad, DR Congo, Mali and Niger) had **a mixture of new importations and continuation of transmission of viruses from importations that occurred prior to 2010**. In Chad, transmission of WPV3 that was first imported in 2007 continued. New importations of WPV3 from Nigeria into Chad, Niger and Mali occurred in 2010, and, further importations of WPV3 into Niger and Mali were again detected in 2011. In Chad imported WPV1 from Borno, Nigeria, was detected in 2010 and transmission continued into 2011. The closest genetic relative of a WPV1 detected in Niger in July 2011 was a virus found in Chad in 2010. Three WPV1 cases in Mali in 2010 represented 3 separate importations linked to the 2009 WPV1 transmission in Burkina Faso and Guinea and the WPV1 2010 transmission in Mauritania, all of which resulted from westward spread of virus from Nigeria.

² Non-endemic countries with new WPV importation cases or outbreaks in 2010 included Congo, Kazakhstan, Liberia, Nepal, Russian Federation, Tajikistan, Turkmenistan and Senegal.

³ Non-endemic countries with only new WPV importation cases or outbreaks in 2011 included China, Côte d'Ivoire, Gabon and Guinea.

The Democratic Republic of Congo (DRC) had WPV1 cases linked to 3 different sources: cases in south east provinces represented uninterrupted transmission of virus imported from India in 2006; cases in Kasai Occidental and Bandundu represented virus that spread from Angola to DRC in 2008 and cases in Kinshasa and Bas Congo were linked to the outbreak in neighbouring Congo which in turn resulted from a separate 2010 importation from Angola.

- Five countries (Angola, Kenya, Mauritania, Sierra Leone, Uganda) had **continued transmission of WPV1 from importations that occurred prior to 2010**, and transmission appears to have been interrupted in two of them (Mauritania and Sierra Leone). Cases in Angola represented continued transmission of virus imported from India in 2007. Transmission of imported WPV1 has persisted in border areas between Kenya and Uganda since 2009, with long gaps in WPV detection evident both in real and virus evolutionary time. Nucleotide sequencing data linked the WPV1 found in Uganda in November 2010 to its closest genetic relative found previously in Kenya in 2009, while a WPV1 found in Kenya in 2011 had a Uganda virus from late 2010 as its closest relative..

aWPV1 detected in a single sewage sample in Aswan, Egypt, in December 2010, was linked to WPV1 circulation in Sudan in 2009 but there has been no further subsequent detection of WPV1 in either location.

Vaccine Derived Polio Viruses (VDPV)

Circulating VDPVs (cVDPV) outbreaks were newly detected in Yemen (VDPV2, 4 cases), Mozambique (VDPV1, 2 cases) and Angola (VDPV2, 1 case linked to an older outbreak in Somalia) in 2011. A VDPV type 2 (VDPV2) outbreak that first started in Nigeria in 2006 continues with 27 and 14 related cases detected in 2010 and in 2011. Single cases related to the Nigeria outbreak were found in Chad and Niger in 2010. VDPV2 outbreaks in Afghanistan and Somalia that started in 2010 and 2008, respectively, continued into 2011. No further VDPV2 or VDPV3 cases related to separate outbreaks in Ethiopia have been reported since 2009 and 2010, respectively. VDPV2 outbreaks in DR Congo and India appear to have been brought under control with no related cases reported since 2010. VDPV2 isolates related to outbreak viruses were found in contacts of AFP cases in various locations, including Nigeria, Somalia and India.

VDPVs of ambiguous origin (aVDPVs). VDPVs that are unrelated to outbreaks and that occur in persons not known to be immunodeficient are considered to be of ambiguous origin (aVDPVs). Since January 2010, aVDPV2 isolates from AFP cases were detected in Chad (1), China (2), DR Congo (2), India (5), Myanmar (1), Nigeria (1), Peru (1), Somalia (1), and Syria (1). A VDPV3 was detected in single AFP cases in China (VDPV3) and Tajikistan in 2010.

A VDPV2 strains were detected in unrelated AFP contacts in Sudan (1) and Nigeria (1) in 2011 and a single aVDPV1 was isolated from a healthy child in Turkey. VDPV2 isolates were also reported from non-AFP sources such as single sewage samples collected in Estonia in November 2010 and Finland in July 2011 and in 5 samples collected in Israel since January 2010. In all three countries the VP1 nucleotide sequences of isolates indicates their continued evolution from viruses previously detected in the same locations, albeit from as yet unidentified sources. A single VDPV1 positive sewage sample was collected in Egypt in February 2010.

VDPVs from immunodeficient persons (iVDPVs). Since January 10, type 2 VDPVs were isolated from immunodeficient persons from China (1), India (1), Egypt (1), Iran (1), Iraq (1), Sri Lanka (1), and Turkey (1). VDPV1 isolates were found in immunodeficient persons in Egypt (1) and Tunisia (1) and VDPV3 strains were isolated in China (1) and Algeria (3) also from samples from immunodeficient individuals. A special multi-country study of immunodeficient persons led to the detection of at least four of the reported iVDPV cases.

VDPVs pending classification. Some VDPVs reported in 2011 were pending categorization and the outcome of follow-up investigations. These included VDPV2 isolates from Nigeria (1), India (1), China (1) and a VDPV3 from China.

Investigation of the role of Non-polio enteroviruses in VDPV emergence. An Institute Pasteur multi-country collaborative study involving 11 laboratories (3 in Europe and 8 in Africa) is being conducted to characterize non-polio enteroviruses (NPEV) and PV isolates from AFP cases and healthy children. Human Enterovirus species C (HEV-C) recombinant nucleotide sequences have been frequently observed in non-structural parts of the genomes of cVDPVs. HEV-C was highly prevalent in Madagascar during the time that VDPV outbreaks occurred and Coxsackie A17 and A13 viruses were the main contributors of the observed recombinant sequences found in cVDPV isolates. Preliminary data were shared from Algeria, Central African Republic (CAR) and Cameroon. In Algeria three persons infected with VDPV3 were identified from the sequencing analysis of PVs. Follow up investigations revealed that the VDPVs were from persons with primary immunodeficiency, 2 of whom were siblings. In CAR, no VDPVs have been found but NPEVs are highly prevalent and the proportion of the contribution of HEV-C varies from year to year. In Cameroon, NPEVs were isolated from 35% of the stools from healthy children between 2008 and 2009 and were highly diverse, with 29 different serotypes identified. HEV-C accounted for 38% of all NPEVs but represented a higher proportion (approximately 50%) of those from the western part of the country that borders Nigeria than from other parts of Cameroon. Future collaborative work is proposed to compare the nucleotide sequences of cVDPVs from Nigeria, NPEVs and PVs from Cameroon and Chad. Chad has had re-established WPV infection and two VDPVs cases reported to date, 1 of which was linked to the Nigeria cVDPV outbreak

GPLN's Quality Assurance Programme

Laboratory Accreditation and Proficiency Tests. The results of the laboratory accreditation and proficiency testing programmes were reviewed during the consultation. Overall, 98.6 % of network laboratories were fully accredited in 2010, as were 96.5 % of those reviewed up to September 2011. PT Panels were distributed to evaluate the accuracy in carrying out 5 laboratory procedures. Summary results are shown in **Table 1**.

Table 1
Summary results of Proficiency Tests

Procedure Evaluated	Passing score	No. of participating laboratories	Score	No. laboratories
Virus isolation in cell culture - traditional test algorithm	≥ 80%	81 (3 reports pending)	100%	68
			80 to 99%	9
			< 80%	1
Virus isolation in cell culture - rapid traditional test algorithm	≥ 90%	66 (1 report pending)	100%	53
			90 to 99%	10
			< 90%	2 (NB. 95% & 100% on repeat)
ELISA-ITD	≥ 80%	15 (2 reports pending)	100%	9
			80 to 99%	2
			< 80%	2 (NB. 1 x 95% on repeat; 1 shifted to rRT-PCR)
Conventional PCR-ITD	≥ 90%	9 (4 reports pending)	100%	2
			90 to 99%	2
			< 90%	1
rRT-PCR ITD	≥ 90%	54 (results available for 46)	100%	43
			90 to 99%	3
			< 80%	0
rRT-PCR VDPV screening	≥ 90%	54 (results for 46)	100%	45
			100%	1
			90 to 99%	0
Nucleotide sequencing	Only qualitative feedback	8		

5 laboratories with performance concerns were identified during the accreditation and proficiency test programmes in 2010: 2 showed inaccuracy in virus isolation; 1 failed the on-site visit; 2 had problems with ITD molecular based testing. Problems have been resolved in all five laboratories.

Implementation of a quality assurance programme for sequencing laboratories has started. The method for serotype specific VP1 sequencing has been published, primers are available to GPLN members and there are plans to make reagents available in a kit format by end of 2011. Standard operating procedures (SOPs) for generating VP1 sequences from untyped isolates containing PV monotypes, homotypic or heterotypic mixtures have been distributed in July 2011. On-site accreditation evaluations were carried out by expert reviewers in laboratories in India, South Africa, Thailand and Tunisia, all of which attained on-site passing scores of ≥ 90 % for their performance. Minor changes to the checklist were recommended by the reviewers to include a requirement for

direct sequencing of heterotypic virus mixtures, referral of homotypic mixtures to global specialized laboratories for characterization and archiving of data for isolates tested in parallel with other laboratories. Areas identified for improvement were dye removal cleanup, sequence editing processes, data management and appropriate archiving of test documents and results. Proficiency testing began in 2011, using the same panel of specimens distributed for evaluation of rRT-PCR ITD performance. Participants were required to sequence individual Sabin virus isolates present in samples and sequencing of virus mixtures was optional. Only qualitative feedback is planned for 2011. Five laboratories that provided results showed high sequence quality and appropriate interpretations.

Capacity building for performing rRT-PCR. The network continued its programme to increase capacity for performing real time PCR procedures for intratypic differentiation (ITD) and screening for VDPVs. Currently 64 laboratories (45% of the network) have ITD capacity, and the majority (54) of them had new rRT-PCR capacity established since mid-2009. Indeed there has been a 100% increase in the total number of laboratories with any ITD capacity since capacity building commenced in 2006. ITD results are now available more rapidly for programme use, VDPV2 detections have increased and there has been a decrease in the demand and cost for inter-laboratory and international shipment of infectious isolates. With the uptake in rRT-PCR testing, there has been a concomitant decline in use and demand for ITD ELISA tests. ELISA use is now only restricted to 13 laboratories (10 of which also do rRT-PCR), mainly for characterizing PV isolates from sewage. Use of molecular procedures for characterizing sewage isolates is currently being evaluated.

Biosafety training. The GPLN developed training materials and launched a biosafety campaign in 2010 with the goal of improving PV containment, reducing incidents accidents and decreasing the risk of PV infections in laboratory workers. Standardized audiovisual training materials are now available in 4 language versions (Chinese, English, French, Russian) and are being used as a cost effective way to reach a wide target audience. To date 6 training workshops have been run in 5 WHO regions and participants have been trained from approximately 50 laboratories. A "training of trainers" approach is being used. Evaluation of the biosafety campaign has started and 26 trainers have reported running 119 training workshops that reached approximately 600 trainees. The impact and changes in individual laboratories are to be more formally evaluated through questionnaires and observations during on-site visits linked to the GPLN's accreditation programme.

Pilot testing of FTA cards for referral of isolates for ITD and sequencing

Experiences with pilot testing of FTA cards for shipping of isolates from 4 sending laboratories (Cameroon, DR Congo, Ibadan-Nigeria and South Africa) to 3 receiving laboratories (in Egypt, South Africa and United States of America) were presented and discussed.

- There was equivalent performance of ITD tests (> 90% concordance in serotype and intratype results in rRT-PCR assays, respectively) for traditionally referred isolates and those spotted on FTA cards.
- Shipping costs were reduced by 50% in Nigeria using FTA cards.
- Total shipping time was generally not reduced and amounted to 5 to 7 days.
- The total processing time of FTA referred samples was longer than that for traditionally referred isolates, because of extra steps required for elution and RNA extraction of samples.
- Use of disposable punchers during sample processing of FTA cards decreased the time spent on cleaning reusable punches to reduce the risk of cross contamination.

- RNA extraction using a commercial kit was more reliable and technically less challenging than an alcohol precipitation procedure.
- There was poor quality of sequences generated from a small number of RNA samples extracted from FTA cards (e.g. < 10% of approximately 200 samples analysed in one receiving laboratory). Various strategies were used to resolve problems that included: (i) use of a 2-step DNA synthesis protocol; (ii) use of a larger volume of cDNA from step (i) as template in the standard amplification reaction; (iii) gel purification of amplicons prior to sequencing; (iv) adjustment to the sequencing protocol to use multiple primers to obtain overlapping fragments to give the full sequence of interest.
- It was noted that the FTA cards are recommended for referral of DNA samples and that most publications mention amplification and sequencing of fragments ≤ 200 base pairs in length whereas the polio samples are of RNA and PCR targets are longer.
- Successful outcomes in transfection experiments conducted in two network laboratories imply that although virus is inactivated and rendered non-infectious for shipment, eluted RNA can be deliberately manipulated *in vitro* and introduced into cells for replication to obtain infectious virus for downstream testing.
- Sequence quality issues could be affected by one or more of the following: virus titre, inadequate drying of cards, delays in shipping, storage temperature of cards prior to or during shipment, lack of addition of desiccant to the shipping envelopes.
- Audiovisual training materials have been produced to demonstrate Standard Operating Procedures for use by FTA card sending and receiving laboratories.

Authentication of identity of cell lines used for PV isolation

Scientists at the National Institute for Biological Standards and Control (NIBSC), United Kingdom, have spearheaded development of a new quality assurance tool in the GPLN for authenticating the identity of cell lines. The test is based on a molecular testing principle (PCR followed by sequencing) and targets the cytochrome C oxidase subunit 1 for identifying the species of origin of the cell line. A very sensitive method has been developed to detect contamination of L20B (mouse cells) with human cells for cell lines commonly used for PV isolation, as well as for detecting contamination with cell lines with origin in other species that may be manipulated in some cell culture preparation laboratories that handle multiple cell lines. Five global specialized laboratories referred cell line samples to NIBSC for testing and all were found to be authentic with respect to the species of origin. Validation of test performance on different rRT-PCR platforms is planned as well as technology transfer and proficiency testing to build capacity to perform the authentication test in designated cell line repository and distribution reference laboratories in different WHO regions.

Revised VDPV screening test kit. CDC continues to monitor the field performance of distributed rRT-PCR reagents and to do further developmental work on the VDPV assay. The current version of the VDPV test has targets in both the VP1 and 3D region of the PV genome. The 3D target permits detection of recombination with any non-Sabin enterovirus, but is not relevant to VDPV detection which relies on a target present in VP1. A test version without the 3D target was produced to improve the robustness of signal strength and reduce production costs. Another modification was made to the PCR cycles such that 15 (for ITD assays) or 5 (for VDPV assays) PCR cycles are run at the start of the assay that use the annealing temperature for degenerate primers followed by an additional 40 amplification cycles using higher annealing temperatures. The performance of the modified test was

validated at CDC-USA and it was confirmed that signal strength was higher, test sensitivity was 10-fold higher (in terms of number of copies of target detected) and a small number of Sabin-like viruses were detected (in mixtures) that had been missed by the older version of the test. Higher signal strength, easier interpretation and high sensitivity were confirmed through testing of 450 Sabin like and 80 VDPV isolates using the modified ITD and VDPV tests under field conditions in Finland, Mumbai-India and South Africa. Distribution of the modified rRT-PCR tests within the GPLN will begin in 2012.

Improving efficiency of testing of sewage samples

There is interest to expand the use of surveillance for PVs in sewage to identify locations in which PV circulate. However current testing approaches:

- are inefficient and resource demanding
- are varied and non-standardized in terms of protocol and reagents used.
- are not readily evaluated by objective criteria to determine comparative accuracy.
- are lengthy with results available in some settings 6 to 8 weeks after sample collection.
- have not been analyzed objectively to document virus recovery at the sewage concentration step or virus isolation rates.

WHO/HQ is conducting a survey to document the sewage testing protocols used in different laboratories with a view to recommending test approaches for use in 3 epidemiology situations: (i) OPV use and active PV transmission; (ii) OPV use and polio free; (iii) IPV use and polio free.

Results of operational research projects conducted in THL-Finland & NIH-Pakistan were presented and discussed. The main findings were:

- 4 methods for sample concentration were compared (i) polyethylene glycol-dextran "2 phase separation" followed by direct inoculation of final concentrate into cell lines (ii) method (i) plus chloroform extraction of concentrate before inoculation; (iii) PEG precipitation, and (iv) filtration. Method (iv) was evaluated in only one test run so far and the other methods were run using sewage samples spiked with a known quantity of virus on 3 to 4 separate occasions. The percent virus recovery was estimated by titration in cell cultures. All methods showed percent virus recovery of 20 to 25%. There was inter-run variability in virus recovery for methods (i), (ii) and (iii). In method (ii) time and speed of centrifugation appeared to influence percent virus recovery.
- A "lab in a bag" approach (in which all required reagents for sample processing are pre-weighed and available in disposable equipment) was compared but found to be slightly less sensitive (23% lower) for virus recovery than the traditional sewage ("two phase" separation) concentration method in Finland. The "lab in a bag" concept shows promise for use in resource poor settings with poor laboratory infrastructure where sewage samples can be concentrated before being referred elsewhere for downstream testing in better equipped laboratories.
- Virus isolation data from Finland obtained from testing 589 sewage concentrates from Egypt were reviewed to determine the potential impact of reducing total observation time in cell cultures. Inoculated RD cultures showed virus cytopathogenic effects (CPE) faster for PV positive sewage samples than inoculated L20B cells. For known PV positive samples, CPE was observed in 92% of RD(A) cultures before 6 days post-inoculation and in 97% of

cultures after post-passage inoculations. In L20B cells, inoculated cultures were generally negative after first inoculation and only 31% of known PV positive samples showed CPE before 6 days post-passage. Both RD(A) and L20B cell lines had passed cell sensitivity quality assurance tests. There is a risk of reducing PV isolation sensitivity in Finland if the total observation time of L20B cell cultures inoculated with sewage samples is shortened.

- Selection of PVs by growth in L20B cells followed by passage into RD(A) or African Green Monkey cells and incubation at 40 C were evaluated as methods to enrich for WPV and VDPVs in sewage concentrates with known NSL or VDPV positive results using traditional cell culture approaches. In initial experiments in both locations, the inoculated concentrates of samples known to be positive for WPV and/or VDPV were correctly flagged by showing cytopathic effect at 40 C. Characterization of isolates by rRT-PCR ITD showed that although there was high concordance of results (> 80%) with those obtained using the traditional cell culture method for samples containing monotypes of NSL or VDPV, not all isolates in samples containing PV mixtures (whether NSL mixtures or VDPV mixtures) could be recovered when using the high temperature protocol. Prospective testing of 39 sewage specimens in Finland showed that the traditional test approach was more sensitive (6/39 found to be VDPV positive) than the high temperature protocol (3/39 were VDPV positive).
- rRT-PCR assays were evaluated for the simultaneous determination of the sero- & intra-type properties of untyped sewage isolates. In Finland, an IPV using country with rare PV detections, the rRT-PCR ITD and VDPV tests worked well. In Pakistan, a WPV endemic country using OPV, invalid rRT-PCR ITD test results were obtained for 40% of untyped isolates and this could be reduced to 12% by the use of purified viral RNA rather than cell culture supernatant.

Summary and recommendations

The Consultation concluded that the GPLN operates with high efficiency. A wide range of projects aimed at improving testing efficiency and biosafety are on track. The following recommendations were made.

1. The GPLN should develop a plan for laboratory surge capacity to support responses to outbreaks or increases in surveillance activities.
2. **Proficiency testing**
 - The GPLN should consider modifying the PT scoring system such that all polio results must be correct to obtain a passing score
 - Laboratories that supply Proficiency Test (PT) panels should request input from WHO laboratory coordinators with respect to when and how remaining PT panels should be shipped. Coordinators should also supply an updated lab contact and address list.
3. **Laboratory Accreditation**
 - Sequencing laboratories should perform a self evaluation by filling in the WHO checklist, to provide baseline Quality Assurance data for planning purposes.
 - There is a need to train additional Subject Matter Experts to evaluate sequencing laboratories for accreditation.

- A scoring system should be developed for the PT to be used for sequencing laboratories.

4. Improvements in rRT-PCR methods

- The new VP1-only VDPV assay should be implemented in the GPLN
- The protocol/package insert in the new kit must explicitly describe the changes in interpretation and include the kit version number.
- The new protocol/package inserts for both ITD and VDPV must explicitly describe the changes to be made to the thermocycling parameters and mention the expectation of occasional uniquely shaped curves that occur with some samples with high RNA concentration and the follow actions to be taken.
- The methods should be validated for use on other real-time PCR platforms used in the network.

5. Cell line authentication

- NIBSC should design a multi-site pilot study with cooperation from other Global Specialized Laboratories to facilitate transfer of cell authentication assays to GPLN cell distribution laboratories. A survey of cell lines used in all network laboratories with information on their susceptibility for infection with poliovirus and other enterovirus should be conducted with the assistance of the regional laboratory coordinators.

6. FTA card experience

- WHO regional laboratory coordinators should compile a list by the end of 2011 of laboratories that have a need to ship isolates beyond their national borders for ITD or sequencing, to estimate the need for use of FTA cards
- The FTA card protocol that incorporates QIAamp RNA extraction, should be finalized and validated in network laboratories in Mumbai-India and South Africa by end 2011.
- Materials and reagents required by FTA sending and receiving laboratories should be added to the WHO supply catalogue by end October 2011.

7. PV detection directly in faecal specimens

- The GPLN should focus on developing direct detection methods for PV screening to identify samples that should be further analyzed and reduce the workload for virus isolation in cell cultures.
- Development work on use of panPV reagents for direct stool screening should be continued, and results analyzed with respect to serotype and WPV vs VDPV vs Sabin.
- Methods in development should be evaluated with fresh stool specimens tested in parallel with virus isolation.
- Internal control (e.g. phage MS2) templates should be used to assess possible sample derived PCR inhibition
- All members of the GPLN with experience in direct detection methods are encouraged to share their protocols and data with WHO to contribute to the work on identifying a reliable, standardized approach for wide scale use in the network.

8. Laboratory safety

- Efforts must be made to choose persons known to have good communication skills and to provide trainers with resources materials at least one week in advance of planned biosafety workshops. Experience shows that the effectiveness of the audiovisual training modules developed for the GPLN is impacted by trainer knowledge, experience and preparation, as well as group size and language issues.
- Additional training materials should be developed to address chemical safety, avoiding contamination in laboratories using molecular methods, laboratory design and layout.

9. Improvements in efficiency and quality assurance of sewage processing methods for environmental surveillance

- Use of WPV1 and WPV3 genotype-specific rRT-PCR for identification of environmental isolates should be evaluated with a view to improving the efficiency of testing. Detection of VDPVs will remain an issue and should be addressed in the future.
- The GPLN WHO/HQ should continue efforts to standardize methods for testing sewage samples among laboratories.
- WHO should develop a proficiency testing plan for sewage processing methods to measure isolation efficiency in each laboratory.
- Comparative data should be gathered documenting virus isolation rates relative to sewage concentration method and volume of extract analyzed.
- The network laboratory in Pakistan is encouraged to share results of parallel testing using two methods of analysis of sewage samples with WHO/HQ by end of November 2011;
- The network laboratory in Ibadan, Nigeria, is requested to retain stored frozen aliquots of sewage concentrates because of their collection in a unique virologic and epidemiologic situation. Such concentrates will be a valuable resource for evaluating test methods.

- 10.** GPLN laboratories are encouraged to seek opportunities through submitting grant applications to support their research project e.g. applications for funding can be made through the Bill and Melinda Gates Foundation Grand Challenges Explorations to support creative projects to improve laboratory methods or logistics.