

Summary report on the

Fourteenth intercountry meeting of directors of poliovirus laboratories in the Eastern Mediterranean Region

Damascus, Syrian Arab Republic
25–28 October 2010



**World Health
Organization**

Regional Office for the Eastern Mediterranean

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1. Introduction

The fourteenth intercountry meeting of directors of poliovirus laboratories in the WHO Eastern Mediterranean Region was held in Damascus, Syrian Arab Republic from 25 to 27 October 2010. Directors of poliovirus laboratories in Egypt, Islamic Republic of Iran, Iraq, Jordan, Kuwait, Morocco, Oman, Pakistan, Saudi Arabia, Sudan, Syrian Arab Republic and Tunisia attended the meeting. Participants also included a scientist from the the National Institute for Biological Standards and Control (NIBSC), United Kingdom and the polio laboratory director from Kenya Medical Research Institute (KEMRI), along with staff from the WHO Regional Offices for Eastern Mediterranean and Africa.

Dr Tahir Pervaiz Mir, Regional Adviser, Polio Eradication Programme welcomed the participants and delivered a message on behalf of Dr Hussein A. Gezairy, WHO Regional Director for the Eastern Mediterranean. In his message, Dr Gezairy commended the excellent work performed by the regional polio laboratory network. He expressed appreciation for the achievements of the polio network laboratories in implementing new methods. Following Dr Gezairy's message, H.E. Dr Saeed Rida, Minister of Health, Syrian Arab Republic, welcomed the participants and emphasized the importance of high routine immunization coverage and ensured their commitment toward polio eradication efforts.

The regional polio network laboratories continue to support the polio eradication initiative through isolation and characterization of polioviruses from faecal specimens from cases of acute flaccid paralysis (AFP) and other supplementary surveillance activities such as the analysis of stool samples from contacts of AFP cases and the processing of sewage samples from environmental surveillance. Regional polio network laboratories also continue to play a critical role in research activities that contribute to the development of improved laboratory techniques for the rapid and accurate detection of poliovirus isolates to be used globally.

Participants in the meeting reviewed the results of AFP surveillance in the Region during the period January 2009 to October 2010. Transmission links between wild polioviruses (WPVs) isolated during that period from both AFP surveillance and supplementary surveillance activities were discussed, as were the detection and properties of vaccine-derived polioviruses (VDPVs). Important issues related to the network's laboratory quality assurance programme were also discussed with the main focus on biosafety aspects related to the work with poliovirus. A WHO biosafety campaign designed to promote awareness of biorisk in poliovirus laboratories was introduced to the group. The campaign consists of six separate modules supported by DVD presentations. The aim was to qualify trainers who can run the campaign in their respective countries. Recommendations were made to sustain and improve the performance in regional laboratories.

2. Summary of discussions

Eastern Mediterranean Region overview: Most of the countries in the Region are polio free. A type 1 outbreak in south Sudan that started in 2008 was stopped over one year ago. Pakistan and Afghanistan are the only endemic countries in the Region. In Afghanistan, circulation is localized and innovative measures are continuously being taken to overcome the situation. In Pakistan 75% polio cases are from KP/FATA where lack of access to children is a major issue because of deteriorating security. Floods created epidemiological imbalance in southern Punjab and northern Sindh. South Sudan, Somalia and Yemen are high risk countries with alarming number of unvaccinated children and adequate proportion of unprotected children.

AFP surveillance indicators at national level are satisfactory but the subnational data analysis is clearly showing the gaps. In 2010, AFP surveillance reviews were conducted in nine countries of the Region. Field and laboratory staff joint efforts have resulted significant reduction

in surveillance process timeline (average days). Contact sampling is a good initiative in the Region particularly in detecting the WPV when the index cases are negative. The laboratory network had marvellous achievements; accreditation, managing extensive workload, containment. The certification process is ongoing with significant progress. The Region will continue efforts to alert countries through the use of a risk assessment model, AFP surveillance reviews and also helping in preparedness to response.

The regional polio network laboratories had excellent achievements in terms of accreditation, the management of extensive workloads and the implementation of poliovirus containment. The workload of the regional polio network laboratories is very high. During 2009 the regional polio network laboratories processed 26 330 specimens from AFP cases and contacts, and the workload continues to be at the same level in 2010. The average time between the receipt of stool samples in the laboratory and reporting the result was 13 days in 2009. Overall, 94% of specimens had culture results within 14 days, 95% had ITD results within 7 days of virus culture positive referral and in 97% of AFP cases, the final laboratory testing results were provided within 45 days of paralysis onset.

The real-time PCR (rRT-PCR) method for rapid characterization of polioviruses was fully implemented in five ITD laboratories. There are a further two laboratories (Morocco and the Syrian Arab Republic) which are in the process of establishing the rRT-PCR method. These laboratories are facing major problems due to the non-availability of rRT-PCR machine.

New LABIFA version 4.1 software was developed to include changes due to the introduction of the rRT-PCR method; it was distributed and installed in regional polio network laboratories. High quality genomic sequencing of poliovirus continued in the Pakistan laboratory, which is greatly helping surveillance activities in Pakistan. Between January 2009

and October 2010, circulating vaccine-derived polioviruses (cVDPVs) were isolated from AFP case and contacts in Afghanistan and Somalia, and one type 2 VDPV was isolated from an AFP case in the Syrian Arab Republic.

The ongoing environmental surveillance in 21 districts with 34 collection sites in Egypt discovered one type 1 aVDPV from a sewage collection site in Helwan. Environmental surveillance was established in priority districts of four provinces of Pakistan in 2009–2010.

Global overview: Between January 2009 and June 2010, the global polio network laboratories (GPNL) tested 258 000 faecal specimens from 130 000 AFP cases during the period January 2009 and June 2010, and approximately 22 250 non-AFP specimens. The majority of all WPVs reported globally were found in the African Region, where 20 countries had WPVs, compared with 3 countries in the Eastern Mediterranean Region and 2 countries in the South-East Asia Region.

In Africa, all WPV1 and WPV3 isolates belonged to West Africa B Polio Virus 1 (WEAF-B PV1) or WEAF-B Polio Virus 3 (WEAF-B PV3) genotypes, respectively, except for South Asia (SOAS) genotype viruses found in Angola (PV1 only), Burundi (PV1 only) and the Democratic Republic of the Congo (PV1 and PV3). There was continued, uninterrupted transmission of indigenous WPV1 and WPV3 in Nigeria. Nine other countries (Burundi, Cameroon, Central African Republic, Guinea, Kenya, Liberia, Sierra Leone and Uganda (2009), Liberia (2010) had new WPV1 importations or outbreaks detected during the reviewed period. In seven countries (Burkina Faso, Benin, Chad, Côte d'Ivoire, Mauritania, Niger and Togo), WPV1 was found in 2009 that was linked to outbreaks following importations in previous years. Most of the latter outbreaks, plus the new ones found in 2009 and 2010 resulted from the westward spread from Nigeria that started in 2008. Two countries (Niger and Chad) that immediately border Nigeria experience repeated WPV

importations. SOAS genotype viruses were responsible for approximately 10% of all WPV cases detected in Africa between January 2009 and September 2010. SOAS PV1 was originally imported from India into Angola in 2007 and an ensuing outbreak has continued without interruption since that time in Burundi and the Democratic Republic of the Congo. WPV3 was found in Angola (2008) and also in the Democratic Republic of the Congo (2009).

In India, the northern provinces of Uttar Pradesh and Bihar accounted for 79% and 16% of all detected WPVs in the country, respectively. WPV1 viruses from these two locations generally grouped into separate transmission chains. Sporadic WPV1 cases found in Delhi and Rajasthan in 2009 were linked to transmission in Uttar Pradesh, whereas those found in Jharkhand and Punjab were linked to viruses in Bihar. In 2010, WPV1 was exported from Bihar to neighbouring Nepal. WPV3 accounted for approximately 90% of all WPVs in India in the reviewed period.

A large WPV1 outbreak was detected in Tajikistan in 2010. The outbreak virus originated in Uttar Pradesh, India, and spread along multiple transmission chains within Tajikistan. The virus was also found in the Russian Federation, where at least 6 importations from Tajikistan occurred, with evidence that at least two importations led to limited spread to close contacts of cases in Russia. Turkmenistan had at least 2 importations of virus from Tajikistan. Epidemiology investigations of some WPV1 cases found in the Russian Federation suggested links to Uzbekistan, although no WPV has been confirmed there.

Outbreaks of circulating VDPVs (cVDPV) of serotype 2 were detected in Afghanistan, India and Somalia. Previously detected cVDPV2 outbreaks continued in the Democratic Republic of the Congo and Nigeria. Ethiopia also had a second VDPV outbreak, but of serotype 3 virus. VDPVs were isolated from 5 immunodeficient persons from 4

countries (Argentina, Colombia, India and Kuwait). Some VDPVs from AFP cases considered to be of ambiguous origin were found through routine surveillance in Angola, Cameroon, Chad, Democratic Republic of the Congo, Ethiopia and in the Syrian Arab Republic. VDPVs were also reported from non-AFP specimen sources: VDPV2 isolated from 4 non-paralysed children in northern India; genetically linked VDPVs of all 3 serotypes in sewage in Finland; VDPV2 in sewage in Israel; VDPV2 in sewage in Tallinn-Estonia; VDPV3 in Mumbai; and VDPV1 in Egypt in 2010.

Despite the increase in workload, especially in endemic countries, a high quality of performance is being maintained by the global poliovirus network laboratories, and laboratories are meeting the needs of the polio eradication programme. New methodologies have been implemented and progress is being made in developing new methods: IgM and IgA ELISA tests for detection of polio antibodies; poliovirus direct detection test algorithm using molecular-based test procedures; methods for rendering isolates non-infectious for shipping; and improvements in the efficiency of testing sewage samples for polioviruses.

The new global polio eradication initiative strategic plan has been launched with implications for the global poliovirus laboratory network: expansion of use of environmental surveillance; involvement in sero-surveys; support to research and product development. Environmental surveillance for polioviruses was initiated in Pakistan and India (Delhi) through the polio eradication initiative and in Australia through a national initiative. A new biosafety campaign has been launched in 2010.

Virus surveillance: Only WPVs of serotypes 1 (WPV1) and 3 (WPV3) were detected in the regional polio laboratory network in 2009 and 2010. Genotypes and transmission links among WPVs are routinely investigated through molecular epidemiology studies based on the analysis of the complete nucleotide sequence of the VP1 region of the

viral genome. The characteristics of WPVs found between January 2009 and September 2010 were summarized at the meeting according to genotype, geographical location and transmission links.

Pakistan/Afghanistan: The Pakistan Regional Reference Laboratory (RRL) is annually processing more than 15 000 stool specimens from AFP cases and contacts, from both Afghanistan and Pakistan. The laboratory is maintaining high standards of laboratory performance indicators. The RRL obtained 100% score in all proficiency testing (PT) panels. As of October 2010, 97 WPVs (76 WPV3 and 21 WPV1) were isolated from all provinces of Pakistan. A total of 19 WPVs (11 WPV1 and 8 WPV3) were isolated from Afghanistan, mostly from southern provinces. Overall non-polio enterovirus (NPEV) rate remained around 20%.

Environmental surveillance was initiated in July 2009 in two mega cities of Pakistan (Karachi and Lahore). In 2010, more collection sites were added from major cities of provinces. Two scientists completed training in advance molecular biology techniques for sequencing of the complete genome and a new bioinformatics tool (MATLAB) was introduced for the sequencing analysis of WPVs. There is increase in workload due to addition of more sites.

Egypt: The Egypt RRL continued to test stool specimen and virus isolates for Egypt, Iraq, Lebanon, Syrian Arab Republic and Sudan. During the past 12 months, the RRL processed 2572 samples (2158 from AFP and 414 from contacts). In 2009 and as of October 2010, the laboratory is maintaining high standards for all laboratory performance indicators. The laboratory performance is monitored through a strict quality assurance programme. The RRL scored 100% in all the PT panels. Environmental surveillance continued with collection of sewage samples from 34 sites in 21 provinces.

South Sudan and Somalia: The KEMRI polio laboratory (KPL) serves Djibouti, Somalia and south Sudan in the Eastern Mediterranean Region, in addition to Kenya and Eritrea in the African Region. Stool specimens submitted to the laboratory from AFP cases and their contacts in the countries of the Eastern Mediterranean Region account for approximately 70% of the laboratory workload. Of the 2514 samples tested in 2010 alone, 1038, 687 and 12 were from south Sudan, Somalia and Djibouti respectively. The KPL is maintaining good laboratory performance indicators. All poliovirus isolates are referred to the RRL at National Institute of Communicable Diseases (NICD), South Africa. There is high concordance of results between KPL and RRL. The overall non-polio enterovirus isolation rate for all countries for 2010 stands at 12.4%; south Sudan and Somalia have rates that are well over the recommended 10%. In 2010, the KPL fully implemented ITD methodologies (conventional PCR and ELISA) and plans are under way to upgrade to real-time PCR in the first quarter of 2011.

Detection of wild polioviruses: In 2009 and 2010, WPVs were detected in Afghanistan, Pakistan and south Sudan. Indigenous WPV1 and WPV3 circulation continued in Pakistan and Afghanistan. In 2010, three clusters of WPV1 and two of WPV3 have been detected to date. WPV1 was found in Sudan and represented continuation of an outbreak that followed importation of virus in 2004, although there had been a long gap in case detection between 2005 and 2008. WPV1 was most recently detected in Sudan in June 2009.

WPV1 Cluster A-3A1A: Sequence analysis of polioviruses reveals circulation of eight different lineages of this cluster in both Pakistan and Afghanistan. The viruses from Afghanistan are from Farah, Kunduz, Nangarhar and Kandahar and are genetically placed in three different lineages. Kunduz and Kandahar viruses are importations from FATA (Mohmand Agency). In 2010, Charasada and Mohmand viruses are the continuation of 2009 Swat viruses. The Nangarhar virus is also an

importation from Swat. Three P1 wild viruses were isolated from North Waziristan (two of them are orphan viruses). North Waziristan viruses are circulation of local virus introduced from Sherani with continued circulation through the low transmission season. Lakki Marwat virus is also continued circulation of Bannu and North Waziristan 2009 viruses. An orphan virus isolated from Killa Abdullah is drawing attention towards circulation of P1 wild virus in Quetta Killa Abdullah block. Five out of 10 orphan viruses found in Pakistan are in this cluster. Environment samples collected from two different sites in Peshawar, i.e. Shaheen Town and Lara Ma near Bakhshoo Pull, show active circulation of WPV1 in Peshawar. Importantly, the virus from Shaheen Town is an orphan virus showing 2.5% divergence with the Afghanistan–Nangarhar virus. Another orphan virus isolated from a sewage sample of Karachi Gulshan Iqbal site indicated 2.3% divergence with Pakistan–Quetta.

WPV1 Cluster A3-D2: This is the most active cluster during 2010 and circulation is continuing in KP (Peshawar, Hangu, Kohat), FATA (Mohmand agency, Khyber, Orakzai), Sindh (Ghotki, Khairpur) and Punjab (Multan, Muzafargarh and Mianwali) and Afghanistan (Farah). Sequencing data of KP and Farah cases show these isolates are closely related to the Rajanpur June 2009 virus and signify the rapid movement of populations between provinces and countries. Viruses were isolated from sewage samples collected from Gulshan Iqbal, Gadaap and Sohrab Goth Karachi presenting continuous virus circulation. A virus of this cluster also isolated from two different sites of Multan (Ali Town and Suraj Miani) is placed in separate lineages. A3-D2 virus was also isolated from sewage samples collected from Gulshan Iqbal, Gadaap and Sohrab Goth Karachi presenting continuous virus circulation. A virus of this cluster was also isolated from two different sites of Multan (Ali Town and Suraj Miani) placed in separate lineages.

WPV1 Cluster B4-A1: A total of three lineages of this cluster are circulating: two orphan viruses belonging to two lineages are circulating

in KP (Bajour and Mohmand) and a third lineage detected in a sewage sample from Shaheen Town Peshawar with 3.4% divergence from its closest match (Khyber 2008).

WPV3 Cluster B1-C5: This is the major active cluster, which has more than 11 lineages with several source reservoirs, circulating during 2010. Three lineages were detected in sewage samples cases from Karachi, which shows that Karachi remains a persistent reservoir for WPV3. The viruses isolated during 2010 from Karachi are related to 2009 local circulating viruses. In KP, five separate lineages are identified representing Bajour, Peshawar, Khyber, Swat and Swabi isolates. Recent viruses detected from Quetta, Killa Abdulla and Chaghi are linked to Quetta viruses.

Afghanistan Block. Quetta-S: Afghanistan has established circulation for more than 12 months. One orphan virus isolated from Chagi is >1.5% divergent and has closest link with Hyderabad virus. In Afghanistan, a virus isolated during 2009 continued its circulation during 2010 in Hilmand, Zabul, Nangarhar and Kandahar. Maywand, Kandahar virus (AFG10-775 AFG/08/10/076 Maywand Kandahar 27-April-2010) is an orphan virus having 2.3% divergence from its closest matches (AFG10-126 AFG/08/10/012 Hilmand 13-Jan-10, AFG09-1592 Kandhar and AFG09-1286 Maywand Kandhar). Virus isolated from Nangarhar is an importation from Peshawar (PAK10-892 PAK/NW/30/10/020 Peshawar 04-Mar-10).

WPV3 Cluster B1-C6A: This cluster was active in Afghanistan during 2010 with isolation of only one orphan virus from southern Afghanistan (Hilmand) having 2.4% divergence from AFG/08/07/689 (Hilmand virus). This cluster was silent in 2009 but during 2008 three viruses were isolated from Farah and Kandhar.

Detection of vaccine-derived polioviruses: In 2009 and 2010, outbreaks of circulating VDPVs (cVDPV) of serotype 2 were detected in Afghanistan and Somalia. In Afghanistan, they were localized to Kandhar (2009) and Hilmand (2010). In Somalia, nucleotide sequencing data from retrospective analysis of isolates provided evidence for cVDPV2 outbreaks in 2008 and 2009 extending into 2010. The 2008 Somalia cVDPVs are not closely related to each other. All, except one, of 2009 Somalia cVDPVs are not closely related to any of the 2008 viruses: Somalia cVDPVs are not closely related to the Ethiopian cVDPVs either, suggesting various independent emergences of VDPVs. One VDPV2 was isolated from an immunodeficient person, a severe combined immunodeficiency (SCID) case from the Islamic Republic of Iran who sought medical care in Kuwait; follow-up samples taken at an interval of one month have been negative so far. The patient is alive and is followed up regularly in Kuwait. One VDPV2 was detected in the Syrian Arab Republic through routine surveillance, but there was no information available about the immune status of this patient. One aVDPV1 was detected from a sewage collection site in Helwan province of Egypt in February 2010. The identity of VP1 sequence to Sabin 2 was 98.9% at the nucleotide level and 98.68% at the amino acid level. Follow-up samples were collected but no more VDPVs were found in environmental samples. Furthermore, all Sabin-like viruses isolated from AFP cases from September 2009 to December 2010 were tested by rRT-PCR and none were characterized as VDPVs.

Improvement in VDPV screening diagnostics and emerging concerns about VDPV definitions: The implementation of rRT-PCR procedures has resulted in more frequent and more rapid VDPV detection, particularly in the polio-endemic regions. There is clear evidence for circulation of type 2 Sabin-derived polioviruses associated with paralytic disease with fewer than 10 changes in VP1, suggesting that viruses with fewer changes may be relevant to polio surveillance and eradication. For this reason, it has been proposed that any type 2

poliovirus with 6 or more changes from Sabin 2 will be considered a “vaccine-derived poliovirus” of programmatic importance. The change in the definition of type 2 VDPV should result in increased sensitivity in detection of circulating viruses.

Environmental surveillance for detection of polioviruses: Environmental surveillance for polioviruses has been implemented in the provinces of Baluchistan (Quetta), Sindh (Karachi), Punjab (Lahore, Multan, Rawalpindi) and KP (Peshawar) in mid-2009 to 2010. Implementation was generally in accordance with the WHO guidelines for environmental surveillance of PV circulation.

In Pakistan, WPV1 and WPV3 viruses were detected in both locations, although with higher frequency in Sindh than in other provinces. In Sindh, WPV3 was detected in sewage in mid-2009 and detection continued for several months into 2010 and was later replaced with WPV1. Characterization of WPV1 isolates from sewage collected in Sindh since August 2009 suggests multiple separate introductions of virus linked to transmission in North West Frontier Province and Punjab. One of these importations led to transmission that continued for at least 2 months; genetically related viruses had been found previously in Punjab and Afghanistan in 2009.

Representatives of a WPV1 lineage were found in sewage collected in Lahore, Punjab, in August 2009, and genetic comparison to its closest related virus suggested a gap of approximately 4 years in detection. This lineage was subsequently identified in an isolate from an AFP cases from the Punjab (DG Khan, 2010). While the characterization of viruses from sewage generates substantial workload in Pakistan, this surveillance approach is providing useful information to guide immunization activities and to focus attention on areas of weak AFP surveillance performance. In 2010, viruses were detected from sewage collection sites of all provinces

at different interval over the period of time and orphan viruses were detected from Karachi, Peshawar, Multan and Rawalpindi.

Looking at the success of implementation of environmental surveillance for poliovirus isolation, the polio eradication initiative in Pakistan is planning to expand the environmental surveillance to other programmatically important districts.

Laboratory accreditation programme: Performance of network laboratories continues to be monitored through an accreditation programme coordinated by the Regional Office. All regional polio network laboratories except Kuwait RRL are fully accredited by WHO as of October 2010. The Kuwait RRL has a long history of being unable to obtain valid ITD test results. The workload is low and all poliovirus isolates are being referred to a global specialized laboratory to provide accurate results for programme use. The accreditation programme for sequencing laboratories is now available for use. An accreditation checklist has been completed and sent to laboratories in October 2010, with comments due by 30 November 2010 and implementation to follow thereafter in 2011. Development of a proficiency test for sequencing is ongoing at the global specialized laboratory in the United States.

Proficiency tests: A proficiency testing programme for the global poliovirus laboratory network is coordinated by WHO in collaboration with two global specialized laboratories in the United States and The Netherlands. Five different PT panels are now provided that are aimed at evaluating 1) accuracy of virus isolation; ITD by 2) ELISA, 3) probe hybridization or 4) rRT-PCR; and 5) rRT-PCR for VDPV screening (newly introduced in 2010).

- All laboratories using the new algorithm that attempted the virus isolation PT in 2009 attained passing scores of > 90%.

- Three laboratories (Egypt, Pakistan and Tunisia) that attempted the ELISA PT in 2009 attained passing scores of $\geq 90\%$.
- Two laboratories (Egypt and Pakistan) that attempted the probe hybridization PT attained passing scores of $\geq 90\%$.
- All five laboratories (Egypt, Islamic Republic of Iran, Pakistan, Oman and Tunisia) that attempted the two rRT-PCR panels for ITD and VDPV screening attained passing scores of $\geq 90\%$.

Protocol for authenticating the identity of cells lines: The global specialized laboratory in the United Kingdom presented the results of work to develop a standardized procedure for authenticating the identity of cell lines used for poliovirus isolation in the global poliovirus laboratory network. Assays based on sequencing a conserved mitochondrial gene fragment (COXA1) and real-time PCR with melt curve analysis have been evaluated and are ready for implementation.

Biosafety campaign: Participants learned of the development, pilot testing and distribution of training materials to be used in a biosafety campaign that has been launched in the GPLN in 2010. A “Training of trainers” workshop for laboratory directors who will act as biosafety focal points (or name a deputy) was held during the meeting. Group discussions were held on the six biosafety modules. Participants identified laboratory malpractices showed during the video sessions and summarized key messages for each of the biosafety modules. The participants showed willingness to replicate similar activity in their own laboratories and also extend this facility to other public health laboratories.

3. Recommendations

1. All laboratories performing ITD testing should use the new ITD test algorithm based on two separate rRT-PCR procedures for ITD and VDPV screening, respectively. Isolates interpreted as NSL in the ITD

test and/or VP1-negative in the VDPV test should be referred for full VP1 nucleotide sequencing.

2. Ongoing environmental surveillance activities in the Region should be further strengthened by seeking more support for laboratories so they can meet increasing needs for testing and possible expansion of sewage collection sites.
3. The current VDPV definition is inadequate to identify all Sabin-related viruses of programmatic importance. Effective immediately, any type 2 vaccine-related poliovirus with 6 or more changes from Sabin 2 will be considered a “vaccine-derived poliovirus” of programmatic importance, regardless of its source; the definition of type 1 and type 3 VDPVs remains unchanged (≥ 10 changes in VP1). The current upper limit of divergence for a VDPV is eliminated. Documentation should be updated as needed.
4. Following guidelines for all relevant laboratories of the GPLN, the standard protocol and laboratory accreditation checklist for poliovirus sequencing, developed by CDC, should be implemented in regional polio network laboratories with sequencing capacity (Pakistan and Tunisia) in 2011. The proficiency testing programme should be implemented as soon as it is available. Technology transfer for standardized reagents and automating procedures for sequence data handling, reporting and mapping should begin in 2010.
5. Contamination of a cell line such as L20B with a different cell line used in the same laboratory could lead to the wrong classification of virus isolates during poliovirus isolation and characterization, resulting in the waste of time and resources. Cell lines used for poliovirus isolation and passage should be authenticated using a standardized procedure. Laboratories that maintain master cell banks for distribution to other network laboratories should refer samples of their cells on FTA cards to NIBSC for authentication. Mycoplasma testing will be performed on the same samples.
6. All staff in regional polio network laboratories, ranging from the support staff and technicians, to biosafety officers and the laboratory

head should complete the six training modules of the GPLN Biosafety Campaign. The laboratory director together with relevant laboratory staff should critically review their own practices in relation to biosafety and make changes and improvements when necessary. Coordination with relevant personnel and local/national authorities at their country institution is essential.

7. Laboratory directors should be fully aware of all aspects of the work carried out in the laboratory naming deputies for selected activities when necessary and critically reviewing all results, e.g. a reduction in NPEV isolations could be due to seasonal or local variations in virus circulation but can also indicate a decrease in the quality of samples sent to the laboratory. Effective and prompt communication should be maintained with the laboratory regional coordinator on technical issues.