Meeting of the Polio Laboratory Network of the WHO European Region (laboratories of the Russian Federation and newly independent states)
Abstract
The regional Polio Laboratory Network assists the Global Polio Eradication Initiative (GPEI) in fighting polio in the WHO European Region through laboratory detection of poliovirus. Fourteen national and subnational (NL and SNL, respectively) polio diagnostic laboratories from seven countries, heads of the regional reference laboratory (RRL) in Moscow, a representative of the RRL in Helsinki and specialists of the WHO Regional Office for Europe participated in the meeting, which focused on implementation of activities on safe laboratory containment of polioviruses (PVs), including the current stage of the plan and problems affecting laboratory performance.

KEYWORDS
POLIOMYELITIS – diagnosis, epidemiology, prevention and control
POLIOVIRUS VACCINE – administration and dosage
POLIOVIRUS CONTAINMENT
LABORATORIES
EPIDEMIOLOGICAL MONITORING

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### Abbreviations

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<tr>
<td>AFP</td>
<td>acute flaccid paralysis</td>
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<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<td>cDNA</td>
<td>complementary chain of deoxyribonucleic acid</td>
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<td>CPA</td>
<td>cytopathogenic agent</td>
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<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>GAPIII</td>
<td>Global action plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use</td>
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<td>GPEI</td>
<td>Global Polio Eradication Initiative</td>
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<td>GPLNMS</td>
<td>global polio laboratory network</td>
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<td>IPV</td>
<td>inactivated poliovirus vaccine</td>
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<td>ITD</td>
<td>intratypic differentiation</td>
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<td>LDMS</td>
<td>laboratory data management system</td>
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<td>NIS</td>
<td>newly independent states</td>
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<td>NL</td>
<td>national laboratory</td>
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<td>OPV</td>
<td>oral polio vaccine</td>
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<td>bOPV</td>
<td>bivalent OPV</td>
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<tr>
<td>mOPV1</td>
<td>monovalent OPV, type 1</td>
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<tr>
<td>mOPV2</td>
<td>monovalent OPV, type 2</td>
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<tr>
<td>tOPV</td>
<td>trivalent OPV</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>PEF</td>
<td>poliovirus essential facility</td>
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<td>PV</td>
<td>poliovirus</td>
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<td>PV2</td>
<td>PV, type 2</td>
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<td>RCC</td>
<td>Regional Certification Commission for Poliomyelitis Eradication</td>
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<td>RNA</td>
<td>ribonucleic acid</td>
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<td>RRL</td>
<td>regional reference laboratory</td>
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<td>RT-PCR</td>
<td>reverse transcription PCR</td>
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<td>SIA</td>
<td>supplemental immunization activity</td>
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<td>SNL</td>
<td>subnational laboratory</td>
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<td>VDPV</td>
<td>vaccine-derived poliovirus</td>
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<td>VDPV1</td>
<td>VDPV, type 1</td>
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<td>VPDV2</td>
<td>VDPV, type 2</td>
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<td>WPV</td>
<td>wild poliovirus</td>
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<td>WPV2</td>
<td>wild poliovirus, type 2</td>
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**Introduction**

Fourteen national and subnational polio diagnostic laboratories (NL and SNL respectively) from the Russian Federation and six newly independent states (NIS) (namely Belarus, Georgia, Kazakhstan, Republic of Moldova, Ukraine and Uzbekistan), heads of the regional reference laboratory (RRL) in Moscow, a representative of the RRL in Helsinki and specialists of the WHO Regional Office for Europe (Regional Office) participated in the meeting.

Dr E. Gavrilin, coordinator of the Polio Laboratory Network, presented the meeting’s programme, goal and tasks, stressing that the main focus should be on implementation of activities on safe laboratory containment of polioviruses (PVs), including the current stage of the plan and problems affecting laboratory performance. Dr G. Lipskaya was elected Chair of the meeting.

**Session 1 – WHO global and regional updates**

Dr Sergei Deshevoi, while presenting a report on global and regional polio eradication status, drew attention to an important event which took place several weeks earlier – the global cessation of oral trivalent polio vaccine (tOPV) and switch to bivalent oral polio vaccine (bOPV), which does not contain type 2 PV. Risk mitigation from tOPV cessation was ensured by introduction of at least one dose of inactivated polio vaccine (IPV) into the immunization schedule, high population immunity to type 2 PV (PV2) and availability of monovalent type 2 OPV (mOPV2) in case of outbreak.

With regard to wild PVs (WPVs), the main goal at the moment is to stop international transmission, which decreased in the last year to cases only between the two remaining endemic countries (Afghanistan and Pakistan). The WHO European Region borders with both of these countries, so there is an ongoing risk of importation. Immunization coverage in the Region is sufficiently high, except for in Ukraine. Last year, highly evolved vaccine-derived type 1 PV (VDPV1) was detected in this country, which necessitated the involvement of international experts to assess the situation, implement supplementary immunization activities (SIAs) and put in place more active acute flaccid paralysis (AFP) surveillance. SIAs were also implemented in 2015 in other countries (Azerbaijan, Georgia, Germany, Montenegro, Russia, Tajikistan). Inter-country and inter-regional exercises to strengthen preparedness for a polio
outbreak were carried out to assess the countries’ readiness to respond in the event of WPV or VDPV identification. The Regional Office developed recommendations for independent implementation of response measures on the national level, which are available on the Regional Office website. In conclusion, Dr Deshevoi stressed that the Regional Certification Commission for Poliomyelitis Eradication (RCC) emphasized the high performance level of the Polio Laboratory Network at its 2016 meeting.

Dr M. Yakovenko presented an overview of poliovirus containment activities in the European Region. In accordance with the Global action plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use (GAPIII), all 53 countries of the Region provided to WHO the required information on national PV inventories, and, thus, confirmed the completion of Phase I containment in their territories. However, data on potentially infectious materials, as well as information on implemented containment activities, were not provided by some countries in the annual update report on polio eradication activities for 2015. The Regional Office requested that special attention be paid to this issue.

Thirteen countries expressed an intention to have a specialized facility to store PV (poliovirus essential facility, PEF), and these countries will need to pass a certification process. Concern was expressed that:

- most countries do not have national legislation to implement containment;
- conditions to maintain balance between containment requirements and continuation of vaccine manufacture are not defined;
- not all countries are capable of ensuring the certification of their PEF.

Dr Yakovenko presented a certification structure, risk assessment principles and measures for risk mitigation.

Based on experience with the certification of the Region’s polio-free status, meeting participants discussed the complexity of containment activities and stressed the need for joint meetings of stakeholders and governmental agencies.

Dr Gavrilin presented an analysis of the European Polio Laboratory Network’s performance and plans for poliovirus containment. He stressed the importance of presenting laboratory data, both in annual reports and as operative information through the laboratory data management system.
(LDMS) and thanked all laboratories for transitioning to the global laboratory polio network management system reporting system (GPLNMS). It was noted with satisfaction that all 48 laboratories of the Region use the new PV study algorithm, which enables them to significantly decrease the time required to obtain results. Supplementary surveillance, enterovirus surveillance (including serous meningitis surveillance) and environmental surveillance (sewage) play a significant role in poliovirus surveillance in the Region; however, AFP surveillance still should be of primary importance.

The main noted challenges in the work of the Polio Laboratory Network were:

- the lack of integration of viral and epidemiological data from enterovirus and environment surveillance;
- frequent change of kits for PV intratypic differentiation (ITD), which requires the availability of compatible equipment and continuous training of personnel.

Concern was also expressed regarding situations in which compliance with containment requirements contradicts with laboratory activity, especially when using virus to assess cell culture sensitivity, for population immunity studies, etc.

Dr Gavrilin presented a scheme of operations of various level laboratories (i.e. implementing virus isolation, ITD, sequencing, PV storage) when handling contaminated material and underlined the need for immediate destruction of unneeded L20B-positive samples or transfer of valuable L20B-positive samples to PEF. It was suggested that the immunity of laboratory workers to PV2 in the specialized laboratory of the United States Centers for Disease Control and Prevention (CDC) and similar laboratories be studied.

When discussing the report, meeting participants noted that it is practically impossible to exclude all risks when implementing PV containment, as it is impossible to stop all work of clinic laboratories and implementation of other laboratory activities which use clinical samples. It is therefore important to know what the real, acceptable risk level is and which biological materials pose a potential risk of spreading PV and should be destroyed. A number of questions were raised on the absence of a list of activities and timing of their implementation in the event of confirmed PV2 isolation.
Dr Deshevoi reported on activities implemented in response to the outbreak caused by circulating VDPV in Ukraine in 2015, including data of an assessment mission carried out six months after the outbreak’s registration. As response measures, three rounds of supplementary immunization were carried out in the country using mOPV1 and tOPV; however, the coverage in each round did not reach 95%. During this time:

- more teachers became aware of the outbreak;
- the number of immunization opponents and immunization refusals decreased;
- the quality of surveillance for AFP cases, their contacts and environment increased.

However, 3–10% children of 0–10 years of age had still not received a single dose of polio vaccine and sensitivity of AFP surveillance did not reach the recommended level of 3/100 000 children under 15 years of age. According to the conclusion of the assessment commission, it is very likely that the VDPV transmission in the country was stopped, but the risk of PV spreading in the country remains.

Dr L. Kozlovskaya presented the results of the RRL in Moscow, including data on virus characteristics in Azerbaijan, Kyrgyzstan, Tajikistan and Turkmenistan, for which the RRL acts as NL. As of the meeting, the RRL had completed the replacement of current cell lines on genetically authenticated cell lines in all of the Network’s laboratories. In 2015, all laboratories successfully implemented professional testing within 14 days using the new algorithm. The RRL receives a large number of samples, acts as a NL for five countries and investigates (L+R+) and (R+L+R+) samples for other countries. About 2000 samples were received in 2015, but the new algorithm enabled a timely provision of results. Using FTA® cards for sending samples demonstrated a good track record, however only three countries implemented this method. Taking into account the large number of samples in which cytopathogenic agents(CPA) being identified were received by the RRL, the main method used for PV ITD is real-time PCR, while partial and full genome sequencing is performed on a sample basis. In 2015, four circulating VDPV1 isolates were identified in samples from AFP cases from Ukraine. VDPV2 with 157 nucleotide substitutions in the VP1 genomic region and about 20% replacements in full genome in comparison with parental vaccine Sabin strain was identified in a Moscow sewage sample. The origin of this virus was not established.
The RRL completed Phase 1 PV containment activities by inventorying WPV, VDPV and vaccine PV2 collections and depositing them for storage in the biosafety department of its own facility.

Dr S. Kajalainen presented the results of the work of the Helsinki RRL. Since 2016 this laboratory has been supervising the work of 16 countries, including 3 NIS: Armenia, Georgia and Ukraine. In 2015 and the beginning of 2016, ITD was performed for more than 300 PV samples, most of which came from Ukraine. All samples were sent to the RRL on FTA® cards within 1–6 days, and all were classified as vaccine related. As part of containment activities, an inventory register and destruction of PV-contaminated materials were completed. Finland does not plan to have a PEF; all significant isolates (WPV, VDPV) were transferred to CDC. Upon the conclusion of the report, the Regional Office expressed its gratitude to the laboratory for its readiness to act as the RRL for a number of NIS countries.

**Session 3 – Report of the national and subnational laboratories**

The NL of Georgia informed the meeting of surveillance results in the country and in Armenia. This was followed by reports from the NLs of Belarus, Kazakhstan, Republic of Moldova, the Federal center for hygiene and epidemiology of Rospotrebnadzor and six SNLs of the Russian Federation, the NL (Kiev) and SNL (Odessa) of Ukraine, and the NL of Uzbekistan.

Their reports presented the results of AFP surveillance and supplementary enterovirus and environment surveillance. In the course of surveillance, laboratories supervise on a regular basis “silent” territories, assess the local efficiency of sewage sample analysis and, when needed, train personnel and change sampling sites. Both virus isolation in the cell culture and real-time PCR are used to detect enteroviruses and determine their serotype. The laboratories noted the efficiency and importance of the cell culture method. Belarus and Russian Federation plan to implement PEF certification and subsequent storage of PV2; other countries reported on PV2 destruction. The main challenges in the work included the need to study a large number of migrants; absence of clear sample selection criteria during enterovirus and environmental surveillance and, as a result, difficulty in comparing results obtained in different territories; absence of antiserum for non-polio enterovirus serotyping; and lower quality of medium provided for cell cultures.
When discussing reports, Dr Deshevoi provided the analysis of annual country reports for environmental surveillance and suggested that surveillance should be considered “well organized” when about 10 sewage samples per 100,000 of population are studied. Dr Gavrilin presented future Network diagnostic standards and informed that nine laboratories in the European Region are involved in ITD. The only high-sensitivity kit for this purpose accredited by WHO is the real-time RT-PCR, made by the CDC and currently approved for use on ABI7500 and RotorGene devices.

**Conclusions**

Based on the presented data, the meeting confirmed the utmost importance of laboratory studies for the implementation of the Global Polio Eradication Initiative (GPEI). The Network laboratories in the attending countries demonstrated a high performance level for 2015–2016.

- All laboratories introduced the new algorithm, which enabled them to significantly decrease the time required for sample investigation.
- All countries achieved a high quality of laboratory performance on polio identification under the AFP surveillance system, and implemented supplementary surveillance of enteroviruses and environmental surveillance.
- All laboratories successfully passed professional testing.
- A timely ITD was arranged for all isolated PVs.
- The LDMS online reporting platform was successfully introduced and used in all laboratories.
- All laboratories provided reports on implementation of Phase 1 of poliovirus containment.

Taking into account that GPEI implementation in 2015 was highly esteemed by the RCC, the meeting expressed its gratitude to all laboratories for their work.

The meeting furthermore drew attention to the following:

1. Taking into account the important role of laboratory analysis in the context of OPV2 cessation, WPV2, VDPV2 and vaccine PV2 containment, national health authorities in the attending countries were requested to continue their support for NLs and SNLs to ensure successful GPEI implementation.

2. AFP surveillance remains the most important component in polio surveillance and should be implemented in strict adherence to all WHO criteria. At the same time, the results of supplementary environmental and enterovirus surveillance provide very important information
for the GPEI that should be taken into account. The meeting encouraged all Network laboratories
to analyse the data of years of supplementary surveillance and assess the efficiency of using
various methods to identify samples positive for enteroviruses.

3. The need to strengthen control over the timeliness and conditions of isolate
delivery to the RRL remains. Sending PV isolates (or samples characterized according to the
new study algorithm, such as L+R+) from NLs to the RRL on FTA®-cards is the most preferable
method, as it optimizes this process and ensures shorter delivery times, cheaper and simpler
procedures. Network laboratories should expand the application of FTA® cards.

4. For an effective organization of further studies of isolates received under polio
surveillance, NLs and SNLs should note the date and delivery method as well as the number of
samples when sending them to the RRL.

5. The high quality of cell cultures used in the laboratories remains important, and
requires regular control of their sensitivity to PV and mycoplasma contamination. To assess cell
culture sensitivity to PV, the RRLs recommend that all Network laboratories refresh
PV reference-strains and are ready to provide them upon request.

6. The meeting gladly noted that all countries have successfully switched to
reporting through LDMS. In the past year, there was an increase in timeliness and accuracy of
information entry, however these indicators still require improvement. Taking into account the
importance of coordinating viral and epidemiological data, the meeting encouraged all countries
to pay special attention to their reporting systems and to ensure stable data provision on a weekly
basis, including mandatory “zero” reporting.

7. While following all poliovirus containment requirements under the GPEI,
Network laboratories expressed their concern about the possible impact of containment activities
on the performance of other programmes involving collection, storage and study of potentially
infectious biological material. In this regard, the meeting requested that WHO carry out an
expert assessment and provide precise recommendations for types of biological samples that bear
minimal risk of potential laboratory spread of PV (e.g. viral, viral RNA, cDNA samples, stool
sample suspensions for ELISA studies, nasopharyngeal swabs).

8. To unify activities implemented in Network laboratories in the event of
PV2 presence in samples, the meeting stated the need for WHO to develop and provide an
appropriate algorithm of activities to all interested parties, including a list of response measures,
their timing, reporting manner and reporting level.
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