Latest developments of the poliomyelitis laboratory network in the Western Pacific Region

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Targeted Disease Control Initiatives of EPI/WPRO

- Maintaining polio free status: China one year polio-free - last wild case in 9 Oct 2011
  - High coverage with polio vaccine: routine and SIA
  - Sustain high quality surveillance for acute flaccid paralysis (AFP) and performance of lab network
  - Laboratory containment of wild poliovirus
- Maternal and neonatal tetanus elimination (MNTE) in five remaining countries:
  - Cambodia, China, Laos, Philippines, Papua New Guinea
  - WHO validated that MNTE in China as of Oct 30 2012

Targeted Disease Control Initiatives of EPI/WPRO

- Twin goals by 2012
  - Measles Elimination: no endemic virus by 2012
    - Accelerated rubella control to achieve and maintain control of rubella and prevention of CRS in the WPR by 2015
    - Achieving and maintaining high population immunity,
    - High quality case-based measles surveillance
    - Accredited measles and rubella laboratory network
  - Hepatitis B Control: 2012 milestone - chronic HBV infection rates <2% among children five years old as interim goal; Final goal of <1% by 2017
    - Achieving high vaccine coverage, including timely birth dose
    - Validation of control by serologic surveys & coverage data
    - Expert panel for verification

Progress since 1988 WHA resolution to eradicate polio

<table>
<thead>
<tr>
<th>Region</th>
<th>Polio-free certificate</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO African</td>
<td>1994</td>
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<tr>
<td>WHO Americas</td>
<td>1994</td>
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<tr>
<td>WHO South-East Asia</td>
<td>1995</td>
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<tr>
<td>WHO Eastern Mediterranean</td>
<td>2002</td>
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<td>WHO Western Pacific</td>
<td>2000</td>
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2011: 650 cases
2012: 202 cases (553 for Jan 1–Nov 27 in 2011)

No new case in India for 23 months

UN General Assembly
Special Event on Polio, September 2012

“Who has made polio eradication a top priority in my second term as Secretary-General. We also committed to tackling malaria, new paediatric HIV infections, maternal and neonatal tetanus, drastically reducing deaths from measles and implementing the global strategy for women’s and children’s health. I have made it publically known in January this year that during my second term as Secretary-General I will wipe off these five major killers, including polio, so I have to keep my word. This is a matter of health and justice. Every child should have the right to start life with equal protection from these diseases. When children are protected from polio, they are better protected against all diseases. To deliver global results, we need global solidarity. That is why I have put the strength of the entire United Nations system behind polio eradication.”

The Polio Emergency 2012-13

WHO Executive Board

“DECLARES polio eradication an emergency for global public health...”

21 January 2012

World Health Assembly

...requests the Director-General to rapidly finalize a polio endgame plan, with a tOPV-bOPV switch.”

25 May 2012
**Activation of emergency centres/procedures**

- Emergency Operation Center (CDC)
- Strategic Health Operations Centre (WHO)

**The Polio 'Endgame' Strategy**

- Wild virus eradication: end-2014
- OPV type 2 cessation: 2015/2016
- Global Certification: end-2018
- OPV type 1+3 cessation: 2019/2020

**WPR Certified Polio-free Region**

- 29 Oct 2000
- Date of onset of last case: 19 March 1997, Cambodia
- Certification of Poliomyelitis Eradication in WPRO

**Wild poliovirus (N =21) and month of paralysis onset**

- Xinjiang Uygur Autonomous Region, China, 2011
- Xinjiang borders 8 countries: Russia, Mongolia, Kazakhstan, Kyrgyzstan, Tajikistan, Afghanistan, Pakistan, India
- 5 rounds of SIA

**Subnational Polio Risk Assessment**

- Western Pacific Region, 2011*

**LEGEND:**

- Low risk
- Medium risk (China, Malaysia)
- High risk (Cambodia, Lao PDR, Papua New Guinea, Philippines)

* Source: country progress reports submitted to RCC17 (Nov 2011)
VDPVs detected in China 2010 - 2012*

LEGEND:
- aVDPV (2010) – 7 cases (Chongqing, Shanghai, Shanxi [2], Tianjin, Tibet, Yunnan)
- aVDPV (2011) – 5 cases (Chongqing, Fujian, Gansu, Shandong, Xinjiang)
- iVDPV (2011) – 3 cases (Guizhou, Ningxia, Shanghai)
- cVDPV (2011) – 3 cases (Sichuan)
- VDPV (2012) – 4 cases (Guizhou, Sichuan [2], Tianjin)

Two type 2 VDPVs from Southern Vietnam in 2012

China CDC conducted the national workshop to introduce the new algorithm in Feb 2012 and real time PCR training in March 2012 (23 provinces)

NPL’s testing samples from AFP and Supplementary surveillance

<table>
<thead>
<tr>
<th>NPL’s testing samples from AFP and Supplementary surveillance</th>
<th>AFP surveillance</th>
<th>Enterovirus surveillance (including HFMD)</th>
<th>Environmental surveillance</th>
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<tbody>
<tr>
<td>VDRL Australia</td>
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<td>NED Japan</td>
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<td>NIH, CDC Korea</td>
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WHO conducts accreditation of 43 polio labs including 31 provincial labs in China

2011-2012 WPR Polio Labnet Updates

- Accreditation status: all 43 network laboratories maintained full accreditation status
- Quality assurance
  - Virus isolation: All 43 polio labs passed 2011 virus isolation PT (12 non-China lab used the new algorithm: Scored 100% except two labs (95% & 90%).
  - ITD: 7 ITD labs passed ITD and VDPV screening PT (100% score) in 2011-2012. 5 labs used real time PCR and 2 labs used conventional PCR. ITD labs will be increased to ~30 by 2013.
  - Sequencing: 5 ITD labs except Malaysia and New Zealand will participate in 2012 polio sequencing PT. All 7 ITD labs will conduct polio sequencing from 2013 ~ sequencing lab accreditation.

Key Development of China Polio Labnet

- Introduction of the New algorithm for virus isolation among 31 provincial labs in China planned from August 2012 (delayed to Jan 2013), will be implemented together with real time PCR for ITD and VDPV screening from 2013
- Timeline for China- Virus isolation within 18 days and ITD within 7 days -> total of 25 days
- China CDC conducted the national workshop to introduce the new algorithm in Feb 2012 and real time PCR training in March 2012 (23 provinces)
- Follow up of polio real time PCR training
  - Real time PCR platform available for most provinces.
  - Implementation steps including proficiency test completed in 23 provincial labs after the training (22 provinces scored 100% for PT)
  - Most provincial labs trained in 2012 will be upgraded to polio ITD labs from 2013 (Henan and Guizhou?, training of 8 remaining provinces planned in 2013)
- Yunnan provincial CDC and China CDC Detected a Myanmar VDPV case in 2012
Introduction of the new algorithm
Biosafety awareness training

Performance of China Polio Labnet during Xinjiang outbreak
- Rapid detection of wild poliovirus detection using molecular method as screening and timely data sharing with WPRO, WHO HQ, US CDC
- Use of molecular detection supplemented by virus isolation of RT-PCR negative samples enabled timely response to the wild poliovirus outbreak from 45-60 days to 3 days
- Excellent collaboration between China CDC and provincial laboratories (Guangdong and Shandong) and regional/global polio labnet
- Use of mobile P3 lab for processing environmental samples in Xinjiang: two wild poliovirus strains detected
- Seroprevalence study in Xinjiang using 2611 convenience samples collected before the outbreak in Xinjiang done after outbreak showed NT Ab titer and GMT were lower among age group of 0-5 years and 15-40 years than 5-15 years and >40 years

Timeliness of ITD Reporting, 2007-2012*

Timeliness of Polio Lab Performance
Western Pacific Region, 2009 – 2012*

18th GPLN consultation, Geneva 2012
Polio Ad Hoc meeting, US CDC 2012
Polio sequencing workshop2012 in US CDC
Hand Foot Mouth Disease in China

- **38th Infectious Disease, Notifiable Communicable Disease in China**

- Subgenotype C4 of EV71 was consistent circulating in China from 1998 to 2008, C4b(1998-2004); C4a(2003-2009)

- Compared to CA16 or other enterovirus, EV71 caused more Severe and fatal cases in China from 2007 to 2009. EV71 is predominant circulating virus at some counties and prefectures from 2008-2009.

- Virulence: No strong association between HEV71 genotype or sub-genotype. However Potential for Emergence of Increasingly EV71 virulence and transmission.

- EV 71 Vaccine in development – China CDC clinical trial

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**Recorded prevalence of EV71 subgenogroups in the Asia–Pacific region**

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**China LabNet for HFMD surveillance activities**

Based on the Polio/measles lab network

- Genotyping for HEV71 and CA16 isolates
- Quality control and Training
- Technical support for provincial lab

- Enterovirus isolation and identification (RT-PCR, real time RT-PCR)
- Quality control and training prefecture lab
- Technical support for prefecture lab

- Collection of specimens
- RT-PCR, real time RT-PCR

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**Polio Labnet Summary**

- 43 fully accredited polio labs in WPR provided timely laboratory data to the programme.

- Expansion of polio ITD labs: China real time PCR training workshops in China conducted in March and regional training planned in Dec 2012

- NIID and VIDRL continued to provide support to process AFP samples from Cambodia, Laos (NIID), Brunei, PNG, PICs (VIDRL)
Some issues identified

- Shipping of polio isolates from the first VDPV case PI HCMC to NIID Japan delayed due to shipping permit problem - response delayed
- Action point: 3 NPLs from Vietnam (2) and Philippines will be upgraded to ITD labs by 2013
- Increased cost to operate additional ITD labs to cover additional kits/reagents for real time PCR: funding gap
- Increased workload to coordinate different categories of polio labs: 43 labs, ~30 ITD labs, 7 sequencing labs; Measles/rubella (n=383) and Japanese encephalitis (n=10~20)
- Involve regional/global lab experts from RRL/GSL for lab accreditation?

WPRO Polio Labnet Plans

- 2nd regional training for real time PCR for ITD and VDPV screening planned in Dec 3-7 2012: Hong Kong, New Zealand, Viet Nam (2), Philippines, Korea
- 3rd round of real time PCR training for 8 remaining provincial labs in 2013?
- Reduced shipping of polio isolates by increasing polio ITD labs
- WPR Labnet meeting for polio, measles/rubella and JE in March 11-15 2013
- Distribution of New lab reporting format in 2013
- Improve the timeliness of reporting polio results from non-AFP samples: environmental surveillance and healthy children survey etc.

Objectives of the training

- Learn the new real-time PCR technique for rapid detection and characterization of polioviruses;
- Familiarize with hands-on practice on real-time techniques for ITD of polioviruses and VDPV screening using real-time PCR platform; and
- Discuss problems and challenges for polioviruses laboratories and provided updates of the GPLN including new laboratory performance indicators.

Attaining and ensuring proficiency in rRT-PCR

Four Steps

- Step 1: At least 4 test runs of 10 samples (Same set) to check for reproducibility of performance - Jan 2013
- Step 2: Retrospective testing (Sabin like) - Feb 2013
- Step 3: Prospective parallel testing of L20B positive cultures and polioviruses (can be done in parallel with step 2 - Feb 2013
- Step 4: Proficiency Testing - March 2013
- Each step will be started after successful completion of one activity (Decided by WHO/USCDC)

Step 1: Testing of Known isolates

- Select from among isolates that have been sequenced
  - NIBSC standard Sabin strains (to represent SL monotypes).
  - Make up samples to represent mixtures using NIBSC strains.
  - Known VDPV (if available).
- Prepare the panel and share information with WHO and CDC before commencing the test.
- Report result of the test within 48hrs after test completion.
- Summary result template, screen shots and work sheets to be shared with WHO/WPRO and CDC for review and feedback

Step 2: Retrospective testing of Sabin-like isolates only

- List Sabin positive isolates from the last three months that are in storage. (Low workload labs: 2006-9 isolates or obtain from other labs, n=~50)
- How to proceed:
  - Set up in batches to maximize efficiency and minimize reagent needs.
  - One isolate per specimen (unless isolate has a PV mixture).
  - Results entered on standardized excel spreadsheet (cumulative)
  - Results sent to CDC, WHO/WPRO for review and feedback.
Step 3: Prospective parallel testing

- Follow flowchart for new algorithm in terms of workflow for ITD Labs:
  - 2 ITD methods will be run (conventional PCR or sequencing, rRT-PCR)
  - 2 VDPV screening methods will be run (Sequencing and rRT-PCR): No need for neutralization test before rRT-PCR for PV mixtures. rRT-PCR VDPV done on all identified SL viruses.
  - Report valid ITD results to the program as usual based on traditional ITD test
  - rRT-PCR and VDPV results sent to WHO/CDC at end of each test run within 48 hours of completion on summary spread sheet.

- Can be done with step 2 retrospective testing

Step 4: Proficiency testing

FINAL:
Implementation of Real-time ITD and VDPV screening as a routine test following success in proficiency test !!!!!!
Real-Time PCR for Poliovirus Identification, Intratypic Differentiation and VDPV Screening

Centers for Disease Control and Prevention Workshop in Manila, Philippines, 03-07 Dec 2012

Advantages of Real-Time PCR
- High clinical sensitivity
- High analytical specificity
- Sample can be either RNA or cell culture
- Can be performed on sample with mixture of viruses
- More rapid results
- Easier to scale for larger number of specimens
- Reduces chances of assay contamination

Real-Time ITD Assays
- Same rationale, targets and number of assays as conventional ITD PCR assays
- PCR assay bench work nearly identical
- Same reporting
- New reagents
- New machines
- New analysis
- New troubleshooting

Diagnostic Questions
- Poliovirus? Yes / No
- What kind of poliovirus?
  - Wild
  - PV1/PV2/PV3
  - VDPV
  - Vaccine-related
- What is it’s relationship to other polioviruses?

Real-time Assays
- Real-time ITD Assays
  - Pan Enterovirus
  - Multiplex Sabin 1,2,3
  - Pan Poliovirus
  - Poliovirus serotype PV1
  - Poliovirus serotype PV2
  - Poliovirus serotype PV3
- Real-time VDPV Screening Assays
  - Sabin 1 VDPV
  - Sabin 2 VDPV
  - Sabin 3 VDPV
- Real-time Genotype Assays
  - Africa PV1 wild
  - Africa PV3 wild
  - Pakistan-India PV1 wild
  - Pakistan-India PV3 wild

† Kilpatrick et al., manuscripts in preparation
* Designed to detect Sabin

RRT-PCR primers and probes

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Pan Enterovirus</th>
<th>Pan Poliovirus</th>
<th>Sabin 1 VDPV</th>
<th>Sabin 2 VDPV</th>
<th>Sabin 3 VDPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan Enterovirus</td>
<td>PV1 (A)</td>
<td>PV2 (A)</td>
<td>PV3 (A)</td>
<td>PV1 (A)</td>
<td>PV2 (A)</td>
</tr>
<tr>
<td>Pan Poliovirus</td>
<td>Sabin 1 (A)</td>
<td>Sabin 2 (A)</td>
<td>Sabin 3 (A)</td>
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<tr>
<td>RVT-PCR primers and probes</td>
<td>AGGGCGCCCTAACTTT</td>
<td>GCGATTGTCACCATWAGCAGYCA</td>
<td>TTAGGTCAGATGCTTGAAAGC</td>
<td>TGCGIGAYACIACICAYAT</td>
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</table>
ITD Algorithm

- CPE positive
  - Culture *
  - Neutralization
  - Performs ITD

- Poliovirus mixtures
- Neutralization
- Performs ITD
  - Sabin-Like
  - Monotypic
  - Non-Sabin Like
  - Monotypic

- Sabin-like result and refer for sequencing
- Non-Sabin like result and refer for sequencing
- Report polio serotype and
  - CPE positive
  - Neutralization
  - Performs ITD

New Real-time ITD Algorithm

- CPE positive
- Neutralization
- Performs ITD
- Neutralization
- Performs ITD
- Performs ITD
- Performs ITD
- Performs ITD

- Non-Sabin Like
- Monotypic
- Non-Sabin Like
- Monotypic

- Sabin-like result
- Performs ITD by ELISA
- Performs rt-PCR
- Performs Assay

Summary

- Real-time RT-PCR assay has been developed to completely replace previous PCR assay for identification, serotyping and ITD of poliovirus isolates
- Evaluation completed with thousands of poliovirus isolates
- Method has been evaluated on different real-time platforms
- Parallel testing and implementation in CDC Diagnostic Lab has been completed
- So Far 87 labs have been certified in the network.

Real-time VDPV Screening Assay

- New assays
- Analogous Reagents and Assays to real-time ITD assays
- Designed to replace ELISA ITD
- Rationale is similar to Sabin ITD PCR
- Need to revise ITD algorithm
- Analogous to ELISA for reporting

VDPVs: July 2009–August 2011

- Type
  - WPV1
  - WPV2
  - WPV3
  - aVDPV
  - aVDPV (AFP patient)
  - aVDPV (Environment)

SOURCE: WHO POLIO LABNET

Circulating Vaccine-derived Poliovirus*, 2000-2012

- Italic indicates vaccine-derived poliovirus (VDPV) is associated with 2 or more cases of AFP
- Poliovirus typing and genotyping
- Figure includes VDPV, WDPV, and aVDPV

*Figures include multiple emergences and transmission chains. **VDPVs due to importation.
Could we have Missed Some VDPVs?

Problems with ELISA ITD method
- Type 2: some antigenic variants are missed
- Sabin 2: antigenic evolution more subtle
- MAD cVDPVs were missed
- Early NIE outbreak cVDPVs were missed
- Type 3: cross-absorbed antisera of variable quality
- Sabin 3-related isolates (identified by PCR) are routinely sequenced

VDPV Assays

Screen for possible VDPVs by targeting a “key” antigenic site in VP1
- S1, amino acid #99
- S2, amino acid #143
- S3, amino acids #285-291

ITD Algorithm

New Real-time VDPV Algorithm

* Includes 2 categories: L20B+RD+, RD+L20B+RD+
Summary

- Real-time RT-PCR assay has been developed to screen for possible VDPVs by targeting VP1.
- The assay replaces the ELISA test for screening of SL viruses following PCR ITD.
- Evaluation completed with more than 1000 poliovirus isolates.
- Parallel testing with ELISA has shown higher sensitivity for cVDPV detection for serotype 2 and equivalent for serotypes 1 and 3.
- Field evaluations have been completed with the real-time ITD assays.
- 87 labs have been certified in the rRT-PCR ITD assay and rRT-PCR VDPV assay.