Do you know where your sample was. . .?

A highly relevant question nowadays. Focusing immunization efforts on the last reservoirs of poliovirus depends heavily on the accuracy and reliability of laboratory information. Every Laboratory should be prepared to answer the inevitable hard questions from the national and Regional immunization coordinators on isolate authenticity. Every Laboratory should repeatedly ask itself these same hard questions. Can a detailed history be traced for every isolate, every sample received in the laboratory, every sample in relation to all others? This may appear to be a lot of effort, but it is a very small effort compared to a sub-national NID or mop-up campaign that need not have been done if accurate information had been available.

Incorrect wild poliovirus identification through contamination from laboratory stocks is no longer a risk. National Laboratories no longer use wild polioviruses as reference or test control strains. Today, the greatest risks of incorrect results are from mislabeled samples and contamination of virus-negative materials with virus-positive materials processed about the same time. The laudable accomplishment of isolating wild poliovirus is overshadowed and often discounted if other materials and samples are contaminated through handling the positive isolate. Laboratory practices and procedures designed to reduce these risks are described in earlier issues of UPDATE: VI(4) and V(1).

Knowing where your sample was. If mislabeling or contamination occurs, it is crucial for the laboratory to be able to quickly identify when, where, and how it may have occurred. Every laboratory should be able to trace every step in handling or manipulating each sample in relation to all other samples. An outside reviewer should be able to re-construct daily events and examine contamination potential for samples handled on the same day. Knowing when a sample was handled and where it was in relation to other samples are key elements for successful re-construction of events. Having a record of any deviation in standard laboratory practice, such as spillage and clean up, also could prove highly useful. The two major components of successful sample tracing are 1) keeping meticulous test records and 2) keeping a daily dairy. Most laboratories do a much better job with the former than the latter.

Keeping meticulous test records. Permanent records of daily observations of inoculated cells and virus typing worksheets are required of all laboratories as part of the accreditation process. These should be standard, detailed laboratory forms that permit recording of samples in the order in which they were handled; listing of all materials used; commenting on events that may impact results, such as spillage, error in inoculation, etc.; and signatures of technical staff to promote accountability. These basic records are essential, but they tell only part of the story. They provide no information on the myriad of other activities that took place before, after, and between tests. That is where the diary comes in.

(Sample continued on page 2)

Inside this Issue

1. Do you know where your sample was. . .?
2. Major personnel changes in the network
3. ERC, Mumbai achieves Global Specialized Laboratory status
4. Post-certification WPR Network maintains performance

Poliovirus Surveillance Report, January - August 2001
Major personnel changes in the network

Dr. Ray Sanders has resigned from his post as global laboratory coordinator effective October 31, 2001. Ray has done a magnificent job in accomplishing what he set out to do. Under his leadership, the laboratory network expanded greatly in efficiency and effectiveness with competence and capacity growing along with its reputation. Ray will continue to serve as the consultant to the Network and work closely with the very able but very overworked, remaining Geneva lab network team of David Featherstone and Chris Wolf.

WHO Geneva has posted the job, which may be found on www.who.int/per/vaccancies/phq01_069e.htm. The job involves managing the global level of the polio laboratory network, ensuring continued development, and strategic planning for laboratory containment. A degree in medicine, science or social sciences with a post-graduate degree in virology, microbiology, public health or epidemiology is required. In addition, the job requires extensive knowledge and experience in virology, demonstrated management ability, good interpersonal communication skills and ability to work technically with partners. Over ten years experience is required at the national or regional level in a virus diagnostic laboratory and over two years experience at the international level in planning, development, financial management and daily operations of a laboratory network. The closing date for applications is 5 October 2001.

Dr. Esther De Gourville, a member of the strong team of regional laboratory coordinators that made Ray look so good, has resigned from her EMRO (Cairo) position to take up a teaching post at the University of West Indies, Port of Spain, Trinidad, her hometown. Based on her demonstrated polio training skills, she will make a highly qualified and exceptionally gifted professor. Esther was the inspiration for the lead article in this issue of the UPDATE. She is dedicated to eradication becoming reality and fully intends to find time between her teaching chores to continue to lend her expertise. Until a replacement is found, EMRO will rely heavily on the Region’s polio laboratory expertise to fill the gaps.

On a more positive note Dr. Hiroyuki Shimizu has assumed the position as head of the Global Specialized Laboratory, Tokyo allowing Dr. Tatsuo Miyamura to

“Micropipettors are likely the most common cause of virus cross-contamination” according to David Featherstone. Micropipettors used in the place of volumetric pipettes must be used correctly to minimize the possibility of cross-contamination. Always change tips to avoid virus carry over. Always use aerosol resistant tips (ARTs) to avoid contamination of the pipettor and subsequent materials. Only the pipettor tip should be inserted into the container. Do not insert the pipettor barrel beyond the neck of the container; use long tips or short containers. Most importantly, use ARTS for all operations in the poliovirus laboratory, including non-infectious. Reducing lab costs by purchasing less expensive non-filtered tips is false economy. Any savings is miniscule compared to the enormous laboratory and program costs of even one incident of virus cross-contamination.

Keeping a daily diary. The diary fills in the details of all laboratory activities that took place between recording incoming samples in the logbook and reporting results. It should not repeat information already recorded on test work sheets. Many activities that might seem trivial at the time could prove highly useful for tracing a problem event. This means that each member of the technical staff keeps a dairy summarizing his/ her main activities each day. Sequential entries include such items as:

- prepared 2 liters of MEM (date);
- removed RD vial 22(6) from nitrogen freezer, thawed, and seeded 2/50 ml flasks
- unpacked stool samples # 15 through 20 from country X and stored in freezer #1, containers were clean, no visible fecal material;
- removed country Y stool extracts (#285 - 287) and X stool extracts (#13-14) from freezer #2, thawed, and inoculated on L20B and RD;
- repassaged all L20B and RD cell cultures inoculated on 10 September 01

Zero contamination and zero misidentification are the Network goals, but should either occur, we must be prepared to respond quickly to determine the origins and ensure that neither reoccur.

Contributing: Dr Esther De Gourville, Regional Laboratory Coordinator, EMRO

Polio Lab Network

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Post-certification WPR Network maintains performance

The last indigenous wild poliovirus in the Western Pacific Region (WPR) was found in 1997. The Region was certified as polio-free in October 2000. Now begins the challenge of maintaining the high performance levels of the Laboratory Network in the absence of wild polioviruses. The Laboratories have continued to do exceptionally well in 2001, with performance indicators above target as seen in the figure below.

To ensure performance remains high, the Network is striving to implement the TCG target of 80% of ITD results available within 60 days of onset of paralysis, developing a plan of action to upgrade the Regional data management system, writing guidelines on internal quality control, introducing a Regional distribution system for critical supplies, and establishing a support system for equipment maintenance.

ERC, Mumbai achieves Global Reference Laboratory status

The reputation of the Enterovirus Research Centre (ERC) is legend throughout the Global Network. During 2000, intratypic differentiation (ITD) tests were performed on poliovirus isolates from 751 AFP cases. In 2001, to date, 680 isolates from 292 cases have been examined by ITD. All tests were completed within the 28 days target with >80% of results released within 2 weeks of receipt of isolates. In fact, 96% of primary isolation results from India, are made available within 3 weeks of stool collection, which says great things for the entire Indian Lab Network.

In addition to its remarkable ITD performance, ERC sequences all wild poliovirus isolates, hosts laboratory training courses for the South East Asian Region, acts as a regional cell bank and distribution center and maintains an environmental surveillance research program. Given its competency, achievements, and global importance, ERC Mumbai was elevated to Global Specialized Laboratory status in October 2000.

In his report to the Government of India, Ray Sanders wrote “….the ERC serves as an example of quality, efficiency, and dedication to purpose from which all other laboratories in the Global Polio Laboratory Network could learn.” Congratulations to Drs. Deshpande, Mandke, and Saxena and all members of the staff who work so hard to make that statement a reality.

(Personnel continued from page 2)

concentrate on his larger responsibilities as head of the Department of Virology, National Institutes of Infectious Diseases. We are pleased to welcome Hiroyuki, who has already demonstrated competence and exceptional dedication to the PEI, at both the Regional and international levels.
### Poliovirus Surveillance Report, January – August 2001

#### Regional Ref. Lab

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<thead>
<tr>
<th>REGION</th>
<th>No. of isolates submitted</th>
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</tr>
<tr>
<td>EMRO</td>
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<tr>
<td>EURO</td>
<td>1358§§</td>
</tr>
<tr>
<td>SEARO</td>
<td>328***</td>
</tr>
<tr>
<td>WPRO</td>
<td>168***</td>
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#### Poliovirus Intratypic Differentiation Results

<table>
<thead>
<tr>
<th>REGION</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
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</thead>
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<td>Sabin</td>
<td>Wild</td>
<td>Sabin</td>
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<tr>
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<tr>
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<td>34</td>
</tr>
<tr>
<td>WPRO</td>
<td>0</td>
<td>73</td>
<td>0</td>
<td>114</td>
</tr>
</tbody>
</table>

#### Notes

- * Specimens
- ** Includes 48 vaccine and 1 wild mixtures
- *** Includes mixtures
- § Bulgarian outbreak: multiple isolates from 4 individuals tested in Rome and Paris
- §§ Total samples and isolates

AFRO: Up to the end of August 2001 17 wild viruses were detected from AFP cases in Nigeria (15), Mauritania (1) and Ethiopia (1). Despite increased surveillance in many countries in the African WHO region only 3 countries have been found with wild virus in 2001 compared with 12 infected countries last year. Hopefully this situation will be maintained over the peak of the polio transmission season, which is over July to September for most of the region.

AMRO: In late 2000 a small number of vaccine-derived type 1 polioviruses were detected from AFP cases in Haiti (1) and Dominican Republic (11). In 2001, up until September, intensified surveillance has detected a further 6 cases in Haiti and 3 in the Dominican Republic.

EMRO: Wild polioviruses were detected from 50 cases in six countries up until the end of August this year, compared to 135 cases from six countries for the same period in 2000. Pakistan and Afghanistan with 35 and 8 cases respectively make up the bulk of cases this year but have shown marked improvement on last year with reduction of 60% and 38% in cases over the same period.

EURO: Extensive countrywide immunisation activities were carried out in Bulgaria in response to the imported cases of the wild polio type 1 found in April 2001. Despite extensive surveillance activities no further wild polioviruses have been found apart from the initial two AFP cases, one contact, and one healthy child.

SEARO: India is the only country with wild poliovirus in the South East Asian region this year and it continues to show good progress resulting from extensive immunisation and surveillance activities. At the end of August 76 wild polioviruses from AFP cases have been detected compared with 134 for the same period last year. All cases this year have been in Northern India, with most in Uttar Pradesh (83%) and Bihar (13%).

WPRO: No evidence of endemic wild poliovirus circulation since March 1997, despite good AFP surveillance in the Region. The Region was certified free of polio in October 2000.