ROTARIANS CONTRIBUTE IN MULTIPLE WAYS TO POLIO ERADICATION

By the year 2005, Rotarians’ contributions to the global polio eradication effort will approach US$400 million. In addition, millions of dollars of “in-kind” and personal contributions have been made by and through local Rotary clubs and districts for polio eradication activities. Hundreds of thousands of Rotarians at the local level have provided support at clinics or mobilized their communities for immunization or polio eradication activities. More than one million Rotarians worldwide have contributed toward the success of the polio eradication effort to date. One hundred and eighteen nations around the world have benefited from PolioPlus grants.

In this issue, we discuss the PolioPlus Partners Program of the Rotary Foundation of Rotary International. The PolioPlus Partners Program was founded in 1995 to support the coordination of polio eradication resources for social mobilization of National Immunization Days (NID), polio medical officers, and poliovirus laboratories, which will continue to contribute to public health long after polio is eradicated.

PolioPlus Partners Help Strengthen the Global Network

The PolioPlus Partners Program of the Rotary Foundation of Rotary International works like a brokerage. Project applications are submitted to the Partners Program for funding. Once the projects have been registered with the PolioPlus Partners Program, they are published in a catalogue known as the Open Projects List. This list is distributed to Rotarians in clubs and districts around the world who may be interested in supporting global polio eradication efforts. Rotarians can review the catalogue of projects and may decide to support an entire project, individual items within a project, or portions of items within a project. By their support they become a “partner” of the project.

The advantage of the PolioPlus Partners Program is that projects are updated and circulated monthly to mobilize support. The PolioPlus Partners Program tries to ensure that all projects are funded in a timely manner; however, there are no guarantees of funding. The staff in the PolioPlus Partners Program works closely with the Regional Laboratory Coordinators to update them regarding the status of registered projects.

Laboratories in the four remaining endemic Regions of WHO have received to date about US$500,000 from PolioPlus Partners.

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Maintaining partnerships once the laboratory has been equipped.

Rotary's commitment to the development of the Global Polio Laboratory network extends beyond financial support.

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**PolioPlus**

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National PolioPlus Chairmen are in many of the polio endemic countries and are available to support the laboratory. They may help in clearing equipment through customs, transporting equipment to the laboratory, or educating local health workers about the importance of submitting stool samples from suspected cases of polio to the laboratory for analysis. If local Rotarians are not already involved in surveillance activities in your area, Rotary International encourages you to contact them. They are an excellent resource of support. You may contact PolioPlus Partners staff to obtain the information for the National or Regional PolioPlus Committee Chairman in your area.

**The WHO role**

Poliovirus laboratory projects must be reviewed and approved by the WHO Regional Laboratory Coordinator before they may be registered with the PolioPlus Partners Program. This ensures that laboratory projects are appropriate within the context of regional and global polio eradication strategies.

Rotarians realize that the development of the global poliovirus laboratory network is a dynamic process. It is understood that as the Network develops, needs may change and projects for laboratories that have already received Rotary support may develop. Rotarians are committed to the achievement of a polio-free world and the development of a Laboratory Network that will help accomplish this and other future public health goals.

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**How can my laboratory apply for support from PolioPlus Partners?**

- Assess the needs for your laboratory. Please refer to the “Polio Lab Network - Quarterly Update Volume III Number 1 1997” for a basic list of equipment, supplies, and reagents needed for National Poliovirus Laboratories. Please consult your Regional Laboratory Coordinator for advice regarding equipment and supplies not included on this list, or regarding specific models of equipment appropriate to local conditions.
- **Complete a PolioPlus Partners Request Form.** This form can be obtained by contacting PolioPlus Partners Program Coordinator Kris Pierotti, Tel: 847-866-3344, Fax: 847-866-0269, E-Mail: pierottk@riorc.mhs.compuserve.com

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**ALL CHINA LABS ACHIEVE 100% ON PT**

Two years ago, the Update reported that all 29 Provincial Laboratories achieved passing scores of >80% on the China 1995 poliovirus proficiency test (PT). This year, we are pleased to report that all 30 Laboratories (now including Tibet), as well as the National/Regional Reference Laboratory in Beijing), not only passed but all achieved scores of 100%. We congratulate Dr. Zhang LiBi, Director, WHO National/Regional Reference Laboratory and the Provincial Laboratories on the remarkable progress.

**Provincial Laboratory Proficiency Test Results, China, 1992-1997**

<table>
<thead>
<tr>
<th>Year</th>
<th>100%</th>
<th>&gt;80%</th>
<th>&gt;60%</th>
<th>&lt;59%</th>
<th>100% score rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1992*</td>
<td>9</td>
<td></td>
<td>11</td>
<td>9</td>
<td>31%</td>
</tr>
<tr>
<td>1993</td>
<td>15</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>52%</td>
</tr>
<tr>
<td>1994</td>
<td>24</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>83%</td>
</tr>
<tr>
<td>1995**</td>
<td>26</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>87%</td>
</tr>
<tr>
<td>1996</td>
<td>25</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>83%</td>
</tr>
<tr>
<td>1997</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100%</td>
</tr>
</tbody>
</table>

* 3 of the 9 did not report results  ** Including the Tibet Laboratory
Mycoplasma Revisited

Mycoplasma contamination of cell culture systems at some time is an inevitable event in the life of a cell culturist. Mycoplasma species can cause serious problems for cell cultures and major aggravation for the culturist. It may be the most common factor leading to poor cell conditions and a subsequent reduction in laboratory performance and virus isolation.

What are the effects of contamination?

Mycoplasmal replication alters host cell metabolism by elaboration of undesirable growth by-products and consumption of essential cell nutrients, especially nucleic acid precursors and amino acids. The effect on cells may be reduced levels of protein, RNA and DNA synthesis, purine metabolism and nucleotide pools. As a consequence, cellular growth may be altered and cell morphology and antigenicity changed by mycoplasmal attachment and exchange of membrane antigens between the host cell and the parasite.

Some continuous cells are more susceptible to the effects of mycoplasmal contamination than others. For some cells, there may be very little visible change, although virus sensitivity may be altered. For other continuous cells, mycoplasmal contamination may alter the growth rate and appearance. The most common visible effects of mycoplasma contamination are “dirty” cells and the shortened length of time that cells can be maintained, resulting in reduced time for virus replication and reduced ability to detect virus CPE.

How can contamination be detected?

Cell cultures may be screened for contamination by direct inoculation onto broth and/or agar enriched for mycoplasmal growth. These methods are useful to detect most, but not all, of the common contaminants. Some strains of mycoplasma are non-cultivable. A medium for the effective isolation of all possible mycoplasmal contaminants does not currently exist.

Can contamination be eliminated?

Treatment of cell cultures to eradicate mycoplasmas is not recommended. It is difficult, expensive, time consuming, and usually unsuccessful. Furthermore, the drastic treatment often required for eradication may alter the cell lines so that they no longer express the desired qualities.

How does contamination occur?

The initial source of mycoplasmal contamination is usually calf serum used for preparation of growth and maintenance media. High grade commercial fetal calf serum that has been screened for mycoplasmas are rarely at fault. The most likely sources of initial contamination are unscreened sera from non-certified commercial or local vendors. However, the most common source of unwanted mycoplasmas in the diagnostic laboratory is cross contamination from other infected cell cultures. Mycoplasma titres in infected cells may range from $10^6$ to $10^8$ organisms per milliliter. Cross contamination occurs most frequently during weekly passage when trypsinized suspensions are repeatedly aspirated and expelled to break up cell clumps. The infected aerosols generated by this process remain suspended in the environment for many hours.

How can contamination be avoided?

Contamination of one cell line with another can be avoided by never passaging more than one at the same time, by using a clean air hood with the appropriate filtration system, and by washing down the bench.
The Pan American Health Organization (PAHO) in 1985 was the first Region to declare a goal of interruption of wild poliovirus transmission. Many of the current global polio surveillance and eradication strategies were pioneered by PAHO, including the concept of a tiered system of poliovirus laboratories based on the complexity of tests performed.

There are 10 Laboratories in the PAHO Network. Laboratories in Argentina, Brazil (3), Colombia, Guatemala, Mexico, Trinidad and Tobago, and Venezuela provide virological support for AFP surveillance in all former polio endemic countries. The Oswaldo Cruz Foundation (FIOCRUZ), Brazil, and the Centers for Disease Control and Prevention (CDC), USA serve as the Regional Reference Laboratories. With years of poliovirus experience, attention to quality assurance, and proven test capabilities, these Laboratories constitute a strong and sophisticated Regional poliovirus surveillance capacity.

The last indigenous wild poliovirus was isolated in Peru six years ago in 1991. Only two wild virus importations have been recorded since then, both in Canada, in 1993 and 1996.

Maintaining AFP surveillance in the absence of wild poliovirus transmission is an increasing challenge for the Region. The following table illustrates the decline in the annual number of AFP cases investigated and the number of samples submitted to the Laboratory Network. The Region is working hard to reverse this trend. The challenge of maintaining high level, long-term surveillance in polio-free Regions underscores the urgency for all Regions to achieve the global year 2000 goals.

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of AFP Cases</th>
<th>No. of Samples</th>
<th>Negative for Enterovirus</th>
<th>Other Enterovirus</th>
<th>Sabin P1</th>
<th>P2</th>
<th>P3</th>
<th>Mixt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>5,385</td>
<td>7,266</td>
<td>5,345</td>
<td>1,693</td>
<td>117</td>
<td>72</td>
<td>28</td>
<td>11</td>
</tr>
<tr>
<td>1995</td>
<td>3,248</td>
<td>3,658</td>
<td>2,815</td>
<td>750</td>
<td>34</td>
<td>23</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>1996</td>
<td>2,929</td>
<td>3,066</td>
<td>2,275</td>
<td>532</td>
<td>18</td>
<td>11</td>
<td>16</td>
<td>14</td>
</tr>
</tbody>
</table>

Mycoplasma

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with disinfectant between each cell passage procedure. The best defense against contamination of continuous cell lines is to begin with a known mycoplasmal-free cell line from a WHO-approved source. From this seed, multiple subcultures are prepared to create a working cell bank in liquid nitrogen. Each batch from the working cell bank should be used for no more than 15-20 consecutive passages.

Protocols for the creation and maintenance of a liquid nitrogen cell bank may be found in the 1997 WHO Manual for the virologic investigation of polio. This edition should now be available in all Network Laboratories.