The major purpose of the WHO Global Action Plan for Laboratory Containment of Wild Polioviruses is to provide a plan to prevent the reintroduction of wild polioviruses from the laboratories of the world. While most laboratories are aware when they are handling known poliovirus infectious materials such as virus isolates, the plan also addresses the more difficult question of potential wild poliovirus infectious materials. These materials are defined as feces, respiratory secretions, and environmental sewage and water samples of unknown origin or collected for any purpose at a time and in a geographic area where wild polioviruses or VDPV were suspected to be present, as well as products of such materials in poliovirus permissive cells or animals.

Since most wild poliovirus infections cause no recognizable paralytic disease, many healthy individuals may have inapparent infections and be shedding wild polioviruses in feces and respiratory secretions. Samples collected from individuals without clinical disease would therefore contain wild polioviruses and if stored frozen may potentially retain infectious wild polioviruses for decades. Often, laboratories that have these specimen collections are unaware that these pose a particular risk in some laboratory procedures. Although this risk is theoretically evident, it has seldom been demonstrated in actual collections.

A small study was undertaken to see if this wild poliovirus risk could be explicitly demonstrated in potential infectious materials. In collaboration with the Viral Gastroenteritis Section at the Centers for Disease Control and Prevention (CDC), two specific stool sample collections were identified that met the definition of potential wild poliovirus infectious materials. These collections were obtained from two different countries in South Asia more than 20 years ago, at a time when wild polioviruses were widespread throughout the entire region. The samples were maintained frozen and had been periodically used for studies of enteric viruses other than enteroviruses. The second collection also included 40 frozen MA104 cell cultures from a previous investigation to isolate enteric viruses. All 40 cultures had been described as containing uncharacterized viruses isolated from other stool samples in the collection.

These collections were analyzed according to standard WHO polio diagnostic protocols as described in the Polio Laboratory Manual (WHO 2001). Stool samples and cell culture dilutions were prepared for inoculation onto both RD(A) and L20B cell culture lines. Inoculations were harvested by day 7 and re-inoculated onto both cell lines. Cell cultures of samples exhibiting positive CPE (cytopathic effect) from the second passage were harvested and tested by enterovirus and poliovirus diagnostic PCR. Cultures positive for poliovirus

(Wild virus continued on page 2)
PCR were tested against serotype PCR primers. ELISA was used as a second test method for serotype and ITD confirmation. Wild poliovirus strains were sequenced. The results are summarized in the table 1.

In this study, polioviruses and non-polio enteroviruses were isolated from both collections indicating that these viruses had survived storage for more than 20 years. The non-poliovirus enterovirus isolation rate from stool samples was 31% (23/75) and 43% (78/183) among the non-polio samples from collections I and II, respectively. The first collection also yielded six wild polioviruses, types 1 and 2. Although these viruses are being characterized further, the type 2 wild viruses appear to be the first ever reported from that country, which now appears to be polio-free. The frozen cell culture materials from the second collection yielded 6 Sabin-like polioviruses.

Contributed by: Mark Pallansch and Michelle Staples, CDC, Atlanta

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**Editorial Note:** The accompanying article by Pallansch and Staples demonstrates that fecal materials collected from endemic countries for other purposes can contain wild polioviruses even though they were collected from individuals without paralytic disease. The percentage of wild poliovirus positive samples in such collections is likely to vary widely, depending on the region, the season of the year, intensity of virus circulation, and subjects’ age and living conditions. For example, the wild poliovirus isolation rates from stools collected in the polio high-season among healthy children mostly under the age of 4 were 8% in Cartagena, Colombia, in 1989 (1) and 19% in Mumbai, India, in 1994 (2).

Importantly, the present report also demonstrates that stool samples inoculated onto permissive cell lines for other purposes have the potential to grow polioviruses. These cytopathic agents often remain unrecognized when they are not relevant to the disease or agents under study. The findings here reinforce the definition of potential infectious as including products of such clinical and environmental materials in poliovirus permissive cells or animals.

The TCG at its April meeting in Geneva recognized the value of Pallansch’s and Staples’ findings and encouraged similar studies by others to add to our understanding of the risks of potential infectious materials. Specifically, the TCG recommended that such studies be completed in mid-2003 in at least two large industrialized countries in the course of establishing national inventories.

The TCG re-affirmed that the critical criterion for defining potential infectivity of a sample is its history, when and where it was collected. Finding wild poliovirus absent in a subset of samples from a large collection does not prove its absence from the entire collection. It provides information on the probable lower range of potential infectivity. Only by testing all potential infectious samples in poliovirus permissive cells is it reasonable to assume that the collection is free of wild poliovirus.

Wild poliovirus transmission is interrupted in EUR

The last indigenous case in the European Region was in Turkey in November 1998. On 21 June 2002, the European Regional Certification Commission declared the Region free of indigenous wild poliovirus transmission. The formal signing of the declaration took place in the Glyptotek, the museum in Copenhagen that houses the more than 3,000 year old Egyptian stele depicting a lame gatekeeper and his family, presumed to be the earliest evidence of polio. The Certification ceremony was seen as a tribute to the extraordinary dedication and hard work of the men and women of EURO headquarters and each of the 51 countries, particularly those most challenged by economic and political strife. Members of the EUR laboratory network were praised for their dedicated efforts and the exceptional quality of those efforts. All 51 laboratories were accredited or provisionally accredited at the time of certification. In addition, wild poliovirus inventories were submitted by 48 of 51 countries (41 complete), an accomplishment in which laboratory staff in many countries played a pivotal role. Virtually all countries are expected to have submitted a complete inventory by the end of 2002.

Certification honored a monumental achievement, but the member states recognized that certification of the Region was only a milestone toward the goal of interrupting wild poliovirus transmission worldwide. The separate incidents of virus importations from northern India that caused an outbreak in Bulgaria and a case of non-paralytic polio in Georgia in 2001 served as reminders that vigilance cannot be relaxed as long as poliovirus circulates anywhere in the world. Maintaining high immunization levels, effective surveillance, and a strong laboratory network were emphasized as essential for ensuring the legacy of that achievement.

Laboratories set new PT records for isolation and typing

The 2001/2002 proficiency-testing (PT) results attest to the global capacity of the laboratory network to isolate and identify polioviruses (Table 2). All 150 laboratories in the network participated and 144 (96%) passed on the first try. In EMR, EUR, and SEAR, all laboratories passed with mean scores above 98%. In AFR and WPR, one laboratory each failed to pass on the first test, but scored 100% on repeat panels. In AMR, 3 laboratories that failed have been given new panels. One did not report by the deadline. AMR is to be commended for re-instituting PT and striving to bring the network to its pre-Certification high performance standards. Experience in AMR demonstrates that the effort required to re-build a network greatly exceeds that required to maintain it.

### PT for Isolation and Typing

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<th>AFR</th>
<th>AMR</th>
<th>EMR</th>
<th>EUR</th>
<th>SEAR</th>
<th>WPR</th>
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<td>1*</td>
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<td>84.5%</td>
<td>98.3%</td>
<td>99.8%</td>
<td>100%</td>
<td>98.2%</td>
<td>97.9%</td>
</tr>
</tbody>
</table>

remarks

*100% on repeat, **failed to report on time or were given new panel

Data per 25-4-2002

Contributed by Harrie van der Avoort, RIVM, Bilthoven
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Polio Laboratory Network

REGION | Number of AFP cases with specimens | P1 only | P2 only | P3 only | NPEV | Enterovirus Mixtures | Results within 28 days | Pending |
-------|-----------------------------------|--------|--------|--------|------|----------------------|-----------------------|--------|
AFR    | 1,961                             | 21     | 14     | 27     | 15%  | 23                   | 93%                   | 5      |
AMR    | 393                               | 0      | 1      | 3      | 13%  | 0                    | 62%                   | 33     |
EMR    | 861                               | 20     | 7      | 14     | 11%  | 25                   | 91%                   | 15     |
EUR    | 484                               | 2      | 2      | 4      | 3%   | 7                    | 75%                   | 191    |
SEAR   | 2,095                             | 56     | 25     | 65     | 22%  | 51                   | 98%                   | 0      |
WPR    | 1,294                             | 22     | 40     | 19     | 6%   | 28                   | 88%                   | 176    |

Regional Ref. Lab

Poliovirus Intratypic Differentiation Results

REGION | No. of cases with isolates submitted | Type 1 | Type 2 | Type 3 | Results within 28 days | Pending |
--------|-------------------------------------|--------|--------|--------|------------------------|--------|
AFR*    | 236                                 | 38     | 79     | 0      | 59                     | 11     | 90                   | 96%    | 0       |
AMR     | 4                                   | 0      | 0      | 0      | 1                       | 0      | 3                    | 50%    | 0       |
EMR#    | 66                                  | 8      | 11     | 0      | 7                       | 4      | 51                   | 86%    | 1       |
EUR##   | 152                                 | 0      | 47     | 0      | 42                      | 0      | 31                   | 51%    | 1       |
SEAR**  | 197                                 | 21     | 73     | 0      | 55                      | 12     | 95                   | 87%    | 0       |
WPR     | 75                                  | 0      | 28     | 0      | 37                      | 0      | 21                   | 95%    | 1       |

* specimen based data
** 51 cases had poliovirus mixtures
# 25 cases had poliovirus mixtures
## poliovirus isolates obtained from all sources (AFP cases and others e.g. meningitis, environment etc.) are included in the tabulated data

AFR: Between 01 January and 31 March 2002, wild viruses were detected from AFP cases in 2 countries: Nigeria (16 cases) and Zambia (2 cases). This compares with the detection of wild viruses in 7 countries in 2001: Algeria (1), Angola (1), Ethiopia (1), Mauritania (1), Nigeria (56), Niger (6), and Zambia (3). Sequence and epidemiological data linked imported viruses to cases detected in Algeria, Mauritania and Zambia in 2001.

AMR: No wild polioviruses were detected between January and March 2002. No vaccine-derived polioviruses (VDPV) have been detected since the type 1 cases were reported in Haiti and the Dominican Republic in the first half of 2001.

EMR: Wild polioviruses were detected in 15 AFP cases between January and March 2002: Afghanistan (1), Pakistan (12), and Somalia (2). Testing of sewage samples as part a supplementary surveillance project in Egypt revealed wild type 1 polioviruses in 5 settlements in the upper Nile.

EUR: This Region was declared free of endemic wild poliovirus circulation in June 2002, with no further evidence of type 1 spread from the independent episodes of virus importation in Bulgaria and Georgia in 2001.

SEAR: India remained the only country with wild polioviruses detected in the Region with 33 cases with onset between January and March 2002. Sequence data continue to show reduction in the biodiversity of virus isolates.

WPR: This Region has been free from endemic wild poliovirus circulation since March 1997. No VDPV has been detected in the Region since type 1 VDPV strains were isolated from 3 AFP cases and one contact in the Philippines in early 2001.