WHO/V&B/99.32
ORIGINAL: ENGLISH
DISTR.: GENERAL

WHO global action plan for laboratory containment of wild polioviruses

DEPARTMENT OF VACCINES AND BIOLOGICALS

World Health Organization
Geneva
1999

The Department of Vaccines and Biologicals
thanks the donors whose unspecified financial support
has made the production of this document possible.

This document was produced by the
Vaccine Assessment and Monitoring Team
of the Department of Vaccines and Biologicals
Ordering code: WHO/V&B/99.32
Printed : December 1999

This document is available on the Internet at:
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Purpose

To provide a systematic, worldwide Action Plan
to prevent reintroduction of wild polioviruses
from the laboratory into the community.

Executive summary

This document provides a systematic, worldwide plan of action to prevent transmission of wild poliovirus from the laboratory into the community.

Once polio is eradicated, the laboratories of the world will be the only remaining source of the virus. Safe handling and, ultimately, maximum containment of poliovirus and potentially infectious materials in the laboratory is crucial.

Until now, poliovirus biosafety concerns have been minimal. Universal immunization with inactivated polio vaccine (IPV) or oral polio vaccine (OPV) has reduced the risk of disease for laboratory workers and the general public. Current technologies and biosafety practices have further reduced the risks of poliovirus contamination of the environment.

The probability of a laboratory-associated poliovirus infection is small, but the consequences of an infection grow greater with time. A chance reintroduction of wild poliovirus from the laboratory into the community after cessation of transmission presents a threat to polio eradication. A chance reintroduction of wild poliovirus after cessation of immunization presents a threat to public health of global proportions.

The world now faces the formidable, but not insurmountable, challenge of locating the many laboratories that have wild poliovirus infectious, or potentially infectious, materials and ensuring that they are adequately contained in the laboratory, rendered non-infectious, or destroyed. The Global Action Plan addresses these responsibilities. It is linked to the major eradication objectives, and consists of three phases.

Pre-Eradication

Safe handling of wild poliovirus infectious or potentially infectious materials (BSL-2/polio)

The Pre-Eradication phase covers the period when wild polioviruses continue to circulate. Three tasks are critical to this phase.

1) Nations must identify and develop an inventory of laboratories that have wild poliovirus infectious materials or potentially infectious materials.
2) Laboratories must institute enhanced biosafety level-2 (BSL-2/polio) procedures for safe handling of all such infectious or potentially infectious materials.
3) Nations must begin planning for implementation of biosafety requirements for Post-Global Eradication.

Completion of all Pre-Eradication tasks is a prerequisite for certification of a region as polio-free.

Post-Global Eradication

High containment of wild poliovirus infectious and potentially infectious materials (BSL-3/polio): To begin one year after detection of the last wild poliovirus

The Post-Global Eradication phase begins one year after detection of the last wild poliovirus anywhere in the world, at which time the probability is high that all human transmission has ceased.

All laboratories possessing wild poliovirus infectious materials or potentially infectious materials must elect one or more of the following three options:

1) Implement containment (BSL-3/polio) procedures, or
2) transfer wild poliovirus infectious and potentially infectious materials to WHO designated repositories, or
3) render such materials non-infectious, or destroy them, under appropriate conditions.

All biosafety actions are to be implemented and documented as complete before global certification of polio eradication can be considered.

Post-OPV Immunization

Maximum containment (BSL-4) of wild poliovirus infectious and potentially infectious materials and high containment (BSL-3/polio) of OPV and OPV-derived viruses: To begin when OPV immunization stops

The Post-OPV Immunization phase begins with the world-wide cessation of OPV administration and the subsequent rapid increase of non-immune susceptible children. The biosafety requirements for wild poliovirus infectious and potentially infectious materials increase from BSL-3/polio to BSL-4, consistent with the increased consequences of inadvertent transmission of wild poliovirus from the laboratory to the community. Biosafety requirements for OPV and OPV-derived viruses increase from BSL-2/polio to BSL-3/polio to prevent reintroduction and potential circulation of these viruses in unimmunized populations. Procedures will be developed to control or destroy unused OPV in clinics, immunization centers, physician's offices, and other sites.

Publication of the plan

This document provides the background, rationale, and strategy to ensure that laboratory biosafety is consistent with the risk that inadvertent reintroduction of poliovirus poses to the community.

Full cooperation and commitment of all nations are essential to achieve wild poliovirus eradication and to implement the Global Action Plan to ensure poliovirus will never be a threat to future generations. This Plan will be in effect 1 January 2000.

Poliomyelitis

Description

Polio, or poliomyelitis, is an infectious disease caused by poliovirus, a member of the genus *Enterovirus*. There are three serotypes of poliovirus: 1, 2, and 3. Humans cells contain specific protein receptors to which poliovirus may attach and thereby enter susceptible cells. The virus infects cells of the oropharynx, the tonsils, the lymph nodes of the neck, and the small intestines. Infection progresses through cycles of virus replication, resulting in destruction of the infected cells. Once infection is established, poliovirus can enter the bloodstream and invade the central nervous system through the blood/brain barrier, by spreading along nerve fibers, or by both routes.

When non-immune persons are exposed to wild poliovirus the results may include inapparent infection without symptoms, mild illness, aseptic meningitis, or paralytic poliomyelitis[1]. Only about 1% of the infections result in recognized clinical illness. The incubation period is 4-35 days, typically 7-14 days. Initial clinical symptoms may include fever, fatigue, headache, vomiting, constipation (or less commonly diarrhea), stiffness in the neck and pain in the limbs. Virus multiplication destroys the motor neurons responsible for activating muscles. These nerve cells do not regenerate, resulting in the inability of affected muscles to function.

Mode of transmission

The virus is transmitted from person to person. Poliovirus can be spread to others by droplets from the upper respiratory tract during the early days of infection. More commonly, infected persons pass large numbers of virus particles through their faeces, from where they may be spread indirectly, or directly to infect others[2].

Poliovirus in nature

Poliovirus in immunocompetent persons is found in the oropharynx for one to two weeks, blood for about one week, and faeces for one to two months after initial infection. In fatal cases, poliovirus may be recovered from faeces, intestinal contents, lymph nodes, brain tissue, and spinal cord tissue. Because less than 1% of infections result in poliomyelitis, many "healthy" children shed virus during periods of high prevalence.

There is no long-term carrier state in infected immunocompetent persons, regardless of the clinical course. However, persistent shedding of oral vaccine-derived poliovirus has been shown to occur in some immunocompromised patients with B-cell deficiencies[3].

Humans are the only animal reservoir for poliovirus, although higher non-human primates may be infected experimentally and sometimes in the wild[4]. Poliovirus in the environment is the direct result of recent poliovirus infections in the human community.

Poliovirus contamination of soil occurs through human defecation near dwellings, crop fertilization with untreated or inadequately treated night soil or sewage, and recycled wastewater for irrigation. Poliovirus in sewage reflects the prevalence of infection in the community. Contamination of surface waters may occur through discharge of untreated or inadequately treated sewage or run off from contaminated soil.

Poliovirus survival

Poliovirus is resistant to inactivation by common laboratory disinfectants such as alcohol and cresols. It is readily inactivated by dilute solutions of formaldehyde or free residual chlorine, ultraviolet light, heat and drying. Inactivation may be slowed by the presence of extraneous organic matter.

Rates of poliovirus inactivation in nature are greatly influenced by the immediate environment. Poliovirus infectivity decreases by 90% in soil every 20 days in winter and every 1.5 days in summer. A similar 90% decrease at ambient temperatures occurs in sewage every 26 days, in freshwater every 5.5 days, and in seawater every 2.5 days[4].

Under stable laboratory conditions poliovirus in clinical or environmental specimens may survive at freezing temperatures for many years, under refrigeration for many months, and at room temperatures for days to weeks. The virus is rapidly destroyed by exposure to temperatures of 50°C or greater, autoclaving, or incineration[1].

Polio vaccines

Protective immunity against poliomyelitis is conferred through immunization or natural poliovirus infection. Immunity is poliovirus serotype-specific. Protection against disease is associated with antibodies that circulate in the blood stream and prevent spread of the virus to the central nervous system. Protection against infection is associated with both circulating antibodies in the blood and secretory antibodies in the gut and upper respiratory tract[5].

Live attenuated oral polio vaccine (OPV) and inactivated polio vaccine (IPV) both protect against disease, but differ in how and to what extent they protect against infection. IPV stimulates protective antibodies in the bloodstream (i.e. circulating immunity), but with only low-level, transient protection against poliovirus infection inside the gut (i.e. secretory immunity). Thus, IPV provides effective individual protection against the disease but incomplete protection against infection with wild poliovirus. In persons immunized with IPV, wild virus can still multiply in cells of the intestines and be shed in stools[6]. Nevertheless, the use of IPV of adequate quality has controlled polio effectively in countries with good sanitation levels.

OPV induces circulating as well as secretory immunity and provides long-term protection against disease and short-term protection against infection. Immunization with OPV creates an effective barrier against wild poliovirus transmission. Vaccine-derived viruses may be shed for weeks in faeces. In about one in every 2.5 million doses administered, the live attenuated vaccine virus can cause paralysis in either the vaccinee or a close contact[6].

Polio eradication

Polio occurred worldwide prior to the advent of immunization in the mid-1950s. Immunization has been highly effective in reducing the number of cases worldwide. Polio can be eradicated by interruption of human transmission through improved routine childhood immunization in many countries and the strategic use of vaccines in the polio eradication initiative.

There is no evidence of a persistent wild poliovirus carrier state or animal or insect reservoirs, and the virus can survive only for finite periods of time in the environment[4]. Higher non-human primates (chimpanzees and gorillas) are susceptible to infection and disease, but these populations are not sufficiently large to sustain poliovirus transmission in the absence of human infections. Humans are the only natural reservoirs of poliovirus. Therefore, once poliovirus is deprived of its human host through immunization, it will rapidly die out[7]. The continued decrease in the incidence of polio in many countries and the progressive disappearance of polioviruses both suggest that the interruption of human transmission, and thus eradication, is within reach[8].

Evidence for laboratory-associated infections

Less than one year after the last case of naturally acquired smallpox in 1977, two cases of smallpox occurred in the United Kingdom. Both were linked to a laboratory in the Birmingham University Medical School. The index case was a medical photographer, who worked in a darkroom located on the floor above the poxvirus research laboratory. The second case was the mother of the photographer. The index case apparently acquired infection through air leaking from the service duct linking the photographer's office to the poxvirus laboratory. Two persons died; the index patient as a result of infection, and the director of the laboratory, who took his own life because of the accident[9].

Polio is not smallpox. The viruses and the epidemiology of the diseases they cause are quite different. As with smallpox, however, transmission of wild poliovirus from the laboratory to the community might occur through contamination of the environment or an infected laboratory worker. Smallpox spreads slowly, is clinically evident, and could be contained through strategic vaccination, but wild poliovirus from the laboratory could spread quickly in an unimmunized population, ultimately creating a public health tragedy of global proportions.

Although theoretically possible, no direct evidence exists for poliovirus transmission to persons outside the laboratory through contaminated laboratory effluents released into sewage, solid wastes transported to landfills, or spent air exhausted to surroundings. Also, no direct evidence exists for the infection of others through contaminated workers' skin or clothing. Such routes are extremely difficult to document against the current background of high levels of immunity acquired through natural infection or immunization. More readily documented are poliovirus infections of laboratory workers, with potential for transmission to the community.

Work in the laboratory with poliovirus was once considered far more dangerous than providing care for polio patients, with a theoretical attack rate of 2 per 50 to 75 laboratory workers[10]. From 1941 to 1976 a total of 12 laboratory-associated poliomyelitis cases, including two deaths, were recorded[11,12,13,14]. Accounts of 7 of the 12 were unpublished. Most cases occurred in the pre-vaccine era and before the advent of cell culture. The five published cases were reported in the 1940s, at a time when an increasing number of investigators had turned to the study of human disease. Laboratory workers were being exposed increasingly to tissues or excreta of humans with poliomyelitis and to primates infected with poliovirus of recent human origin.

The first report of a laboratory-associated infection was published in 1941 and described a case of poliomyelitis most likely acquired through washing and grinding infected tissues in preparation for inoculation into monkeys[15]. Two years later, two laboratory workers were accidentally infected with the prototype Lansing (Armstrong) strain while attempting to infect mice[16]. Two additional reported cases of poliomyelitis in laboratory workers were fatal: one in the United States[10] and the second in South Africa[17].

No cases were reported during the next 10 years[18]. In a recent review of potential risks for laboratory-associated infection, polioviruses are not even mentioned[19]. The paucity of reports of laboratory-associated poliomyelitis since vaccines were introduced testifies to the effectiveness of vaccines and vastly improved laboratory facilities, technologies, and procedures. By inference, poliovirus infections would also be expected to be rare among laboratory workers.

Despite the advances in biosafety over the past 40 years, recent evidence indicates that the potential nevertheless exists for transmission of poliovirus from the laboratory to the community. In 1992, a wild-type 1 strain used for IPV production was documented as being transmitted from a worker in a vaccine production facility to his 18-month-old son[20]. The boy had been suffering from gastroenteritis when, by chance, the wild IPV seed virus was isolated from his stool. In another incident, a child was reported to be infected with a prototype strain of type 3 commonly used in laboratories for research or vaccine production. The source of this infection was not determined[20].

These cases demonstrate that reintroduction of wild poliovirus from the laboratory to the unimmunized community remains a serious and unacceptable risk.

Although IPV is highly effective in preventing disease, its use cannot be assumed to prevent silent infection among laboratory workers. OPV provides a more effective barrier but silent infections may still occur. Thus, in the absence of fully effective vaccines, extraordinary biosafety precautions must be taken to prevent infection of laboratory workers and the risk of subsequent spread to the community.

Definitions of poliovirus

Polioviruses are defined by standard neutralization tests with specific antisera. The three poliovirus serotypes form a unique genetic group of human enteroviruses that initiate infection by binding to a specific cellular receptor (PVR:CD155). Other enteroviruses may occasionally be associated with cases of acute flaccid paralysis, but they are not polioviruses and they do not bind to PVR.

Wild polioviruses have the capacity to circulate indefinitely within susceptible human populations. Molecular studies have shown that the capsid sequence lineages of wild polioviruses are maintained along chains of transmission, while the noncapsid and noncoding sequences may be exchanged by recombination with other enteroviruses during circulation. Thus, the identification of sequences outside of the capsid region as “poliovirus” may be arbitrary. Important determinants of the attenuation phenotype reside in the capsid regions of OPV strains, and these determinants are not known to occur in the capsid sequences of wild polioviruses.

The distinction between wild and OPV strains is not based on neurovirulence. Some field isolates and reference strains have low neurovirulence when measured in experimental animals, but are known to be genetically similar to circulating viruses associated with paralytic disease. Candidate attenuated strains that are not approved for use in oral polio vaccines by national control authorities are regarded as wild polioviruses.

Definitions of poliovirus are presented in Box 1.

Box 1: Definitions of poliovirus

Polioviruses: human enteroviruses that exist as three well-defined serotypes, which infect cells via a specific receptor (PVR:CD155).

Wild polioviruses: field isolates and reference strains derived from polioviruses known or believed to have circulated persistently in the community.

Oral poliovirus vaccine strains: attenuated polioviruses approved for use in oral vaccines by national control authorities.

Vaccine-derived polioviruses: progeny of approved oral poliovirus vaccine strains.

Wild poliovirus infectious materials

Wild poliovirus may be present in faeces and throat specimens; less commonly in blood; and rarely in cerebrospinal fluids from patients with non-paralytic as well as paralytic infections. In fatal infections, wild poliovirus may be present in faeces, intestinal contents, lymph nodes, brain tissue, and spinal cord tissue. Poliovirus may be found in blood during the first week of infection, before neutralizing antibodies appear, but is rarely found in blood after onset of clinical signs of central nervous system involvement. All such clinical materials from persons known or suspected to be infected and have been treated and stored under conditions known to preserve the virus are defined as infectious, even though the presence of virus may not have been confirmed.

Other infectious materials are wild poliovirus isolates, reference strains, and all products of the laboratory that meet the definitions of wild poliovirus (Box 1). Also included are environmental sewage or water samples known or suspected to be contaminated, infected laboratory animals, and materials from infected animals.

Infected non-human primates and transgenic mice pose a biosafety risk in that the virus may be shed and transmitted to susceptible humans. Transgenic mice infected with poliovirus should be maintained according to World Health Organization (WHO) recommendations[21].

Definitions and examples of infectious materials are presented in Boxes 2 and 3 respectively.

Box 2: Definitions of wild poliovirus infectious materials

- **Infectious clinical materials**: all clinical and investigative materials from confirmed or suspected cases of poliomyelitis.
- **Infectious research materials**:
  - All poliovirus derivatives produced in the laboratory that have capsid sequences derived from wild polioviruses.
  - Full length poliovirus RNA or cDNA containing capsid sequences derived from wild poliovirus.
  - Cells persistently infected with poliovirus strains whose capsid sequences are derived from wild poliovirus.
- **Infectious environmental materials**: all sewage or water samples known or suspected to contain wild polioviruses.
- **Infectious animals**: any experimental animal infected with a strain containing capsid sequences derived from a wild poliovirus, especially PVR transgenic mice infected with wild poliovirus.

Box 3: Examples of wild poliovirus infectious materials

- **Throat, faecal, blood, and cerebrospinal fluid specimens from suspected or confirmed polio cases collected for:**
  - Laboratory diagnosis
  - Poliovirus epidemiologic studies
- **Autopsy/biopsy specimens (unfixed) from suspected or confirmed polio cases**
- **Stocks of wild virus**
  - Prototype strains used as controls
  - Isolates
  - Proficiency test panels
  - Seeds for inactivated vaccines
- **Research laboratory materials with wild poliovirus capsid sequences**
  - Poliovirus derivatives
  - Full length poliovirus RNA or cDNA
  - Infected cells
- **Samples of environmental sewage and water known or suspected to be contaminated with wild poliovirus**
- **Specimens from laboratory animals infected with wild virus (non-human primates, transgenic mice)**

Potentially infectious materials

Clinical and environmental materials compatible with the potential presence of poliovirus collected for any diagnostic or research purposes at a time and in a geographical area of wild poliovirus endemicity must be considered potentially infectious.

All such clinical and environmental materials maintained in the laboratory under conditions known to preserve poliovirus must be carefully evaluated for potential infectivity. Examples include faecal specimens or respiratory secretions collected for any purpose, including epidemiologic surveys.

Each collection must be assessed to determine the likelihood of the presence of wild polioviruses, based on treatment and storage history, the country of origin, the year, the time of the last indigenous wild poliovirus isolates in that country, and the type of specimen. Frozen stool samples from young children during endemic periods would likely have the highest levels of infectious polioviruses. Routinely collected serum specimens and cerebrospinal fluids are not likely to contain sufficient levels (if any) of poliovirus to cause infection and are not considered infectious.

Clinical or environmental materials stored without refrigeration for three months or more, refrigerated for one year or more, heat inactivated, treated with a disinfectant known to inactivate polioviruses, or tested and found negative for the presence of enteroviruses are not considered infectious or potentially infectious for wild poliovirus.

Definitions and examples of potentially infectious materials are presented in Boxes 4 and 5.

Box 4: Definition of potentially infectious laboratory materials

Potentially infectious laboratory materials: clinical materials such as throat swabs and faeces; and environmental samples collected for any purposes at a time and in a geographical area where wild poliovirus was known or suspected to be present and maintained under conditions known to preserve polioviruses.

Box 5: Examples of potentially infectious materials collected at a time and in a geographical area where wild poliovirus was known to have been present *

- **Clinical materials**
  - Faeces
  - Throat swabs
- **Environmental samples of sewage and untreated water**
- **Laboratory products**
  - Untyped enterovirus-like cell culture isolates
  - Undifferentiated poliovirus isolates

* Considered as non-infectious are such materials stored without refrigeration for three months or more, refrigerated for one year or more, heat inactivated, treated with antiviral disinfectants, or previously tested and found negative for the presence of enteroviruses.

Agencies/institutions and laboratories with wild poliovirus infectious and/or potentially infectious materials

Infectious materials

Laboratories possess wild poliovirus infectious materials for numerous reasons. Many diagnostic and public health laboratories keep poliovirus isolates and clinical specimens for documentation of past investigations of endemic or imported cases of poliomyelitis. Some maintain multiple virus strains for test controls, reference purposes, or their historic value. Educational institutions have wild polioviruses for teaching exercises. Virus research laboratories retain poliovirus stocks or infectious materials for studies on the biologic, biochemical, or genetic properties of the virus. Other research laboratories store potentially infectious materials as documentation of completed studies or for future studies. Some environmental laboratories retain contaminated materials or wild poliovirus reference for testing the effectiveness of virucidal compounds. Vaccine producers have wild strains for the production of IPV or for testing the quality of OPV. National Control Laboratories may have similar strains.

The identification of laboratories with wild poliovirus infectious materials presents a formidable, but not insurmountable challenge. Channels for identifying laboratories with wild poliovirus in developed countries are available, and include national health and research infrastructures, laboratory registries, accrediting bodies, professional organizations, and national and institutional biosafety networks.

All of these channels might not be available in developing countries. However, the number of biomedical laboratories in developing countries with long-term storage capacities is considerably fewer and usually known to national authorities and WHO.

Laboratories most likely to have wild poliovirus infectious materials are present or past poliovirus/enterovirus laboratories, WHO Poliovirus Network Laboratories, poliovirus vaccine production laboratories, and diagnostic laboratories (see Boxes 6 and 7).

Poliovirus/enterovirus laboratories: Laboratories actively working with poliovirus constitute a relatively small number of the total microbiology laboratories worldwide. Most such laboratories are known through Ministries of Health, professional societies, the poliovirus research community, WHO reports of wild poliovirus isolates, and scientific publications.

The WHO Global Polio Laboratory Network: This network consists of over 140 National Laboratories, Regional Reference Laboratories, and Specialized Reference Laboratories established to facilitate poliovirus surveillance worldwide. The National Laboratories (and sub-national laboratories in many countries) test stool specimens from cases of acute flaccid paralysis to detect poliovirus and identify serotypes. The Regional Reference Laboratories confirm the identity of polioviruses isolated by the National Laboratories and determine whether the viruses are wild or vaccine derived. A Regional Reference Laboratory may also serve as the National Laboratory for its own country and/or other countries that do not have their own laboratories. The Specialized Reference Laboratories perform various reference activities, including genomic sequencing of epidemiologically important poliovirus isolates. Sequencing serves as a method of “fingerprinting” polioviruses to provide definitive information for distinguishing between imported and indigenous cases, estimating temporal linkage between isolates, and identifying laboratory contaminants

The laboratories of the WHO network are useful resources for advice on other laboratories in the nation or region that might possess wild polioviruses or infectious materials. The WHO laboratories also serve as models in the application of appropriate procedures for safe handling and containment of wild polioviruses.

Poliovirus vaccine production laboratories: IPV and OPV production laboratories are few in number and known to national regulatory authorities and WHO.

Diagnostic and other laboratories: Some virus laboratories not identified above may have worked with polioviruses/enteroviruses in the past or occasionally perform poliovirus diagnostic tests, research, or teaching exercises. These laboratories may have wild poliovirus stocks and infectious materials in frozen storage. Such laboratories may be found in numerous organizations, including public health institutions, national control agencies, clinical facilities, commercial services, and research and academic institutions. Some national, international, private, or industrial culture collections have wild polioviruses. Such organizations may be located through the international society of culture collections.

Box 6: Agencies/Institutions with laboratories that might possess wild poliovirus infectious and/or potentially infectious materials

- **Biological control sgencies**
  - National/provincial
- **Biomedical research institutions**
  - National/provincial/commercial/non-profit
- **Culture collections**
  - National/institutional
- **Environmental agencies**
  - National/provincial/local
- **Hospitals**
- **Military agencies**
  - Health/research
- **Producers**
  - Biologicals/vaccines
- **Public health agencies**
  - National/provincial/local
  - Food safety
- **Universities**

Potentially infectious materials

Most challenging to identify are laboratories with clinical, epidemiological, research, or environmental specimens collected for other purposes at a time and in a geographical area of wild poliovirus endemicity and are potentially contaminated.

In developed countries, potentially infectious materials will be found in research laboratories with international programs. In developing countries, the laboratories most likely to possess such materials should also be identifiable based their respective research programs. However, the absence of such materials in other laboratories, regardless of size, cannot be assumed. The search for potentially infectious materials must include all medical/biological laboratories that maintain such materials under conditions known to preserve polioviruses (boxes 4 and 5).

Agencies/institutions and laboratories that might possess wild poliovirus infectious and/or potentially infectious materials are listed in boxes 6 and 7.

Box 7: Laboratories that might possess wild poliovirus infectious and/or potentially infectious materials

- **Microbiology laboratories***
  - Control
  - Diagnostic
  - Production
  - Research
  - Teaching
- **Pathology laboratories****
- **Gastroenterology laboratories****
- **Nutrition laboratories****
- **Environmental laboratories****

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* Includes bacteriology, mycology, parasitology, and virology.

** Includes types of laboratories listed under Microbiology as might apply.

Surveying laboratories and establishing inventories of wild poliovirus infectious and/or potentially infectious materials

Implementation of the plan in the Pre-Eradication phase of the Global Action Plan requires two critical steps: surveying all medical/biological laboratories that might possess wild poliovirus infectious and/or potentially infectious materials and establishing a global inventory system for laboratories that retain such materials.

The purpose of the Global Survey is to establish a Global Inventory of laboratories with wild poliovirus infectious and/or potentially infectious materials, describe the Global Action Plan to ensure safe handling of all such materials and to effect disposal of those materials no longer needed by the laboratory.

The Global Survey is hierarchical, beginning with notification by WHO and proceeding through Ministries of Health, agencies and institutions, and to laboratories. Because many laboratories that might possess such materials are outside the health sector, completion of the survey will require Ministries of Health to enlist the cooperation of other ministries, including Education, Defense, and Environment.

The purpose of the inventories is to document location and type of wild poliovirus infectious and/or potentially infectious materials being retained; to meet the country requirements for Regions to be certified as polio-free; and to maintain a current list of laboratories to be notified to initiate containment procedures one year after detection of the last wild poliovirus.

Data for the inventory system are obtained from the Global Survey, beginning with a thorough search by each laboratory for any materials that meet the definition of wild poliovirus infectious or potentially infectious materials. Data from the laboratories are submitted by parent agency/institutions to the National Inventory maintained by each country. Data from the National Inventory are provided to the National Committee for the Certification of Eradication of Poliomyelitis as well as to the appropriate WHO Regional Office.

Further guidance on conducting surveys and establishing inventories is provided in the document entitled Guidelines for Implementing the Pre-Eradication Phase of the Global Action Plan for Laboratory Containment of Wild Polioviruses: Surveying Laboratories/Establishing Inventories which may be obtained by writing to the Global Polio Laboratory Coordinator, Vaccines and Biologicals, Global Poliomyelitis Eradication Initiative, 20 Avenue Appia, CH-1211 Geneva 27, Switzerland.

Biosafety requirements

The basic principle of biosafety is to ensure that the microbiological techniques of the worker and the design, construction, and safety features of the laboratory are consistent with the risk of the infectious agent to the worker and the community.

Conventionally, the relative hazards of infectious agents are classified according to risk groups: 1 through 4. Risk group 1 represents the lowest level of risk to the laboratory worker and the community, and risk group 4 represents the highest. Four biosafety levels (BSL) correspond to these four risk groups[22]. The biosafety requirements become progressively more stringent as the risk increases (Box 8).

Wild polioviruses are classified as risk group 2. The rationale for the minimal biosafety levels is the near universal immunization of the population with OPV and/or IPV. Biosafety Level 2 (BSL-2) is the currently recommended minimal standard for all countries. To ensure safe handling of wild polioviruses and potentially infectious materials as eradication nears (Pre-Eradication), BSL-2, hereafter referred to as BSL-2/polio, should be enhanced by specific practices described in this document (Box 10).

When polio eradication is achieved (Post-Global Eradication), wild poliovirus in the laboratory constitutes a special category, that is, with little or no risk to the immunized worker, but a potential threat to successful eradication if transmission occurs in the community. The required biosafety level for wild poliovirus infectious and potentially infectious materials increases from BSL-2/polio to BSL-3/polio (high containment).

When immunization stops (Post-OPV Immunization), the number of unimmunized, susceptible persons in the world will grow rapidly. Possible transmission of wild poliovirus from the laboratory to the community becomes a public health threat of global proportions. The biosafety requirements for wild poliovirus infectious and potentially infectious materials increase correspondingly from high containment (BSL-3/polio) to maximum containment (BSL-4). A corresponding increase in biosafety requirements from BSL-2/polio to BSL-3/polio will be required for OPV and OPV-derived viruses to reduce the theoretical risk of virus circulation in an unimmunized population.

The major criteria for BSL-2/polio, BSL-3/polio, and BSL-4 are summarized in Box 9.

Box 8: Risk groups and biosafety levels[22]

Risk group	Level of risk	Description of risk group	Biosafety level (BSL)
1	No or very low level of individual and community risk	A microorganism that is unlikely to cause human or animal disease.	Basic – BSL-1
2	Moderate individual risk, low community risk	A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection limited.	Basic – BSL-2
3	High individual risk, low community risk*	A pathogen that usually causes serious human or animal diseases but does not ordinarily spread from one infected individual to the other. Effective treatment and preventive measures are available	High Containment – BSL-3
4	High individual and community risk*	A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available	Maximum Containment – BSL-4

* Wild polioviruses represent a unique risk. When eradication is achieved (Post-Global Eradication), they constitute little or no individual risk to the immunized individual, but an increasing community risk. When OPV immunization stops (Post-OPV immunization), they constitute little or no individual risk to the immunized worker, but an exceptionally high risk to the unimmunized community.

Box 9: Summary of biosafety levels for wild poliovirus infectious and/or potentially infectious materials

	Eradication phase		
	Pre-Eradication BSL-2/Polio	**Post-Global Eradication BSL-3/Polio**	**Post-OPV Immunization BSL-4**
Good microbiological techniques (Annex 1)	+	+	+
Personnel			
- Immunized	+	+	+
- Medical assessment		+	+
- Protective laboratory clothing	+	+	+
Facility			
- Separation of laboratory		+	+
- Restricted access	+	+	+
- Water resistant surfaces		+	+
- Sealable for decontamination		+	+
- Negative pressure		+	+
- HEPA exhaust filters		+	+
- BSC* I or II	+	+	
- BSC* III or positive pressure suits			+
- Autoclave:			
on site	+		
in room		+	
double-ended			+
- Liquid effluents treated			+
Wild poliovirus			
- Stored securely with controlled access and used only when essential	+	+	+
Listed on National Inventory	+	+	+

* Biological safety cabinets.

Pre-Eradication

Safe handling of wild poliovirus infectious or potentially infectious materials (BSL-2/polio)

The purpose of increasing the biosafety requirements for wild polioviruses from the current BSL-2 to BSL-2/polio is to reduce further the risk of transmission from the laboratory to the community at a time when polio is decreasing or no longer occurring in many areas of the world.

BSL-2 consists of the practice of good microbiological technique in a basic microbiology laboratory, as described in the 1993 WHO *Laboratory Biosafety Manual.* Included in good microbiological technique are safe laboratory practices, safe shipment of specimens and laboratory materials[23], appropriate procedures for disinfection and sterilization, and the use of equipment designed to eliminate or reduce hazards (Annex 1).

The basic microbiology laboratory consists of a facility with an autoclave on site and a class I or II biological safety cabinet for containment of all potential infectious aerosols. A mechanical ventilation system with inward directional airflow is also desirable (Annex 2).

Additional requirements that constitute BSL-2/polio for wild polioviruses or potentially infectious materials include: discontinuing the non-critical use of wild polioviruses; disposing of non-essential infectious or potentially infectious materials; keeping accurate records on wild poliovirus stocks, storing polioviruses and infectious materials in secure locations; using only designated strains or non-infectious inactivated materials when wild poliovirus antigens are required and restricting access to the laboratory to only those person who need to work with wild polioviruses and are appropriately immunized. The BSL-2/polio laboratory is described in Box 10.

> *Pre-Eradication requirements for the laboratory*
>
> *All laboratories working with wild poliovirus infectious or potentially infectious materials should immediately implement BSL-2/polio requirements and apply for listing in the National Inventory.*
>
> *Laboratories no longer wishing to retain wild polioviruses should either destroy all infectious and potentially infectious materials by autoclaving or incineration (Annex 3), or transport selected materials according to WHO recommendations (Annex 4) to an interim WHO-designated Repository*.*

* For designated repositories contact the Global Polio Laboratory Coordinator, Vaccines and Biologicals, Global Poliomyelitis Eradication Initiative, 20 Avenue Appia, CH-1211 Geneva 27, Switzerland.

Box 10: Biosafety level (BSL)-2/polio requirements

- Good microbiological techniques are practiced (Annex 1).
- Facility meets standards for basic BSL-2 laboratory (Annex 2).
- Access to laboratory is restricted.
- Persons entering the laboratory have been fully immunized against polio.
- Use of wild polioviruses is discontinued where attenuated vaccine polioviruses, inactivated antigens, or non-polio enteroviruses may serve the same purposes, for example, as challenge viruses in neutralizing antibody tests.
- All poliovirus stocks and potentially infectious materials are disposed of when there are no programmatic or research needs for retention.
- An internal control system is implemented for all wild polioviruses retained in the laboratory (current inventory, good record keeping).
- Wild polioviruses are stored in separate, secure areas with limited access.
- Only viruses that are readily identifiable by molecular methods are used if wild virus reference strains or working stocks are required.
- Appropriate sterilization and/or incineration is used for disposing of wild polioviruses, infectious materials and potentially infectious materials (Annex 3).

Post-Global Eradication

High containment of wild poliovirus infectious and potentially infectious materials (BSL-3/polio): To begin one year after detection of the last wild poliovirus

The purpose of BSL-3/polio is to further reduce the risk of transmission from the laboratory to the worker and/or the community at a time when wild polioviruses no longer circulate anywhere in the world, but universal immunization continues. The BSL-3/polio laboratory incorporates all of the good microbiological practices described for the BSL-2/polio laboratory. Additional major facility requirements include separation of the laboratory from common hallways; double door entry; self-closing, lockable doors; windows, if any, permanently sealed; water resistant surfaces for cleaning; room sealable for decontamination; negative pressure to environment; HEPA exhaust filters; and an autoclave in the laboratory, preferably double-ended. A complete list of facility requirements for BSL-3 is described in the WHO *Laboratory Biosafety Manual*[22]. All infectious or potentially infectious materials must be autoclaved or chemically treated before disposal. Spilled infectious materials must be treated with disinfectant as recommended[22] and strict care must be taken that no infectious or potentially infectious materials are discharged into the sanitary sewer. All laboratories retaining or working with poliovirus infectious or potentially infectious materials under BSL-3/polio conditions must be listed on the designated Agency/Institutional and National Inventories. A summary of the design and equipment for the high containment laboratory is given in Box 11.

Post-Global Eradication requirements for the laboratory

All laboratories wishing to retain wild poliovirus infectious or potentially infectious materials must begin implementing BSL-3/polio containment procedures as soon as possible, but at the latest one year after detection of the last wild poliovirus. All biosafety actions are to be implemented and documented as complete before global certification of polio eradication can be considered.

*Laboratories wishing to qualify as a BSL-3/polio facility and retain wild poliovirus infectious materials must be listed on **Agency/Institutional, National and Regional Inventories.***

Laboratories not wishing to convert to BSL-3/polio containment must render as non-infectious or destroy all wild polioviruses and potentially infectious materials by autoclaving or incineration (Annex 3).

Alternatively, to arrange for transfer and storage of selected materials (Annex 4), laboratories may contact a WHO-designated BSL-3/polio repository.*

Box 11: The high containment facility (BSL-3/polio): Summary of laboratory design and equipment[22]

In addition to all requirements for the BSL-2/polio facility:

- The laboratory is separated from the areas that are open to unrestricted traffic flow within the building.
- Access doors are self-closing and self-locking.
- The surfaces of walls, floors and ceilings are water-resistant and easy to clean.
- The laboratory room is sealable for decontamination. Air-conducting systems are constructed to permit gaseous disinfection.
- Windows are locked and sealed.
- Anti-backflow devices are fitted to the water supply.
- Negative pressure is maintained in the facility by a mechanical, individual, inwardly directed, HEPA-filtered supply, and a HEPA filtered exhaust air system.
- An autoclave for decontamination of infected waste material should be available in the laboratory room.

* For designated repositories contact the Global Polio Laboratory Coordinator, Vaccines and Biologicals, Global Poliomyelitis Eradication Initiative, 20 Avenue Appia, CH-1211 Geneva 27, Switzerland.

Post-OPV Immunization

Maximum containment (BSL-4) of wild poliovirus infectious and potentially infectious materials and high containment (BSL-3/polio) of OPV and OPV-derived viruses: To begin when OPV immunization stops

The biosafety requirements for wild poliovirus infectious and potentially infectious materials increase from BSL-3/polio to BSL-4 when immunization stops. The increased containment level is consistent with the potential consequences of an inadvertent transmission of wild poliovirus from the laboratory to a rapidly increasing non-immune population.

BSL-4 may be achieved by a primary containment system consisting of positive pressure ventilated suits or a closed system biological safety cabinet (BSC)-III. The BSL-4 suit laboratory requires a special design to provide suit ventilation, a chemical shower, and a special waste disposal system. The construction and operation of such a facility is expensive, complex, and represents a major investment.

Those laboratories that already meet the BSL-3/polio requirements can achieve BSL-4 by installing a BSC-III, singly or in a chain and meeting the additional requirements for the maximum containment laboratory as outlined in Box 12. BSL-4 requirements are described in the *WHO Laboratory Biosafety Manual*[22].

Additional security requirements for polioviruses include the following:

- Controlled laboratory access through locks, keys and security clearance of all qualified employees.
- Records kept in files and computers are locked. Records include validation of security procedures, and entries to laboratories.
- Viruses are maintained in locked laboratories and locked freezers. Locked inventories are maintained with documentation and accountability.
- Domestic and international shipment procedures are consistent with WHO Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens[6]. Personnel are trained to use appropriate procedures for both incoming and outgoing shipment, prepare the required documentation, and file the appropriate permits.
- Administrative controls consist of a designated safety officer, designated biosafety committee, and evidence that all laboratory personnel are immune to poliovirus.

In the post immunization phase, the biosafety requirements for OPV and OPV-derived viruses increase to BSL-3/polio based on the theoretical risk from circulation of these viruses in unimmunized populations.

Post-OPV Immunization requirements for the laboratory

All laboratories working with wild poliovirus infectious or potentially infectious materials should immediately implement maximum containment (BSL-4) requirements upon notification that OPV immunization has stopped. All laboratories working with OPV viruses should implement high containment (BSL-3/polio) procedures. IPV vaccine production facilities should implement maximum containment and will be assessed on a facility by facility basis. Only laboratories meeting these requirements will be permitted to retain or work with wild polioviruses and potentially infectious materials.

BSL-3/polio laboratories not wishing to convert to BSL-4 may retain OPV and OPV-derived viruses but must dispose of all infectious and potentially infectious materials by autoclaving or incineration (Annex 3), or transport selected materials according to WHO recommendations (Annex 4) to an interim WHO designated Repository.*

Box 12: The maximum containment facility (BSL-4): Summary of laboratory design and equipment [22]

In addition to all requirements for the BSL-3/polio facility:

- Entry and exit of personnel and supplies are through an airlock or pass-through system. On entering, personnel should put on a complete change of clothing; before leaving, they should shower before putting on their street clothing.
- All fluid effluents from the facility are rendered safe before final discharge.
- A double-door, pass-through autoclave is available for sterilization of laboratory clothing, waste and materials.
- An efficient primary containment system is in place, consisting of one or more of the following:
  - Class III biological safety cabinets
  - Positive-pressure ventilated suits. A special chemical decontamination shower is provided for personnel leaving the suit area.
- Airlock entry ports or dunk tanks are installed for specimens and materials.

* For designated repositories contact the Global Polio Laboratory Coordinator, Vaccines and Biologicals, Global Poliomyelitis Eradication Initiative, 20 Avenue Appia, CH-1211 Geneva 27, Switzerland.

Special biosafety considerations

Vaccine production laboratories: IPV is produced with non-attenuated wild strains. Maximum containment of wild polioviruses and potentially infectious materials in IPV production facilities presents special challenges because of large volumes and high concentration of viruses. Each facility must be reviewed on an individual basis by national authorities in collaboration with WHO to establish procedures that reflect current risks.

Public health and clinical diagnostic laboratories: Surveillance for poliovirus will be active for many years after interruption of wild virus transmission and cessation of OPV immunization. Diagnostic testing will continue in designated laboratories under BSL-2/polio conditions. Tests will be performed using vaccine virus and non-infectious poliovirus products as controls in Post-Global Eradication and Post-OPV Immunization, respectively. Surveillance tests in the laboratory for the presence of poliovirus in clinical or environmental specimens do not constitute a greater risk to the community than that already occurring in the community if poliovirus is found to be present.

The biosafety requirements for all types of laboratories are summarized in Box 13.

Box 13: Requirements for laboratories having or working with polioviruses

			Eradication phase		
			Pre-Eradication Wild virus circulating	**Post-Global Eradication** No wild virus circulating for at least one year	**Post-OPV Immunization** OPV stopped
All laboratories	OPV vaccine/vaccine-derived virus		BSL*-2/polio	BSL-2/polio	BSL-3/polio
	Wild virus		BSL-2/polio	BSL-3/polio	BSL-4
Special circumstances	Public health and clinical (diagnostic tests only)		BSL-2/polio	BSL-2/polio**	BSL-2/polio†
	Vaccine production	OPV	BSL-2/polio	BSL-2/polio**	BSL-3/polio
		IPV	BSL-2/polio	BSL-3/polio	BSL-4‡

* Biosafety level (see Box 9).

** No live wild virus controls used in diagnostic or reference tests.

† No live virus controls used in diagnostic tests.

‡ Maximum containment in vaccine production facilities will be addressed on a facility-by-facility basis.

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Annex 1: Good microbiological techniques[22]

- Specimens are handled safely
- No mouth pipetting is permitted
- Pipettes and pipetting aids are used safely
- Dispersal of infectious materials is avoided
- Contact of infectious materials with skin and eyes is avoided
- Ingestion of infectious materials is avoided
- Separation of serum is carried out safely
- Centrifuges are used safely
- Homogenizers, shakers and sonicators are used safely
- Tissue grinders are used safely
- Refrigerators are maintained and used safely
- Ampoules containing infectious materials are opened safely
- Infectious materials are stored safely
- Precautions are taken with blood and other bodily fluids
- Specimens and infectious materials are shipped safely
- Appropriate disinfection and sterilization are carried out
- Hands are washed between procedures and prior to leaving laboratory
- Laboratory gowns are worn for work in laboratory
- Storage of food or drink in the laboratory or any storage receptacle containing infectious materials is prohibited
- Eating, drinking, and smoking in the laboratory is prohibited

Annex 2: The basic biosafety level 2 (BSL-2) facility[22]

- Ample space is provided for the safe conduct of laboratory work and for cleaning and maintenance.
- Walls, ceilings and floors are easily cleanable.
- Illumination is adequate for all activities.
- Storage space is adequate to hold supplies for immediate use.
- Hand washing basins, with running water, if possible, are provided in each laboratory room, preferably near the door.
- An autoclave (or suitable pressure cooker) is available in the same building as the laboratory.
- Facilities for storing outer garments and personal items for eating and drinking are provided outside the working areas.
- A good-quality and dependable water supply is available. There are no cross-connections between sources of laboratory and drinking-water supplies.
- A standby generator is desirable for the support of essential equipment such as incubators, biological safety cabinets, freezers, and the like.
- Pipetting aids are available to replace mouth pipetting.
- Biological safety cabinets are available for:
  - Procedures with high potential for producing aerosols, including centrifugation, grinding, blending, vigorous shaking or mixing, sonic disruption, and opening of containers of infectious materials whose internal pressure may be different from the ambient pressure.
  - Handling high concentrations or large volumes of infectious materials.
- Centrifuges with sealed safety buckets are available for centrifuging high concentrations or large volumes of infectious materials in the open laboratory. These buckets must be loaded and unloaded in a biological safety cabinet.
- Screw-capped tubes and bottles are available to hold positive specimens and cultures.
- Autoclaves are available to sterilize contaminated material.

Annex 3: Methods for disposal of poliovirus infectious or potentially infectious materials[22]

Sterilization (Use of autoclaves)

Moist steam under pressure is the most effective method of sterilization of laboratory materials.

- All cultures and contaminated materials should normally be autoclaved in leakproof containers, e.g., autoclavable, color-coded plastic bags, before disposal.
- Plastic bags should be opened so that steam will penetrate to their contents.
- After being autoclaved, the materials may be placed in transfer containers for transport to the incinerator or other point of disposal.

Incineration

- Incineration is the method of choice for final disposal of contaminated waste, including carcasses of laboratory animals, preferably after autoclaving. Incineration of infectious materials is an alternative to autoclaving only if:
  - the incinerator is under laboratory control;
  - the incinerator is provided with an efficient means of temperature control and a secondary burning chamber.
- Materials for incineration, even if they have first been autoclaved, should be transported to the incinerator in bags, preferably plastic.
- Incinerator attendants should receive proper instructions about loading and temperature control.

Final disposal

The disposal of laboratory and medical waste is subject to various national regulations. In general, ash from incinerators may be treated in the same way as normal domestic waste and removed by local authorities. Autoclaved waste may be disposed of by off-site incineration or in licensed landfill sites.

Annex 4:
Requirements for safe transport of wild poliovirus infectious or potentially infectious materials

Transport of Wild Poliovirus Infectious or Potentially Infectious Materials should follow IATA transport regulations for Infectious substances affecting humans.

The following instructions are excerpted from the *Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens*, WHO, 1997*. Please refer to the complete document when making arrangements for transport of wild poliovirus and potentially infectious materials.

The current packaging requirements for infectious substances consist of a triple system described as follows and shown in the accompanying figures.

Basic triple packaging system

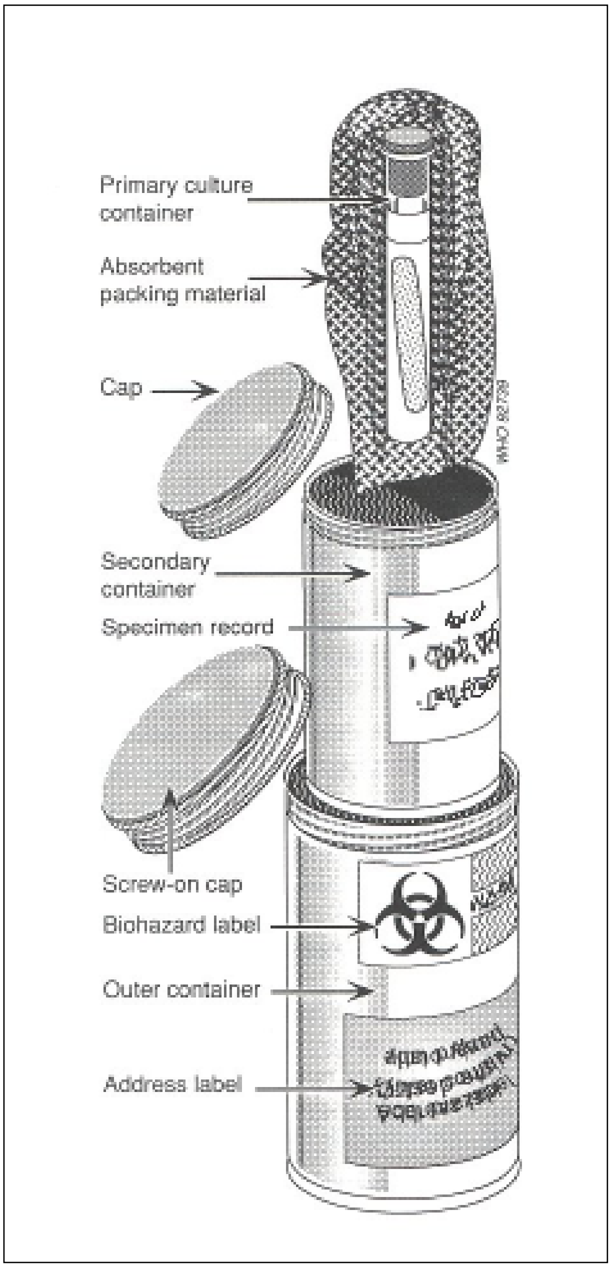
The system consists of three layers as follows.

1) Primary receptacle. A labeled, watertight, leak-proof receptacle containing the specimen.
2) Secondary receptacle. A second, durable, watertight, leak-proof receptacle to enclose and protect the primary receptacle(s).
3) Outer shipping package. An outer shipping package which contains the primary and secondary receptacles.

Specimen data forms, letters and other types of information that identify or describe the specimen, shipper, and receiver should be taped to the outside of the secondary receptacle.

This document is also available on the Internet at http://www.who.int/emc/biosafety.html.

Triple packaging system



Hand carriage of infectious substances is strictly prohibited by international air carriers, as is the use of diplomatic pouches.

If transport is by passenger aircraft, the maximum net quantity of infectious substances that can be contained in an outer shipping package is 50 mL or 50 g. For transport by cargo aircraft or other carriers, the limit per package is 4 L or 4 Kg.

Labeling of the outer package for shipment of infectious substances must include the following elements:

1) The International Infectious Substance Label.
2) An address label with full information.
3) Required shipping documents – these are obtained from the carrier and are fixed to the outer package.
4) An import and/or export permit and/or declaration if required.
5) If the outer package contains primary receptacles exceeding 50 mL in combination at least two “Orientation Labels” (arrows) must be places on opposite sides of the package showing correct orientation of the package.

It is the sender’s responsibility to ensure the correct designation, packaging, labeling and documentation of all infectious substances and diagnostic specimens.

Efficient transport and transfer of infectious materials require good coordination among the sender, carrier and receiver (receiving laboratory) to ensure that the material is transported safely and arrives on time and in good condition. Such coordination depends upon well-established communication among the three parties and a partner relationship.

All have specific responsibilities to carry out in the transport effort.

The sender

The sender has the following responsibilities:

1) makes advance arrangements with the receiver of the specimens, including investigating the need for an import permit;
2) makes advance arrangements with the carrier to ensure:
   - that the shipment will be accepted for appropriate transport
   - that the shipment (direct transport if possible) is undertaken by the most direct routing, avoiding arrival at weekends;
3) prepares necessary documentation including permits, dispatch and shipping documents; and
4) notifies the receiver of transportation arrangements once these have been made, well in advance of the expected arrival time.

The carrier

The carrier is responsible for the following:

1) providing the sender with the necessary shipping documents and instructions for their completion;
2) providing advice to the sender about correct packaging;
3) assisting the sender in arranging the most direct routing and then confirming it;
4) maintaining and archives the documentation for shipment and transport;
5) monitoring the required holding conditions of the shipment while in transit; and
6) notifying the sender of any anticipated (or actual) delays in transit.

The receiver

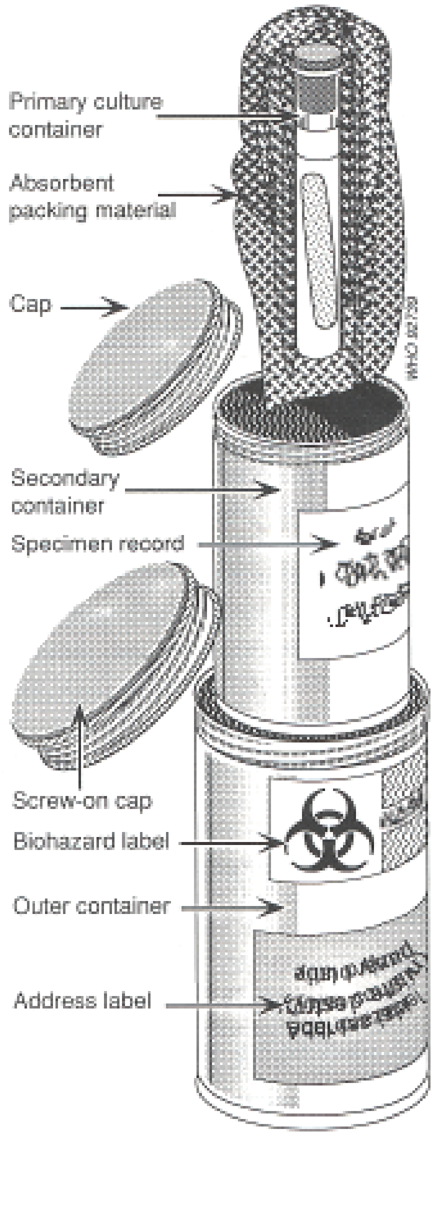
The party receiving infectious materials is accountable for the following:

1) obtaining the necessary authorization(s) from national authorities for the importation of the material;
2) providing the sender with the required import permit(s), letter(s) of authorization, or other documents) required by the national authorities;
3) arranging for the most timely and efficient collection on arrival; and
4) immediately acknowledging receipt to the sender.

Shipments should not be dispatched until:

- advance arrangements have been made between the sender, carrier and receiver
- the receiver has confirmed with the national authorities that the material may be legally imported
- the receiver has confirmed that no delay will be incurred in the delivery of the package to its destination.

Detailed information on response and emergency safety measures in transport-associated accidents can be found on pages 52-54 of *WHO's Laboratory Biosafety Manual* [22].


Primary culture container
Absorbent packing material
Cap
Secondary container
Specimen record
Screw-on cap
Biohazard label
Outer container
Address label