Welcome to Module 4 of this program on the laboratory approach to the diagnosis of smallpox. This Module comprises an overview of specimen collection.

Module 4 of this series discusses the appropriate specimen types and collection methods that are useful for diagnosing orthopoxvirus infection and other rash illnesses in humans. Collection of non-human and environmental samples is reviewed. We will learn about the regulations and required procedures for packaging and shipping infectious materials. We will also learn about the chain of custody procedures required to protect the integrity of samples for law enforcement.
Module 4 Unit 1: Specimen Collection

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At the conclusion of this Module, participants will be able to complete the following learning activities:

- Describe the types of specimens that should be collected for orthopoxvirus laboratory testing.
- Describe appropriate safety procedures for specimen collection and handling.
- Outline packaging and shipping procedures that comply with IATA/DOT regulations for transporting infectious substances.
- Outline the steps required to maintain a defensible chain of custody from collection to final disposition of samples.

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Unit 1 of this Module will discuss considerations for collecting specimens from patients with symptoms suggestive of a poxvirus infection. Procedures to collect specimens from humans and animals will be described, and environmental sampling requirements will be discussed.

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Modules 2 and 3 introduced the clinical rash algorithm and its applications to enhance rapid diagnosis of smallpox and smallpox look-alike diseases. The clinical-rash algorithm helps triage cases with varying levels of concern for smallpox-like disease, coupling appropriate testing and public-health responses to each level of suspected disease. This significantly decreases the odds of a false-positive laboratory result for smallpox virus, and ensures that clinical and laboratory resources are used prudently. The clinical-rash algorithm also prompts healthcare providers to consider alternative diagnoses, particularly chickenpox, thus providing a framework for appropriate diagnostic testing. Since its inception, this process has demonstrated clinical value, contributing to the diagnosis of several cases of smallpox look-alike disease that might otherwise have remained undiagnosed.
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The algorithm provides significant diagnostic benefits even in the absence of smallpox and encourages careful diagnosis of other rash illnesses such as varicella zoster or vaccinia. The use of such an algorithm minimizes the number of cases that require intensive investigation and focuses attention where it is most needed and justified. This is an important consideration for both laboratory and clinical settings. It provides a more rapid, thorough response to highly suspect cases.

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Laboratory-testing guidelines have been developed by the Centers for Disease Control—CDC—to complement the clinical febrile vesicular-rash algorithm. The laboratory algorithm (Laboratory Testing for Acute, Generalized Vesicular or Pustular Rash Illness in the United States) introduced and discussed in detail in Module 3 provides a standardized approach to rapidly triage and test specimens for the possible presence of variola virus, the infectious agent that causes smallpox. It also provides a logical progression of testing if the case patient is not considered to be at high risk for smallpox.

Testing for orthopoxviruses and common smallpox look-alike diseases may include Direct Fluorescent Antibody testing, PCR, electron microscopy, virus and bacterial cultures, immunohistochemistry, and serology.

Additional information can be found at the CDC website.
In the United States and its territories, a suspected case of smallpox must be reported immediately to the appropriate local, state, or territorial health department. The public health laboratory should also be consulted. After their review, if smallpox is still suspected, the CDC Emergency Operation Center will be alerted. Approval must be obtained before shipping clinical specimens from a potential smallpox patient to CDC. As a true smallpox case would raise immediate concerns about potential terrorism, law enforcement chain of custody procedures and documentation should be implemented for specimen collection and transfer. Chain of custody is discussed in Unit 3 of this Module. The public health Laboratory Response Network laboratory can also provide guidance on chain of custody protocols.

If you have questions about disease caused by another orthopoxvirus or another poxvirus, contact the Poxvirus Program at CDC.

More detailed information for managing a suspect smallpox case can be found in the CDC Smallpox Response Plan and Guidelines (Version 3.0).

As also discussed in Module 3, this is the testing algorithm that should be used when there is suspicion of a smallpox vaccine adverse event or infection from a monkeypoxvirus or other orthopoxvirus. Clinical diagnosis of a possible vaccinia-associated adverse event would share some features with the febrile vesicular rash algorithm for diagnosing smallpox. In the event of a smallpox event, vaccination would be used as a control measure; therefore differentiation between a vaccine adverse event and smallpox infection would be a priority.

Along with clinical signs and symptoms, recent smallpox vaccination history or contact with a vaccinee should be considered. Travel history, exposure to exotic animals, or other appropriate epidemiologic links should also be considered. Many of the laboratory testing requirements for vaccinia and monkeypox infection are very similar to those for variola. Specimens should be referred to CDC for monkeypox-specific confirmatory testing.

Detailed specimen collection guidelines for vaccinia virus and monkeypox virus can be found on the CDC website. Specimen collection from animals will be discussed later in this unit.
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The quality and reliability of all diagnostic testing is dependent on the proper selection, collection, and transport of clinical specimens. Clinical material for laboratory testing should be collected:

- From sites associated with the presence of infectious agents;
- With adequate volume of material to perform all required tests;
- Within the timeframe that infectious agents or antibodies are likely to be present;
- Using containers designed to maintain survival or stability of the suspected agents, prevent leakage, and loss of material; and
- With proper technique to minimize contamination of specimens, and to protect the healthcare worker collecting the specimens.

Testing specimens that are improperly collected, stored, or transported can result in false-negative results, compromising patient management and disease control efforts.

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Healthcare workers collecting specimens from patients with suspect variola infection should take proper precautions to prevent disease transmission. Modes of smallpox transmission described here were observed during the smallpox eradication era. Smallpox typically spread among close contacts of another smallpox infected person. Epidemiologic studies suggested that those at highest risk for disease had spent significant time within 6 to 7 feet of another smallpox case. The disease is most commonly transmitted by virus-laden airborne droplets, but not as fine particle aerosols. A fitted respirator NIOSH N-95 mask or better should provide substantial protection from inhalation of droplet aerosols and airborne infection. There is no carrier state. Smallpox was less frequently transmitted from fomites, although smallpox virus may be relatively stable in certain environments. It was not recognized to have been transmitted by ingestion of contaminated food or water.
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As part of the Smallpox Response Plan and Guidelines (Version 3.0), the infection control guidelines would guide the public health response and many of the federal, state, and local public health activities required in the event of a smallpox outbreak. There are two sections in the infection control guidelines.

Part One of the Isolation and Quarantine Guidelines describes the infection control measures for healthcare and community settings. Part Two includes the required quarantine measures that would form an important part of a response to a smallpox emergency.

The Decontamination Guidelines include medical decontamination protocols, which utilize low- to intermediate-level chemical germicides or EPA-registered detergent disinfectants to clean surfaces potentially contaminated with smallpox virus.

All of these guidelines can be found at the CDC smallpox website links shown here.

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A variety of infection control measures can be used to decrease the risk of smallpox transmission. The CDC recommends personal protective equipment consistent with Contact and Airborne Precautions, in addition to Standard Precautions, for contact with patients with known or suspected smallpox.

Personal protective equipment, including gloves, gowns, masks, and protective eyewear should be worn in combination to provide barrier protection.

Disposable latex or vinyl gloves must be changed between patients and hands should be washed after gloves are removed.

Disposable gowns especially treated to make them impermeable to liquids will provide greater protection to the skin.

The latest recommendation for respiratory protection equipment is N95 (N category at 95% efficiency) or higher and high-efficiency particulate air (HEPA) filter respirators certified by the CDC National Institute for Occupational Safety and Health (NIOSH).
Wearing masks and eye protection such as goggles in combination protects the mucous membranes of the eyes, nose, and mouth from contact transmission of the smallpox virus. Used patient-care articles should be enclosed in containers or disposable bags to prevent inadvertent exposures to patients and personnel and to prevent contamination of the environment.

CDC’s latest requirements for personal protection can be found in the Guideline for Isolation Precautions in Hospitals

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Smallpox vaccination within the previous 3 years is generally considered to provide a good level of protection from possible smallpox infection. Ideally, specimen collection for suspected cases should be performed by successfully vaccinated personnel wearing appropriate barrier protection, including gloves, gown, shoe covers, and respiratory protection. Respiratory protection is currently recommended for all personnel whether vaccinated or not. Masks and eyewear or face shields should be used if splashing is anticipated. If vaccinated personnel are not available to help collect specimens, otherwise healthy persons using appropriate Personal Protective Equipment (PPE) could collect smallpox specimens safely, just as they might with other infectious diseases. Persons with contraindications to smallpox vaccination should avoid specimen collection since post-exposure vaccination would be required if smallpox diagnosis is confirmed. Fit-tested N95 masks and other appropriate PPE should be worn by individuals caring for suspected patients. Contact your State Health Department for appropriate instructions.
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Collection methods are essentially the same for specimens suspected to contain a varicella zoster virus or an orthopoxvirus such as variola, vaccinia, or monkeypox. The best specimens for many of the vesicular rash diagnostic laboratory tests are the “roofs” or crusts from the lesions, which often contain large amounts of virus. Vesicular fluids from the lesions are also convenient sources of diagnostic material. Whichever tests are considered for diagnosis, roofs and vesicular fluids from multiple lesions should be sampled to provide adequate material for testing. Collection of biopsies can be done with local anesthetic if a histopathologic exam is being considered. Histopathology is especially important for successful diagnosis of several of the orthopox look-alike syndromes. Blood samples should be collected if serologic testing is indicated. Cerebral spinal fluid (CSF) may be collected if encephalitis is suspected.

The chart shown here, summarizing specimen types, collection methods, and diagnostic assays for the detection of poxviruses from human specimens, can also be found on the CDC website.

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Materials useful for collection of lesion or vesicular fluid specimens for laboratory testing include:

- A disposable scalpel (1) No. 10 blade;
- Several sterile 26-gauge needles;
- Sterile dry polyester or Dacron swabs;
- A plastic specimen container with formalin for the lesion biopsy;
- A punch biopsy collection tool;
- Sterile plastic vials for specimens (without any viral culture medium or fluid); and
- Clean Microscope slides and slide storage container.

If available, electron microscopy (EM) grids are also useful for specimen collection and facilitate EM examination of materials for the presence of orthopoxvirus particles.
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Prior to specimen collection, clean the skin site with an alcohol wipe, and allow the alcohol to dry completely. To collect materials from vesicles, use a scalpel or needle to unroof the vesicle. Place the skin or scab that constitutes the roof of the lesion into the sterile plastic vial. This specimen should be sent dry without any viral culture medium or fluid. A scalpel may be more useful for removing the roof of a vesicular or pustular lesion and then placing it into the sterile plastic vial.

Be sure to collect the scabs or roofs from multiple lesions. Avoid using glass vials due to risk of breakage in transport.

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You may also scrape the base of the vesicle with a blunt end of a scalpel or wooden applicator and smear some of this material onto a microscope slide.

Touch preps should be prepared by repeatedly touching a clean microscope slide to the base of the unroofed lesion. If a slide is not available, swab the base of the lesion with a polyester or Dacron swab. Place the swab in a sterile vial; do not use transport media.

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If available, an electron microscopic grid should then be carefully touched 1 time to the base of the unroofed lesion. Grids should be touched to the lesion with the shiny side of the grid down, coming into direct contact with the base of the lesion. Note how the grid forceps hold the grid towards the edge and not in the middle.

Two or three different grids should be touched to the base of the lesion. Using a different touch pressure for each grid allows increasing amounts of material to be collected. EM grids from multiple lesions will also provide more material for reliable examination.
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Allow the slides and EM grids to air dry for 10 minutes. Then place the slides in a plastic slide holder for shipping. Do not store slides from different patients together. Store EM grids in the appropriate EM grid box. Note the number of the slot on the grid box where the EM grid used for the touch prep is stored.

Be sure to label all specimens and containers with the date of collection, patient information, specimen source and other appropriate information. Unlabelled specimens cannot be accepted for testing.

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A biopsy of a separate intact lesion, with the roof of the lesion still on, should be obtained using a punch biopsy tool as shown here. It is recommended that a punch biopsy be performed on 2 separate lesions. Place one in a container with formalin and the other in a sterile, plastic tube (without any viral media or fluid). Alternately, split a single punch biopsy specimen in half and place one half in formalin and the other half in the sterile, plastic tube. The formalin-fixed specimen can be used for histopathology, while the other, non-fixed specimen can be used for DNA detection or virus isolation.

Serum can also be collected if it is considered diagnostically useful. Serum is relatively stable at refrigerator temperatures or frozen. However, if serum cannot be separated from red blood cells, such whole blood should not be frozen prior to transport. More details about the utility of serology are provided in Module 5.

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The CDC has provided a fact sheet outlining a step-by-step protocol for collecting specimens when smallpox is suspected. The fact sheet is available at the CDC smallpox website.

A video from CDC, developed for healthcare professionals, illustrates the most appropriate procedures for collecting VZV skin lesions and blood specimens. As noted earlier, collection of specimens from rash lesions for varicella and orthopoxviruses is essentially the same.
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Submission of uniform patient information along with the samples is critical for ensuring that the specimen can be processed expeditiously and that the appropriate individuals will be notified when diagnostic results are obtained.

Patient and specimen data include:
- Demographic information;
- Clinical history such as date of fever and rash;
- Types and quantities of lesions;
- Varicella (chickenpox) exposure and vaccination history along with a possible history of smallpox vaccination; and
- Specimen information.

Reporting results to healthcare providers, and the appropriate public health infrastructure is obviously critical. To help ensure that results are available in a timely manner, it is important to provide contact information for the submitting clinician and health officials. If needed, the Poxvirus Program will contact the person submitting the specimen for additional information by phone.

The appropriate specimen collection forms can be obtained from the state public health laboratory or CDC. The forms MUST accompany any specimens shipped to a public health LRN laboratory or to the CDC. When sending clinical specimens to CDC for poxvirus diagnostics, please enclose an accessioning form for each individual case submitted.

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Specimens should be transported to the appropriate laboratory ASAP.

Specimens collected from patients with poxvirus infections can be stored at 4°C if they will be shipped to a reference center within 24 hours. However, -70°C or dry ice should be used if specimens must be stored for more than 24 hours before shipment. Changes in the pH of specimens, such as those caused by dry-ice vapors, can be avoided by using a gasketed, or o-ring-containing, screw cap vial. Avoid using specimen containers with loose-fitting lids such as rubber stoppers or containers that might break during transit.
Suspect vaccinia adverse event samples should be sent to the closest state or regional Laboratory Response Network (LRN) laboratory capable of performing orthopoxvirus testing. Contact the director of your state public health laboratory for specific shipping address information. Consider obtaining this local contact information before beginning large-scale vaccination programs.

Specimens considered at high risk for smallpox must be shipped directly to CDC and to LRN reference labs with smallpox testing capacity. Chain of custody documentation should be included. As part of the febrile vesicular rash illness algorithm, state health departments should be in contact with CDC regarding submission of high-risk specimens. Note that approval must be obtained before clinical specimens from a potential smallpox patient are shipped to CDC. Specific directions on transporting the packaged specimens to CDC will be given at the time of consultation.

More information on packaging and shipping is provided in Unit 2 of this Module. Most commercial shippers and regulatory groups have explicit instructions and specific procedures for shipping clinical specimens at room temperature, $4\,^\circ\mathrm{C}$, or $-70\,^\circ\mathrm{C}$. Additionally, special packaging and shipping regulations apply when transporting infectious materials.

In the event of a zoonotic outbreak or a suspected act of terrorism, public health laboratories may need to rule out or identify orthopoxviruses in non-human samples. These requests may be for testing of non-human animal specimens or “environmental” samples. General guidance regarding collection of specimens from these sources is discussed here. Additional detailed guidance would be provided by CDC and state health or veterinary officials based on the specific scenario.
Variola virus had no non-human reservoirs. Other orthopoxviruses are zoonotic, as discussed in Module 2. During May and June 2003, the first cluster of human monkeypox cases in the United States was reported. This outbreak in the American mid-west was a reminder that zoonotic infections can be sources of emergent infectious diseases of humans as well as associated non-human species. Most human case-patients with this febrile vesicular rash illness were believed to have acquired the infection from prairie dogs. The prairie dogs became ill after contact with various exotic African rodents shipped from Ghana to the United States in April 2003. Culture and polymerase chain reaction (PCR) performed on various tissues demonstrated monkeypox virus in two rope squirrels, one Gambian rat, and three dormice. Early in the 2003 monkeypox outbreak, the epidemiologic association between captive prairie dogs and human poxvirus disease was an important factor for ruling out possible smallpox, even before laboratory testing.

These photos show the human lesions caused by exposure to infected prairie dogs. Patients A and B were bitten and scratched by a monkeypox-infected prairie dog. Patient C’s pre-existing scratch was exposed to an infected prairie dog.

Even though epidemiologic links suggested the human disease was not smallpox, public health laboratories were required to respond to what turned out to be an outbreak of a related human orthopoxvirus infection. This experience helped to validate the value of “generic” diagnostic orthopoxvirus protocols, used in concert with species-specific tests. In addition, as part of this public health response to monkeypox infections, public health laboratories were expected to test samples from non-human sources as part of an effort to understand the introduction and transmission of disease.

In general, collecting lesion samples from non-human animal sources is similar to obtaining those samples from humans. However, infection in non-human species does not always include obvious lesions, and careful collection of infected tissues from animals may require non-traditional resources, such as veterinary assistance and appropriate BSL containment facilities for necropsy. In the absence of BSL-3 necropsy facilities, it may not be prudent to do full necropsies on suspect virus-infected animals, which can have extremely high levels of infectious virus.
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Animals or animal tissues that are submitted for diagnostic testing should be accompanied by the animal submission form. The CDC or state health department will provide appropriate forms. Required information includes:

- The city, state, and name(s) of the animal’s owner, or location where the animal was found if there is no owner;
- The animal species and date of birth if known;
- The source of animal purchase; and
- The specimen type and method of collection.

Linkage to a possible human case of disease should be noted along with other possible contacts to the animal. Any history of the animal’s condition should be provided as well, especially any symptoms the animal may have exhibited.

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As with analysis of human clinical samples, the positive predictive value for meaningful diagnosis increases significantly if there is reason to suspect infection. When present, visible lesions were excellent sources of monkeypox DNA, antigens, and viral particles. Additional tissues, or pools of various tissues, which included lung, liver, and spleen were often useful for PCR detection of monkeypox virus DNA and for immunohistochemical detection of viral antigens. Skin biopsies, absent visible lesions, from acute non-human monkeypox virus-infected animal species proved useful for detection of monkeypox virus by PCR. At present, it is unclear and perhaps unlikely that a single tissue source, other than skin lesions when present, will always constitute the optimal tissue for poxvirus analysis. Research testing for generic orthopoxvirus-genus specific IgG antibodies is available. However, absence of meaningful positive and negative serologic controls complicates interpretation of serologic results in non-human species.

This chart, summarizing specimen types, collection methods and diagnostic assays for the detection of poxviruses from animal specimens, can be found on the CDC website.
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Since the anthrax events of 2001, law enforcement, hazardous-material first-responders, and public health laboratories have played a joint role in sampling and testing suspicious substances and other environmental samples for the detection of infectious agents. If environmental contamination with variola or other biothreat agents is suspected, law enforcement should be contacted to perform a risk assessment. Sample collection should only be performed by trained first-responders wearing appropriate personal protective equipment. Chain of custody protocols should be implemented for all samples collected.

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Test results must be interpreted carefully, and in the context of the law enforcement risk assessment. In the absence of suspicion of compatible disease, the expectation for a positive test result from the environmental samples approaches zero. The probability of a false positive result, even if very small, may be greater than the positive predictive value for a true positive test result. This underscores a key difference in the conceptual frameworks for clinical test analysis of disease as compared with forensic environmental sampling and testing.
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The chart shown here, Laboratory Testing for Environmental Samples in the United States, presents an orthopoxvirus testing algorithm for environmental or other non-clinical samples. These samples should not be processed by clinical laboratories. After explosives, radiation, and toxins have been ruled out by law enforcement, the environmental samples are sent to the LRN Reference Labs for PCR testing and Electron Microscopy (if available) with CDC consultation only.

If orthopoxviruses are ruled out, the need for further testing should be assessed in conjunction with law enforcement agencies. Orthopoxvirus-negative results should be reported to other groups investigating the specimens as soon as possible.

If testing results suggest a non Variola orthopoxvirus, refer to CDC for confirmatory testing.

If an orthopoxvirus is identified and there is a possibility that it may be variola, refer immediately to CDC and to the nearest reference LRN laboratory with variola testing capacity for confirmatory testing.

Details for performance and interpretation of each assay are specified in each LRN procedure.
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As noted previously, testing of environmental samples, in the absence of suspicion of clinical disease, raises concerns regarding the predictive value of apparently positive laboratory results. Special care must also be exercised to exclude the possibility that a negative orthopoxvirus DNA result, originating from a poorly characterized environmental sample, is the result of the presence of a test inhibitor. Quality Assurance & Quality Control (QA/QC) criteria for heterogeneous environmental samples can be complex and may not account for all confounders impacting test results.

So, even though the diagnostic tests described in the orthopoxvirus diagnostic algorithm are potentially useful for identification of virus in environmental substances, significant differences exist in how test results must be evaluated. Persons requesting such services from public health laboratories need to be aware that reasonable expectations for interpretation of test results will typically be much better for clinical samples collected from persons with a suspicion of infection than from most environmental samples.

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All clinical samples and environmental samples should be transported to the laboratory ASAP to ensure reliable test results. Primary containers should be properly packaged and transported at appropriate temperatures, depending on the specimen type. Specific US and international packaging and shipping regulations apply when transporting infectious materials. Most commercial shippers as well as the regulatory groups have explicit instructions on specific procedures for shipping materials at room temperature, 4 C, or -70 C. More information on packaging and shipping is provided in Unit 2 of this Module.
Module 4 Unit 2: Packaging and Shipping

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Unit 2 of this Module will discuss requirements for packing and shipping clinical specimens and other samples from sources with suspected poxvirus infection.

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It is critical to submit uniform patient information along with the samples. This information ensures that the specimen can be processed expeditiously and that the appropriate individuals will be notified when diagnostic results are obtained.

Patient and specimen data include:

- Demographic information;
- Clinical history such as the date of fever and rash onset;
- Types and quantities of lesions;
- Varicella (chickenpox) exposure and vaccination history along with a possible history of smallpox vaccination; and
- Specimen information.

Reporting results to health care providers and the appropriate public health infrastructure is obviously a critical feature of diagnosis. Be sure to include the name of the specimen submitter and 24/7 contact information to ensure that results can be made available in a timely manner. When necessary personnel from the Centers for Disease Control Poxvirus Program will contact the person submitting the specimen for additional information by phone.

When submitting non-human animal or environmental samples, relevant information must also be provided, using the appropriate forms as designated by local health authorities or the CDC.

If illegal or terrorist acts are suspected, chain of custody should be implemented and appropriate documentation included with all specimens.

The appropriate specimen collection forms can be obtained from the state public health laboratory or CDC. The forms MUST accompany any specimens shipped to a public health LRN laboratory or to the CDC. When sending clinical specimens to CDC for poxvirus diagnostics, please enclose an accessioning form for each individual case submitted.
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Formalin-fixed tissue and electron microscope grids must be shipped at room temperature. They should never be allowed to freeze.

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All other virus-containing material should be stored and shipped frozen on dry ice. If overnight transportation to the testing laboratory can be arranged, specimens may be shipped refrigerated instead of frozen.

Virus-containing materials include:
- The roof of the lesion;
- Scrapings of the base of the lesion;
- Microscope slide touch preps; and
- Non-formalin fixed punch biopsy.

Serum, if collected, should be shipped frozen or at 4°C. If it is not possible to separate serum from whole blood on site, ship whole blood at 4°C.

Keep all virus-containing material out of direct sunlight; the virus and DNA material are sensitive to ultraviolet light and exposure may decrease the ability to culture virus.

As stated in the diagnostic testing algorithms, specimens with high suspicion of smallpox diagnosis must be shipped directly to CDC and to LRN reference labs with smallpox testing capacity. As part of the febrile vesicular rash illness algorithm, state health departments should be in contact with CDC regarding any such specimen and its transport.

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When materials known or suspected to contain infectious substances are transported, they are regulated as hazardous materials by the United State Department of Transportation (DOT), foreign governments, and the International Civil Aviation Organization. International and domestic transport regulations for infectious substances are designed to prevent the release of these materials in transit. These regulations protect the public, workers, property, and the environment from the harmful effects that may occur from exposure to these materials. Non-compliance with these regulations can result in significant penalties and fines.
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Infectious substances may exist as cultures or patient specimens, such as body fluids, tissues and lesions. Infectious substances are Class 6, Division 6.2 hazardous materials. They must be packaged, marked, and labeled as such for transport.

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Most clinical or diagnostic specimens are now classified as Biological substances, Category B. These are infectious substances that will not cause permanent disability or fatal disease in humans when an exposure occurs. Category B specimens are shipped under the UN 3373 designation. Category B specimen shipping guidelines are generally appropriate for specimens submitted for vaccinia virus testing.

A category A infectious substance is a material known or suspected to contain an infectious agent that is transported in a form capable of causing permanent disability or life-threatening or fatal disease to otherwise healthy humans or animals when an exposure occurs.

Category A infectious substances are assigned to identification number UN 2814 for substances that can cause disease in humans, or in both humans and animals. UN 2900 designation is used for substances that cause disease in animals only.

Specimens that may contain variola or monkeypox, or are suspected to contain unknown poxviruses (such as animal specimens), must be shipped as infectious substances in accordance with applicable regulations. The CDC website, and websites of relevant regulatory authorities discussed in this unit, should be checked regularly for the most up to date information.
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Regulations for transport of biological agents are designed to protect the public and workers in the transportation chain from exposure to any agent in the package. Protection is achieved through:

- Requirements for rigorous packaging that will withstand rough handling and contain all liquid material within the package without leakage to the outside;
- Appropriate labeling of the package with the biohazard symbol and other labels to alert workers in the transportation chain that the package contains hazardous materials;
- Documentation of the hazardous contents, should such information be needed in an emergency; and
- Training all persons in the transportation chain to familiarize them with the hazardous contents so they can respond appropriately to emergencies.

It should be noted that when these requirements are met, properly packaged, even potentially dangerous pathogens can be handled quite safely without additional precautions.

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Training is an essential element in maintaining a safe regulatory regime. All those involved in preparing or transporting dangerous goods must be properly trained to carry out these responsibilities prior to shipping. Also, if you are a shipper, the carrier relies on your ability to properly package, label, and declare goods. It is the responsibility of the shipper to receive training on the proper packaging, documentation, and shipping requirements in order to comply with the IATA and DOT regulations. This unit provides a general overview of the packaging and shipping regulations for infectious substances. It does not replace the detailed training and testing required for responsible persons.
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Shipments of category A infectious substances require Class 6.2 UN specification packaging, which consists of a primary receptacle, water-tight secondary packaging, and durable outer packaging. Class 6.2 UN specification packaging has been rigorously tested. It meets both DOT and IATA standards for packaging containing category A infectious substances assigned to UN2814 and UN2900. Those packaging materials and the required labels are available from a variety of commercial suppliers. Air carriers will not accept specimens that are improperly packaged or labeled, and fines may be imposed on shippers failing to follow these regulatory requirements.

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Shipments of category B infectious substances must be packed in triple packaging consisting of a primary receptacle, leak-proof or sift-proof secondary packaging, as appropriate, and a rigid outer packaging. Packaging for Category B infectious substances that are assigned to UN3373 must be capable of passing drop tests as outlined in 49 CFR. Class 6.2 UN specification packaging is not required, but it can be used since it exceeds the regulatory requirements for packaging category B materials. Packaging materials and required markings for category B infectious substances are also available from commercial suppliers.

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Detailed packaging instructions are provided by the various transportation authorities. IATA Dangerous Goods Packing instruction 650 applies to Biological substances, category B. IATA Dangerous Goods Packing instruction 620 applies to Infectious substances affecting humans and Infectious substances affecting animals. These instructions are available at the IATA website.
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Triple packaging is required for Category A and B substances. The packaging must consist of three components:

- The primary receptacle(s);
- Secondary packaging; and
- Rigid outer packaging.

Primary receptacles must be packed in secondary containers in such a way that, under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

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The primary receptacle contains the specimen or the potentially infectious substance, such as a microscope slide holder, plastic specimen tubes with lesions or swabs, or EM grids. This receptacle must be sealed and, as an extra precaution, adhesive tape or other appropriate sealing material must be added to ensure that leakage does not occur. The entire contents of the primary receptacle is considered potentially infectious. If there are multiple primary receptacles, they must be separated or individually wrapped to prevent breakage. Liquid specimens should have enough absorbent material outside the primary container to absorb the entire contents if the primary receptacles were to break.

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After the primary receptacles are sealed and wrapped with absorbant material, place them in the secondary container to protect them against damage and leakage. Add enough filler so that the primary receptacles fit snugly into the secondary container. If the bottom of the primary receptacle isn’t wrapped, put padding in the bottom of the secondary container. If necessary, also add padding to the top to absorb any shock to the outer shipping container during transit.
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The secondary packaging is placed into a rigid outer container. The outer container must be strong enough to withstand the packaging testing as outlined in the regulations for the appropriate infectious material. The manufacturer will affix a Class 6.2 UN specification mark to the outer packaging that is manufactured to UN specifications. An itemized list of contents must be enclosed between the secondary and outer containers. The maximum quantity of Category A infectious material that can be shipped via cargo aircraft in one package is 4L or 4kg. The maximum quantity of Category A infectious material that can be shipped via passenger aircraft is 50mL or 50g. The maximum quantity for Category B materials is 4L or 4kg for both passenger and cargo aircraft.

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Packages containing Category A agents require specific hazard labels and markings. Be sure the following are affixed to the outer package:

- The UN certification marking that is affixed by the manufacturer;
- Orientation arrows affixed to two opposite sides of package;
- Infectious Substance or Class 6.2 hazard label. Adjacent to this add “Infectious substance, affecting humans” and UN2814; and
- A Cargo Only label for contents over 50mL or 50g.

For transport by an IATA-member airline, add the name and 24-hour telephone number of a responsible person as an emergency contact, and affix the air waybill and other required documents to the package per the carrier’s instructions.

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Category B packages must have a UN3373 marking affixed to one surface. Include the proper shipping name, “biological substance, category B”. The name and phone number of a responsible person must be placed on the outer packaging of materials transported by motor vehicle or US mail. For air transport, the name and phone number can be listed on an air waybill that accompanies the package. Air waybills are not required for the air transport of category B infectious substances. However, when used, they must be completed according to the IATA Dangerous Goods Regulations and any requirements of your air carrier. Additionally, the Class 9 label and net quantity must be included if packaged in dry ice.
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If dry ice is used as a refrigerant for packages shipped by IATA-member airlines, follow IATA Dangerous Goods Packing Instruction 954. This requires that the packaging be designed and constructed to permit the release of carbon dioxide gas, so as to prevent a build up of pressure that could rupture the packaging. In practice, the dry ice is often placed inside a Styrofoam-lined box, which holds a class 6.2 UN specification packaging containing an infectious substance. When packed as just described, the Styrofoam-lined box is referred to as an overpack. When using an overpack, be sure to completely and appropriately mark and label the inside UN specification packaging. Transfer the infectious substance hazard markings and labels to the outside of the overpack. Add the text Overpack to the outside. Also add the Class 9 hazard label. Adjacent to the hazard label, place the proper shipping name and ID number for dry ice, which are *dry ice* and UN8145, respectively, and the weight of the dry ice used as the refrigerant.

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Specific packaging instructions for dry ice are provided by the US Department of Transportation, United States Postal Service and IATA.

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All couriers and air carriers should be reputable and experienced in transporting material of this nature. If relevant, ensure that the operators are able to carry packages containing dry ice. Packages for air transport of infectious materials must be consigned as cargo – samples are not permitted on aircraft in hand luggage or checked baggage or carried on the person.
Slide 23 of 24:
This unit provided a general overview of packaging and shipping requirements to ensure safe transport of specimens and other samples intended for poxvirus testing. The regulations may change, so the websites of the relevant regulatory agencies should be reviewed regularly. Shippers are required to maintain staff trained in proper packaging and shipping procedures.

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Additional information about shipping infectious substances is available at the websites of the regulatory agencies.

The Department of Transportation. 49 CFR Part 171-180, Hazardous Materials Regulations, applies to the shipment of infectious substances in commercial transportation into, out of, within, and through the United States.

Technical Instructions for the Safe Transport of Dangerous Goods by Air provided by the International Civil Aviation Organization (ICAO). These instructions apply to the shipment of infectious substances by air and are recognized in the DOT and other regulatory agencies as a standard for air transport.

Dangerous Goods Regulations are issued by the International Air Transport Association (IATA). These regulations are based on the ICAO Technical Instructions, and must be followed by IATA-member airlines, including FedEx and UPS.

IATA has also developed an abbreviated guidance document to assist shippers with the classification and proper packaging of patient specimens and cultures. It is available at the IATA website.
Module 4 Unit 3: Chain of Custody

Slide 2 of 11: This unit will introduce you to the concept of chain of custody for specimens and samples collected when bioterrorism is suspected. Proper chain of custody procedures for documenting the collection, possession, or custody of samples submitted for laboratory testing will be discussed.

Slide 3 of 11: What exactly is chain of custody? In simple terms, it is the written documentation of the collection, transfer, receipt, analysis, and final disposition of the samples collected.

More specifically, the chain of custody is the methodology used to track and maintain control and accountability of all evidentiary items. This includes initial collection of the evidence through the final disposition of the specimens. Both law enforcement and public health personnel must provide accountability at each stage of collecting, handling, testing, storing, and transporting the evidentiary items, and reporting any test results. Failure to properly maintain the chain of custody may prevent the evidence in question from being introduced at trial.

Slide 4 of 11: When illegal activities or an act of terrorism is suspected, the importance of chain of custody cannot be minimized. It is imperative to keep accurate written records that show who had access to the samples at all stages from collection to the final disposition. The only way to be sure samples have not been tampered with is by ensuring their integrity. Legally defensible results can only be assured if a proper, written, chain of custody record is kept. Of course, the use of proper analytical methods and techniques is also important. A proper, written, chain of custody procedure should be followed for all samples when the results may become part of litigation.

Slide 5 of 11: The chain of custody process includes all of the physical controls used to secure and limit access to the evidence, including laboratory samples, as well the documentation of the transfer and storage of the evidence and laboratory samples. Chain of custody must account for the whereabouts and security of evidence at all times.
Slide 6 of 11:
This is a graphic representation of the steps that a sample goes through when it is sent to the laboratory. Each step is a link in the chain of custody, from the time a sample is collected all the way to its final disposition. The chain of custody starts at the very beginning when a sample is collected. The individuals responsible for collecting the specimen are responsible for filling out the chain of custody form, and ensuring security of the sample while it is held in the field. The chain of custody form moves with the specimen through its entire life cycle. All transfers of custody of the sample must be documented on the form.

The sample is then transported to the laboratory. If bioterrorism is suspected, the sample will be transported by law enforcement. If a patient presents to a health care facility with symptoms associated with an agent of bioterrorism, such as smallpox, the chain of custody for the specimen should begin ASAP in the emergency department or patient care room.

The transfer of every specimen to the designated laboratorian should be documented on the chain of custody form, and the specimen should be kept in a lockable area to prevent potential tampering. Any transfers from person to person required for specimen processing must be documented on the chain of custody form. After the sample is analyzed, the results are reported out. Final disposition of the sample takes place at the appropriate time, in consultation with law enforcement and public health officials. The sample may be destroyed, saved, or sent to another laboratory for further testing. Final disposition is also documented on the chain of custody form.

It is very important to document on the chain of custody form all transfers of the sample from person to person. Additionally it is best to limit the number of people who have access to the sample. This will help to reduce the chances of sample tampering or contamination.

Slide 7 of 11:
Clinical samples may be received and processed by the laboratory before there is any suspicion that an agent of bioterrorism may be involved. The standard practices and documentation for ensuring the integrity of clinical samples can be used for chain of custody. Healthcare providers routinely document the collection of samples in the patient chart. Couriers document transfer of specimens, and the laboratory routinely logs in the receipt of all specimens.
Slide 8 of 11:
Specimen information such as the date and time of collection, date and time of processing, and persons receiving or processing the sample may be captured and stored using electronic Laboratory Information Management Systems. This tracking information can also be used to support chain of custody.

Electronic data must be secure and archived for long-term accessibility.

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The custodian of the samples is responsible for the security of samples. The custodian ensures that specimens are properly packaged, using tamper detection seals when possible; provides a secure area for holding the specimens; and maintains the chain of custody form and logs of all specimen handling. Since all information is discoverable in court, the custodian must be able to testify to the whereabouts and security of the samples at all times. The laboratory should assign a custodian to the samples immediately upon receipt.

Slide 10 of 11:
This is an example of a generic chain of custody form. The top part identifies the type of sample, who collected it, and other information identifying the sample. The middle section documents the person-to-person transfer of the sample. For example, the person who delivers the sample to the laboratory signs in the “released by” section and the laboratorian who takes custody of it signs in the “received by” section. The bottom half of the form identifies and documents the final disposition of the sample. Careful documentation throughout this process is very important. Other approved forms may be provided by local law enforcement, FBI, or Hazardous Material response personnel or the LRN laboratory.

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In conclusion, the chain of custody documents all the steps in the transport and handling of a sample. It extends from the initial collection, through the laboratory for analysis to its final disposition. It is important to follow the chain of custody procedures, especially the careful documentation on the chain of custody form. Properly followed procedures, along with this written documentation, help to ensure that the results from the sample analysis will be legally defensible in court, if needed.