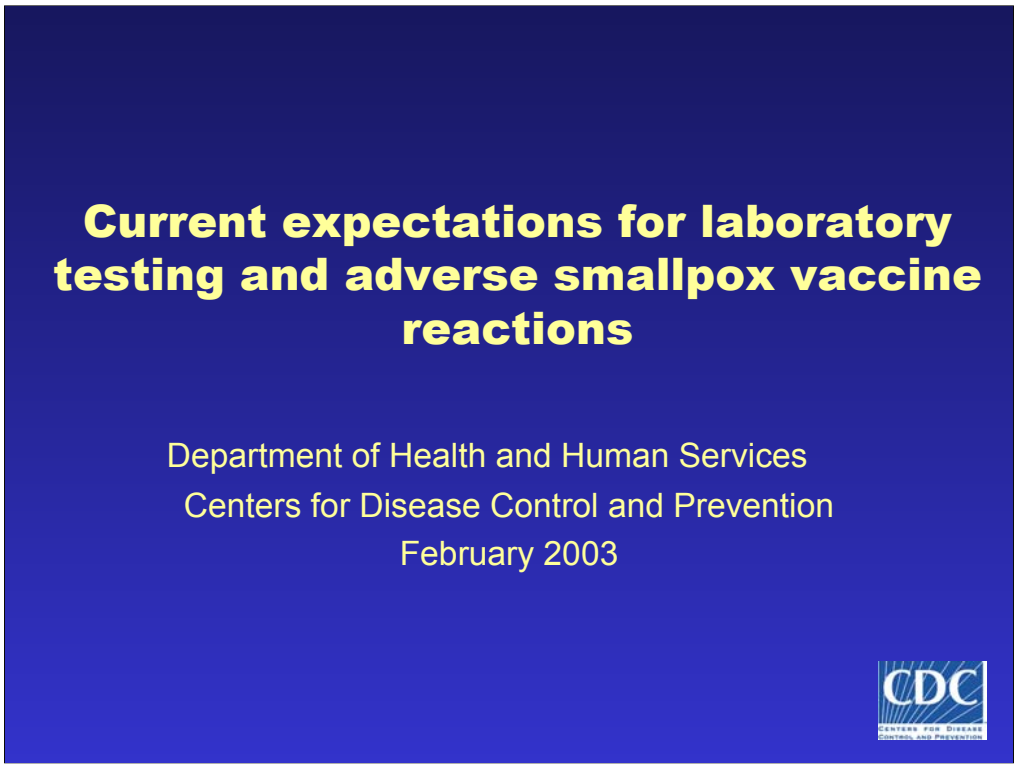


Drs. Rotz and Lane have presented a comprehensive range of clinical issues associated with smallpox vaccination.

Today, I will be discussing the use of laboratory tests, primarily targeting poxviruses, to evaluate adverse events which may be associated with vaccination with vaccinia vaccine, and will also discuss laboratory diagnostic issues in differentiating vaccinia virus from variola virus, the causative agent of smallpox.

Poxviruses

- Two Subfamilies:
  - *Chordopoxvirinae* (vertebrate poxviruses)
    - Orthopoxvirus (variola, vaccinia, cowpox, monkeypox, raccoonpox, camelpox, skunkpox, volepox, ectromelia, taterapox)
    - Others
  - *Entomopoxvirinae* (insect poxviruses)

As background there are a large number of poxviruses currently recognized to exist in nature including poxviruses in both vertebrates and arthropods.

Today we're only concerned with those that belong to the closely related orthopoxviruses, especially variola, the agent of smallpox, and vaccinia, the virus that's used for smallpox vaccination.

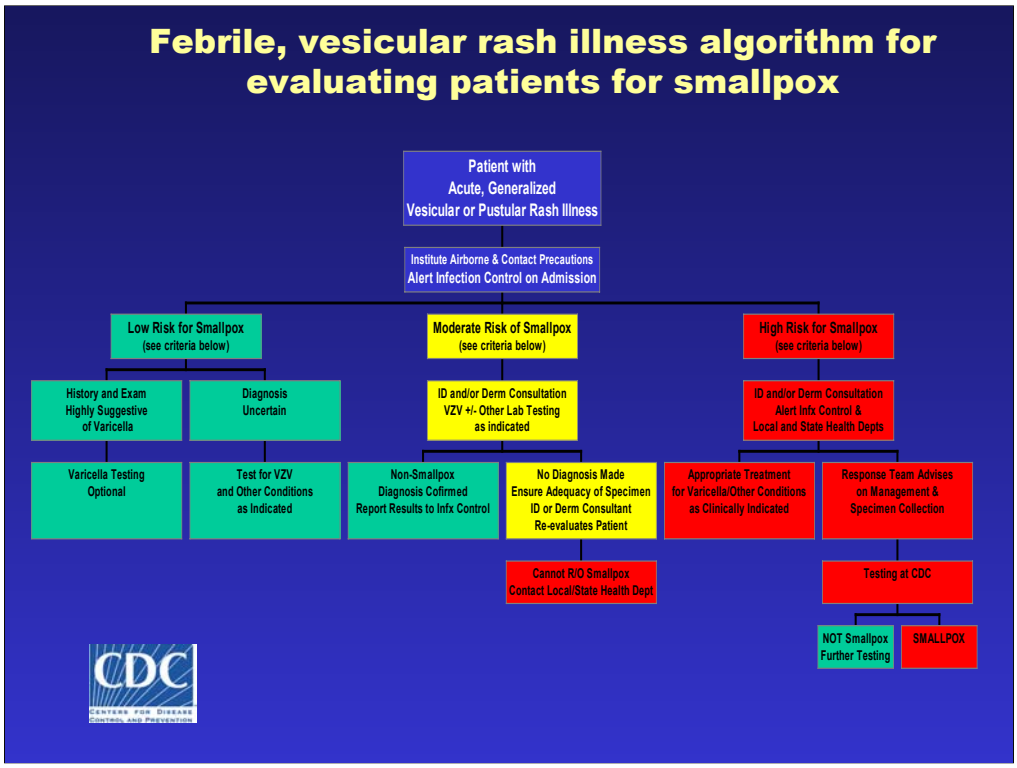
Characteristics of Orthopoxviruses

- Host ranges vary
  - Variola vs vaccinia
- Antigenically similar; serologic cross reactivity

One of the interesting features of orthopoxviruses is their varied host range. Variola only infects humans, whereas other closely related viruses have wider host ranges, such as vaccinia.

The reasons for these differences are not completely understood. Importantly these viruses are genetically and antigenically very similar and provide cross protective immunity from infection.

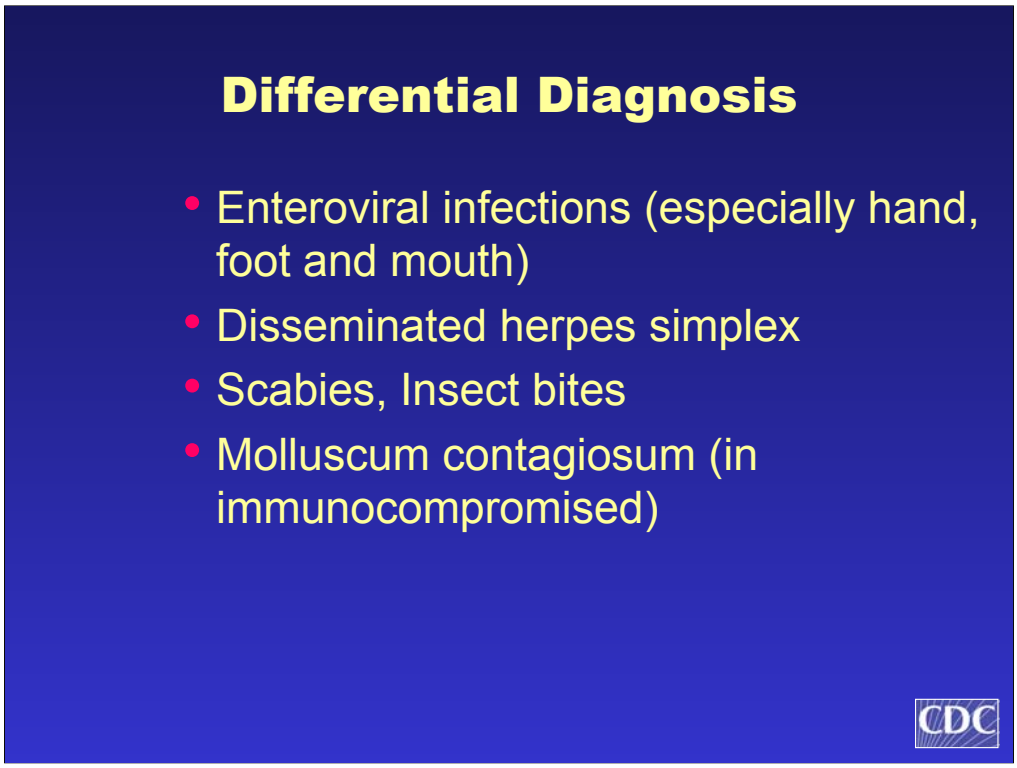
Hence the basis for vaccination with vaccinia to prevent smallpox..



The febrile vesicular rash algorithm, reviewed in other broadcasts, provides a framework to think about the need for testing for orthopoxviruses in clinical samples. The algorithm was specifically designed to discriminate infection with variola virus (smallpox) from other illnesses (bacterial, viral, immune mediated) which may manifest with similar vesicular pustular rash.

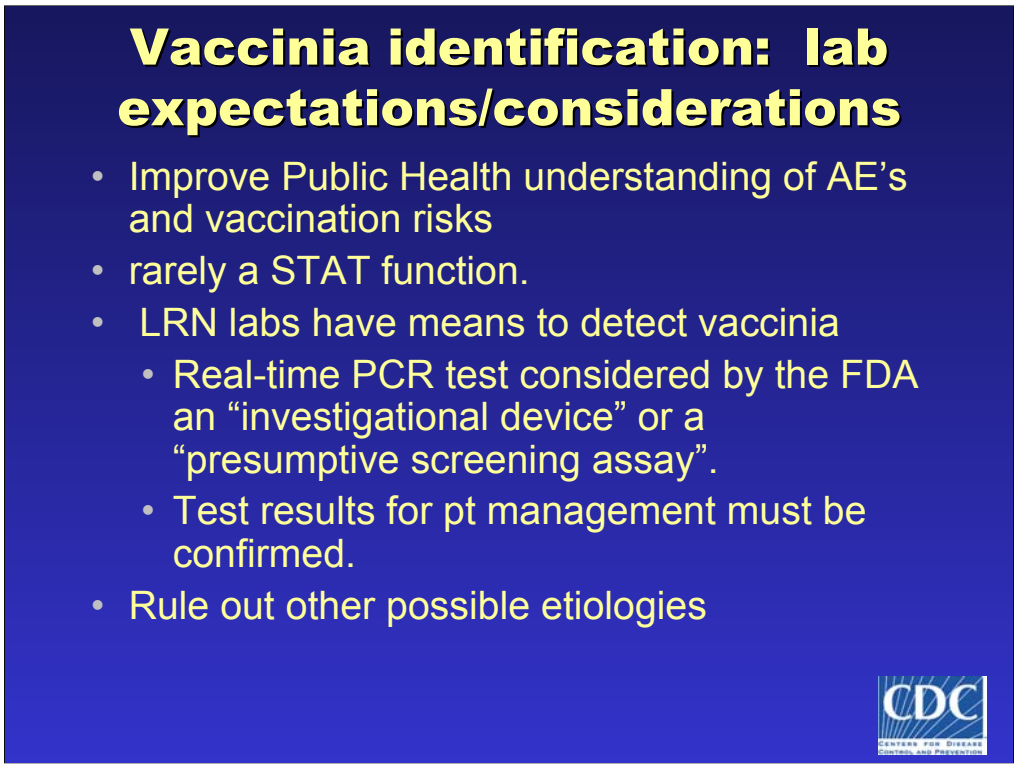
Certain vaccinia (smallpox vaccine) related adverse events could fall into this algorithm. An important clinical clue will be the presence of recent vaccination, or contact with an individual who has been vaccinated. Clinical correlation of differential diagnosis, with appropriate laboratory testing will be critical for public health understanding and evaluation of adverse events associated with vaccination, as well as differentiation of smallpox from other diseases which may be clinically confused with smallpox. In addition, this will indicate the need for appropriate specimen sampling and collection.

The communication between the clinical and epidemiology staff personnel, with the laboratory, will be critical in quickly performing appropriate laboratory testing to confirm the etiology of such rashes.



As discussed earlier by Dr. Rotz, generalized rashes can be associated with vaccinia (smallpox) vaccination. These are some of the most common, and usually benign side effects associated with vaccinia (smallpox) vaccination. Both clinical and laboratory approaches may be needed to differentiate the causes of these rashes.

Especially in those cases where a contact of a vaccinee develops a rash, alternative etiologies, such as chickenpox should be considered.



The confirmation of the various etiologies of adverse events associated with vaccinia (smallpox) vaccination, including those rashes which are ultimately determined not to be related to the vaccine (such as chickenpox, herpesvirus infections, etc.) will be important for our public health understanding of adverse events and vaccination risks.

In most cases this will not be a "STAT" function, unless potential therapeutic options for other causes are being considered.

Specifically, if a case of encephalitis is associated with vaccination, it will be important to rule out (or in) other treatable causes such as herpes encephalitis. Historically, post vaccinial encephalitis was a diagnosis of exclusion.

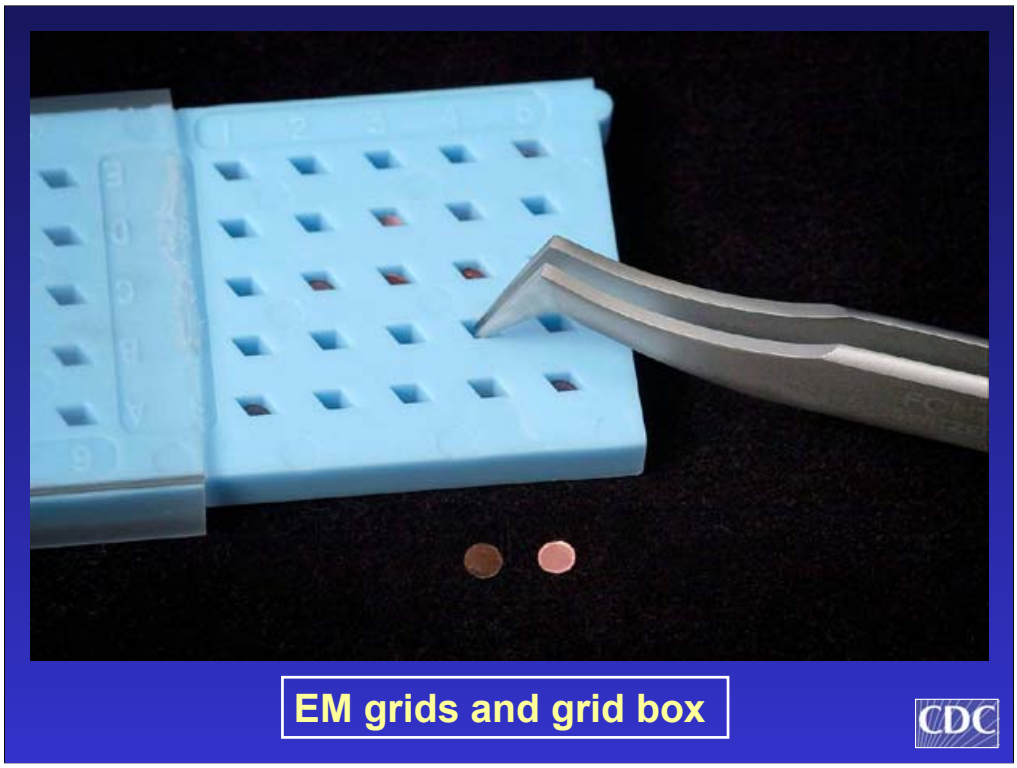
Currently, the Laboratory Response Network labs have a test to detect vaccinia within clinical specimens. The test, and the nature of the Laboratory Response Network will be reviewed shortly. The assay is currently considered investigational pending FDA review. If the test is to be used to affect patient management, results should be confirmed with additional tests. Currently the CDC can support confirmatory testing.

Specimen collection

- Specimen collection for skin lesion specimens associated with vaccination, of high suspicion for vaccinia
  - http://www.bt.cdc.gov/agent/smallpox/vaccination/vaccinia-specimen-collection.asp
- Specimen collection for suspect smallpox specimens (similar)
  - www.bt.cdc.gov/agent/smallpox/response-plan/files/guide-d.pdf
  - http://

To collect specimens when vaccinia is suspected, please review the website listed here. A similar set of guidelines is also available for collection of specimens from a patient suspected to have smallpox.

These are examples of materials useful for collection of specimens for laboratory testing including the plastic vials in which crusts would be shipped to the LRN site for analysis.



Another component of specimen collection would be electron microscope grids and forceps. Pictured here is a close-up of the EM grids and the box, demonstrating shiny and dull sides of the grid.

Specimen collection

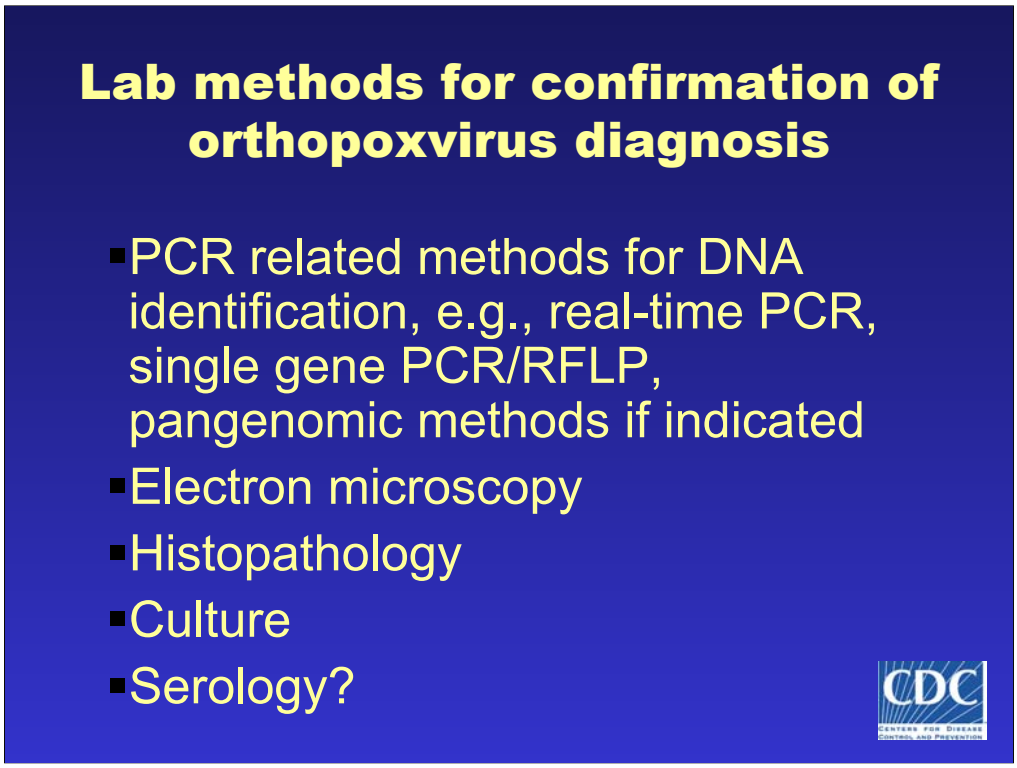
Detailed description, with pictures on webcast of December 5 and 6, 2002

To review:

http://www.bt.cdc.gov/agent/smallpox/training/webcast/dec2002/index.asp

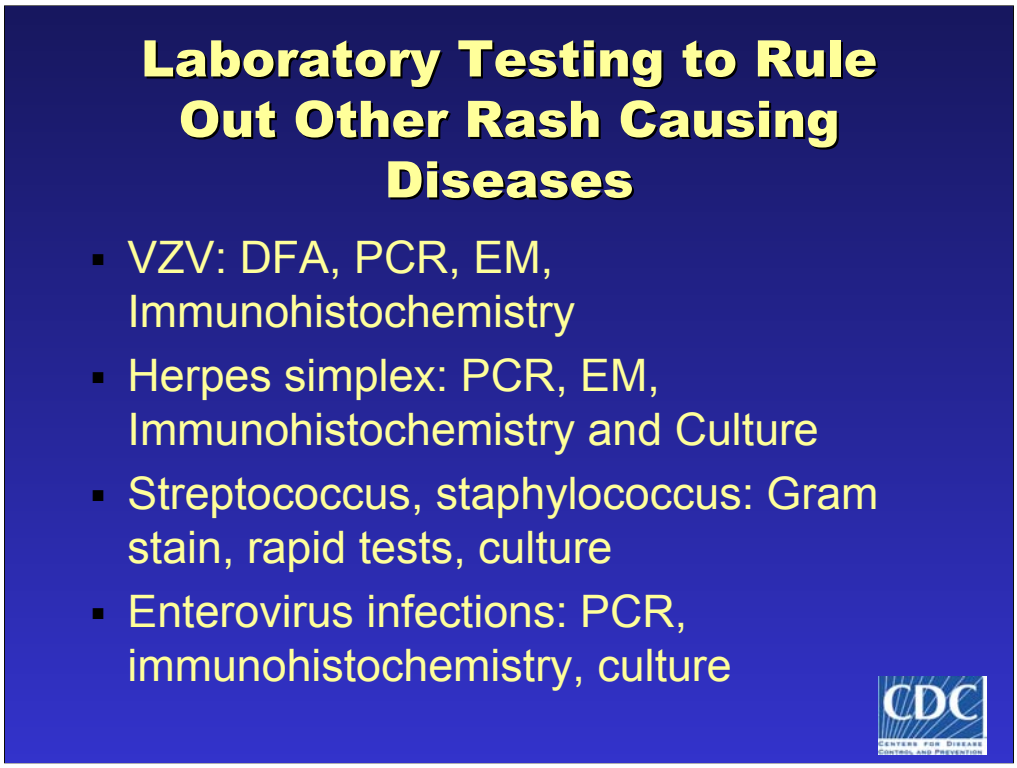
Go to: Smallpox Vaccination Laboratory Support module

Specific and detailed instructions on specimen collection, accompanied with pictures, were reviewed in the December 5 and 6 webcast. The link to that webcast is provided here, and should be reviewed if the viewer is unfamiliar with its content.



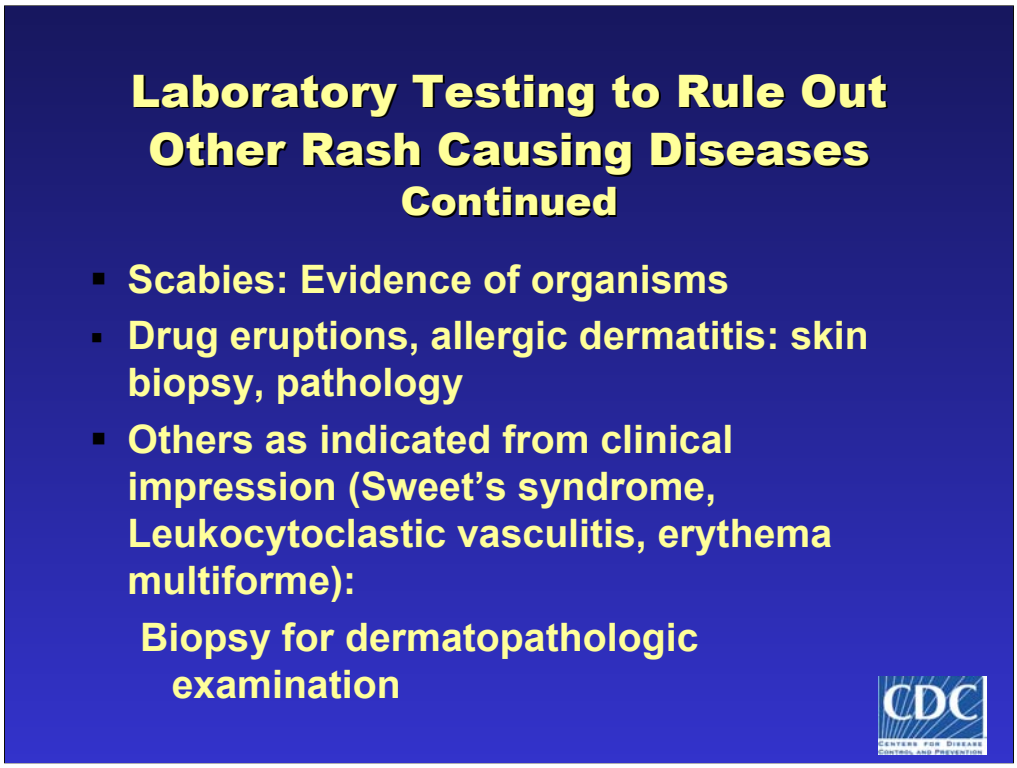
These are the methods currently used at the CDC to confirm and to identify an orthopoxvirus infection, whether vaccinia, monkeypox, variola (smallpox).

Serologic methods may help to evaluate the extent of an immune response but will not be helpful to determine if replicative vaccinia is the cause of an vaccine associated adverse event.

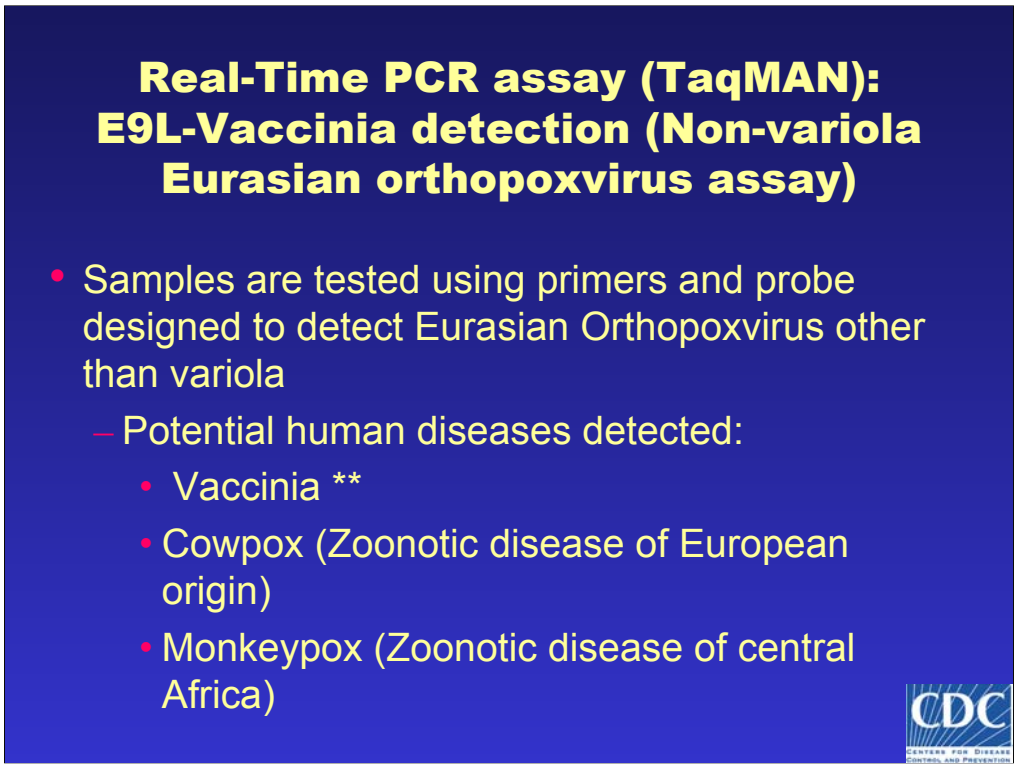


Lab tests should be used, as clinically indicated, to evaluate other causes of adverse events associated with vaccination. Some approaches to laboratory testing are listed in this and the following slide.

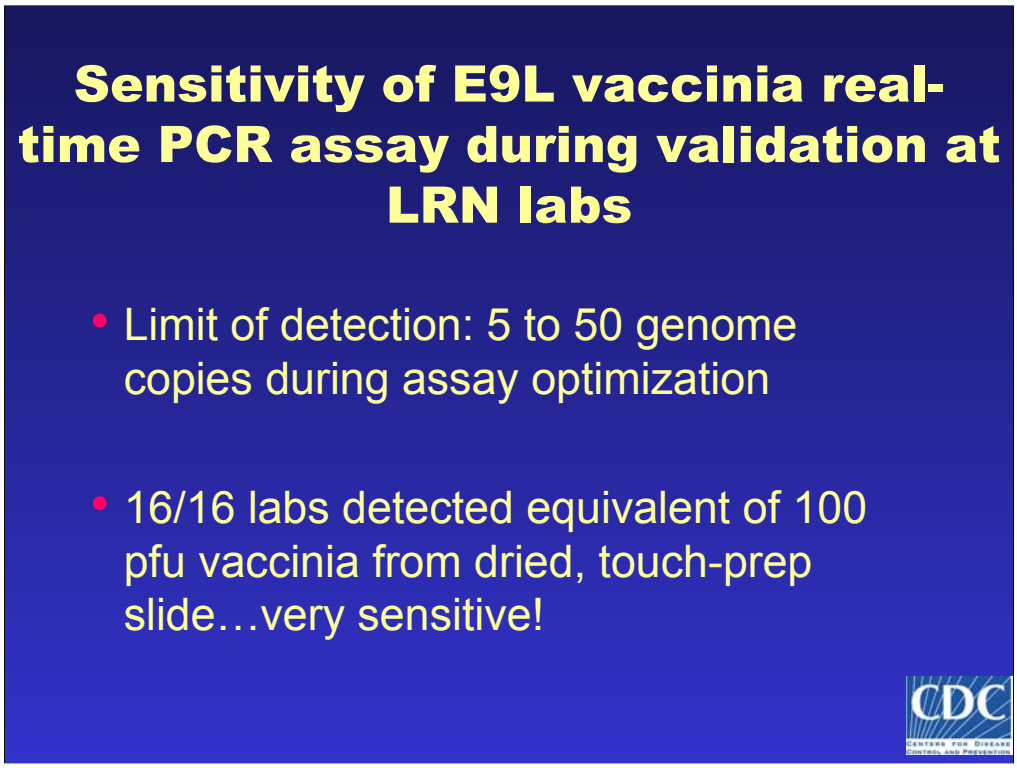
The detection and diagnosis of herpesviruses is discussed in depth in the course "Smallpox and Vaccinia Laboratory Testing" found on our website. Standard methods can be used to evaluate specimens for the presence of bacteria or enteroviruses.



Note that in many cases, a biopsy, for dermatopathologic examination will be an important diagnostic to evaluate the causes of adverse events associated with vaccination.

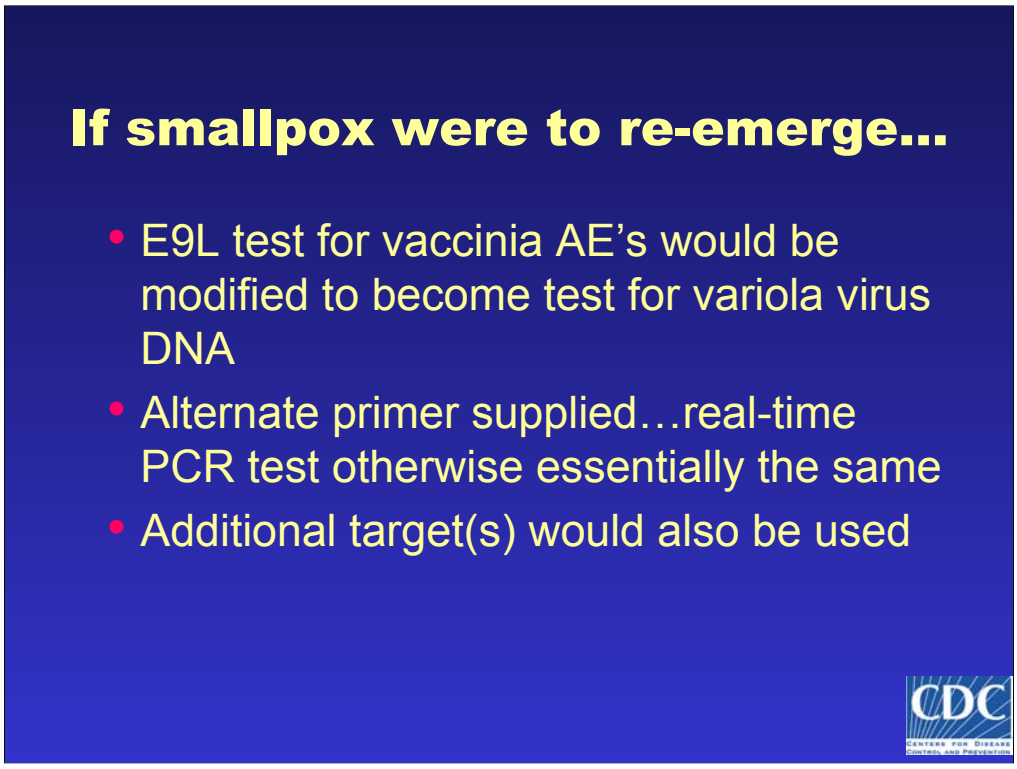


The real-time PCR assay that is currently available at LRN laboratories for analysis of vaccinia adverse reactions detects the presence of the DNA polymerase gene of vaccinia virus, the so-called E9L gene. This assay will also detect cowpox virus and monkeypox viruses, two viruses that do not occur naturally in North America. From a North American diagnostic screening perspective, this assay can be considered diagnostic for vaccinia infections.

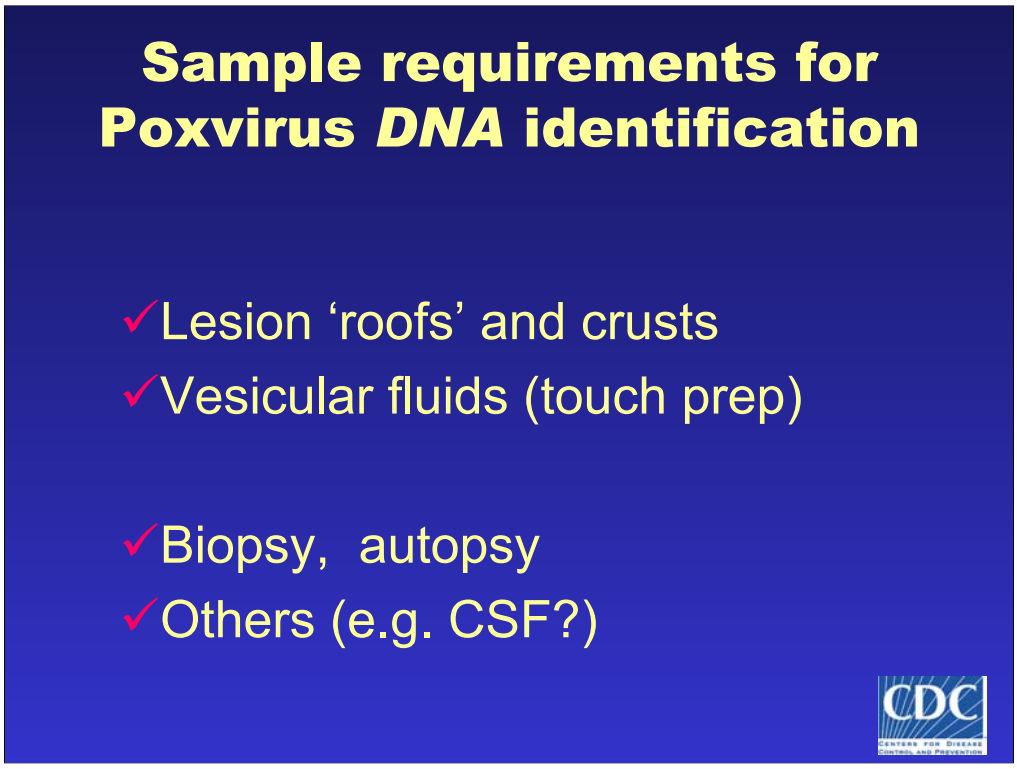


After assay optimization between 5 to 50 copies of vaccinia could be detected on various TaqMAN real-time PCR platforms. The assay has been extensively tested against variola and does not detect any of the 45 variola strains it was tested against.

Thus a positive result is indicative of the presence of an orthopoxvirus other than variola, most likely vaccinia in the U.S. When evaluated outside of the CDC, the assay and its operators were able to reproducibly detect 100 infectious particles of vaccinia in a simulated clinical specimen. Thus, the assay appears quite robust.



An almost identical assay will also detect the DNA of variola virus and therefore can be used for the diagnosis of smallpox. Both assays incorporate internal controls and a wide variety of similar assays, targeting additional DNA signatures, are currently being evaluated and readied for deployment. This is very much an ongoing process.

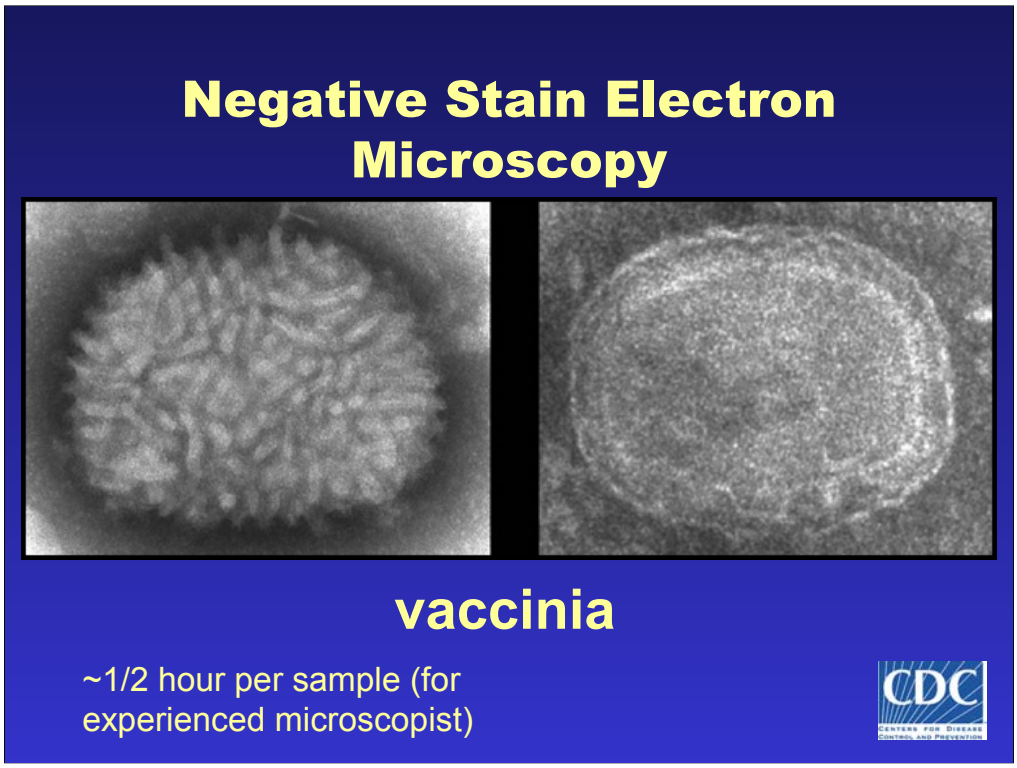


The best specimens for many of the orthopox laboratory tests are the "roofs" or crusts from the lesions which contain large amounts of orthopoxvirus material. Vesicular fluids from the lesions are also convenient sources of diagnostic material.

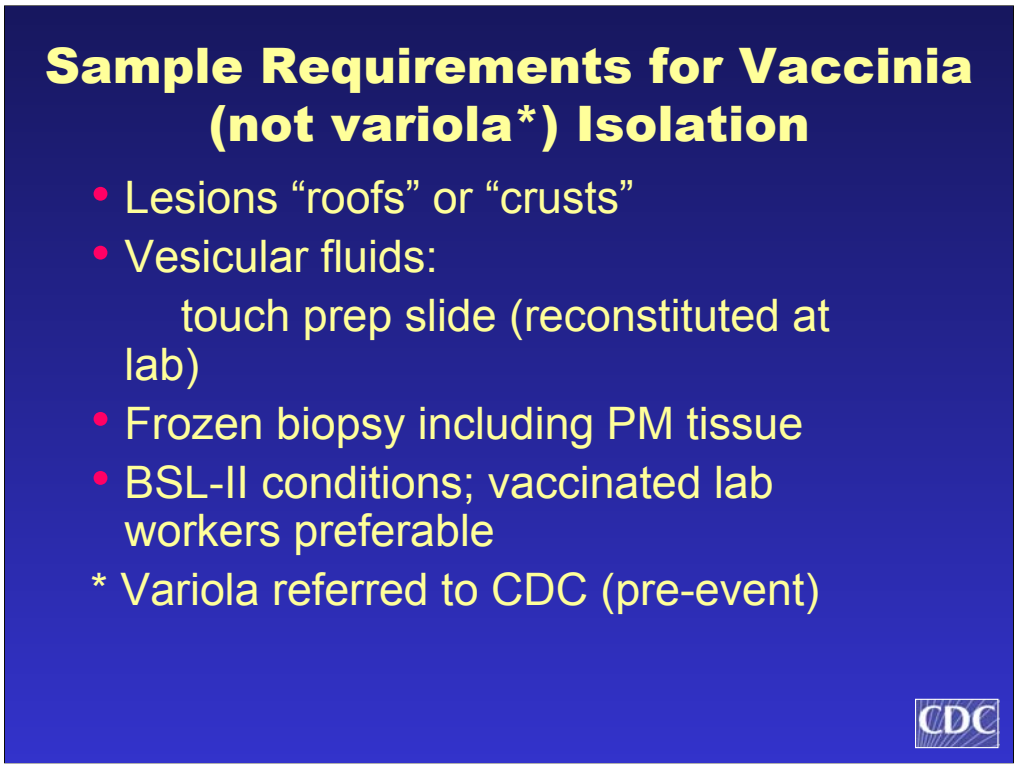
Vesicular fluids are also good starting materials for electron microscopy. Whichever tests are considered for diagnosis, multiple lesions should be sampled for both roof of lesions and vesicular fluids from the lesions since not all lesion specimens are easy to identify. Biopsy material can be used for viral identification.

For non-dermatologic adverse event evaluation, appropriate material should be taken to evaluate for potential other etiologic causes of the adverse event, such as CSF for hepresvirus testing in suspect post vaccinial encephalitis. In addition, these materials can be tested for the presence of vaccinia.

For electron microscopy, both lesion roofs, scab or crust material, or vesicular fluid can be used.

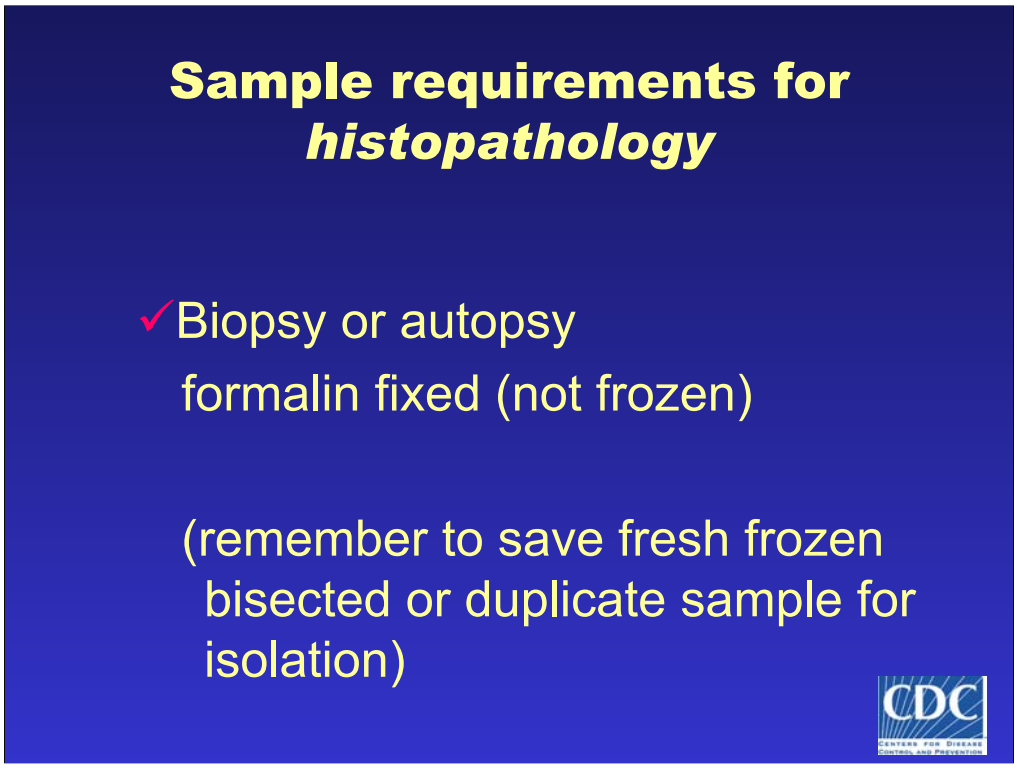


This is an example of a negatively stained electron micrographic preparation of vaccinia virus. The EM process may be very quick for a skilled observer and can be used to differentiate generic orthopoxviruses from other groups of viral agents. However, it cannot differentiate between variola and vaccinia.

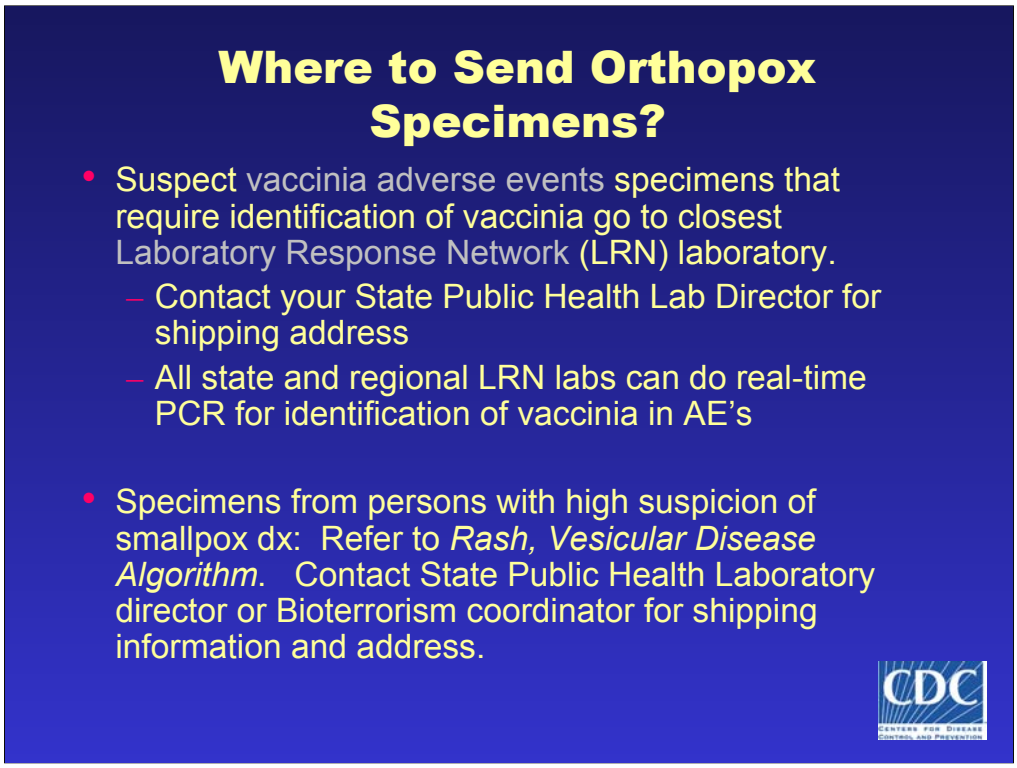


The same specimens can be processed for viral propogation in tissue culture. The use of tissue culture to propogate the virus can also be valuable to facilitate more detailed analysis of specimens, in addition to demonstrating the infectious organism.

For vaccinia isolation, the use of BSL-2 conditions, with vaccinated personnel is sufficient biosafety. If a specimen is suspected to contain variola, the state health department should be contacted, who will contact CDC.



Collection of biopsies can be done with local anesthetic if histopathologic exam is considered. It is especially important for successful diagnosis of several of the orthopox look-alike syndromes.



Specimens from individuals with suspect vaccinia adverse events, requiring identification of vaccinia, should go to the closest laboratory response network laboratory. Your state public health lab director should be contacted. For specimens with a high suspicion of smallpox contact your state public health department, who will contact the CDC.

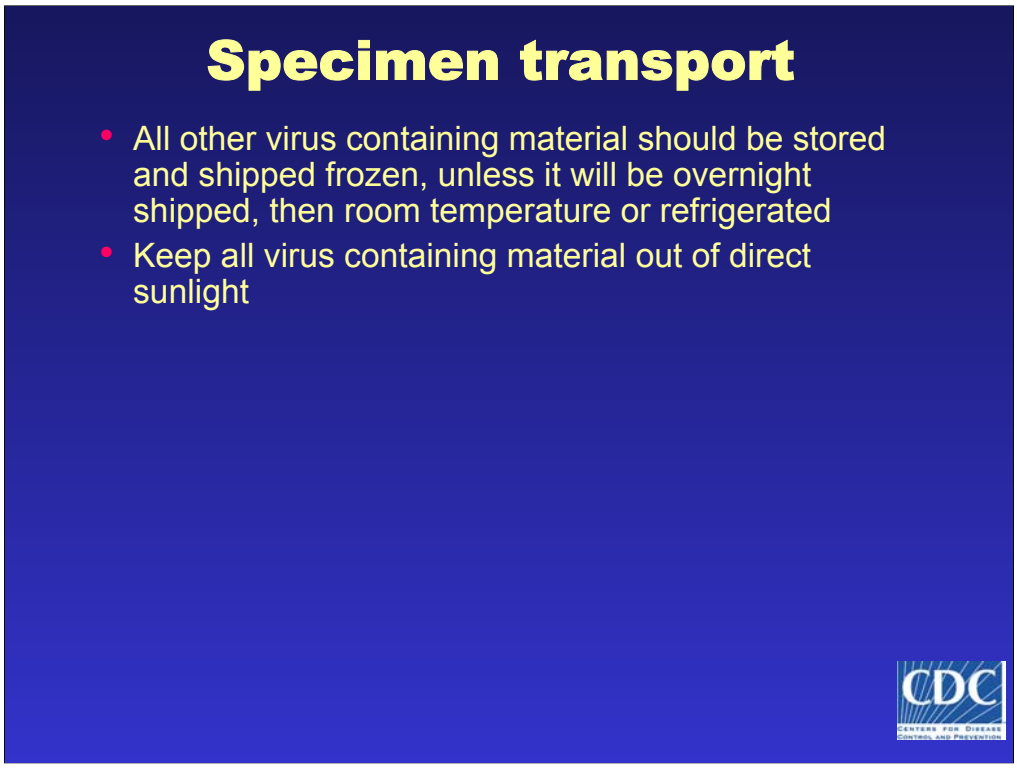
Specimen transport

- Standard dx specimen shipping guidelines available (subject to change): http://www.bt.cdc.gov/labissues/PackagingInfo.pdf
- Serum, if collected, should be refrigerated and shipped
  - If spun and separated on site, freeze
- Formalin fixed material should be shipped at room temperature DO NOT FREEZE
- EM grids should be shipped at room temperature

For vaccinia testing, standard shipping guidelines are appropriate. Standard diagnostic specimen shipping guidelines are available at the website listed on the slide here (www.bt.cdc.gov/labissues/packaginginfo.pdf). Serum, if collected, should be shipped frozen. If it is not possible to separate the serum from the blood on site, you can send the whole blood, but refrigerate it.

Formalin-fixed tissue must be shipped at room temperature. It is not to be frozen. Electron microscopic grids must be shipped at room temperature

All other virus-containing material must be stored and shipped frozen. Transportation overnight can be arranged and in that case refrigeration is not necessarily required. Keep all virus-containing material out of direct sunlight..



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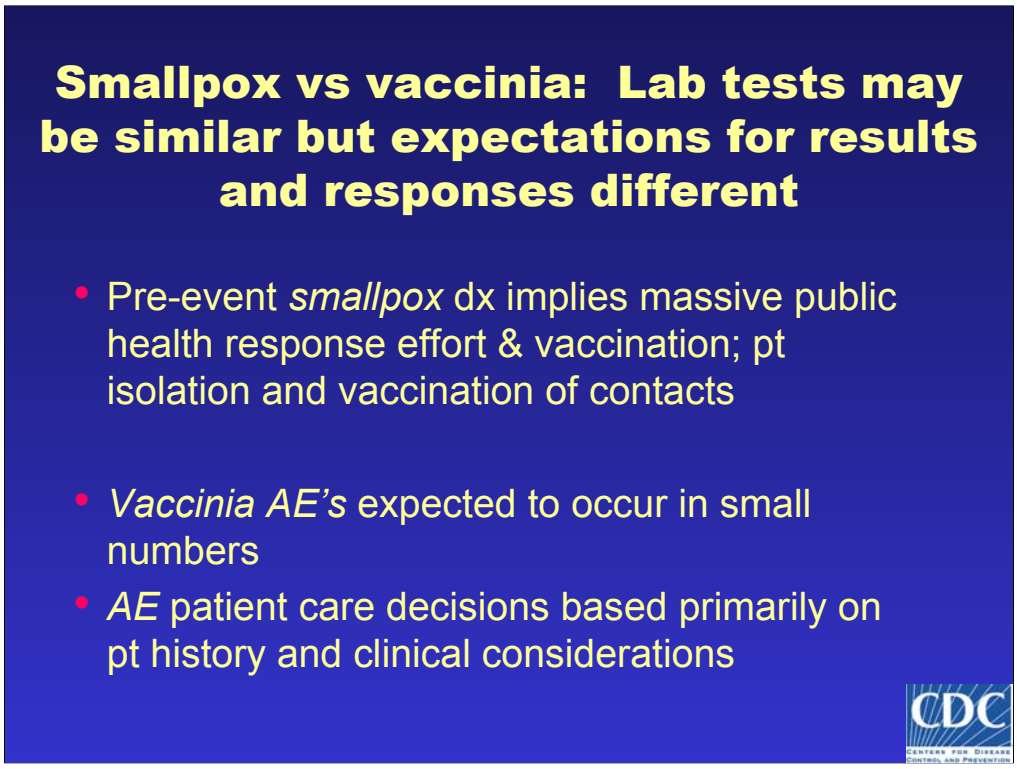

The types of tests used for vaccinia and variola detection are very similar, thus a lab that is proficient in vaccinia diagnostics will likely be capable, with appropriate biosafety conditions, of variola diagnostics.

For More Information

- CDC Smallpox website
  www.cdc.gov/smallpox