CDC National VZV Laboratory Services

National Center for Immunization an Respiratory Diseases

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CDC National VZV Laboratory

The National VZV Laboratory was established in May 1998. It currently operates within the National Center for Immunizations and Respiratory Diseases, Division of Viral and Diseases. This laboratory supports surveillance efforts toward monitoring the impact of varicella vaccination in the U.S. population, including outbreak investigations, confirmation of disease, vaccine adverse events, duration of immunity, breakthrough infections with wild-type virus, and disease susceptibility.

CDC National VZV Laboratory Services

The National VZV Laboratory can provide several types of VZV-specific testing free-of-charge to state and local public health departments who require confirmatory evidence for VZV infection or confirmation of atypical herpes zoster cases, and to physicians and scientists participating in various epidemiologic and laboratory-based studies.

Specimens may be submitted for suspected vaccine-related adverse events, including herpes zoster in a person who received varicella or zoster vaccine, a varicella-like rash with more than 50 lesions 7-42 days after vaccination, any serious adverse event (pneumonia, ataxia, encephalitis) occurring after varicella vaccination, and suspected secondary transmission.

- **IgG whole infected cell ELISA.** The version of this assay developed at the National VZV Laboratory compares very favorably with other similar assays (99.7% sensitive and 100% specific compared with the assay used by the California Department of Health Services). While it is probably not sufficiently sensitive to detect all seroconversions to vaccination, the assay reliably detects seroconversion to natural infection.
- **gpELISA.** An ELISA method developed at CDC using highly purified VZV glycoproteins obtained through a material transfer agreement with Merck and Co. This method is more sensitive than whole infected cell ELISA, reliably detects vaccine seroconversion, and compares favorably with the FAMA assay. All specimens received for serology are first tested using whole infected cell ELISA, and all specimens with negative or equivocal results are retested using gpELISA.
- **IgM capture ELISA.** We have developed a capture ELISA that reliably detects VZV-specific IgM antibody in serum. As with the IgG ELISA, this assay is routinely performed in the laboratory and is available without charge for outbreak studies, and confirmation of recent VZV infection in clinical cases of suspected VZV. Commercially available IgM assays are not reliable. False positives may occur due to interference by IgG.
- **IgG avidity.** This assay compares a result obtained using the standard ELISA protocol side-by-side with a duplicate test plate pretreated with diethyl amine, a reagent that reduces the binding potential of

antibody. Low-affinity antibodies in serum do not bind antigen in the presence of this agent. Since early antibodies produced in a response tend to have overall lower affinity for antigen (low-avidity antibody) and since these are supplanted over time with antibodies that have increased affinity for antigen (high avidity), the presence of low-avidity antibody in a serum specimen is a reliable indicator of primary infection.

- **FRET PCR assays.** We routinely perform four different realtime Forster Resonant Energy Transfer (FRET)–based PCR methods using the LightCycler platform. Each of these assays targets different vaccine-associated, single nucleotide markers in the VZV genome. All four of these methods confirm the presence of VZV DNA in a specimen. We test for markers in VZV open reading frames ORF38 and ORF54 (these markers discriminate J strains, like those from which the Oka vaccine virus was derived, from other wild-type strains) and for two vaccine strain–specific markers in ORF62. Together, these methods are used to confirm VZV infection and to robustly discriminate vaccine strain from wild-type strains.
- **VZV genotyping.** We have developed a genotyping method for VZV that involves amplifying and sequencing three short regions in VZV ORF21, ORF22, and ORF50 that reliably discriminates all seven genotypes that have been identified thus far. We have also developed a microarray DNA resequencing chip that allows us to determine 84,400 base pairs of sequence even on non-viable specimens for which limits in DNA sample size have previously been an insurmountable obstacle to large-scale VZV sequencing.

Guidelines for Collecting and Shipping Specimens.

Collecting and Shipping Blood for VZV Serologic Assays

There are two ways to prepare specimens of peripheral blood suitable for testing at CDC using VZV-specific serologic assays.

First Method: Blood spot method

1. Prick the subject's finger, using a lancet.
2. Collect a sufficient quantity of blood onto both of the defined areas on the filter strip so that the spot expands to the circular border. (Filter strips will be made available to state and local public health laboratories and to the varicella surveillance project office in your area on request.)
3. Permit the specimen to air dry (do not allow strips to come into contact with each other while wet).
4. Once the blood specimen has completely dried, place each filter strip in a separate, sealable plastic bag. **Important: Specimens must be permitted to air dry completely before placing them inside a separate, sealable plastic bag. Otherwise, bacterial or fungal growth can occur, destroying the specimen.**

Dried blood specimens should be stored at ambient temperature; there is no need to refrigerate or freeze specimens prepared in this fashion. Specimens should be mailed to the laboratory by regular postal service (unless a result is urgently required) at the earliest opportunity.

Second Method: Preparation of serum from whole blood

1. Collect whole venous peripheral blood in serum separator vacutainer tubes.
2. Permit the specimen to fully clot by standing at room temperature for at least 30 minutes.
3. After the clot has formed, tubes can be centrifuged at approximately 200 x *g* for 5 minutes.
4. The clot will have passed to the bottom of the tube and the serum fraction will be at the top, with the separator plug as a barrier between the two fractions. The serum fraction can simply be aliquoted into sterile, 0-ring seal freezing tubes using a sterile pipet.
5. Freeze serum specimens at −20°C.

Ship specimens by overnight mail on sufficient dry ice to keep them frozen for 3 days. Frozen specimens obtained for larger studies may be kept indefinitely at −20°C, accumulated, and sent in batches to CDC, depending on preference.

Collecting and Shipping Specimens for VZV PCR/Genotyping

To make a laboratory diagnosis of VZV infection using polymerase chain reaction (PCR) method, the presence of the virus DNA should be demonstrated in tissues, vesicular fluid, maculopapular lesions, or crusts from lesions. The following methods are recommended:

Polyester swab method

(Best suited to sampling vesicular lesions)

1. A sterile needle should be used to unroof the top of the vesicle.
2. A sterile swab[†] is then used to vigorously swab the base of the lesion—applying enough pressure to collect epithelial cells without causing bleeding—and collect vesicular fluid. It is important to collect infected epithelial cells from the base of the lesion because they usually contain a significant amount of virus.

   [†]We recommend swabs made from synthetic fibers, such as polyester, because it is difficult to elute virus from cotton swabs, and wooden swab supports usually absorb extraction buffer and inhibit PCR.
3. Swabs must be placed individually into separate, empty tubes to avoid contamination. Place swabs directly into tubes—**Do not place transport medium into the tube; the specimen MUST be kept dry.** Tubes must be individually labeled and must be resistant to breakage.
4. See shipping instructions below.

Glass slide method

(This method is critical for the collection of material from maculopapular lesions)

1. Rake the edge of the slide over the selected lesion, abrading the lesion with sufficient vigor to ensure that skin cells are gathered onto the slide. Use a sterile polyester swab to scrub the abraded lesion and (using the same swab) collect the material collected on the edge of the slide. Note: with young children, it may be less stressful if you ask them to help with this. If more than one lesion is sampled, a separate swab should be used for each one.
2. Insert the swab into a tube and close it (many swabs are provided with a tube that includes a label for marking the specimen).
3. Ship in a padded envelope. The swab for each sampled lesion must be placed in a separate swab tube, but multiple tubes can be shipped in the same envelope. Dry maculopapular lesion material is stable for several weeks at ambient temperature.
4. See additional shipping instructions below.

Collecting crusts (scabs)

Crusts are also excellent samples for PCR detection of VZV DNA. Crusts can be lifted off the skin (a glass slide is useful for this purpose) and transferred directly into break-resistant, snap-cap or screw-top tubes. See shipping instructions below.

Collecting other specimen types

For some disease presentations with a suspected VZV etiology (e.g., meningitis, multifocal organ damage), samples of cerebral spinal fluid (CSF), blood, or biopsy tissue may also be shipped. Blood and CSF can be shipped on cold packs or frozen. Biopsy tissue is preferred shipped frozen and, if available, unfixed. See additional shipping instructions below.

Handling and shipment

Dried specimens for PCR can be stored at ambient temperature indefinitely, although we prefer to receive specimens as soon after collection as possible. Do not refrigerate or freeze dry specimens intended for testing by PCR. Specimens can be mailed by regular post unless a result is urgently required. **Do not suspend specimens in transport medium: they should be shipped dry.**

In rare cases involving severe complications or death, other types of specimens (e.g., biopsied tissue, cerebrospinal fluid, peripheral blood) may be sent to the National VZV Laboratory for PCR testing. When possible, liquid specimens should be shipped frozen.

Sources of Suitable Supplies

- **Freezing vials**: 2.0-ml polypropylene vials are available from a number of companies, including Nalgene Labware (#5000-0020), Wheaton Science Products (#985916), Corning (#430659, 431386), and Nunc (#347627).
- **Plastic re-sealable bags** *(for containment of blood spot pads and PCR swab tubes to reduce risk of cross-contamination):* 8" x 8" or larger bags are available from Daigger & Company, Inc (#HX28281D) and Fisher Scientific (#01-816-1E).
- **Swabs with tubes***:* a single-unit, polyester swab with tube and slide on cap is available from Epicentre Biotechnologies (#QEC091H). Suitable swab tubes are also available on request from the CDC National VZV Laboratory.
- **Filter blot pads***:* these pads are made to custom specifications for the CDC National VZV Laboratory. On request, CDC will supply to local and state health departments and to the varicella surveillance project office in your area.

These items are available through distributors of scientific laboratory products, such as Fisher Scientific and WVR International.

Specimen Collection Form

Specimens submitted to CDC for VZV testing will require 2 forms to be completed

- the CDC specimen submission form (CDC Form 50.34) and
- the VZV specimen collection form.

The VZV specimen collection form can be accessed through CDC Form 50.34. If VZV is selected for testing within CDC Form 50.34, the link for the VZV specimen collection form will be provided.

Additional Information

- Video, Collecting Specimens for Varicella Zoster Virus (Chickenpox & Shingles) Testing http://www.cdc.gov/shingles/lab-testing/collecting-specimens.html
- Shingles (Herpes Zoster) Diagnosis & Laboratory Testing http://www.cdc.gov/shingles/hcp/diagnosis-testing.html
- Shingles (Herpes Zoster) http://www.cdc.gov/shingles/index.html
- Chickenpox (Varicella) Interpreting Laboratory Tests http://www.cdc.gov/chickenpox/hcp/lab-tests.html
- Chickenpox (Varicella) http://www.cdc.gov/chickenpox/index.html
- Contact National VZV Laboratory at 404-639-0066/404-639-2192 (phone) or dss1@cdc.gov/kjr7@cdc.gov (email).