Centers for Disease Control and Prevention
National Center for Immunizations and
Respiratory Diseases
National VZV Laboratory
Atlanta, GA USA

Laboratory Services Provided Through the CDC National VZV Laboratory

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Establishment of the National VZV Laboratory. The National VZV Laboratory was established in May 1998 in the Centers for Disease Control and Prevention. This laboratory supports surveillance efforts towards monitoring the impact of varicella vaccination in the U.S. population including outbreak investigations, confirmation of disease (including breakthrough infections), vaccine adverse events, and disease susceptibility.

Services available through the VZV laboratory. The National VZV Laboratory is able to provide several types of VZV-specific testing free-of-charge for state and local public health officials who require confirmatory evidence for VZV infection, and for physicians and scientists participating in various epidemiologic and laboratory-based studies. Specimens may also be submitted by public and private providers for suspected vaccine adverse events including herpes zoster in a vaccinee, rash with more than 50 lesions 7-42 days after vaccination, any serious adverse event (pneumonia, ataxia, encephalitis) post vaccination, and suspected secondary transmission.

IgG whole infected cell ELISA (WC 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 to the assay in use by the California Department of Health Services). While it is probably not sufficiently sensitive to detect all seroconversions to vaccination (since serum antibody levels tend to be lower among vaccinees than in naturally infected persons), it reliably detects seroconversion to natural infection.

gpELISA. An ELISA method was 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 is thought to compare favorably to the FAMA (Fluorescent Antibody to Membrane Antigen) 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. Commercially available IgM assays are not reliable. False positives may occur due to interference by IgG, a problem eliminated by the capture assay format.

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. It can be performed using either WC ELISA or gpELISA (based on strength of test result). Low affinity antibodies in serum no longer bind antigen in the presence of this agent. Early antibodies produced in response to either primary infection or vaccination tend to have overall lower affinity for antigen (low avidity antibody); then over time these antibodies are supplanted with antibodies that have increased affinity for antigen (high avidity). The presence of low avidity antibodies in a serum specimen is a reliable indicator of recent 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 ORF 38 and ORF 54 (these markers discriminate wild-type J strains from wild-type strains of other genotypes), and for two vaccine-strain-specific markers in ORF 62. Together, these methods are used to confirm VZV infection and to reliably 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 open reading frames ORF21, ORF22, and ORF50 that reliably discriminates all seven genotypes that have been identified to date. We are currently developing a microarray DNA resequencing chip that will enable 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.

Preparation and shipping of specimens for diagnosis.

Serology. There are two ways to prepare specimens of peripheral blood suitable for diagnosis at CDC using VZV-specific serologic assays. Where feasible, serum is preferred since the typically larger samples allow for broader testing and, where needed, retesting.

(1) Preparation of serum from whole blood.

1. Collect 10 ml of 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 service 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. Specimens should be shipped frozen, preferably on dry ice, by overnight mail service.

(2) 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 different strips to come into contact with each other while wet.

4. Place the strips in a sealable plastic bag. *Specimens must be permitted to air dry completely before placing them inside a plastic bag. Otherwise, bacterial or fungal growth can occur, destroying the specimen.* Once the blood specimens have completely dried, it is acceptable to bundle them with a rubber band and place them in a single bag. 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.

PCR/Genotyping. To make a laboratory diagnosis of VZV infection using polymerase chain reaction (PCR) method, the presence of the virus DNA can be demonstrated in tissues, vesicular fluid or crusts from lesions. We recommend the following methods for the collection of specimens for PCR testing; collection on glass slides tends to provide a better specimen, particularly in the case of mild disease as commonly occurs with breakthrough infection in vaccinees. As noted below, scabs generally contain sufficient viral DNA for amplification and as such are also useful specimens.

Polyester swab method:

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. Collection of infected epithelial cells in the base of the lesion is important 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 support of swab usually absorbs extraction buffer and inhibits PCR.

3. Place swab into empty tube directly. DO NOT PLACE TRANSPORT MEDIUM INTO THE TUBE; THE SPECIMEN MUST BE KEPT DRY. To avoid contamination each swab must be placed directly into individual tubes and labeled (tubes must be resistant to breakage).

Glass slide method:

1. Rake the edge of the slide over the selected lesion to disrupt it.

2. Press the flat surface of the slide against the opened lesion, rocking it back and forth several times. With young children, it may be less stressful if you ask them to help with this.

3. Air dry the specimen.

4. Ship in a container that protects against breakage. Cardboard mailers are the only container we've found that permit glass slides to pass through the mail unscathed. Plastic slide mailers have never worked well.

Crusts (scabs)

Crusts are also suitable for PCR detection of VZV DNA. Crusts can be transferred directly into breakage-resistant snap-cap or screw top tubes.

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.

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

Sources of suitable supplies

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

Freezing vials: 2.0 ml polypropylene vials are available from a number of companies, including Nalgene (#5000-0020), Wheaton (#985731), Corning (#430659), Nunc (#347627), Costar (#2028), Sarsdedt (#72.694.006).

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; Daigger & Co., Inc (#HX28281D), Fisher (#01-816-1E).

Swabs with tubes: Catch-All Sample Collection Swab. Foam tip polyester with reclosable tube. Epicentre (Madison, WI). Suitable swab tubes are available on request from the VZV lab at CDC.

Filter blot pads: Made to custom specifications for the VZV lab at CDC. CDC will supply to local and state health departments and to the varicella surveillance project office in your area on request.