

# ***Varicella zoster virus***

## **Laboratory case definition**

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *varicella zoster virus*.

**Authorisation:** PHLN

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## **1 PHLN summary laboratory definition**

### **1.1 Condition:**

Varicella (chicken pox); herpes zoster (shingles)

#### **1.1.1 Definitive Criteria**

*Varicella zoster virus* (VZV) infections (chickenpox, herpes zoster) have characteristic clinical presentations and are usually diagnosed clinically. However, laboratory diagnosis is recommended when the clinical picture is atypical or complicated. Diagnostic methods used in the laboratory include:

- Detection of VZV by nucleic acid amplification testing (NAAT), or direct immunofluorescence assay (DFA); or
- Seroconversion with VZV-specific IgG antibody; or
- Isolation of VZV by cell culture, now rarely performed

VZV NAAT, DFA and/or cell culture are also unable to differentiate varicella from herpes zoster, which requires either clinical correlation and/or antibody testing.

#### **1.1.2 Suggestive Criteria**

- Detection of VZV specific IgM antibody by enzyme immunoassay, or another validated serological assay.

### 1.1.3 Special Considerations / Guide for Use

Laboratory confirmation is useful for atypical presentations of VZV, particularly in vaccinated and immunocompromised persons, and to distinguish between herpes simplex virus (HSV) and VZV infection in situations where cutaneous rashes may resemble one another, such as genital HSV and zoster in the ano-genital area. Direct antigen detection may be performed when there is cutaneous manifestation of disease, and where fluid and/or scrapings from the base of fresh vesicles are obtained for testing. The diagnosis of zoster *sine herpete* (zoster in the absence of rash), which may occur in immunocompromised persons, is more challenging as infection may be difficult to confirm in the laboratory.

## 2 Introduction

*Varicella zoster virus* (VZV), also known as human herpes virus 3 (HHV-3), is a highly communicable *alpha-herpesvirus*, which causes varicella (chickenpox) and herpes zoster (shingles). It has a double-stranded DNA genome approximately 125Kb in length, within a 100nm icosahedral nucleocapsid surrounded by a lipid envelope, giving a total virion size of approximately 150-200nm.

*Varicella* is a common, and usually self-limited childhood disease causing fever and a generalised pruritic vesicular rash after which VZV establishes life-long latency in the dorsal root ganglia of the spinal cord. Unvaccinated children usually acquire *varicella* between five to 10 years of age, with the highest rates of hospitalisation occurring in children under the age of four. VZV has a worldwide geographic distribution, but infection is more prevalent in temperate climates with a strong seasonality, occurring most often during late winter and spring<sup>1</sup>. VZV is one of the most contagious infectious diseases, with a  $R_0$  (reproductive number) between 3.7 – 5.0. It is spread by inhalation of respiratory aerosols, and to a much lesser extent aerosolised virus particles from vesicular skin lesions. After an incubation period of approximately two weeks, a characteristic cropping vesicular rash moves from the scalp and trunk to the periphery accompanied by mild fever. Lesions evolve from vesicles and begin to crust over within eight to 12 hours.

Complications include skin infection, benign cerebral ataxia in children, post infections encephalitis in adults, and pneumonitis. Immunocompromised persons, neonates, adults and pregnant women may be at increased risk of severe disease. Neonates in particular are at increased risk if primary maternal infection occurs four days before to 48 hours after birth, due to potential exposure to viraemia prior to development of maternal antibody.

Herpes zoster (HZ) occurs from the reactivation of latent VZV, with a lifetime risk of reactivation of approximately 50%. HZ causes a localised, painful, vesicular rash involving one or more adjacent dermatomes, most commonly one or more adjacent unilateral thoracic dermatomes. Disseminated disease may occur in immunocompromised hosts with the rash involving multiple non-contiguous dermatomes, central nervous and pulmonary systems. HZ is typically preceded by a one to four day prodrome of pain and paraesthesia in the affected dermatome(s). HZ is uncommon before the age of 12 years, and most cases occur in persons over the age of 40. The incidence of herpes zoster increases with age, and is increased in immunocompromised persons.

Post herpetic neuralgia occurs in up to 50% of persons following HZ, characterised by persistent severe pain in the affected dermatome(s). Other central nervous system (CNS) complications include

meningitis, encephalitis, and post-infectious strokes from granulomatous angiitis. Herpes zoster ophthalmicus may occur with infection of the ophthalmic division of the trigeminal nerve.

Maternal varicella during the first and second trimesters of pregnancy may result in congenital infection among a minority of offspring. Congenital varicella syndrome, which is characterised by cicatricial skin lesions, limb deformities, a variety of ocular abnormalities, and CNS disease appears to occur in less than 5% of infants from varicella-infected pregnancies. If there is significant exposure to VZV during pregnancy in a woman with no history, or an uncertain past history of varicella or VZV vaccination, then serology for VZV IgG should be performed urgently. Pregnant women who are seronegative should be assessed for passive immunisation with VZV immunoglobulin (ZIG), or for therapy with oral acyclovir, depending on the time that has elapsed since significant exposure. VZV vaccine is not recommended during pregnancy.

Varicella-containing vaccine is recommended for children aged 12 months to <14 years (one but preferably two doses) and for all non-immune adolescents  $\geq 14$  years of age and adults (two doses)<sup>2</sup>. Other groups where varicella vaccine is recommended include non-immune healthcare workers, childhood educators and carers, persons who work in long-term care facilities and household contacts of people who are immunocompromised. Monovalent (e.g. Varilrix® and Varivax®) and combination varicella vaccines which also contain *measles-mumps-rubella viruses* (e.g. Priorix-tetra® and ProQuad®) are available.

The live zoster vaccine (Zostavax®) is the only zoster vaccine currently on the National Immunisation Program (NIP) in Australia<sup>2</sup>. As of November 2018, the PBAC did not recommend the listing of the recombinant VZV glycoprotein E antigen (AS01B adjuvanted) vaccine (Shingrix®) on the NIP. Zoster vaccine is recommended for adults aged  $\geq 60$  years of age, and adults aged  $\geq 50$  years who are household contacts of a person who is immunocompromised. People who have received the varicella vaccine previously are not recommended to receive zoster vaccine.

Both varicella and zoster vaccines are derived from the Oka strain of VZV (vOka)<sup>3</sup>. Vaccine failure is known as 'breakthrough varicella' and is defined as a case of wild-type varicella post vaccination. Breakthrough varicella infections are generally mild with fewer lesions than natural infection, but can be contagious<sup>4</sup>. Cases of varicella or HZ occurring in vaccinated persons can be caused by either wild-type or vaccine virus. Monitoring of these and other potential vaccine-related complications, as well as transmission events, requires that the vaccine strain be differentiated from wild-type viruses, which may be determined by strain-specific NAAT or sequencing methods. These methods have classified wild-type strains into clades separated by geographic regions. Detection and quantitation of viremia during VZV infections are potentially useful for diagnostic, prognostic and therapeutic monitoring purposes<sup>5</sup>.

## 3 Tests

### 3.1 Clinical specimens

Swabs taken from the base of fresh vesicular lesions are suitable for NAAT, and virus genotyping of amplicons, DFA and culture. Ideally, swabs in viral transport media should be used for sampling. Rayon or Dacron-tipped swabs with plastic-coated shafts can also be used. After sampling, the swab

should be placed immediately in viral or universal transport medium and stored at 4°C until processed. Cerebrospinal fluid (CSF) can also be used for testing in the appropriate clinical situation. Acceptable specimens for quantitative real-time polymerase chain reaction (rt-PCR) analysis also include whole blood or plasma, serum or peripheral blood mononuclear cells (PBMCs), or CSF.

## 3.2 Nucleic Acid Detection

### 3.2.1 Polymerase Chain Reaction (PCR)

PCR allows the rapid and highly sensitive detection of VZV from clinical specimens in the clinical laboratory. As for other PCR, two chemistries are currently used for the amplification and detection of VZV gene targets; sequence-specific probes and intercalating dsDNA fluorescent dyes. Nucleic acid extraction may be performed separately, or using automated platforms that integrate nucleic acid extraction, amplification and detection in the one instrument.

### 3.2.2 Qualitative and quantitative PCR

Qualitative rt-PCR is used for the detection of VZV, whilst quantitative rt-PCR may be used for the detection and quantitation of VZV, but is generally reserved for specific settings, and not as widely available as qualitative testing. Monitoring of VZV viral load in blood and CSF may be useful in complicated cases which fail to respond to antiviral treatment.

### 3.2.3 Test Details

Gene specific primers and FRET (fluorescent resonance energy transfer) labelled probes or alternatively, a dsDNA intercalating fluorescent dye followed by melt curve analysis can be used for both the qualitative detection and quantitation of VZV. Suitable gene targets often used in PCR detection include ORF 29, 62, glycoprotein 19 and polymerase gene<sup>6-9</sup>.

For quantitation, a standard curve comprising plasmid constructs containing the VZV gene target of interest is used as a standard and incorporated into each PCR test assay. Hence, the VZV genome equivalents (geq) of the test samples are interpolated from the standard curve.

### 3.2.4 Test Performance

Sensitivity of PCR for detection of VZV significantly exceeds that of other methods and approaches 100%. Gene specific probe based assays have shown higher sensitivity to detecting VZV DNA than fluorescent dye based methods<sup>7,9</sup>. However, the use of fewer than ten copies of target may produce inconsistent results due to the random distribution inherent in dilutions of samples with low copy numbers. The specificity of each NAAT assay is dependent on the primer/probe design, but is generally very high.

### 3.2.5 Predictive Values and Relevant Populations

The predictive value of a positive result is high in symptomatic individuals. In asymptomatic and immunocompromised individuals, predictability may be compromised by the absence of detectable or low VZV viral loads. A significant proportion of patients with uncomplicated varicella have DNA detectable in blood and CSF during the acute phase of illness. A smaller proportion of zoster patients have detectable viremia.

### 3.2.6 Suitable Test Acceptance Criteria

Varicella-zoster positive control, a negative control and a non-template control should be included in each assay. If processed together, care should be taken to ensure that CSF specimens are not contaminated by skin specimens.

### 3.2.7 Suitable Internal Controls

An amplification control sequence is incorporated in the same tube to ensure that any VZV-negative samples do not contain PCR inhibitors and as a check on DNA sample integrity.

## 3.3 VZV-Specific Antibody Detection

Serological diagnosis is important for determining the immune status of an individual to VZV. Suitable specimens are serum or plasma samples (EDTA/heparinised/citrated plasma). Samples should be stored for not more than 3 days at 2-8°C. Paired acute and convalescent sera are used for the diagnosis of primary varicella infection, but this is less reliable for herpes zoster where specific antibodies are already present. For this reason, collection of the first sample as soon as possible after the onset of the rash is important to show a rising titre. However, cross reactivity between HSV and VZV can make the interpretation of results difficult.

Recent infection is suggested by the detection of serum VZV-specific IgM antibodies, however this alone is not diagnostic of acute varicella infection due to biological false positives or non-specific reactivity due to other infectious or non-infectious conditions, recurrence of IgM during zoster reactivation and/or prolonged detection following recent infection. The antibodies occur within days of onset of infection and to a lesser extent after further exposure. Immunity to varicella is relatively rarely an indication for a laboratory test, in part because of the high predictive value of a history of varicella, and the relatively high prevalence of immunity in those without such a history. This may change as varicella vaccine displaces naturally acquired immunity. In addition, post-immunization serological testing for immunity is not generally indicated because of the high level of immunity conferred by the vaccine – 95% of adults seroconvert using high quality tests after receiving two doses of vOka vaccine.

In addition, VZV IgG assays used in clinical practice are not as sensitive as those used for vaccine studies, and undetectable IgG following appropriate immunisation in an immunocompetent individual does not necessarily indicate vaccine failure. Assessing for immunity is most important in high risk individuals, such as pregnant women and immunocompromised (mainly to assist in management in the event of an exposure), and in healthcare workers with uncertain immune status.

### 3.3.1 Test Details

The fluorescent antibody to membrane antigen (FAMA) assay is the accepted gold standard serological assay to determine immunity/protection against VZV. However as FAMA is labour intensive, it is rarely performed (and available) in routine clinical laboratories. In healthy persons with a FAMA titer of  $\geq 1:4$ , <2% developed varicella after a household exposure to VZV, compared to an attack rate of 59% in persons with a FAMA titer of  $< 1:4$ <sup>10</sup>. By contrast, FAMA does not perform well in determining protective immunity in immunocompromised persons.

Whilst commonly available, commercial VZV-specific tests may lack sensitivity in detecting antibodies against VZV (81). Examples of three commonly used commercial assays include:

1. Enzyme-linked immunosorbent assay (ELISA) – (e.g. Enzygnost, Siemens, Germany)
2. Chemiluminescent microparticle immunoassay (CMIA) – (e.g. LIAISON, DiaSorin, Italy)
3. Enzyme-linked fluorescent immunoassay (ELFA) – (e.g. VIDAS, bioMérieux, France)

ELISAs and CMIA are generally performed by many laboratories in a high-throughput automated fashion, and in general, are the preferred diagnostic test for determining serum antibodies against VZV. They are typically based on detection of antibody to whole antigen extracts prepared from VZV-infected cells. The performance of ELISAs and CMIA in determining IgG post vaccination vary greatly, and false negative results may occur in up to 35% of sera tested. In addition, false positive results may also occur in up to 10% of varicella-susceptible individuals<sup>11</sup>.

Glycoprotein ELISAs are more sensitive, but previous criticisms are that the tests are too sensitive in that they may detect very low levels of VZV antibodies that may mis-identify persons with insufficient antibodies to confer protection against VZV<sup>10</sup>. Laboratories that may not have the capacity perform ELISA or CMIA may choose to screen samples initially with ELFA. As the analytical sensitivity of ELFA is less compared to ELISA and CMIA, equivocal sera or sera which require confirmation may be re-tested using the latter methods.

A less common technique for identifying VZV antibodies is the indirect-immunofluorescence assay (IIFA) in which bound antibody is detected by a fluorescent-labelled anti-immunoglobulin. The stained VZV-infected cells are examined using fluorescent microscopy and the antibody titre determined by the highest dilution of a serum sample that allows the clear detection of fluorescence.

VZV IgG avidity testing is potentially capable of determining if a rash consistent with VZV-infection is due to a primary infection or reactivation of latent virus. Persons infected in the past have high affinity IgG antibodies that bind to the antigen compared to persons with more recent infection. However, IgG avidity testing is not commercially available, and rarely performed in the routine laboratory.

### 3.3.2 Test performance

The performance of VZV-specific serology varies widely and is dependent on the type of assay used, the reference standard the assay is being compared to, and the population tested. In a study evaluating three commercial VZV-IgG ELISA (Enzygnost anti-VZV/IgG, Euroimmun anti-VZV and Serion Classic VZV IgG) against FAMA, the sensitivity and specificity of the assays ranged from 90.5% - 99.2% and 89.4% - 100%, respectively. A total of 638 sera collected from children, blood donors, VZV vaccine recipients and hematopoietic stem cell transplants were tested. The Enzygnost and Euroimmun assays are based on highly purified viral proteins, but the Serion assay is VZV glycoprotein based<sup>12</sup>. In another study detecting VZV-IgG in sera collected from hospital patients, the sensitivity of fifteen commercial ELISAs ranged from 69.6% - 97.4% when compared to time resolved fluorescence immunoassay (TRIFA)<sup>13</sup>. The least sensitive assays were however the most specific. Using TRIFA as the reference, one study determined that the sensitivity/specificity for CMIA and ELFA was 67%/100% and 54.5%/97.9% respectively in detecting VZV-IgG in pregnant women without a history of varicella infection<sup>14</sup>.

Test method	Analytical sensitivity	Analytical specificity
ELISA	69.6% - 99.2%	89.4% - 100%
CMIA	67%	100%
ELFA	54.5%	97.9%

\* equivocal results were considered negative

### 3.3.3 Predictive Value and Relevant Populations

Relevant populations for testing, apart from clinical indications, include possible screening for prenatal/antenatal care programmes, and determining immunity status in healthcare workers.

### 3.3.4 Suitable Reference Controls

Human serum containing IgG and IgM antibodies to VZV antigens is provided as a reference within commercial kits.

### 3.3.5 Suitable External (QAP) Programme(s)

A QAP for VZV-specific antibody detection is available through the Royal College of Pathologists of Australasia.

## 3.4 Direct Immunofluorescence Assay

Specific fluorescent-antibody detection of VZV antigen is a rapid, simple and practical method for early diagnosis. Swabs or scrapings from lesions are spread over a 10 mm diameter area in 50 mL of saline on a clean microscope slide and air dried. The slides are fixed in cold acetone for 10 minutes and stained with a fluorescein-labelled conjugate (monoclonal antibody - VZV glycoprotein complex gp98-gp62) containing Evans Blue counterstain (e.g., MeriFluor VZV, Meridian Bioscience, Inc., Cincinnati, USA). Slides are read using fluorescent microscopy. At the time of writing, there is no monoclonal antibody detecting VZV that is registered on the Australian Register of Therapeutic Goods (ARTG), hence it is anticipated that laboratories performing immunofluorescence assays are importing the reagents outside the ARTG.

### 3.4.1 Test Sensitivity

The sensitivity and negative predictive value of the DFA is reported in the range of 97-98% when compared to cell culture<sup>15</sup>. In another study of 60 samples, VZV was detected in 34 (77%) and 44(100%) using DFA and an in-house developed PCR<sup>16</sup>, respectively.



### 3.4.2 Test Specificity

When clinical diagnosis is used as the gold standard, DFA is 100% specific for VZV.

### 3.4.3 Suitable Test Acceptance Criteria

Staining of a second cell smear with a bivalent fluorescein isothiocyanate-conjugated mouse monoclonal antibody specific for both *herpes simplex virus* types 1 and 2 (HSV-1, HSV-2).

### 3.4.4 Suitable Reference Controls

Incorporation of appropriate positive VZV and HSV antigen control slides.

## 3.5 Cell Culture

Viral isolation in conventional cell culture was previously considered to be the definitive diagnostic test for the detection of VZV, but it no longer performed by the majority of routine diagnostic laboratories. The growth of VZV in culture can be difficult and turnaround times are too slow, hence results are not available in clinically relevant timeframes. Tubes of human diploid lung fibroblasts (e.g., MRC-5; ATCC, Rockville, USA) are inoculated with 0.2 mL of clinical specimen and incubated at 37°C and examined daily for cytopathic effects (CPE) for the first 7 days and then every other day for 21 days. Shell vial cultures can also be performed, with no compromise in sensitivity of test performance. Direct immunofluorescence is used to confirm suspected VZV isolates. Note comment above about the lack of a monoclonal antibodies for confirmation of VZV on the ARTG.

### 3.5.1 Test Sensitivity

Viral culture has a reported sensitivity ranging from 49.4%<sup>15</sup> to 65%<sup>17</sup>. The sensitivity of VZV culture is also dependant on the speed of processing after specimen collection and the age of the sampling lesion as VZV is labile and difficult to culture from lesions that are over 5 days old<sup>18</sup>. Suggestion has been made that the administration of antiviral therapy prior to sampling may slow down the propagation of VZV in culture.

### 3.5.2 Test Specificity

VZV culture is a presumptive test based on observation of focal CPE in MRC-5 cells. CPE appears as small groups of swollen, granular, refractile cells progressing linearly along the long axis of the fibroblasts. VZV CPE can then be confirmed with PCR or DFA.

## 4 Typing & subtyping

### 4.1 Genotyping Methods

VZV infection caused by wild-type or vaccine strains of the virus can be differentiated by genotyping. Three methods are in current use to geographically distinguish between the vOka and the following VZV wild-type stains: African/Asian (A), European (B), European (C) and Japanese (pOka, European) (J). The varicella vaccine strain vOka (Varivax® and Varilrix®) and VZV wild-type strain 'Dumas' (NCBI Genbank Accession No. X04370) are used as laboratory reference control strains.



### 4.1.1 Multi-SNP (Single Nucleotide Polymorphism) genotyping of strains

SNP genotyping of VZV strains is achieved by the detection of gene mutations by allelic discrimination using gene specific probes (dual labelled)<sup>19</sup> or the High Resolution Melt (HRM) analysis of a temperature shift in a melt curve detected by a dsDNA saturating fluorescent dye<sup>20</sup>. The five VZV gene targets, ORF 1, 21 37, 60 and 62 can be used to discriminate vOka from wild-type strains.

### 4.1.2 DNA sequence variation in ORF 22

This genotyping method is based on DNA sequencing of a short region in VZV ORF 22. Four polymorphic positions in a 447-bp fragment of ORF 22<sup>21</sup> distinguish European strains from Japanese (J) and mosaic (M1-M4) strains.

### 4.1.3 PCR-RFLP

PCR followed by restriction fragment length (PCR-RFLP) analysis of two polymorphic loci – a PstI restriction site in ORF 38, and a BglI restriction site in ORF 54<sup>22,23</sup>. This method separates VZV wild-type isolates from the Oka vaccine strain. The vaccine virus strain is PstI<sup>-</sup> BglI<sup>+</sup> at these loci.

### 4.1.4 Whole genome sequencing

Whole genome sequencing of VZV can be performed, however, is generally limited to reference laboratories. In general, most laboratories that perform genotyping do so using a limited set of targeted SNPs, although such an approach may not accurately discriminate some clades by investigating SNPs that are not clade specific.

## 5 Laboratory Nomenclature for National Database Dictionary

SNOMED CT concept	Code
Varicella zoster virus infection (disorder)	309465005
Varicella zoster virus (organism)	19551004
Varicella zoster virus nucleic acid assay (procedure)	398503008
Varicella zoster virus antigen (substance)	260214004
Varicella zoster virus IgM antibody (substance)	710704003

SNOMED CT concept	Code
Varicella zoster virus IgG antibody (substance)	703486008

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