Laboratory Diagnosis of Viral Congenital Infections

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HERPES VIRUSES
VIRAL STRUCTURE

Core: Consists of a single linear molecule of dsDNA in the form of a torus.

Capsid: Surrounding the core with a 100 nm diameter and 162 capsomeres.

Tegument: Consists of viral enzymes.

Envelope: Outer layer composed of altered host membrane and a dozen of viral glycoproteins.
**Virus Structure**

Enveloped, slightly pleomorphic
Spherical
120 – 200 nm in diameter

**Capsid**
**Envelope**
**Tegument**
**Genome**
double stranded DNA per virion
- Primary infection - first contact with HSV
- Latent infection - persistent virus in root ganglia
- Reactivation - production of infective virus by latently infected cell
- Recurrence - clinically apparent disease produced by reactivation
CLASSIFICATION
(Herpesviridae)

• **Alphaherpesvirinae**
  – Herpes simplex virus type 1 HSV-1
  – Herpes simplex virus type 2 HSV-2
  – Varicella-zoster virus VZV

• **Betaherpesvirinae**
  – cytomegalovirus CMV
  – Human herpesvirus type 6 HHV-6
  – Human herpesvirus type 7 HHV-7

• **Gammaherpesvirinae**
  – Epstein-Barr virus EBV
Pathogenesis

Entry by skin or mucous membranes

- viral multiplication
- lysis of cells
- vesicles
- ulcers

- sensory nerve
- root ganglia
- latency

- REACTIVATION

- COLD FEVER SURGERY UNKNOWN
TIMES OF TRANSMISSION

HSV of the newborn is acquired during one of three distinct time intervals:

1. Intrauterine (in utero 5%)
2. Per partum (perinatal 85%)
3. Postpartum (postnatal 10%)
Cultures from skin lesions, mouth, nasopharynx, conjunctiva, urine, stool/ano-rectum and CSF.

Serologic tests should not be relied on

PCR testing for CSF HSV DNA is the diagnostic method of choice for HSV encephalitis
• EM detection
• Stained smears - for multinucleated giant cells with intranuclear inclusions
Laboratory diagnosis of HSV

Direct staining
Tzanck test
Immunostaining
HSV isolation
Serology
PCR
Tzanck test

Cell scrape from base of the lesion
smear on slide

Staining
Wright-Giemsa, Giemsa

Ballooning cell with intranuclear inclusion
multinucleated cell
Tzanck test

Multinucleated cell
Immunofluorescent staining

Cell scrape, smear
fix in cold acetone

down

rabbit anti-HSV Ig
donw

goose anti-RaIg conjugated
with fluorescein dye
donw

mount with glycerine buffer
Specimen collection

Samples: vesicle fluid, lesion swab

Transport media

Smear on slide
Transport media

Isotonic solution or culture media

Protein
- bovine serum albumin
- bovine serum

Antibiotics
- streptomycin
- penicillin
- gentamycin

Anti-fungus
- amphotericin B
Viral isolation

Specimens

Cell culture (human diploid cells, Vero cells, Hela cells)

Cytopathic effect
(rounded, enlarged and multinucleated cell)

Identification or typing

*Immunofluorescent staining
HSV Cytopathic effect

Normal cells

CPE
Serological test for HSV infection

Immunofluorescent staining

Complement fixation test

ELISA: IgM capture test
IgG test
HSV-I & II ANTIBODIES
detected by immunofluorescence

Both HSV-I and HSV-II diagnostic kits are offered by this company. In most cases, the serologic method employed permits differentiation of the two herpes simplex viruses. The limitations of this procedure, as in other conventional procedures, may appear in very low titered sera or double infections with both HSV-I and II. IgM antibodies can be detected by substituting antihuman IgM (FITC) in place of the regularly provided antihuman globulin. The 96-test kits do not need to be shipped with dry ice and they have a 12-month shelf life. Pictured is a HSV-2 positive reaction (250x).

Immuno-Diagnostic Products Inc, PO Box 193.
HSV serology

**Primary infection**

Pair serum: acute & convalescent serum

IgG assay *rising titer* → ≥ ~ times

*seroconversion*

Single serum: IgM assay

**Recurrent infection**

not useful; multiple reactivation
IgM capture ELISA

Substrate+chromogen

Enzyme labeled anti-viral antibody

HSV antigens

Tested sera (IgM)

Anti-M chain capture Ab
Polymerase chain reaction

Samples
infected cell, vesicle fluid, CSF

↓
DNA extraction

↓
PCR solution
(buffer, dNTP, Taq DNA pol, primers)

↓
Amplify 20-30 cycles

Multiplex primers;
- cutaneous group; HSV, VZV
- lymphotropic group; CMV,

Detection:
- gel electrophoresis
- dot blot hybridization
- *restriction fragment length polymorphism
Varicella Zoster Virus

- VZV is a DNA virus
- Belongs to the Herpes Virus Family
- Causes two clinically distinct forms of disease.
  - Chicken-pox (Varicella)
    - Primary infection
    - Usually in childhood
  - Herpes Zoster (shingles)
    - Secondary manifestation of an earlier infection
    - Later in life
Pain and hyperaesthesia
Pathogenesis

- Highly contagious infectious agent
- Easily cultured from skin lesion of patients
- Transmitted from person to person by direct contact with the vesicular fluid of the skin lesions and/or by secretions from the respiratory tract
Rare disease during pregnancy in most industrial countries (protected by IgG antibodies)

Only 3–4% of women were found to be susceptible to primary varicella-zoster virus (VZV) infection (Germany)

Average incidence of varicella in pregnant women: 0.7-3 per 1000 pregnancies

Usually mild clinical course, but may occasionally lead to serious maternal and fetal diseases during pregnancy
Laboratory diagnosis of VZV

Direct staining

Samples → Infected cell scrape

Tzanck test → ballooning cell with intranuclear inclusion
multinucleated cells

Immunostaining: fluorescent staining
Serological test of VZV

ELISA with VZV specific antigen

IgG    seroconversion
       rising Ab titer $\geq \alpha$ times

IgM    detected both
       chickenpox & zoster
Isolation of VZV

Nasal/throat washing vesicle fluid

Inoculate promptly

Human diploid cell culture

0-6 weeks

CPE
ballooning, multinucleated cell

Identification: IF
Polymerase chain reaction

**Single/Nested PCR**

- using primer common with HSV
- detected both VZV & HSV

**Multiplex PCR**

- using mix primers HSV + VZV + ....
Cytomegalovirus Infections in Pregnant Women and Neonates
Human Cytomegalovirus

herpesvirus

betaherpesvirinae subfamily

CMV infected cells may become enlarged (cytomegalia), showing intranuclear inclusions.
**Virus Structure**
Enveloped, slightly pleomorphic
Spherical
120 – 200 nm in diameter

**Capsid**
**Envelope**
**Tegument**
**Genome**
double stranded DNA per virion
TRANSMISSION

Transmitted through infected bodily fluids that come in contact with hands and then are absorbed through the nose or mouth of a susceptible person.
Transmission can also occur
  – congenitally
  - by sexual contact
  - through blood transfusion
CMV may be shed in the bodily fluids
  urine
  saliva
  blood
  semen
  breast milk

The shedding of virus
  - intermittent
  - without signs
  - without causing symptoms.
CMV infection
High-risk groups:

(1) infection to the unborn baby during pregnancy

(2) infection to people who work with children

(3) immunocompromised person:
   a) organ transplant recipients
   b) human immunodeficiency virus (HIV)
   C) undergoing hemodialysis
   d) patients with cancer
The primary infection presents as mononucleosis-like syndrome which soon resolves.

Most of them asymptomatic for life.
Routes of Transmission to Newborn

1- Antenatal (in utero)
   Primary or recurrent maternal infection

2- Perinatal (during delivery)
   Contact with infected cervical secretions

3- Postnatal (after birth)
   Breast milk
   Human – infant contact
   Multiple blood transfusions
Serum and CSF investigation

• **Serum**
  - Complete blood count, platelet count
  - Liver transaminase level, bilirubin level

• **CSF**
  - CSF cell count, protein and glucose level
Laboratory Diagnosis

- Virus isolation from urine or saliva within 3 weeks of birth
- Presence of CMV IgM from the blood
- Antigenemia
- PCR
Diagnosis of Congenital CMV Infections

• Isolation of CMV from urine or other body fluid (CSF, blood, saliva) in the first 21 days of life is considered proof of congenital infection
• Serologic tests are unreliable; IgM tests currently available have both false positive and false negative results
• PCR may be useful in selected cases
CPE observed in fibroblast cell culture
CMV Cytopathic effect
Detection: screening for maternal CMV infection

- CMV IgG antibody – sensitive and specific screen for past infection
- CMV IgM antibody – variable sensitivity and specificity
- Antibody avidity testing can increase accuracy of detection of primary infection
- No test for immune mothers who will transmit
Congenital CMV infections

Low IgG avidity is linked to primary infection
Abnormal Maternal CMV Screening
CMV IgG: positive
CMV IgM: positive

CMV IgG: negative
CMV IgM: negative

CMV-specific IgG and IgM by EIA
CMV-specific IgG avidity by EIA
CMV-specific IgM by immunoblot

CMV uninfected
No further evaluation

CMV IgG: positive
IgG avidity index: high
CMV IgM: negative

Latent CMV infection
No further evaluation

CMV IgG: positive or seroconversion
IgG avidity index: low
CMV IgM: positive

Primary CMV infection
Invasive follow-up

Uncertain serologic results

Undefined CMV infection
Noninvasive follow-up

CMV IgG: positive
IgG avidity index: high
CMV IgM: positive

Recurrent CMV infection
Rubella virus

- Fetal infection with rubella virus: an important preventable cause of congenital malformations and mental retardation.

- Rubella (German or 3-day measles) benign, self-limiting, acute rash illness principally affecting children and young adults.

- A woman acquires rubella early in pregnancy, particularly during the first 3 months, the infection often spreads to the fetus, and may cause abnormalities.
Family Togaviridae

The name is derived from the Latin word, *toga*, meaning "coat," which refers to the fact that the icosahedral virion has an envelope.

It is thought that the capsid contains 32 capsomeres.

The nucleic acid is single-stranded RNA.
**Togaviridae**

1. *Alphavirus*
   a) Sindbis
   b) Semliki Forest
   c) Venezuelan equine encephalitis
   d) Eastern equine encephalitis
   e) Western equine encephalitis
   f) Chikungunya

2. *Rubivirus*
   a) Rubella virus (German Measles)
A. specimens for isolation
• specimens for rubella virus isolation should be collected as early as possible after the onset of illness, preferably within 3-4 days after symptom appear
• the virus is most readily isolated from throat and nasal secretions, but CSF, urine, and tissue specimens are also useful in special circumstance

B. specimens for serological diagnosis
• most rubella serodiagnostic tests require the use of whole serum
lab diagnosis

C. virus isolation and identification
- isolation in cell culture
- identification of isolates

D. serodiagnosis
- HI
- EIA (IgG, IgM)

E. PCR
Congenital **Rubella** Syndrome
Laboratory Criteria for Diagnosis

- **Isolation of rubella virus**, or
- **Demonstration of rubella-specific immunoglobulin M (IgM) antibody**, or
- **Infant rubella antibody level that persists at a higher level and for a longer period than expected from passive transfer of maternal antibody** (i.e., rubella titer that does not drop at the expected rate of a twofold dilution per month).
- **PCR positive rubella virus**
Pattern of Viral Excretion and Infant’s Antibody Response in Congenital Rubella

![Graph showing viral excretion and antibody response in congenital rubella](image)
Typical Serological Events following acute rubella infection

Note that in reinfection, IgM is usually absent or only present transiently at a low level.
Interference assay

Specimen (Positive Rubella virus)

Cell culture

+Echovirus

No CPE

Identify

Specimen (Negative Rubella virus)

Cell culture

+Echovirus

CPE
Rubella antibody detection

- Hemagglutination inhibition test (HI)
- Complement fixation test (CF)
- Enzyme immunoassay (EIA) or ELISA
- Rubella-specific IgM antibody assay
- IgG avidity assay
Method: Hemagglutination inhibition test

1. Treatment of serum

Serum

+ Heparin-MnCl₂ or 25 % HCl treated Kaolin

15-20 min, RT

Add 50 % washed Pigeon RBC

1-2 hr. 4°C

Supernatant

30 min, 56°C

Treated serum (dilution 1:4)
4. **Test procedure**

2-fold dilution of acute and convalescent serum in microtiter “V” plate

↓

Add **Rubella Ag (4–8 HA units)**

(Except serum-RBC and RBC control wells)

↓

45–60 min, 4 °C

Add 0.4 % Pigeon RBC

↓

60 min, 4 °C

Reading the result (HI titer)
Hemagglutination

(No Hemagglutination)

Hemagglutination inhibition
Acute serum titer = 16

Convalescent serum titer = 64
IgM EIA

- Generally 1 Week after rash – at least 1 month

- IgM Result alone cannot be considered absolute proof of primary Infection:
  1. IgM response after primary infection may be prolonged
  2. In some reinfections rubella IgM becomes positive again
  3. False positive: RF( IgM Class)

Heat treatment of sera
Heterophil Abs
Interference by parvoviral infection
IgG Avidity Assay

- Low Avidity IgG  Primary infection
- High Avidity IgG  Reinfection
• IgM

• Sensitivity & Specificity

<table>
<thead>
<tr>
<th>IgM</th>
<th>Positive:</th>
<th>Low avidity IgG</th>
<th>Primary infection</th>
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<tbody>
<tr>
<td></td>
<td>No Primary infection</td>
<td>High avidity IgG</td>
<td>Reinfection</td>
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Properties of Parvoviruses

- **Structure**
  - Icosahedral
  - 18-26 nm diameter
  - Single-stranded DNA, 5.6 kb
  - Two proteins
  - Nonenveloped

- **Classification**
  - Parvoviridae (vertebrates)
    - *Parvovirus*
    - *Erythrovirus*: parvovirus B19
    - *Dependovirus* (requires helper virus, such as an adenovirus)
  - Densovirinae (insects)
Parvovirus Infections in Humans

• Diseases
  – Fifth disease (cutaneous rash)
  – Transient aplastic crisis (severe acute anemia)
  – Pure red cell aplasia (chronic anemia)
  – **Hydrops fetalis** (fatal fetal anemia)
  – B19 virus most common

• Fifth Disease
  – Targets red blood cell progenitors
  – Pain in joints
  – Results in lysis of cells, thus depleting source of mature red cells
  – Anemia ensues
  – Rarely fatal and without complications

**Hydrops fetalis** (parvovirus B19)
Laboratory Diagnosis of Parvovirus B19

• During pregnancy, the laboratory diagnosis of maternal parvovirus B19 infection relies primarily on IgG and IgM antibody testing, although PCR assays may also be helpful in certain situations.

• Parvovirus B19 is difficult to culture.
Maternal parvovirus infection

- Circulating IgM antibodies can be detected approximately 10 days after exposure and just prior to the onset of symptoms; they may persist for three months or longer.

- B19 IgG antibodies are detected several days after IgM and usually persist for years; they are a marker of past infection.

- However, reliance on a negative IgM serologic result alone can be misleading in a patient with a significant exposure history, because in some instances maternal IgM levels may be below the detection limit. In such cases, polymerase chain reaction can be useful.
Fetal parvovirus infection

- Polymerase chain reaction (PCR) is a sensitive method to detect small amounts of B19 DNA.
- Use of this technique on amniotic fluid is the method of choice to make the fetal diagnosis.
- Another option is to obtain fetal blood for B19 IgM.

Other methods
- Other techniques such as electron microscopy, detection of viral DNA, and probe hybridization assays for nucleic acids are available but typically are not necessary to detect acute maternal infection.
APPROACH TO PATIENT EXPOSED TO B19

• Pregnant women who are exposed to or have symptoms of parvovirus infection should have serologic testing for IgG and IgM antibodies.

• Past infection
  • A positive IgG antibody and a negative IgM indicates maternal immunity.
  • thus, the fetus is protected from infection.