laboratory methods in diagnosis of viral infections in immunocompromised patients

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complications such as infection and allograft rejection, which are related by immunosuppressive therapy, remain major causes of morbidity and mortality following solid organ transplantation.

Epidemiologically, some viral infections are the result of community exposures (influenza, adenovirus), whereas some are commonly transmitted with the allograft (cytomegalovirus, Epstein-Barr virus), and others are the result of more distant exposures reactivated in the setting of immune suppression (chicken pox and varicella zoster as shingles)
## Timing of Pathogens Post-BMT

<table>
<thead>
<tr>
<th>Sources</th>
<th>Phase I, Pre-engraftment &lt;30 days</th>
<th>Phase II, Post-engraftment, 30-100 days</th>
<th>Phase III, Late Phase, &gt;100 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactivation</td>
<td>HSV</td>
<td>CMV+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neutropenia, mucositis and acute GVHD</td>
<td>Impaired cellular immunity And acute and chronic GVHD</td>
<td>Impaired cellular and humoral immunity and chronic GVHD</td>
</tr>
<tr>
<td>Community Acquired</td>
<td>HHV-6</td>
<td>Parvo B-19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Respiratory and Enteric Viruses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Environmentally Acquired</td>
<td>Facultative GNR</td>
<td></td>
<td>Encapsulated bacteria</td>
</tr>
<tr>
<td></td>
<td>Gl Tract Streptococci sp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Staphylococcus epidermidis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>All Candida sp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aspergillus sp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pneumocystic carinii</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Toxoplasma gondii</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Correlate of immunity to be checked, reached and documented

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Indication for determination of specific antibody titres</th>
<th>Specific antibody (IgG) and unit</th>
<th>Interpretation of serological analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>During end-stage organ disease</td>
<td>At pre-transplant (listing)</td>
<td>After catch-up immunization (pre- or post-transplant)</td>
</tr>
<tr>
<td><strong>Tetanus</strong></td>
<td>Yes, if history unclear ($)</td>
<td>If unknown serology</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Haemophilus influenzae type b</strong></td>
<td>Yes (children &lt; 5 years) ($)</td>
<td>Yes (if unknown serology in children &lt; 5 years)</td>
<td>Yes (children &lt; 5 years)</td>
</tr>
<tr>
<td><strong>Hepatitis B</strong></td>
<td>Yes (#, &amp;!)</td>
<td>Yes, if unknown serology</td>
<td>Yes (#)</td>
</tr>
<tr>
<td><strong>Measles</strong></td>
<td>Yes</td>
<td>Yes, if unknown serology</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Rubella</strong></td>
<td>Yes</td>
<td>Yes, if unknown serology</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Varicella</strong></td>
<td>Yes</td>
<td>Yes, if unknown serology</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* If history unclear, check antibody titre 4 weeks after a booster dose to define whether further doses are needed.
* # Check anti-HBs IgG titre if last dose given ≤ 5 years ago, or 4–12 weeks after completion of primary series or a booster dose;
* & Include HBsAg and anti-HBc to exclude current/past infection.
* ¥ In immunosuppressed SOT patients, the unknown contribution of immune memory requires regular booster doses to maintain anti-HBs titers ≥ 10 IU/l at all time in patients at risk of exposure.

* Measles and VZV IgG, by commercially used tests; if positive= immune, if negative or doubtful: send serum for analysis by a more sensitive test [30] to the Laboratoire de Vaccinologie des Hôpitaux Universitaires de Genève.

** Loss of pre-existing immunity to measles / VZV may occur in SOT patients.
## Herpesviridae

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Growth &amp; Cytopathology</th>
<th>Latent infections</th>
<th>Genus</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alphaherpesvirinae</strong></td>
<td>Short, cytolytic</td>
<td>Neurons</td>
<td>Simplexvirus</td>
<td>HSV-1 HSV-2</td>
</tr>
<tr>
<td><strong>Betaherpesvirinae</strong></td>
<td>Long, cytomegalic</td>
<td>Glands, kidneys</td>
<td>Cytomegalovirus</td>
<td>CMV</td>
</tr>
<tr>
<td></td>
<td>Long, lymphoproliferative</td>
<td>Lymphoid tissue</td>
<td>Roseolovirus</td>
<td>HHV-6 HHV-7</td>
</tr>
<tr>
<td><strong>Gammaherpesvirinae</strong></td>
<td>Long, lymphoproliferative</td>
<td>Lymphoid tissue</td>
<td>Lymphocryptovirus</td>
<td>EBV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rhadinovirus</td>
<td>Kaposi' sarcoma virus</td>
</tr>
</tbody>
</table>
• **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
HSV-1

• Primary infection occurs in oral mucosa
  – 30% people get clinically apparent cold sores
  – 90% healthy people have been infected with HSV-1
• Virus then travels along trigeminal nerve to ganglion in most those infected
• 70% cases of HSV-1 encephalitis already have antibody present suggesting reactivation of virus most common mechanism
• Why HSV-1 reactivates not known
• In children, HSV-1 encephalitis occurs during primary infection
Complications

• **Meningitis**-- infection of the sheaths and membranes (meninges) covering the brain and the spinal cord.

• **Encephalitis**-- acute inflammation of the brain, commonly caused by a viral infection by insect bites or food and drink

• **Eczema herpetiform**-- widespread herpes across the skin

• **Keratoconjunctivitis**-- Infection of the eye

• **Prolonged, severe infection** in immunosuppressed individuals

• **Pneumonia**

• **Infection of the trachea**

• **Keratitis**-- Corneal infection, irritations, and inflammations
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

\[ \downarrow \]

Staining
Wright-Giemsa, Giemsa

\[ \downarrow \]

Ballooning cell with intranuclear inclusion
multinucleated cell
Tzanck test

Multinucleated cell
Immunofluorescent staining

Cell scrape, smear
fix in cold acetone

↓

rabbit anti-HSV Ig

↓

goat anti-RaIg conjugated
with fluorescein dye

↓

mount with glycerine buffer
Virologic Tests
(continued)

• **Antigen detection (DFA or EIA)**
  – Fairly sensitive (>85%) in symptomatic shedders
  – Rapid (2-12 hours)
  – May be better than culture for detecting HSV in healing lesions

• **Cytology (Tzanck)**
  – Insensitive and nonspecific and should not be relied on for HSV diagnosis
Specimen collection

Samples:
- vesicle fluid
- lesion swab

Transport media

Smear on slide
Virologic Tests

- **Viral culture (gold standard)**
  - Preferred test if genital ulcers or other mucocutaneous lesions are present
  - Highly specific (>99%)
  - Sensitivity depends on stage of lesion; declines rapidly as lesions begin to heal
  - Positive more often in primary infection (80%–90%) than with recurrences (30%)
  - Cultures should be typed

- **Polymerase Chain Reaction (PCR)**
  - More sensitive than viral culture; has been used instead of culture in some settings
  - Preferred test for detecting HSV in CNS
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
Who Is a Candidate for HSV Serologic Testing?
Special populations

- Pregnant women
- Patients prior to transplant or starting immunosuppressive therapy
- Patients with HIV infection
Serological test for HSV infection

Immunofluorescent staining

Complement fixation test

ELISA :  IgM capture test
          IgG test
HSV serology

Primary infection

Pair serum: acute & convalescent serum

IgG assay *rising titer \rightarrow > \alpha \text{ 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

Detection:
• gel electrophoresis
• dot blot hybridization
• *restriction fragment length polymorphism

Multiplex primers;
• cutaneous group; HSV, VZV
• lymphotropic group; CMV,
Cytomegalovirus

• Member of the herpes virus family (EBV, varicella-zoster, herpes simplex)

• Worldwide seroprevalence 30-100%

• Found in body fluids
  – Blood, saliva, urine, breast milk
Types of CMV Infection

• **Primary infection**  
  (asymptomatic to mononucleosis like syndrome in immune competent individuals)

• **Latent infection**  
  (presence of viral genome in mononuclear leukocytes, endothelial cells, and organs in the absence of active replication of infectious virus)

• ** Reactivation**

• **Reinfection**
Laboratory Diagnosis

1. **CMV antigenaemia test** - widely used in many European countries. CMV antigens at the surface of polymorphonuclear leukocytes are detected by immunoperoxidase or immunofluorescence techniques. A result can be obtained within 4 to 6 hours but the technique is very tricky.

2. **Polymerase chain reaction** - becoming the method of choice in a few laboratories, had been reported to carry a higher prognostic value for CMV disease than the Detection of Early Antigen Fluorescent Foci (DEAFF) test. Potential problems with sensitivity.

3. **Serology** - not reliable in general but occasionally, rises in IgG titre and the presence of IgM may be seen.

4. **viral culture** can be insensitive

5. **Histopathology**
Detection of CMV Infection

- **Immune status:** serology (IgG)
- **Active infection (viremia)**
  - Histology
  - Viral culture
  - Shell vial culture
  - Antigenemia assay
  - CMV PCR (qualitative/quantitative)
Serology

- Serologic tests for antibody to CMV are useful for determining whether a patient had CMV infection in the past, a determination of great clinical importance for organ and blood donors, and in the pretransplant evaluation of prospective transplant recipients.

- Antibody tests are not useful in the diagnosis of CMV disease in the immunocompromised host.
Treatment of CMV Infection in Allogeneic Bone Marrow Transplant Patients

• Ganciclovir/Foscarnet

• Two major treatment approaches
  – Prophylactic treatment – treat all patients at engraftment
  – Pre-emptive treatment – monitor patients for viremia and treat when infection detected

• Goal: prevent CMV disease
CMV Monitoring

• Patients monitored every 1-2 weeks for CMV viremia
  – Shell vial cultures
  – Antigenemia assays
  – CMV PCR

• Incidence of CMV viremia may vary depending on monitoring strategy
PCR Based Screening Methods

- PCR is more sensitive than shell vial or antigenemia assays
- Some patients may be pre-emptively treated unnecessarily using PCR strategies
- Quantitative PCR may be more sensitive than qualitative PCR
Tissue culture cells are grown on coverslips on the bottom of shell vials.

CMV in Shell Vial Culture

Molecular Methods

- Real-time PCR
- PCR…
- COBAS Ampliprep/Taqman CMV Test

After treatment: < 137 IU/ml CMV DNA

Specimen: Whole Blood with EDTA
CMV Monitoring /Treatment Strategy

Quantitative CMV PCR (weekly)

- **CMV PCR ≥ 10,000 copies/ml**
  - Treat with once daily ganciclovir (5mg/kg/day)

- **CMV PCR < 10,000 copies/ml**
  - Monitor

Successful treatment defined as CMV PCR < 2000 copies/ml
Comparing culture and molecular testing

<table>
<thead>
<tr>
<th></th>
<th>Culture</th>
<th>Molecular</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>Fair</td>
<td>3- to 5-fold greater than culture</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>Very high</td>
<td>Very high</td>
</tr>
<tr>
<td><strong>Vulnerability to</strong></td>
<td>Sensitive to extreme temperatures and drying</td>
<td>Viable specimen not required</td>
</tr>
<tr>
<td><strong>transport problems</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Typing</strong></td>
<td>2nd step often required</td>
<td>Incorporated in initial procedure</td>
</tr>
<tr>
<td><strong>Turnaround time</strong></td>
<td>2-3 days</td>
<td>4-8 hours</td>
</tr>
</tbody>
</table>
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
Serological test of VZV

ELISA with VZV specific antigen

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

**IgM**  
detected both  
chickenpox & zoster
Laboratory diagnosis of VZV

Direct staining

Samples → Infected cell scrape

Tzanck test → ballooning cell with intranuclear inclusion
                multinucleated cells

Immunostaining: fluorescent staining
Isolation of VZV

Nasal/throat washing vesicle fluid

↓

Inoculate promptly

Human diploid cell culture

↓

 gratuites 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

$\text{HSV} + \text{VZV} + \ldots$
**HHV 6 and 7**

**Acute/primary infection**

- new human herpes viruses (HHV)
- **HHV-6 and -7**
  - both members of the *Roseolovirus* genus of the β-herpesviruses.
  - T-lymphotropic but can infect other cell types
  - primary infections are associated with *roseola infantum* (*exanthem subitum* or 6th disease)
HUMAN HERPES VIRUSES-6

• HHV6
  – Worldwide
  – virus replicates in T and B cells
  – infection occurs in first 3 years of life
  – Clinical  Exanthem subitum (roseola infantosum)
    • mild acute febrile illness
    • incubation period of 2 weeks
    • fever lasts several days
    • macular papular rash appears within 2 days of fever
  – 85% of adults carry virus in saliva
HUMAN HERPES VIRUSE-7

• HHV7
  – isolated from CD4 positive cells
  – virus present in saliva of >75% of adults
  – role in disease unclear
  – Evidence of infection present (seroconversion)
Human Herpes Virus 6 (HHV6)

- HHV6 associates with febrile convulsions in children under 2 years of age.

- It is a cause of meningitis and encephalitis in immuno-competent as well as immunocompromised patients.

- In the bone marrow transplant recipient, encephalitis presentation occurs between 10 days to 15 months (median 45 days) after transplantation.
Diagnosis of HHV-6/-7 Infection

- **Virus Isolation**
- **Serological Assays**
- **Genomic Detection by PCR**
  - Numerous PCR primer sets available for HHV-6
  - Quantitative PCR assay - persistence of a high HHV-6 load in the absence of apparent disease
  - Multiplex PCR method - simultaneous detection of HHV-6 and HHV-7
## Studies of HHV-6 after BMT

### TABLE 1. Prospective studies on HHV-6 reactivation after BMT

<table>
<thead>
<tr>
<th>Detection method</th>
<th>Sample</th>
<th>No. of patients</th>
<th>Incidence of active HHV-6 infection (%)</th>
<th>Observed disease</th>
<th>HHV-6 variant</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>PBMC, BALF</td>
<td>41</td>
<td>46</td>
<td>Vascular endothelial damage, GVHD</td>
<td>Not determined</td>
<td>389</td>
</tr>
<tr>
<td>PCR</td>
<td>PBMC, plasma</td>
<td>92</td>
<td>42.5</td>
<td>Myelosuppression, fever, delayed platelet and neutrophil engraftment</td>
<td>Not determined</td>
<td>177</td>
</tr>
<tr>
<td>PCR</td>
<td>PBL</td>
<td>22</td>
<td>60</td>
<td>Delayed platelet engraftment</td>
<td>B</td>
<td>266</td>
</tr>
<tr>
<td>PCR</td>
<td>PBL, plasma</td>
<td>61</td>
<td>28</td>
<td>Fever, engraftment failure</td>
<td>B</td>
<td>62</td>
</tr>
<tr>
<td>PCR</td>
<td>PBMC</td>
<td>37</td>
<td>Not given</td>
<td>Delayed platelet and granulocyte engraftment</td>
<td>B</td>
<td>421</td>
</tr>
<tr>
<td>PCR</td>
<td>PBL, oral lavage fluid, urine</td>
<td>57</td>
<td>60</td>
<td>Acute GVHD</td>
<td>90% B, 10% A</td>
<td>428</td>
</tr>
<tr>
<td>PCR, IHC</td>
<td>PBL, skin</td>
<td>57</td>
<td>Not given</td>
<td>GVHD</td>
<td>Not determined</td>
<td>15</td>
</tr>
<tr>
<td>qPCR</td>
<td>PBL, CSF</td>
<td>74</td>
<td>78</td>
<td>Delayed platelet engraftment</td>
<td>Not determined</td>
<td>245</td>
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<tr>
<td>qPCR</td>
<td>PBMC</td>
<td>20</td>
<td>Not given</td>
<td>Rash and fever (2/20)</td>
<td>Not determined</td>
<td>79</td>
</tr>
<tr>
<td>VI</td>
<td>PBMC</td>
<td>82</td>
<td>38</td>
<td>Rash</td>
<td>B</td>
<td>447</td>
</tr>
<tr>
<td>VI</td>
<td>PBMC</td>
<td>22</td>
<td>Not given</td>
<td>Skin rash</td>
<td>Not determined</td>
<td>450</td>
</tr>
<tr>
<td>VI</td>
<td>PBMC</td>
<td>26</td>
<td>46</td>
<td>None</td>
<td>B</td>
<td>197</td>
</tr>
<tr>
<td>VI</td>
<td>PBMC and/or bone marrow</td>
<td>23</td>
<td>48</td>
<td>Skin rash</td>
<td>Not determined</td>
<td>436</td>
</tr>
</tbody>
</table>

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*a* RT-PCR, reverse transcriptase PCR; *qPCR*, quantitative (real-time) PCR; *VI*, virus isolation; *IHC*, immunohistochemistry.

*b* PBL, peripheral blood lymphocytes; BALF, bronchoalveolar lavage fluid.

*c* Patients in this study were all HCMV seronegative.

*d* GVHD, graft-versus-host disease.
# HHV-6 after SOT

## TABLE 2. Prospective studies on HHV-6 reactivation after SOT

<table>
<thead>
<tr>
<th>Transplant type</th>
<th>Detection method</th>
<th>Sample</th>
<th>No. of patients</th>
<th>Incidence of active HHV-6 infection (%)</th>
<th>Observed disease</th>
<th>HIV-6 variant</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>PCR</td>
<td>PBMC</td>
<td>21</td>
<td>0</td>
<td>No disease</td>
<td></td>
<td>298</td>
</tr>
<tr>
<td>Kidney</td>
<td>PCR</td>
<td>PBMC</td>
<td>107</td>
<td>Not given†</td>
<td>No disease</td>
<td>Not determined</td>
<td>320</td>
</tr>
<tr>
<td>Kidney</td>
<td>qPCR</td>
<td>PBMC</td>
<td>52</td>
<td>Not given†</td>
<td>No disease</td>
<td>Not determined</td>
<td>205</td>
</tr>
<tr>
<td>Kidney</td>
<td>VI, serology</td>
<td>PBMC</td>
<td>65</td>
<td>Not given†</td>
<td>None</td>
<td>Not determined</td>
<td>455</td>
</tr>
<tr>
<td>Kidney and/or liver</td>
<td>VI, serology</td>
<td>PBMC</td>
<td>32</td>
<td>Not given†</td>
<td>None (unless concomitant HCMV infection)</td>
<td>Not determined</td>
<td>162</td>
</tr>
<tr>
<td>Kidney and/or pancreas</td>
<td>PCR, serology</td>
<td>Urine, serum</td>
<td>30</td>
<td>Not given†</td>
<td>Fever</td>
<td></td>
<td>335</td>
</tr>
<tr>
<td>Liver</td>
<td>IHC</td>
<td>Liver tissue, PBMC</td>
<td>32</td>
<td>Not given†</td>
<td>Acute liver failure</td>
<td>Not determined</td>
<td>155</td>
</tr>
<tr>
<td>Liver</td>
<td>IHC</td>
<td>PBMC</td>
<td>34</td>
<td>29</td>
<td>HCMV disease</td>
<td>Not determined</td>
<td>222</td>
</tr>
<tr>
<td>Liver</td>
<td>qPCR</td>
<td>PBMC</td>
<td>200</td>
<td>28</td>
<td>Opportunistic infections, HCMV disease</td>
<td>Not determined</td>
<td>171</td>
</tr>
<tr>
<td>Liver</td>
<td>qPCR, serology</td>
<td>PBMC</td>
<td>33</td>
<td>Not given†</td>
<td>HCMV disease</td>
<td>Not determined</td>
<td>281</td>
</tr>
<tr>
<td>Liver</td>
<td>qPCR</td>
<td>PBMC</td>
<td>88</td>
<td>Not given†</td>
<td>HCMV disease</td>
<td>Not determined</td>
<td>173</td>
</tr>
<tr>
<td>Liver</td>
<td>PCR, VI, serology</td>
<td>Plasma</td>
<td>47</td>
<td>Not given†</td>
<td>Fever</td>
<td>B</td>
<td>452</td>
</tr>
<tr>
<td>Liver</td>
<td>VI</td>
<td>PBMC</td>
<td>80</td>
<td>Not given†</td>
<td>CNS disease, fungal infections</td>
<td>Not determined</td>
<td>340</td>
</tr>
<tr>
<td>Liver</td>
<td>Serology, IHC</td>
<td>PBMC</td>
<td>51</td>
<td>Not given†</td>
<td>Graft dysfunction</td>
<td>B</td>
<td>233</td>
</tr>
<tr>
<td>Liver</td>
<td>qPCR</td>
<td>PBMC</td>
<td>60</td>
<td>Not given†</td>
<td>Graft rejection</td>
<td>Not determined</td>
<td>150</td>
</tr>
<tr>
<td>Liver</td>
<td>Serology, Serum</td>
<td>Smear</td>
<td>247</td>
<td>Not given†</td>
<td>HCMV disease</td>
<td>Not determined</td>
<td>108</td>
</tr>
<tr>
<td>Liver</td>
<td>PCR</td>
<td>PBMC</td>
<td>46</td>
<td>Not given†</td>
<td>Increased severity of HCV-related fibrosis or hepatitis</td>
<td>Not determined</td>
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<tr>
<td>Liver</td>
<td>VI</td>
<td>PBMC</td>
<td>51</td>
<td>Not given†</td>
<td>Increased severity of HCV-related fibrosis</td>
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<td>357</td>
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<tr>
<td>Liver heart/lung</td>
<td>VI, PCR</td>
<td>PBMC, BALF</td>
<td>30</td>
<td>Not given†</td>
<td>Higher mortality rate, fungal infections</td>
<td>B</td>
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</tbody>
</table>

* Study population restricted to hepatitis C virus-positive patients undergoing liver transplantation.

† qPCR, quantitative PCR; VI, virus isolation; IHC, immunohistochemistry.

‡ BALF, bronchoalveolar lavage fluid.

§ Unclear whether PCR was able to discriminate active from latent infection.

† HCV, hepatitis C virus.
Epstein-Barr Virus

- Epstein-Barr virus (EBV) is a human herpes DNA virus.
- It is estimated that 95 percent of the world population is exposed to the virus.
- In Infectious Mono the virus affects B-lymphocytes.
- There are two techniques used to identify EBV; immunofluorescence and complement fixation.
Epstein-Barr Viral Infection

• It is a systematic immune complex disease of soluble and tissue-fixed antigen involvement characterized by fever, fatigue, chills, headache, myalgia, skin rash, splenomegaly and cervical adenopathy.

• EBV infected B-lymphocytes express a variety of “new” antigens encoded by the virus. Infection with EBV results in expression of:
  1. Viral Capsid Antigen (VCA)
  2. Early Antigen (EA)
  3. Nuclear Antigen (NA)

Each antigen expression has corresponding antibody responses.
Epstein-Barr Virus (VCA)

- **Viral capsid antigen (VCA)** is produced by infected B cells and can be found in the cytoplasm.
- **Anti-VCA IgM** is usually detectable early in the course of infection, 4 to 7 days after onset of signs and symptoms, but it is low in concentration and disappears within 2 to 4 months.
Epstein-Barr Virus (EA)

- **Early antigen (EA)** is a complex of two components, **early antigen-diffuse (EA-D)**, which is found in both the nucleus and cytoplasm of the B cells, and **early antigen-restricted (EA-R)**, which is usually found as a mass only in the cytoplasm.

- **Anti-EA-D** of the IgG type is highly indicative of acute infection, but it is not detectable in 10% to 20% of patients with IM. EA-D disappears in about 3 months; however, a rise in titer is demonstrated during reactivation of a latent EBV infection.

- **Anti-EA-R IgG** is not usually found in young adults during the acute phase. Anti-EA-R IgG appears transiently in the later convalescent phase. In general, anti-EA-D and anti-EA-R IgG are not consistent indicators of the disease stage.
Epstein-Barr Virus (EBNA)

- Epstein-Barr nuclear antigen (EBNA) is found in the nucleus of all EBV-infected cells. Although the synthesis of NA precedes EA synthesis during the infection of B cells, EBV-NA does not become available for antibody stimulation until after the incubation period of Infectious Mono, when activated T lymphocytes destroy the EBV genome-carrying B cells. As a result, antibodies to NA are absent or barely detectable during acute IM.
- Anti-EBNA IgG does not appear until a patient has entered the convalescent period. EBV-NA antibodies are almost always present in sera containing IgG antibodies to VCA of EBV unless the patient is in the early acute phase of IM. Patients with severe immunologic defects or immunosuppressive disease may not have EBV-NA antibodies, even if antibodies to VCA are present.
Epstein-Barr Virus (EBNA)

- Under normal conditions, antibody titers to NA gradually increase through convalescence and reach a plateau between 3 and 12 months postinfection. The antibody titer remains at a moderate, measurable level indefinitely because of the persistent viral carrier state established following primary EBV infection.

- Test results of antibodies to EBV-NA should be evaluated in relationship to patient symptoms, clinical history, and antibody response patterns to EBV-VCA and EA to establish a diagnosis.
Laboratory Diagnosis

• The 3 classic criteria for laboratory confirmation
  1- lymphocytosis
  2- the presence of at least 10% atypical lymphocytes on peripheral smear
  3- a positive serologic test for Epstein-Barr virus (EBV).
Common laboratory findings in patients with infectious mononucleosis include marked lymphocytosis (>50% leukocytes) with atypical lymphocytes. The detection of at least 10% atypical lymphocytes on a peripheral-blood smear in a patient with mononucleosis has a sensitivity of 75% and a specificity of 92% for the diagnosis of infectious mononucleosis.
• Complete blood count
  – 40-70%, **Leukocytosis**  
    (WBC 10,000-20,000 cells per cm³)
  – 80-90% of patients have **lymphocytosis**,  
    with greater than 50% lymphocytes. Lymphocytosis is  
    greatest during 2-3 weeks of illness and lasts for 2-6  
    weeks.
  – 20-40% of the lymphocytes: **atypical lymphocytes > 10%**;
  – Mild thrombocytopenia
• Liver function tests
  – 80-100% of patients: elevated LFT
  – Alkaline phosphatase, AST and bilirubin peak 5-14 days after onset
  – 95% of patients: elevated LDH
  – most liver function test results are normal by 3 months.
<table>
<thead>
<tr>
<th></th>
<th>Acute illness</th>
<th>Convalescent period</th>
<th>Recovery and lifelong</th>
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<tbody>
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<td>HA</td>
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<td>-</td>
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<tr>
<td>Anti-VCA IgG</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Anti-VCA IgM</td>
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<td>+/-</td>
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<tr>
<td>Anti-EBNA IgG</td>
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</tr>
<tr>
<td>Anti-EA IgG</td>
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<td>+</td>
<td>-</td>
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جدول تغییرات سرولوژیک بیماری های مرتبط با EBV

<table>
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<tr>
<th>تستها</th>
<th>Anti-EBNA</th>
<th>Anti-EA</th>
<th>Anti-VCA</th>
<th>هتروفیل</th>
<th>بیماری بیماری</th>
<th>مونونوکلئوز عفونی حاد</th>
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<td>+++</td>
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MONO-Spot TEST

The presence of IgM HA in IM serum causes agglutination of red blood cells from species other than humans.

🔹 Heterophile antibody tests are negative in 25% of patients during the first week of infection and in 5 to 10% during or after the second week.
🔹 Heterophile antibody tests are positive in only 25 to 50% of children under 12 years of age.
Real-time PCR

- Real-time PCR is when the amplified DNA is detected as the reaction progresses in real time. Test has 95% sensitivity and 97% specificity for primary EBV infection.
- Is expensive and not commonly used in clinical practice.
- Test can be useful for diagnosis of serologically indeterminate EBV infections.
HUMAN HERPES VIRUSE-8

• HHV8

• Kaposi sarcoma- associated Herpes Virus (KSHV)
  – detected in epithelial cells of Kaposi sarcoma
  – also present in semen
  – postulated as cause of Kaposi sarcoma

Diagnosis

• Serological Assays (ELISA)
• Genomic Detection by PCR
• Indirect IF