Serological Diagnosis of Epstein Barr Virus

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SEALS Serology

Epstein Barr Virus (EBV)
- Family Herpesviridae, subfamily gammaherpesvirinae, genus lymphocryptovirus
- ds DNA enveloped virus
- Nucleocapsid 100-110nm in diam; with 162 capsomers
- Asymmetrical material surrounding capsid designated the tegument (structures between the capsid & envelope)
- Envelope containing viral glycoprotein spikes on its surface
- Membrane is derived by budding of immature particles through cell membrane and is required for infectivity
- Genome is linear ds DNA molecule with 172 kbp
- Viral genome does not normally integrate into cellular DNA but forms circular episomes which reside in the nucleus
- Genome is large enough to code for 100-200 proteins but only a few have been indentified

History of Serological testing for EBV
- 1932 Paul & Bunnell (sheep RBC’s)
- 1975 Monospot (horse RBC’s)
- 1966 EBV IgG & IgM IFA; cultivation of EBV infected lymphoid cell lines
- 1985 EBV EIA; EBV antigens from infected cells purified under solid-phase absorption
- 1986 EBV EIA; polypeptides with immunodominant epitopes prepared by recombinant technology

Epstein Barr Virus (EBV)
- 2 peaks of infection: young pre-school (1-6) and adolescents/young adults (14-20)
- Estimated 80-90% of adults are seropositive for EBV
- Infectious mononucleosis (IM)
- Chronic active EBV
- Burkitt’s Lymphoma
- Nasopharyngeal Carcinoma
- Lymphoproliferative disorders (immunocompromised)
- X-linked lymphoproliferative syndrome
- Oral hairy leukoplakia, diffuse polyoidal lymphomas, chronic interstitial pneumonitis in AIDS patients

Epstein Barr Virus (EBV)
- Symptoms:
- Sore throat (80-90%)
- Lymphadenopathy (cervical) present in majority of cases and may last several weeks
- Splenomegaly (50-60%)
- Hepatomegaly (15-25%)
- Jaundice (5-10%)
- Pharyngitis & palatal petechiae (grey-white membrane) first week
- Fever first 2 weeks
- Immunocompromised: GI symptoms, renal graft rejection/failure, lymphoproliferative disease, lymphoma
Testing for Heterophile Antibodies
Paul Bunnell + Monospot
IgM class, not EBV specific
90-98% sensitive in adults
Negative early in infection
May remain positive for up to 6-12 months
10-20% adults and up to 50% young children never develop heterophile Ab (false negative)
3-7% false positive rate due to long-term persistence of Ab
2-3% false positive results in patients with autoimmune diseases
Detected in other mononucleosis illnesses (primary CMV, Hep A, HIV, lymphoma)
Heterophile Ab detection + atypical lymphocytes support lab diagnosis of EBV

EBV Viral capsid antigen
Synthesized late in the lytic cycle
A complex of at least 7 structural proteins and glycoproteins making up the viral capsid.
Incl.
gp125 - major capsid protein
p18 - minor tegument protein

EBV VCA IgG ANTIBODY
- Appears early in primary EBV infection
- 4-7 days after symptoms
- May precede EBV VCA IgM: uncertainty in diagnosis from a single sample
- Usually persists for life
- EBV VCA IgG: acute, convalescent or past phase of infection
- High levels in Burkitt’s lymphoma and NPC

EBV VCA IgM ANTIBODY
- Indicator of recent primary EBV infection
- EBV VCA IgM usually present from 2-4 months after primary EBV infection
- Can be delayed, even absent in a small number of primary EBV infection in adults
- May persist for several months (10% for 6-8 months) after infection
- May re-appear in reactivation of EBV
- Cross-reactivity with other Herpesviruses (VZV, HSV)
- False-negative in other acute viral infections (HIV, Parvovirus B19) and patients with IgM RF.
- False-negative in excess IgG/Co-specific IgG blocking attachment sites
- Reactivation in Hepatitis A infection

Epstein Barr Virus Nuclear Antigen (EBNA)
Complex of at least 6 proteins (EBNA -1, -2, -3A, -3B, -3C & -LP)
EBNA-1 thought to be essential for maintenance of episomal state of EBV in infected cells and binds to the origin of replication
EBNA-1 expressed in all known virus carrying cells; expression may be lost when lytic cycle ensues

EBV EBNA-1 IgG ANTIBODY
Late (latent phase protein) marker of primary EBV infection, although may be present soon after onset of IM
Appears 3-6 months following infection; marks transition from acute to convalescence; indicates past or resolving infection
Peak 3-12 months post infection, declines but remains detectable indefinitely
Up to 6% of infections never develop EBNA-1 IgG antibody (higher in immunocompromised) (Bauer 1994)
In severely immunocompromised patients, EBNA-1 IgG may decline to low or undetectable levels in response to increase in productive EBV replication
EBV EBNA-2 IgG ANTIBODY
- EBNA-2 IgG antibodies appear early in EBV infection
- May be present in up to 30% of individuals at time of onset of disease
- Presence of EBNA-1 IgG and absence of EBNA-2 IgG excludes primary infection
- Ratio of EBNA-1 Abs vs EBNA-2 Abs used for serodiagnosis of EBV reactivation
- No commercial assays for EBNA-2 Ab available

EBV EBNA IgM ANTIBODY
- Indicator of recent primary EBV infection
- EBNA IgM usually present from 2-4 months after primary EBV infection
- May persist for several after infection
- May re-appear in reactivation of EBV
- Cross-reactivity with other Herpesviruses (VZV, HSV)
- False-positive in other acute viral infections (HIV, Parvovirus B19) and patients with IgM RF
- False-negative with excess IgG/co-specific IgG blocking attachment sites

EBV EA (Early Antigen) ANTIBODY
- EA is a complex of proteins only expressed in infected cells undergoing lytic cycle
- Early antigen/diffuse (EA/D) & Early antigen/restricted (EA/R)
- EA/D Abs rise during acute infection and fall to undetectable levels within 3-6 months
- EA/R remain elevated for up to 2 years
- 30-70% of patients with acute EBV develop EA/R and EA/D Abs
- High levels of EA/R detected in Burkitt’s lymphoma
- High levels of EA/D IgG and EA/D IgA in NPC

EBV VCA IgA & EA/D IgA Abs
- Induced during acute primary EBV
- Persistent high levels in NPC
- Negative predictive value/Sensitivity approx. 97%
- Positive predictive value 0.5-2% VCA IgA in NPC (high risk populations)
- Positive predictive value VCA IgA + EA/D IgA in NPC rises to 20%
- Used for screening in very selected groups (middle aged to elderly Southern Chinese with family history of NPC)
- Rising titres indicate progression or relapse

EBV VCA IgG Avidity Index
- Can distinguish recent from past or reactivated infection particularly where VCA IgM persists long-term
- B cells switch from IgM to IgG isotype in vivo; the first IgG Abs produced are of low avidity
- Later IgG Abs mature through somatic hypermutation in the IgG DNA-encoded region and B cell clones end up producing relative higher avidities
- AI: ratio between urea-treated and non-urea-treated sample
- Improved sensitivity for diagnosis from 93.5% to 100%
- AI: 54% at 6 weeks
- AI: 82% at 28 weeks

EBV Western Blot
- Classical lyate blot assays with EBV transformed cells
- Line blot assays with recombinant antigens incl. p72 (EBNA-1), p18 (VCA), p23 (VCA), p54 (EA), p138 (EA)
- Detects EBV specific antibodies to multiple EBV-specific antigens simultaneously
- Useful confirmatory method
OTHER EBV SEROLOGY
- EBV VCA IgA: BL, NPC
- EBV EA (D/R) IgG, IgA: BL, NPC
- EBV Western Blot: Confirmatory method
- EBNA-2 IgG: Primary infection, Reactivation

EBV serology
- Dubbo study: 28 well characterised EBV cases
- Specificity: 30 cases previous EBV & recent HIV, CMV or Hepatitis A (FP EBV serology)

The Dubbo Cohort Study
Serological responses

Serological responses (IgG VCA avidity)

<table>
<thead>
<tr>
<th>Sample</th>
<th>VCA IgG</th>
<th>VCA IgM</th>
<th>EBNA IgG</th>
<th>EBNA IgM</th>
<th>VCA VCA avidity (mean % and SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hap A</td>
<td>15 (100)</td>
<td>12 (80)</td>
<td>15 (100)</td>
<td>12 (80)</td>
<td>66 (5.5)</td>
</tr>
<tr>
<td>HIV</td>
<td>14 (100)</td>
<td>5 (36)</td>
<td>14 (100)</td>
<td>10 (71)</td>
<td>88 (2.8)</td>
</tr>
<tr>
<td>CMV</td>
<td>6 (100)</td>
<td>1 (16)</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>96 (3.2)</td>
</tr>
<tr>
<td>Combined</td>
<td>35 (100)</td>
<td>10 (29)</td>
<td>35 (100)</td>
<td>28 (80)</td>
<td>94.3 (4.4)</td>
</tr>
</tbody>
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VCA IgG (gp125) & EBNA-1 IgG

Age 2 - 50 yrs (n=3317)

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<thead>
<tr>
<th>VCA IgG</th>
<th>EBNA IgG</th>
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<tbody>
<tr>
<td>+</td>
<td>59%</td>
</tr>
<tr>
<td>-</td>
<td>6%</td>
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<tr>
<td>IgG</td>
<td>11%</td>
</tr>
<tr>
<td>-</td>
<td>24%</td>
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</table>
VCA IgG (gp125) & EBNA-1 IgG

**Age 10 - 20yrs (n=552)**

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<thead>
<tr>
<th></th>
<th>VCA IgG</th>
<th>EBNA</th>
<th>IgG</th>
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<tbody>
<tr>
<td>+</td>
<td>53%</td>
<td>6%</td>
<td>15%</td>
</tr>
<tr>
<td>-</td>
<td>26%</td>
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**Age > 50 yrs (n=747)**

<table>
<thead>
<tr>
<th></th>
<th>VCA IgG</th>
<th>EBNA</th>
<th>IgG</th>
</tr>
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<tbody>
<tr>
<td>+</td>
<td>76%</td>
<td>11%</td>
<td>8%</td>
</tr>
<tr>
<td>-</td>
<td>5%</td>
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EBV VCA p18 IgG ANTIBODY

- Highly immunogenic in humans
- Recognised by healthy EBV-seropositive persons worldwide
- Found in ‘most’ EBV carriers (Wout 1993)
- A late marker of EBV infection (Hinderer 1999)
- Not lost during immunosuppression (Bauer 2001)
- Does not appear to have sequence homologues to other human herpesviruses

Previous EBV Infection

53 Samples

<table>
<thead>
<tr>
<th>gp125 IgG</th>
<th>p18 IgG</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg</td>
<td>Pos</td>
<td>52</td>
</tr>
<tr>
<td>Neg</td>
<td>Neg</td>
<td>1</td>
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</tbody>
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Anti EBV VCA p18 in recent infection

VCA IgM pos / EBNA IgG neg (n=32)
12/32 anti p18 neg.

VCA IgM pos / EBNA IgG neg/Avidity<60%. (n=12)
5/12 anti p18 neg.

EBV VCA p18 IgG ANTIBODY

CONCLUSIONS

- EBV VCA p18 IgG EIA appears more sensitive than EBV VCA gp125 IgG EIA (except early acute EBV)
- EBV VCA p18 IgG EIA agrees better than EBV VCA gp125 IgG EIA with EBNA IgG EIA (52/53)
- EBV VCA p18 useful to assist in the determination of EBV immune status
EBV summary

VCA-IgM antibody appears in both primary infection & reactivation of EBV.

VCA IgG antibody appears early in primary infection and should last for life.

Low avidity IgG antibody only appears in primary EBV infection & increases to approx 80% by 6 months.

EBNA IgG antibody appears after about 3 months & should last for life.

The combination of low VCA IgG avidity with positive VCA IgM & negative EBNA IgG is 100% specific for the diagnosis of primary EBV infection.

EBV Serology

- EBV VCA IgG: Negative
- EBV VCA IgM: Negative
- EBNA IgG: Negative
- EBV AI: N/A

No evidence of past infection. If early in course of illness, repeat blood. Suggest EBV VCA p18 IgG.

EBV Serology

- EBV VCA IgG: Positive
- EBV VCA IgM: Negative
- EBNA IgG: Negative
- EBV AI:<60%

Probable acute EBV, suggest repeat

EBV Serology

- EBV VCA IgG: Negative
- EBV VCA IgM: Negative
- EBNA IgG: Negative
- EBV AI: N/A

Early EBV or False Positive
Suggest EBV VCA p18 IgG Ab
Suggest repeat blood
EBV Serology

- EBV VCA IgG: Positive
- EBV VCA IgM: Negative
- EBNA IgG: Negative
- EBV AI: >60%

Probable past infection

EBV Serology

- EBV VCA IgG: Positive
- EBV VCA IgM: Negative
- EBNA IgG: Positive
- EBV AI: >60%

Past EBV

EBV Serology

- EBV VCA IgG: Negative
- EBV VCA IgM: Negative
- EBNA IgG: Positive
- EBV AI: N/A

Probable past infection

EBV Serology

- EBV VCA IgG: Positive
- EBV VCA IgM: Positive
- EBNA IgG: Positive
- EBV AI: >60%

Past infection (≥3 months)

EBV Serology

- EBV VCA IgG: Positive
- EBV VCA IgM: Positive
- EBNA IgG: Negative
- EBV AI: <60%

Acute EBV