Epstein-Barr virus (EBV) is a herpes virus that infects predominantly human B cells and is one cause of mononucleosis like symptoms. The rapid Mono Spot test detects a heterophile antibody – a crossreacting IgM antibody which is not specific to EBV itself. It is reasonably sensitive in older children but is not reliable in younger children, particularly those under 5 years. Therefore CMH offers testing to antibodies to EBV itself: IgM and IgG to the viral capsid antigen (VCA), IgG antibody to early antigen (EA) and IgG antibody to the EBV nuclear antigens (EBNA). The pattern of reactivity of a single acute serum can usually determine if the patient has never been infected (currently susceptible), has had a recent primary infection (may explain the current symptoms), or has recovered from an infection in the past (unlikely to be associated with current symptoms). Reactivation of EBV occurs frequently but rarely causes clinically apparent symptoms. Chronic active EBV infection is very rare and remains a controversial topic.

IgM to the VCA appears early in infection and disappears in 4-6 weeks. IgM to VCA may be difficult to detect in children < 4 years of age. IgG to VCA appears in the acute phase, peaks at 2-4 weeks, declines slightly but persists for life. IgG to EA appears in the acute phase and generally falls to undetectable levels in 3-6 months, but persists in ~ 20% of patients for an extended period of time. Antibody to EBNA is not seen in the acute phase but slowly appears over 2-4 months and persists for life.

A primary infection is best characterized by the presence of IgM and/or IgG to VCA and the absence of IgG to EBNA. There will be a rising or high level of IgG to VCA over the first month or two. Antibodies to EA will also be present in most patients.

A past infection is characterized by the presence of IgG to EBNA and the absence of IgM to VCA. IgG to VCA will also be present. Antibodies to EA may or may not be present.

Reactivation of EBV is characterized by prolonged persistence of antibodies to EA and EBNA or a rise in antibody to EA and a drop in antibody to EBNA. The relationship of these antibody patterns to chronic active infection is controversial. Some experts do not believe that chronic symptoms (> 6 months) can be due to chronic active EBV infection.

The CMH Immunology Laboratory has acquired a new instrument, the Mago Plus, made by Diamedix which we will use to do EBV serology (also called antibodies or titers) testing in a different manner. The reports of results will look different. Furthermore, after consultation with the Section of Infectious Diseases, we have decided to make changes in the EBV panel. Antibodies to EA will not be included in the routine panel but will be available as an independent order. This newsletter will discuss these changes.

In the past we tested for EBV antibodies using indirect immunoflourescence (IF) on cells. This was a manual, multi-step assay read subjectively. Because of the complexity and amount of work involved we were only able to run this test once a week.

The new assay is automated (done by the instrument). Genetically synthesized EBV antigens (VCA, EA or EBNA) are bound to microwells, patient serum is added and antibody, if present, will bind to the antigen. After washing, anti-human IgG (or anti-human M) is added and binds to the antibody to EBV if present. The resultant complex is visualized in a manner which permits spectrophotometer reading as an absorbance.
Results will be reported differently. Titers will not be reported. An Index Value, discussed below, will be reported for all tests. The absorbance of the patient’s specimen is compared to the mean absorbance of a cut-off calibrator:

\[
\text{Absorbance of Sample} = \text{Index} \quad \text{Index Value:} \quad < 0.90 \quad \text{No Detectable Antibody} \\
\text{Mean Absorbance of} \quad 0.9-1.09 \quad \text{Equivocal} \\
\text{Cut-Off Calibrator} \quad >1.09 \quad \text{Detectable Antibody}
\]

While the magnitude of the Index Value does not correspond to a titer and is not a direct indication of the amount of antibody present, high is still high and low is still low. A change from “No Antibody Detected” to “Detectable Antibody” is considered seroconversion even if the Index value shows a minimal change. When both specimens have an Index Value interpreted as “Detectable Antibody,” a 1.5 -2.2 fold increase in the Index Value corresponds to ~ 4 fold increase in antibody level. The maximum Index Value is ~ 7. In our preliminary studies the Index Values ranged from 0.17 to 8.39. Clinical correlation has been excellent.

**Reporting Format**

<table>
<thead>
<tr>
<th>Current IF Assay</th>
<th>New Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM anti VCA</td>
<td>&lt; 1:10, &gt; 1:10 Index (see above)</td>
</tr>
<tr>
<td>IgG anti VCA</td>
<td>Titer Index (see above)</td>
</tr>
<tr>
<td>IgG anti EA</td>
<td>Titer Index (see above)*</td>
</tr>
<tr>
<td>IgG anti EBNA</td>
<td>&lt; 1:10, &gt; 1:10 Index (see above)</td>
</tr>
</tbody>
</table>

* IgG to EA will NOT be in Routine Serology (Antibodies) Panel. In the past the standard EBV serology panel consisted of the 4 antibodies listed above. After a review of the results and consultation with the Infectious Disease Section, we have decided to remove IgG anti EA from the panel. It will be orderable as a separate test. IgG to EA can be found in patients who are in the acute phase of the disease and have recovered. IgG to EA can sometimes just add confusion so it will no longer be in the standard panel.

The new assay will be easier to perform and, therefore, we plan to perform it twice a week. You will be notified by Meditech email when the changes will be implemented.

Reference: [www.cdc.gov/ncidod/diseases/ebv/htm](http://www.cdc.gov/ncidod/diseases/ebv/htm)