Serological studies have shown that the clinical onset of NPC is preceded by the appearance of a high antibody titer of IgA to viral capsid antigens and early antigens. The titers increase with the total tumor burden and the antibodies decline with the response to therapy. In patients with confirmed clinical remission elevation of IgA serological titers is highly significant for prediction of relapse 7,8,9.

PRINCIPLE OF THE TEST

Purified EBV-VCA antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the EBV-VCA IgA specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgA specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

MATERIALS PROVIDED

1. Microwell strips: EBV-VCA antigen coated wells.  (12 x 8 wells)
2. Absorber solution: Black cap.  1 vial (22 ml)
3. Washing concentrate 10x: White cap.  1 bottle (100 ml)
4. TMB chromogenic substrate: Amber bottle.  1 vial (15 ml)
5. Enzyme conjugate: Red color solution.  1 vial (12 ml)
6. Negative control: Range stated on label.  Natural cap.  1 vial (150 µl)
7. Cut-off calibrator: Yellow cap.  IgA index = 1  1 vial (150 µl)
8. Positive control: Range stated on label.  Red cap.  1 vial (150 µl)
9. Stop solution: 2 N HCl.  1 vial (12 ml)

STORAGE AND STABILITY

1. Store the kit at 2-8°C.
2. Always keep microwells tightly sealed in pouch with desiccants. We recommend you use up all wells within 4 weeks after initial opening of the pouch.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light during storage or usage.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found nonreactive for HIV, Hepatitis B virus, and other infectious agents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus, or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control / National Institutes of Health manual, “Biosafety in Microbiological and Biomedical Laboratories.” 1984

2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION AND HANDLING

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2 - 8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.

**PREPARATION FOR ASSAY**

1. Prepare 1x washing buffer. Prepare washing buffer by adding distilled or deionized water to 10x wash concentrate to make a final volume of 1 liter.
2. Bring all specimens and kit reagents to room temperature (20 - 25°C) and gently mix.

**ASSAY PROCEDURE**

1. Place the desired number of coated strips into the holder.
2. Prepare 1:20 dilutions by adding 10 µl of the samples, negative control, positive control, and calibrator to 200 µl of absorbent solution. Mix well.
3. Dispense 100 µl of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 µl of absorbent solution in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
4. Remove liquid from all wells. Repeat washing three times with washing buffer.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
7. Dispense 100 µl of TMB Chromogenic Substrate to each well and incubate for 30 minutes at room temperature.
8. Add 100 µl of 2 N HCl to stop reaction. Make sure there are no air bubbles in each well before reading.
9. Read O.D. at 450 nm with a microwell reader.

**CALCULATION OF RESULTS**

1. Calculate the mean of duplicate calibrator value x_c.
2. Calculate the mean of duplicate positive control, negative control, and patient samples.
3. Calculate the EBV-VCA IgA Index for each determination by dividing the mean values of each sample by calibrator mean value, x_c.

Example of typical results:
- Calibrator O.D. = 0.718, 0.704 x_c = 0.711
- Patient sample O.D. = 0.991, 0.956 x_s = 0.974
- EBV-VCA IgA Index = 0.974 / 0.711 = 1.37

**QUALITY CONTROL**

The test run may be considered valid provided the following criteria are met:
1. If the O.D. value of the reagent blank against air from a microwell reader should be less than 0.150.
2. If the O.D. value of the calibrator is lower than 0.250, the test is not valid and must be repeated.
3. If the O.D. value of the patient sample is lower than 0.050, the sample is considered negative.
4. The EBV-VCA IgA Index for negative and positive control should be in the range stated on the labels.

**INTERPRETATION**

Negative: EBV-VCA IgA Index of 0.90 or less is seronegative for IgA antibody to EBV-VCA virus.
Equivocal: EBV-VCA IgA Index of 0.91 - 0.99 are equivocal. Sample should be retested.
Positive: EBV-VCA IgA Index of 1.00 or greater.

**PERFORMANCE CHARACTERISTICS**

Histogram:

516 random samples are determined with DIAGNOSTIC AUTOMATION Microwell ELISA EBV VCA IgA. The test results are computed as IgA Index using a chosen reference serum as IgA Index 1. The distribution of frequency versus IgA Index value is presented as following:

**REFERENCES**

8. Shimakage M; Dezawa T; Chatani M. Proper use of serum antibody titers against Epstein-Barr virus in nasopharyngeal carcinoma: IgA / virus capsid antigen for

### SUMMARY OF ASSAY PROCEDURE

<table>
<thead>
<tr>
<th>Step</th>
<th>(20-25°C Room temp.)</th>
<th>Volume</th>
<th>Incubation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample dilution 1:20 = 10 µl / 200 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Diluted samples, calibrator &amp; controls</td>
<td>100 µl</td>
<td>30 minutes</td>
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<tr>
<td>3</td>
<td>Washing buffer (3 times)</td>
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<tr>
<td>4</td>
<td>Enzyme conjugate</td>
<td>100 µl</td>
<td>30 minutes</td>
</tr>
<tr>
<td>5</td>
<td>Washing buffer (3 times)</td>
<td>350 µl</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>TMB Chromogenic Substrate</td>
<td>100 µl</td>
<td>30 minutes</td>
</tr>
<tr>
<td>7</td>
<td>Stop solution</td>
<td>100 µl</td>
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</tr>
<tr>
<td>8</td>
<td>Reading OD 450 nm</td>
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**Date Adopted**

<table>
<thead>
<tr>
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<td>DA-EBV,VCA IgG-2008</td>
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**ISO 13485-2003**

Revision Date: 5/14/08