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Section: <b>Serology Manual</b>	Subject Title: <b>Epstein Barr Virus Serology</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: March 14, 2001	
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## **EBV VCA IgG**

### **I. Introduction**

This test is used to detect the presence of EBV Viral Capsid Antigen (VCA) IgG antibodies in human serum using an ELISA system. In general, antibodies to EBV VCA appear within the first week after infection. The presence of EBV VCA IgG antibodies may indicate recent or past infection with EBV.

### **II. Specimen Collection and Processing**

- a. Approximately 10 ml of blood is collected in a red-topped tube.
- b. Centrifuge the tube at 2000 rpm for 10 minutes.
- c. Transfer serum into 2 ml storage tube and store in designated rack in 4° C fridge.

### **III. Procedure**

Behring 2000 Analyzer

#### **i) Reagents:**

#### **From EBV Kit (OW1S 15)**

1. Sample Buffer.
2. Working Conjugate Solution: 0.25 ml of Anti-Human IgG into 1 bottle of 12.5 ml Conjugate Buffer.
3. Anti EBV Reference P/N.

From Supplementary Reagent Kit ( OUV P 17):

4. Wash Solution: 100 ml of Wash Solution + 1900 ml of distilled H<sub>2</sub>O. Store at 4°C. Fill up Wash Solution in Behring 2000 when needed
5. Working Chromogen Solution: 1 ml of chromogen TMB + 10 ml of Buffer/ Substrate TMB. Rinse and keep container when test is done.
6. Stop Solution.

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**ii) Method:**

1. Turn hard drive, and Behring 2000 Analyser 'ON'.
2. Double click on ' Short Cut to BEP 2000'.
3. Enter Technologist's initials, click on 'Don't know Password'.
4. The machine will start 'Initialization' and check all instrument functions.  
The results of this check are then displayed on the screen.
5. Check DH<sub>2</sub>O and Buffer level by opening the bottom part of the Analyzer.
6. Perform 'Washer Assay 'every **morning**:
  - a. Click on ' New Worklist '
  - b. Click on ' Add Plate'.
  - c. Click on ' Add Assay'.
  - d. Highlight ' DB-Washer.asy', click on 'Open', click 'OK'.
  - e. 'Lot specific values for plate 1', click 'OK'.
  - f. Click on 'Green arrow' to start.
  - g. 'LOAD', click 'OK'.
  - h. '? Are you sure that the resources have been loaded in the correct positions? Click ' Yes'.
  - i. 'Load Plate', load a **clean** microtiter plate into a metal tray carrier. Open the door on then far right, and load the carrier into Behring 2000, the two pins on the right side of the carrier should fit into the two rings in the Behring 2000. Close door. Click 'OK'.
  - j. 'Are you sure plate layout correct?', click 'Yes'
  - k. 'Result already exist. Replace?', click 'Yes'.

When finished, 'Remove plate and carrier from system', remove plate, and visually check strips 1-6 for equally distributed liquid; Strips 7-12 for complete aspiration. click 'OK'. Discard buffer in sink and rinse plate wit DH<sub>2</sub>O, air-dried

7. Perform' Validation Assay 'every **Friday**:
  - a. Remove Reagent Rack #5, and re-load into analyzer.
  - b. Remove Reagent Rack # I. Open lids and stoppers from Reference solution A, B, and C. and Load into Rack #1. Load Reagent Rack # I into Analyser.
  - c. Remove Control Rack ' R', and re-load into Analyser.
  - d. Click on ' New Worklist '
  - e. Click on ' Add Plate'.
  - f. Click on ' Add Assay'.
  - g. Highlight ' DB-Validation.asy', click on 'Open'.
  - h. 'Set Up Panel', click 'OK'.

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- i. “ Lot specific vales for plate 1’, click ‘OK’, unless a new lot #.  
If this is a new lot, just highlight the area that needs changes, and type in new Value for: lot #, upper margin, lower margin,  $\alpha$ , and  $\beta$ , and ‘save’.
  - j. Click on ‘Green arrow’ to start.
  - k. ‘ Load’, click ‘OK’.
  - l. ‘ ? Are you sure that the resources have been loaded in the correct position’, click ‘Yes’.
  - m. ‘Load Plate’, load a **NEW** microtiter plate (clear one with strips) into a metal carrier. Open the door on the far right, and load the whole tray into Behring 2000, the two pins on the right side of the carrier should fit into the two rings in the Behring 2000.
  - n. Under ‘Plate 1’, enter ‘ **validyear/month/date**’ e.g. **valid030106**. Click ‘OK’.
  - o. ‘Are you sure plate layout correct?’, click ‘ Yes’.
  - p. When finished, remove plate and carrier from system’ appears, click ‘OK’. Remove plate and visually check strips 9-10 for equally distributed liquid, strips 11-12 for complete aspiration.
  - q. Print report and checked that ‘ V.C. Passed ‘ printed on right top corner of the report. File report in ‘Behring 2000 Validation Assay ‘ binder.
8. Perform testing:
- a. Remove Reagent Rack #5. L-load Sample Buffer, Working Chromogen Solution, and Stop Solution into rack, and re-load into Analyzer.
  - b. Remove Reagent Rack # I . Load Working Conjugate Solution into rack, and reload into Analyser.
  - c. Remove Controls Rack ‘ R’. Load Anti-EBV Reference P/N into rack, and re-load into Analyser.
  - d. Load 1<sup>st</sup> rack of specimens (1-20) into Analyser with bar-codes facing the Bar-code reader. Do the same for the subsequent rack(s) (21-40, 41-60).
  - e. ‘Patient Editor’ will appear with the specimen #s. Check that specimen # s are correct .If there is a blank space, click on it and enter the # manually, use ‘TAB’ to move to another row. Click on the column header, highlight ‘DBA EBV-G’. Click on the first space and hold , then drag the mouse down the whole column.
  - f. A ‘√’ (check mark) will appear beside each specimen. Click ‘ Close’ to save.
  - g. If there is a next rack of specimens, the next page of specimens will appear shortly. Repeat step ‘e’.

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- h. Click on 'New Worklist'.
- i. Click on '+' beside Plate 1 : EBV IgG appears.
- j. Click on folder beside EBV IgG.
- k. 'Plate format': will also show the total # of specimens to be tested and # of strips needed. Load EBV stripes onto the plate. Load plate onto plate carrier.
- l. Click 'OK'.
- m. 'Lot Specific Values For Plate 1'. Check the Lot #. Click 'OK'.
- n. Click on the green→. Initialization will start immediately.
- o. 'LOAD'. Load a Dilution Plate into top left hand corner. . If you hover the mouse around the circle ,it will tell you that this is the Anti-EBV Reference P/N. Click on the circle, and drag it over to the correct position on the Rack 'R'.  
(Make sure that the arrow is pointed at the center of the square/circle).

If a 'Grey Pipette tip box ' appears, it is to load a new set of tips into that space. (Noted the size of tip required: Yellow for 300ul, Grey for 1100 ul.)Load tips.

Once finished with loading, click 'OK'.

- p. 'Are you sure that The Resources have been loaded in the correct positions? ', click 'Yes'.
- q. The analyzer will check the levels of the reagents.
- r. 'LOAD PLATE' appears. Under '**Plate ID**', enter **EBVyear/month/date (EBV030106)**.
- s. Load plate carrier into Analyser. Click 'OK'.
- t. 'Are you sure plate layout is correct?', click 'OK'.
- u. 'Downloading Progress' will appear, and then the 'Orange square' will also appear , the testing will start.
- v. To see what stage the test is at: close the inside window on the screen. Click on "+" beside 'Work 2'.
- w. Click on 'Schedule'. You can see the red line move across the screen with each step.
- x. When finished, 'Remove plate & Carrier from the system', remove plate and click 'OK'.
- y. Click on 'Print Report'.
- z. Re-capped all reagents, and place the whole kit in the refrigerator.

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**iii) Validation:**

Each pair of Reference P/N wells must reach or exceed a value of 0.5  $\Delta A$ :  
 $\Delta A$  Reference P/N  $\geq 0.5$

**iv) Interpretation of Results:**

Positive:  $\Delta A$  sample  $> 0.200$

Negative:  $\Delta A$  sample  $< 0.100$

Equivocal:  $0.100 \leq \Delta A$  sample  $\leq 0.200$

**v) Reporting**

**Positive by EIA**

**Negative by EIA**

**Equivocal by EIA**

**vi) Quality Control**

A reference P/N control must be run at the start and at the end of the each run.

Run External Control Lot 20030509(pooled positive sera) with each new lot.

If external control result is negative, the test is invalid. Inform Charge/senior technologist, and repeat testing. Results filed in Reagent Lot Binder.

CAP provides external proficiency testing.

**vii) Reference**

Package insert from Dade-Behring,,Enzygnost Anti EBV/IgG,May,1999.