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Section: <b>Serology Manual</b>	Subject Title: <b>BIO-RAD HBsAg</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: January 5, 2004	
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## HBsAG & HIV 1/2

### **I. Introduction**

Genetic Systems™ HBsAg EIA 2.0 is a qualitative enzyme immunoassay for the detection of Hepatitis B Surface Antigen (HBsAg) in human serum and plasma, and also in cadaveric serum specimens.

Genetic Systems™ HIV-1/HIV-2 Peptide EIA 2.0 is the Genetic Systems Corporation qualitative enzyme immunoassay for the detection of Human Immunodeficiency Virus type 1 and /or Human Immunodeficiency Virus type 2 in human serum and plasma, and also in cadaveric serum specimens.

These kits are only used for postmortem specimens forwarded from the following Eye Banks:

- a. Eye Bank of Canada ( Ontario Division).
- b. New Brunswick Eye bank.
- c. Lions Eye Bank Manitoba & Northwest Ontario  
and  
Tissue Lab (Hospital For Sick Children).

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

- a. Blood or serum collected by Eye Banks
- b. If blood tube received, centrifuge at 3000 rpm for 10 minutes.  
Transfer approximately 1-1.5 ml serum into 2 ml storage tube, give blood tube/serum to AXSYM bench for HCV Ab testing.

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### III. Procedure

#### **P-Lab Analyzer**

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

- |                                     |  |
|-------------------------------------|--|
| 1. HBsAg Conjugate Concentrate      | 11. HIV ½ Peptide coated<br>Microwell Plates |
| 2. HBsAg Cojugate Diluent           | 12. HIV ½ Peptide Specimen<br>Diluent        |
| 3. AntiHBsAg Microwell Strip Plates | 13. HIV1/2 Peptide Conjugate<br>Concentrate  |
| 4. HBsAg Negative Control           | 14. HIV ½ Peptide Conjugate<br>Diluent       |
| 5. HBsAg Positive Control           | 15. HIV 1 Positive Control                   |
| 6. HBsAg Low Positive Control       | 16. HIV 2 Positive Control                   |
| 7. EIA Wash Solution                | 17. HIV Negative Control                     |
| 8. EIA Chromogen Reagent            |  |
| 9. EIA Chromogen diluent            |  |
| 10. EIA Stopping Reagent            |  |

##### **ii) Method:**

HBsAg & HIV ½ can be run simultaneously in the P-Lab analyzer.

- Before beginning EIA assay:**  
Allow all components to reach room temperature.  
Prepare wash solution:  
Add 100 ml of 25x wash solution to 2400 ml of distilled water.  
Dilute each of HIV 1 Positive Control ,HIV2 Positive Control and Negative Control 1:10 in a test tube: 50 ul control + 450 ul of HIV ½ specimen diluent.  
Fill up P-Lab wash bottle. Place bottle in 'Buffer #1' position in P-Lab.
- Turn hard drive and monitor. Wait for software to be fully loaded before turning the P-Lab 'ON'.
- Type in 'Qw' as username and password, 'Enter'.
- Click on third square from the left: '**open session**'.. Click on '**New**',  
Type in assay & date performed, e.g. Biorad 040528(BioRad tested May 28,04). Note session # e.g. '31'. Click on writer Name, enter initial.  
Click 'OK'.
- The screen will show e.g. **BioRad0400528#31**.ssn highlighted , click 'OK'.
- Click on 5 th square from the left: '**Profile Include**', click on 'BioRad HIV no Dilution #2 prf',click 'OK'.
- Click on 7th square from the left : '**Sample programming**'.  
First click on '**Clear sample rack**', then click on sample code, and enter lab # ,and load specimens onto specimen rack at the same time. Also

load second half of the rack with dilution tubes( only for HIV ½ only).

Once finished, double click on ‘Small Rack’ icon in top left corner,

“X’s should appear next to all lab #s, click ’done’.

8. The screen will show plate format ( # of wells needed). Take out the required # of strips and wells. Load HBsAg plate onto the **left** side in the P-Lab, HIV plate onto the right side in the P-Lab. Click on ‘done’.
9. Click on 8 th square from the left : ‘**Setup Entry**’. Click on HBsAg, then Click on pulldown arrow under ‘Lot’.. If it is a new lot, Click on ‘Lot #’,type in new lot #.Then click on‘**Change Date**’ and specify kit expiration date if needed (always change year first). Then do the same for HIV.Click ‘OK’.
10. Click on last square ‘ **Start Session**’, ‘Save document: using the new file...’will appear, click ‘OK’.
11. **Profile-vial locations for controls or standards and reagents’** will appear. Right click on each colored position, will show where to load each diluent, controls, conjugate, substrate and stop solution. Click ‘continue’, and wait for instrument initialization.

<u>Position</u>	<u>Reagent &amp; Controls</u>
R3	HIV Sample Diluent
R4	HIV Working Conjugate
R5	HBsAg Working Conjugate
R6	Working Chromogen
R7	Stop Solution
2	Diluted HIV 1 Positive Control
4	Diluted HIV 2 Positive Control
6	Diluted HIV Negative Control
7	HBsAg high Positive Control
8	HBsAg Low Positive Control
9	HBsAg Negative Control

Prepare **Fresh (same Day) Working Conjugate** as follows (always make 1 extra strip):

Number of Strips	40X conjugate(ul)	Conjugate Diluent(ml)
1	10	1.0
2	20	2.0
3	30	3.0
4	40	4.0
5	50	5.0

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Prepare **Fresh (Same Day) Working Chromogen** as follows (always make 1 extra strip):

Number of Strips	Chromogen Reagent(ul)	Chromogen Diluent(ml)
1	10	1.0
2	20	2.0
3	30	3.0
4	40	4.0
5	50	5.0

11. Click on 2<sup>nd</sup> square from the left ' **Maintenance** '. Click on ' **Start** ' to start up ' **Self Test** '. Machine will do all checks, then asks: ' **Print Self Test report** ', click on ' **Yes** '. Staple printout with day list.
12. Click on ' **Daily Maintenance** ' **Tab**: click on ' **Fill syringes** ', check for bubbles in syringes, and if no bubbles present, click ' **No** '.
13. Click on ' **Fill lung** ', screen will ask you to open lid. Open lid, and click on ' **OK** '. Water level should fill up to just under the lower black line. Click 1-2 times to Add 250ul of water each time to raise the level to just above the lower black line. Close lid and click on ' **OK** '.
14. Click on ' **Buffer 1 prime** ', listen to the noise to make sure that the vacuum is working.
15. Click on 3<sup>rd</sup> square ' **Tips** ' if need to replace pipette box.  
Click on 6<sup>th</sup> square from the left to ' **execute** ' the run, will ask to put sample rack in, click ' **OK** '. The P-lab will start processing. Click on ' **Time chart** ' to see what stages the testing is at.
16. After assay is finished, " **Session terminated** " will appear on screen, click ' **OK** '.
17. Click on ' **Maintenance** ', and click on " **Endwork** ". Will ask to fill in buffer 2 with DH<sub>2</sub>O, open lid, make sure there is enough DH<sub>2</sub>O in the bottle, also change buffer 1 bottle to DH<sub>2</sub>O bottle. Click ' **OK** '. The machine is rinsing the tubing with DH<sub>2</sub>O. Next will ask to ' **empty waste** ', press on " **RED Button** " on the side of the machine, and hold it until almost all fluid is drained off and bubbles appear.  
Empty waste. Click ' **OK** '. ' **Empty tips** ', click ' **OK** '. ' **Don't forget to shut off P-Lab** ', click ' **OK** '.
18. Click on left top corner ' **File** ', and ' **Exit** ', will ask ' **exit from P lab Processor?** ' click ' **Yes** '.
19. Click on 4<sup>th</sup> box from the top : ' **Open Results** '. Click on ' **New Result** '. Then click on your session and ' **OK** '.

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20. Click on 3<sup>rd</sup> box from the left, ‘ **report**’, click on ”HBsAg’, and also the square with the ‘**printer**’. Click on ‘HIV ½’, and also the square with the ‘**printer**’. Report will be printed out.
21. Close all windows by clicking on ‘**X**’ at top right corner three times.
22. Turn off hard drive, monitor, P-lab and printer.

#### iv) **Validation**

##### **HBsAg**

1. Calculate the Mean Absorbance of the Negative Control(NCX):  
Sum of the absorbance values of the three negative controls divided by three .  
Each individual absorbance must be >0.000 AU ,and < or = to 0.100 AU. One Negative Control value may be discarded if it is outside this range.The NCX may be calculated from the two remaining absorbance values
2. Calculate the Mean Absorbance of the Positive Control(PCX):  
Sum of the absorbance values of the two Positive controls divided by two .  
The PCX must be > or = to 1.00 AU.  
Each individual absorbance must be within the range of 0.65 to 1.35 times the PCX.  
No Positive Control absorbance value may be discarded.
3. Calculate the Mean Absorbance of the Low Positive Control(LPCX):  
Sum of the absorbance values of the two Low Positive controls divided by two.  
The LPCX must be Positive, > or = to the assay Cut-off value.  
Each individual absorbance must be within the range of 0.65 to 1.35 times the PCX.  
No Positive Control absorbance value may be discarded.
4. Cut off value =NCx (negative control mean)+0.70.

##### **HIV ½**

5. Calculate the Mean Absorbance of the Negative Control(NCX):  
Sum of the absorbance values of the three negative controls divided by three .  
Each individual absorbance must be >0.020 AU ,and < or = to 0.140 AU. One Negative Control value may be discarded if it is outside this range.The NCX may be calculated from the two remaining absorbance values
6. Calculate the Mean Absorbance of the HIV 1Positive Control(HIV-1 PCX):  
Sum of the absorbance values of the two HIV-1 Positive controls divided by two .  
The HIV-1PCX must be > or = to 0.900 AU.  
Each individual absorbance must be within the range of 0.65 to 1.35 times the PCX.  
No Positive Control absorbance value may be discarded.

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7. Calculate the Mean Absorbance of the HIV-2 Positive Control(HIV-2 PCX):  
Sum of the absorbance values of the two HIV-2 Positive controls divided by two.  
The HIV-2 PCX must be Positive, > or = to 0.700.  
Each individual absorbance must be within the range of 0.65 to 1.35 times the HIV-2 PCX.  
No Positive Control absorbance value may be discarded.
8. Cut off value = NCx (negative control mean)+0.240.

If any control fails, the test is invalid. Should inform Senior/Charge technologists and repeat testing.

**v) Interpretation of Results:**

1. Absorbance value is < cut off: reported as Negative.
2. Absorbance value is > or =cut off: send to PHL for confirmation.

**vi) Quality Control:**

Two Positive Controls, two Low Positive Controls, and three Negative Controls must be included with each HBsAg run.

Two HIV-1 Positive Controls, two HIV-2 Positive controls, and three Negative Controls must be included with each HIV1/2 Run

Run Accurun 5100 as external control for both HBsAg and HIV ½ with each new lot. Result entered in Reagent Lot Binder.

All positive specimens are sent to PHL for confirmation.

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**vii) Reference:**

Package insert from Bio-Rad Human Immunodeficiency Virus Types 1 and 2(synthetic Pepide).  
Revised September 2000.

Package insert from Bio-Rad Human Immunodeficiency Virus Types 1 and 2(synthetic Pepide).  
Revised September 2000.

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