

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/v11</b>	Page 1 of 2
Section: <b>Serology Manual</b>	Subject Title: <b>Table of Contents</b>	
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**SEROLOGY MANUAL**  
**TABLE OF CONTENTS**

**SEROLOGY TESTS:**

C. difficile TOXIN A/B .....	3
AXSYM System for HBsAg, HBsAb, HBeAg, HBeAb, HBcAb-IgM, HBcAb-Total, HAV-IgM, HCVAb, CMV IgG, HIV-1/2, Rubella IgG.....	8
AXSYM HEPATITIS B SURFACE ANTIGEN CONFIRMATORY ASSAY .....	17
HEPATITIS VIRUS SEROLOGY .....	21
HBsAG & HIV 1/2.....	30
EBV VCA IgG .....	37
HUMAN T-LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1) EIA .....	42
INFECTIOUS MONONUCLEOSIS HETEROPHILE ANTIBODIES .....	47
SYPHILIS SCREENING.....	49
VARICELLA-ZOSTER VIRUS IgG .....	52
WEST NILE ( FLAVIVIRUS) IgG.....	56
WEST NILE (FLAVIVIRUS) IgM .....	61
<b>MOLECULAR TESTS:</b>	
CHLAMYDIA TRACHOMATIS AND NEISSERIA GONORRHOEAE.....	66
HEPATITIS B (HBV) DNA.....	72
HEPATITIS C RNA PCR.....	77
WEST NILE VIRUS RT PCR.....	84

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/v11</b>	Page 2 of 2
Section: <b>Serology Manual</b>	Subject Title: <b>Table of Contents</b>	

**APPENDICES:**

Appendix I Serology Test Schedule .....94

Appendix II - Send-Outs.....96

Appendix III Shipment of Parvovirus PCR Samples.....98

Appendix IV The Thermo-Cycler Gene Amp PCR System 9600.....99

Appendix V Entering Serology Refer-Out Results .....104

Appendix VI BDProbeTec ET Workflow.....107

Appendix VII Autoverification Process.....109

Appendix VIII Autoverification for the Probetec.....112

Appendix IX Shipment of Parvovirus PCR to HSC .....114

Appendix X Posting of AxSYM results.....115

Appendix XI Looking Up Previous Hepatitis Results in Vista .....116

Appendix XII Printing of Pending List..... 117

Appendix XIII Wipes Test for ProbeTec® ..... 118

Record of Edited Revisions..... 119

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/01/v01</b>	Page 1 of 5
Section: <b>Serology Manual</b>	Subject Title: <b><i>C. difficile</i> Toxin A/B EIA Test</b>	
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### ***C. difficile* TOXIN A/B**

#### **I. Introduction**

*C. difficile* Tox A/B is an enzyme immunoassay for the detection of toxins A and B produced by toxigenic strains of *C. difficile*. It can be used to detect toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease.

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

Collect stool specimen in clean Starplex container, and send to the Virology laboratory as soon as possible.

Stool collected in Enteric Transport Medium, or in SAF is not suitable for this assay.

- a. Dispense 600 ul of diluent into 2 ml microtube.
- b. Add 150 ul of liquid stool (mix stool well first) or approx. 6 mm diameter of solid stool.
- c. Vortex for 10 seconds, spin for 5 minutes at # 14 in Eppendorf microcentrifuge. Load onto Behring 2000 Analyser sample rack according to worklist for testing.

#### **III. Procedure**

Behring 2000 Analyzer

##### **i) Reagent Preparation:**

1. Prepare Behring Wash Solution as needed. e.g. 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.
2. Prepare *C. difficile* working buffer: 50 ml of 20X Wash Buffer Concentrate + 950 ml of D H<sub>2</sub>O. Store at 4 °C. Fill up *C. difficile* working buffer in Behring 2000 Analyser when needed.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/01/v01</b>	Page 2 of 5
<b>Serology Manual</b>		

3. Pour Conjugate into Behring medium -sized container and labeled as 'Conjugate'.  
 Pour Substrate into Behring large-sized container and labeled as 'Substrate'.  
 Pour Stop Solution into Behring medium-sized container and labeled as 'Stop Solution'.  
 All these reagents can be stored in the respective containers in 4<sup>0</sup>C refrigerator.  
**Use new containers for each new Lot #.**
4. Bring microwell pouch and the above mentioned containers to room temperature before use.
5. Empty waste, change D H<sub>2</sub>O and clean control bottles / replace with new controls every Friday.

**ii) Assay 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: see EBV testing, page 21,step 6.**
7. Perform 'Validation Assay' every **Friday: see EBV testing, page 21,step7.**

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/01/v01</b>	Page 3 of 5
<b>Serology Manual</b>		

8. Performing testing:
  - a. Remove Reagent Rack #5, and re-load into analyzer.
  - b. Remove Reagent Rack # I, and re-load Reagent Rack # I into Analyzer.
  - c. Remove Control Rack 'R' and re-load into Analyzer.
  - d. Load 1<sup>st</sup> rack of specimens (1-20) into Analyzer 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 'C.diff toxin'. Click on the first space and hold , then drag the mouse down the whole column. A '✓' (check mark) will appear beside each specimen. Click 'Close' to save. If there is a next rack of specimens, the next page of specimens will appear shortly. Repeat step 'e'.
  - f. "Lot specific vales for plate 1', click 'OK', unless a new lot #. If it is a new lot #, just highlight the lot #, type in new lot #. Do the same for expired date.
  - g. 'Plate format': will also show the total # of specimens to be tested and # of wells needed. Load *C. difficile* wells onto the plate including controls. Fill in unused space with blanks. Load plate onto plate carrier.
  - h. Click 'OK'.
  - i. Click on the green→. Initialization will start immediately.
  - j. 'LOAD': Purple squares and circles represent Reagents/controls. If you hover the mouse around the squares or circles, it will tell you what it is. Click on the Square, and drag it over to the correct position on the Rack. (Make sure that the arrow is pointed at the center of the square/circle). Chromogen (Substrate) is in the Rack #5, Conjugate & Stop Solution are in Rack # I, and Controls are in Rack # R. If a 'Grey/Green Pipette tip box' appears, it is to load a new set of tips into that space. (Noted the size of tip required: Yellow for 300 µl, Grey for 1100 µl). Load tips. Once finished with loading, click 'OK'.
  - k. 'Are you sure that The Resources have been loaded in the correct positions?', Click 'Yes'.
  - l. The analyzer will check the levels of the reagents.
  - m. 'LOAD PLATE' appears. Under '**Plate ID**' enter **cd year/month/date (cd030106)**.
  - n. Load plate carrier into Analyser. Click 'OK'.
  - o. 'Are you sure plate layout is correct?', click 'OK'.
  - p. 'Downloading Progress' will appear, and then the 'Orange square' will also appear, the testing will start.
  - q. To see what stage the test is at: close the inside window on the screen. Click on "+" beside 'Work 2'.
  - r. Click on 'Schedule'. You can see the red line move across the screen with each step.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/01/v01</b>	Page 4 of 5
<b>Serology Manual</b>		

- s. When finished, 'Remove plate & Carrier from the system', remove plate and click 'OK'.
- t. Click on 'Print Report'.
- u. Repeat any specimen that has 'Clot detected' the next working day.
- v. Re-capped all reagents, and place the whole kit in the refrigerator.

#### **IV. Assay validation**

Positive Control should read >0.500 at 450/620nm

Negative Control should read < 0.080 at 450/620 nm

#### **V. Interpretation of Results**

Spectrophotometric Dual Wavelength (450/620 nm)

Negative = OD <0.08

Positive = OD >= 0.08

#### **VI. Reporting**

Positive: *Clostridium difficile* toxin detected

Negative: *Clostridium difficile* toxin not detected

If patient is positive for *C. difficile* toxin, phone result to ward, and also phone INFECTION CONTROL NURSE at UHN (TG, TW & PMH), or CHC (in-patient only).

#### **VII. Quality Control**

Negative and positive controls must be included with each run. Refer to a senior technologist if control results are outside of limits or for any other problems with running or reporting the assay.

Run external control (Bartels,lot #G-666 C.difficile Toxin Control) with each new lot. Result filed in External Control Binder. . If result is negative, the run is invalid. Inform Charge/senior technologist, and repeat testing.

CAP provides external proficiency testing.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/01/v01</b>	Page 5 of 5
<b>Serology Manual</b>		

### VIII. References

1. Package insert from TechLab, an ELISA for the detection of *Clostridium difficile* Toxin A and B.
2. BEP®2000 Quick Reference Guide, Dade Behring Marburg GMBH.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/02/v03</b>	Page 1 of 9
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**AXSYM SYSTEM FOR HBsAg, HBsAb, HBeAg, HBeAb, HBcAb-IgM, HBcAbTotal,  
HAV-IgM, HCVAb, CMV IgG, HIV-1/2, Rubella IgG**

**I. Introduction**

The Abbott AxSYM System is a fully automated random access analyser that utilizes an enzyme-linked immunoreacting substance (antibody, antigen or hapten) adsorbed onto microparticles to detect the primary interaction of antibody or antigen. The reaction mixture is transferred to an inert glass fibre matrix to which the microparticles bind irreversibly. The immune complex on the glass fibre matrix is detected by alkaline phosphatase-labelled conjugate.

**II. Specimen Collection and Processing**

Blood is collected (5 mL for adult and 1 mL for neonates) in a serum separator tube, Sodium heparin tube, Sodium citrate tube, ACD tube, CPDA-1 tube or EDTA tube. Sample is centrifuged (3,000 g x 10 minutes) and the serum is stored refrigerated until testing. After testing is completed, specimens are stored at -70<sup>0</sup>C for 10 years for bone marrow and bone bank patients and 3 months for other patients.

**III. Procedure**

**Reagents**

Reagents, calibrator and control packs for the assays are stored refrigerated. All other reagents are stored at room temperature, except MUP (Solution 1) which is stored refrigerated. Once MUP solution is opened, it is only good for 2 weeks.

**AxSYM Operation**

**[A] Daily Maintenance**

- 1) Check inventory and waste containers. Flush tubings.
- 2) Scan reagent packs. Add additional reagent packs and calibrate (if necessary) sufficient for the days work.
- 3) Probe clean to be done at the end of the shift.
- 4) Initial the maintenance log book.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/02/v03</b>	Page 2 of 9
<b>Serology Manual</b>		

**[B] Assay Procedure for Patient Sample (Primary tube)**

- 1) Check that label on tube contains ordered tests as per the requisition and the name on the label matched the name on the tube. The bar code label is centered and vertical on the tube.
- 2) Remove the cap. Check for and remove any bubbles in the serum. Ensure that the level of serum is adequate.
- 3) Load the samples and place the segments in the sample carousel.
- 4) Load the appropriate assay reagents in the reagent carousel. (Remember to open reagent bottle #4 on the reagent pack, if present).

**[C] Assay Procedure for Patient Sample (Sample cup)**

- 1) From the MAIN MENU, select "ORDERLIST",select F6 "Patient".
- 2) Scan LIS #, type in "Name",and then press "TAB".
- 3) Type S/P location of the sample cup.
- 4) Select F6 - "ADD", continue the above steps for the next sample cup.
- 5) Select F1 - "EXIT" once all the patients have been entered.
- 6) Print the displayed orderlist using the PRINT KEY. Verify sample cups are in correct location.
- 7) Load the samples.
- 8) Check for bubbles in the sample and place the segments in the sample carousel.
- 9) Load the appropriate assay reagents in reagent carousel. (Remember to open reagent bottle #4 on the reagent pack, if present).
- 10) PRESS "RUN".

**[D] Assay Procedure for Control Sample**

- 1) From the MAIN MENU, select "ORDERLIST".
- 2) Select F5 - control.
- 3) Select the assay for the control by touchscreen.
- 4) Type in the location of the control sample S/P.
- 5) Select positive/or negative control by touchscreen.
- 6) Select F6 - "ADD". Continue for next control follow.
- 7) Select F1 - "EXIT" Repeat control for next assay or Press "ABBOTT LOGO" TO "MAIN MENU".
- 8) Review the displayed orderlist. To print the orderlist, select the PRINT KEY.
- 9) Load the control samples.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/02/v03</b>	Page 3 of 9
<b>Serology Manual</b>		

- 10) Check for bubbles in the sample and place the segments in the sample carousel.
- 11) Load the appropriate assay reagents in reagent carousel. (Remember to open reagent bottle #4 on the reagent packs, if present).
- 12) Press "RUN".

**INDEX CALIBRATION: HBsAg, HBeAg, HBeAb, HBcAb-IgM, HBcAb-Total, HAV IgM Ab, HCVAb, HIV-1/2**

Index Calibration procedure requires only one sample cup. Check required volume on printed orderlist.

- 1) Order Cal from the MAIN MENU, select "ORDERLIST".
- 2) Select F4 - "CAL".
- 3) Select the assay to be calibrated using the touch screen.
- 4) The S/P is automatically assigned. Change the S/P location if necessary.
- 5) Enter reagent lot number and expired date (found on bottle).
- 6) Press F6 - "ADD".
- 7) Press Abbott logo (a) if no other assay calibration is required.
- 8) Print the displayed orderlist using the "PRINT KEY".
- 9) Mix the calibrator and place appropriate volume in the sample cup.
- 10) Check for bubbles in the sample and place the segments in the sample carousel.
- 11) Load the appropriate assay reagents in reagent carousel. (Remember to open reagent bottle #4 on the reagent pack, if present).
- 12) Press "RUN".

**MASTER CALIBRATION: HBsAb, CMV IgG Ab, Rubella IgG Ab**

Master calibration requires two calibrators in separate S/P locations. Check required volume on printed orderlist.

- 1) From the MAIN MENU, select "STORED RESULTS".
- 2) Select calibration review.
- 3) Select F4 - NEW LOT.
- 4) Enter the master curve information by selecting - F4 "SCAN CURVE".
- 5) Scan the bar code label, top and bottom.
- 6) Select F6 - SAVE.
- 7) Master Cal is performed through the calibration orders and calibration type screen.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/02/v03</b>	Page 4 of 9
<b>Serology Manual</b>		

- 8) Go to the MAIN MENU, select "ORDERLIST".
- 9) Select F4 - Cal.
- 10) Select the assay to be calibrated by using the touchscreen.
- 11) The S/P for two master cal samples is automatically assigned. Change the S/P location if necessary.
- 12) Enter master cal lot number and expiry date (number on box).
- 13) Make sure calibration type is Master Cal, if not enter "MASTER CAL" by touchscreen.
- 14) Select F6 - "ADD".
- 15) Select F1 "ABBOTT LOGO" if no further request is needed.
- 16) Print the displayed orderlist using the "PRINT KEY".
- 17) Dispense master calibrators into two sample cups. AUSAB calibrators must be microcentrifuged for 10 minutes on maximum speed before using.
- 18) Check for bubbles in the samples and place the segments in the sample carousel.
- 19) Load the appropriate assay reagent in the reagent carousel. (Remember to open reagent bottle #4 on the reagent pack, if present).
- 20) Press "RUN".

**[E] Daily Clean-up Procedure**

- 1) Ensure that all reagents have been removed and scanned.
- 2) Clean probe.

**[F] Weekly Maintenance**

- 1) MEIA & FPIA Verification
- 2) Clean sample segments and sample cup adaptors.
- 3) Clean matrix cell & processing carousels.
- 4) Clean air filters.
- 5) Clean outside of probes
- 6) Clean wash cups/ waste stations.
- 7) Clean dispenser nozzles and baseplate.
- 8) Flush pumps and syringes.
- 9) Weekly Archive patient results (must be performed before 1,500 records).

**[G] Monthly Maintenance**

- 1) Tubing decontamination.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/02/v03</b>	Page 5 of 9
<b>Serology Manual</b>		

#### IV. Results

Results should be sorted and viewed by S/P to ensure that all tests were completed then resorted and printed.

- 1) From the MAIN MENU, select "RESULTS".
- 2) Select F5 - SORT.
- 3) Select "S/P" by touchscreen, "OK".
- 4) Scroll down the list checking that every S/P with a sample has a result.
- 5) Select F5-SORT.
- 6) Select "SID" OK. Once sorted, select F2-Select All and PRINT.
- 7) Select "Print a listing of all selected results", "OK" using touchscreen to activate printing.
- 8) After successful printing of results, select F3 "Release Results". "Are you sure? - Yes".
- 9) Check print-out for low level positive CMV, Rubella and enter the low level message in the LIS. Check for reactive HB<sub>s</sub>Ag, HB<sub>c</sub>Ab, HCVAb, HIV 1/2, for which repeat testing or reflex testing may be necessary.
- 10) Initial and give print-out to senior technologist for verifying.

**Note:**

HB<sub>c</sub>Ab: Repeat all reactive results for HB<sub>c</sub>Ab. After repeat testing complete.  
Select the appropriate result for 8HBC test from keypad in LIS.  
(Exception: HB<sub>c</sub>Ab reflex testing (8HBC2) performed on HB<sub>s</sub>Ag reactive sample for which HB<sub>c</sub>Ab (8HBC) was not originally ordered as outlined below.

HB<sub>s</sub>Ag: Perform reflex confirmatory testing and HB<sub>c</sub>Ab on all reactive HB<sub>s</sub>Ag samples except for patients with previously confirmed positive HB<sub>s</sub>Ag.  
Preliminary report of stat requests will be phoned as "presumptive positive".

## 2 Reporting Results

### i) **HB<sub>s</sub>Ag Assay Cut-off: >2.0 S/N**

Reactive and confirmed, HB<sub>c</sub>Ab reactive: POSITIVE

Reactive and previously confirmed positive: POSITIVE

Reactive and non-confirmed: Negative

<b>HB<sub>s</sub>Ag S/N</b>	<b>HB<sub>c</sub>Ab Result</b>	<b>Action Required</b>
≤2	+ or -	Report: Negative
2-5	+	Send to PHL
>5 -10	+	Perform Confirmatory Testing
2 – 10	-	Send to PHL
>10	-	Perform Confirmatory Testing
>10	+ S/CO <0.2	Report: Positive
>10	+ S/CO >0.2	Perform Confirmatory Testing

### ii) **HB<sub>s</sub>Ab Assay Cut-off: >10.0 IU/mL**

Reactive: POSITIVE

Negative: Negative

### iii) **HB<sub>c</sub>Ab Assay Cut-off: <1.0 S/CO**

Repeatable reactive: POSITIVE

Non-reactive: Negative

Non-repeatable reactive: NEGATIVE\*

### iv) **HB<sub>e</sub>Ab Assay Cut-off: >1.0 S/CO**

Reactive: POSITIVE

Negative: Negative

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/02/v03</b>	Page 7 of 9
<b>Serology Manual</b>		

**v) HBeAg Assay Cut-off: >1.0 S/N**

Reactive: POSITIVE, repeat specimen with value of >1.0 and <10.

Negative: Negative

**vi) HBcAb-IgM Assay Cut-off: >0.80 Index**

Reactive: POSITIVE

Negative: Negative

**vii) HBcAb-Total Assay Cut-off: <1.0 S/CO**

Reactive: POSITIVE

Negative: Negative

Non-repeatable reactive: NEGATIVE\*

**viii) HAV-IgM Assay Cut-off: >1.20 index**

Reactive: POSITIVE

Grey-zone Reactive 0.8 - 1.2 Index: Inconclusive. Please send repeat specimen.

Non-reactive: Negative

**ix) HCV Ab Assay Cut-off: >1.0 S/CO**

1. If S/CO > 10, reflex to 8HCA2. Report positive if repeatably reactive.
2. If S/CO < 10, enter in the LIS as "To PHL @ MOHC". Send to PHL next day. Preliminary report of STAT testing will be phoned as "Presumptive Positive".

Grey-zone 1.6-2.0 S/N: NON-REACTIVE

Non-reactive: NON-REACTIVE

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/02/v03</b>	Page 8 of 9
<b>Serology Manual</b>		

**x) CMV IgG Antibody Cut-off: >15 AU/mL**

Positive (>25 AU/ml) : POSITIVE  
 Positive (15-25 AU/mL): POSITIVE (Low Level)  
 Negative (< 15 AU/ml): Negative

**xi) Rubella IgG Antibody Cut-off: >10.0 IU/mL**

Positive (>15 IU/mL): POSITIVE  
 Positive (10-15 IU/mL): POSITIVE (Low Level)  
 Greyzone (5-9.9 IU/mL): Negative  
 Negative: Negative

**xii) HIV 1/2 Assay**

\*Reactive: Enter in the LIS as "TO PHL". Send to PHL next day for confirmation.

Negative: Negative

\*Review with Charge Technologist before reporting.

**VI. Quality Control**

**Abbott Controls:**

Positive and negative controls must be run for the following assays once per 8 hour shift:  
 HCV Ab , CMV Ab, and Rubella IgG, .

Positive and negative controls must be run for the following assays once per 24 hour shift:  
 HBsAg, HBsAb, HAV IgM, HBeAg, HBeAb and HIV ½

Results are verified by a senior technologist and filed in AXSYM printout log.

**External Controls:**

Run Positive External Control with each new reagent lot.

Accurun 5100 is for :HBsAg, HIV ½, HBcore Ab,HCV Ab, and CMV IgG

Accurun 51 is for HBeAg, HbcIgM, and HAVIgM

Accurun 52 is for AUSAB, and HBeAb

Accurun 140 for Rubella IgG

Viroclear is used as negative control for all except Rubella IgG

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/02/v03</b>	Page 9 of 9
<b>Serology Manual</b>		

All stock Accuruns are stored at  $-70^{\circ}\text{C}$  freezer (MIFTJ).

External Controls are verified by a senior technologist and filed in External QC binder.

If any external control result is wrong, the test is invalid. Inform Charge/senior technologist, and repeat testing.

CAP, QMP-LS, and LCDC provide external proficiency testings

Calibrations:

Run calibration for each new reagent lot or when control(s) fail.

Failed QC:

Test is invalid without satisfactory QC results.

- a. Do not release reagent lot for use pending resolution of QC error.
- b. Inform charge/senior technologist.
- c. Record in Reagent Log Chart, Instrument Maintenance Log if microscope/incubator is involved in the failure (and Incident Report if necessary).
- d. Re-run failed control materials in parallel to fresh controls to evaluate the QC material itself.
- e. If the re-run shows the old QC material still fails and fresh QC is satisfactory, the error may be attributed to the old QC material itself and the reagent is satisfactory.
- f. If the re-run shows both the old and fresh QC materials fail (or other QCs not satisfactory), the error may be attributed to the reagent then the reagent cannot be released for use.

**VII. Reference**

AXSYM operational manual and manufacturer's package insert for the appropriate assay.

(Abbott Laboratories, Diagnostics Division, Abbott Park IL 60064, USA)

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/03/v01</b>	Page 1 of 4
Section: <b>Serology Manual</b>	Subject Title: <b>AxSym HBsAg Surface Antigen Confirmatory Assay</b>	
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## **AXSYM HEPATITIS B SURFACE ANTIGEN CONFIRMATORY ASSAY**

### **I. Introduction**

AXSYM HBsAg Confirmatory assay is a Microparticle Enzyme Immunoassay (MEIA) used for the detection of HBsAg in human serum or plasma by means of specific antibody neutralization.

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

A minimum of 300 µL of preliminary positive HBs Ag patient sample in a sample cup is required.

### **III. Procedure**

#### **i) Reagents**

AxSYM Confirmatory Kit  
AxSYM HBs Ag Kit

#### **ii) Method**

Use the assigned "H" segment for the confirmatory test.

Using the following chart for the number of samples to be confirmed, add appropriate volume of reagents to the corresponding sample cups at position 1 to position 5. Patient sample starts at position 6 onward.

#### **Segment "H"**

S / P	1	=	Reagent A
S / P	2	=	Reagent B
S / P	3	=	Dilution Reagent
S / P	4	=	Index Calibrator 9use cal. Received with reagent kit)

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/03/v01</b>	Page 2 of 4
<b>Serology Manual</b>		

S / P 5 = HBs Ag Positive Control

S / P 6 = Patient sample

Sample Segment Position	Reagent or Sample	Reagent Color	For Cal/ Control Only	Minimum Volume Required (µL)				
				No. of Patient Samples				
				1	2	3	4	5
1	Reagent A	Violet	100	150	200	250	300	350
2	Reagent B	Yellow	150	200	250	300	350	400
3	Dilution Reagent	Natural	0	500	875	1250	1625	2000
4	Index Calibrator*	Green	270	270	270	270	270	270
5	Positive Control**	Blue	250	250	250	250	250	250
6	Patient Sample			275	275	275	275	275
7	Patient Sample				275	275	275	275
8	Patient Sample					275	275	275
9	Patient Sample						275	275
10	Patient Sample							275

\*270 µL = 8 drops

\*\*250 µL = 7 drops



TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/03/v01</b>	Page 4 of 4
<b>Serology Manual</b>		

#### **IV. Results**

- 1) From the MENU, select "RESULTS"
- 2) Highlight all control and patient results associated with the confirmatory run
- 3) Select "PRINT", choose "Print a formatted result for each displayed test"
- 4) Select "OK" by touch screen

#### **V. Reporting Results**

HBsAg Confirmatory (8CON) testing results are entered into the LIS using the keypads but do not print on the report. The repeat HBcAb (8HBC2) results also do not print, but are automatically posted to the LIS. These confirmatory and repeat HBcAb (8HBC2) results are used to edit, if necessary, the HBsAg (8HAG) and HBcAb (8HBC) results which will print on the report.

##### **HBsAg Confirmatory Assay**

% Neut or % Neut Dil Positive: Confirmed Positive  
 % Neut AND % Neut Dil Negative: Not Confirmed

##### **HBcAb (8HBC2) Assay**

Reactive:      Reactive  
 Negative:     Negative

Refer to AXSYM portion of manual for guidelines on reporting HBsAg, and HBcAb based on reflex testing.

#### **VI. Quality Control**

A positive control confirmation should be included on each run.

#### **VII. Reference**

AXSYM operational manual and manufacturer's package insert. (Abbott Laboratories, Abbott Park, IL 60064, U.S.A.)

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/04/v01</b>	Page 1 of 9
Section: <b>Serology Manual</b>	Subject Title: <b>Hepatitis Virus Serology</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: March 14, 2001	
Approved by: Laboratory Director	Revision Date: September 20, 2001	

## HEPATITIS VIRUS SEROLOGY

Although there are many infectious causes of hepatitis, the majority are caused by hepatitis A, B and C viruses. Acute Hepatitis A is diagnosed by detection of IgM antibodies. Anti-HAV IgM becomes positive just before the development of clinical hepatitis and remains positive for at least 4 months after infection. There is no chronic carrier state for Hepatitis A. Detection of total antibodies to Hepatitis A indicates immunity due to either past infection or immunization.

Hepatitis B is diagnosed by either detecting hepatitis B surface antigen (HBsAg) which indicates the presence of infectious virus, (HBcAb-IgM) **or** Anti-hepatitis B surface antibodies (HBsAb) which indicate immunity due to either past infection or immunization. Anti-hepatitis B core IgM antibodies indicate acute infection and HBcAb-Total indicates previous or current infection. HBsAg should be cleared within 6 months of acute infection. Persistence of HBsAg beyond 6 months is consistent with chronic hepatitis B infection. Some of these tests may occasionally be performed on a STAT basis because of concern regarding transmission of the virus to a susceptible individual following exposure (i.e. needlestick, newborn) to infected blood/body fluids and the need to prevent the disease by administering vaccine and/or immunoglobulin.

Hepatitis due to the delta virus is rare in Canada. It only occurs in association with patients who are positive for hepatitis B surface antigen. Requests for this virus should be forwarded to the Public Health Laboratory.

Hepatitis C (HCV) is a blood borne virus closely associated with blood transfusion and intravenous drug use. The presence of antibodies to HCV indicates that an individual may have been infected with HCV, may harbour infectious HCV and/or may be capable of transmitting HCV infection. The following requests will be handled in our laboratory:

Hepatitis B surface antigen	HBsAg
Hepatitis B surface antibody	HBsAb
Hepatitis B core antibody	HBcAb
Hepatitis B e antigen	HBeAg <sup>1</sup>
Hepatitis B e antibody	HBeAb <sup>1</sup>
Hepatitis A IgM antibody	HAV-IgM <sup>2</sup>
Hepatitis A Total antibody	HAV-IgG

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/04/v01</b>	Page 2 of 9
<b>Serology Manual</b>		

- Note:** 1) These tests will be performed on request only if the patient is HBsAg positive.  
2) Perform only HAV-IgM if type of Hepatitis A test is not specified.

**Table 1. Tests performed as per designated categories**

Clinical Category	Test Performed			
	HBsAg	HBsAb	HAV-IgM	HCVAb
Hepatitis B Screen	X			
Hepatitis A Screen			X	
Hepatitis B Immune Status		X		
Needlestick				
- Patient (source)	X			X
- Staff (exposed)		X		X
Pre/postnatal				
- Mother	X			
Hepatitis Screen	X		X	X

For additional requests within the above categories, consult with the charge technologist or medical microbiologist. In the absence of one of the above clinical categories, do the tests as requested.

**Table 2. Guidelines for STAT Testing**

Clinical setting	Serum tested	Time frame from exposure to report	Days of week	Call-back
Neonate	Mother	< 12 hours	All	No <sup>1</sup>
Prenatal	Mother	< 12 hours	All	No <sup>1</sup>
Needlestick/ mucosal exposure <sup>2</sup>	See below	< 72 hours	All	No <sup>1</sup>
Renal dialysis	Patient	< 12 hours	All	No <sup>1</sup>

- 1) May require call back on weekends if time from exposure to reporting exceeds 12 hours. Must be approved by microbiologist on-call.
- 2) Perform HBsAg and HCVAb on the source. Test HBsAb and HCVAb on the employee. Whichever of these arrives first is to be tested STAT. If both arrive at the same time, test both simultaneously. If the source HBsAg is positive, test the employee STAT. If the source is negative, test employee in the next routine run.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/04/v01</b>	Page 3 of 9
<b>Serology Manual</b>		

## **HUMAN IMMUNODEFICIENCY VIRUS(HIV) ½ SEROLOGY**

HIV testing will be done on patient (source) involved in ' Needle Stick Incident' as 'STAT', so appropriate prophylaxis can be given in case it is positive. It is also done for transplant patients (donor & recipient). Two tubes of blood should be received. If the result is positive, send the unopened tube to PHL. The positive result will be reported as 'sent to PHL for confirmation'. Specimen will be sent out as 'STAT' the next working day to PHL. Put specimen with PHL HIV form in a separate brown bag, clearly marked as 'STAT'. Call 416-340-6022 to inform PHL HIV lab that this specimen is coming as 'STAT', and ask to call result to Lab when it is available.

### Daily Maintenance:

1. Empty Waste Containers first thing in the morning or at the end of the day.  
Open the Waste and Supply Center Doors (DO NOT OPEN THIS DOOR WHEN THE AXSYM IS RUNNING), then open the Interior Waste Door :
  - a. Remove Consumable waste
    - i. Remove biohazard bag from the consumable waste container and dispose it into Biohazard waste box.
    - ii. Place a new biohazard bag in the container.
  - b. Remove Liquid Waste
    - i. Hold down the tubing to the Liquid Waste Container while pressing the metal tab on the side of the clip.
    - ii. Remove the Liquid Waste Container.
    - iii. Transfer liquid waste into decontamination containers near the sinks. Fill container with 4/5 full of waste and 1/5 full of Javex. Label date on the lid.
    - iv. Rinse container with a little bit of Javex, and then lots of H<sub>2</sub>O. Wipe dry outside of container.
    - v. Reattach the tubing by pressing the tubing assembly until it locks into place.

Close the Interior Waste Door, and close the Waste and Supply Center Doors.
2. Update Waste Levels:
  - a. Select Inventory from the Main Menu.
  - b. Select F4- Waste.
  - c. Select F3- Empty Waste.
  - d. Select F6- Save.
  - e. Exit to Main Menu.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/04/v01</b>	Page 4 of 9
<b>Serology Manual</b>		

3. Update Matrix cells and Bulk Solutions:
  - a. Matrix Cells:
    - i. Visually check inventory level.
    - ii. If level is around or less than 100, add a box by removing the plastic cover, and the paper tabs in the middle of the box. Place the box between the plastic walls at the top of the Matrix Cell Hopper. Rest the box ends firmly on the roll bars and apply pressure to the center of the box. The Matrix cells pour into the Hopper. Make sure that no Matrix Cells lie sideways.
    - iii. Update inventory:
      1. Select Inventory in Main Menu.
      2. Select F2- Matrix cell.
      3. Select F3- Add a box.
      4. Select F6-Save.
      5. Exit to Main Menu.
  - b. Bulk Solutions:
    - i. Open Waste and Supply Center Doors:
      1. Remove the bulk solution that you are replacing.
      2. Load the replacement solution bottle and screw on the cap.
      3. Close the Waste and Supply Center Doors.
    - ii. Update inventory:
      1. Select Inventory from Main Menu.
      2. Select F5- Bulk Solutions
      3. Select F2- Replace Solution 1  
F4- Replace Solution 3  
F5- Replace Solution 4
      4. F6- Save, Exit to Main Menu.
4. Flushing Pumps and Syringes:
 

Select Maintenance from the Main Menu.  
 Select Prime and Flush.  
 Enter 1 under sampling syringe flush and Processing syringe flush.  
 Select F2- Perform Maint.  
 Check for leaks or bubbles in the tubings.  
 Exit to Main Menu.
5. After loading all reagent packs, press F5-Scan pack,  
 Press 'Inventory'.  
 Press F3-'Reagent Load List'. Check to see how many tests are left in each reagent pack.  
 To print a copy, Press 'Alt' and 'PRINT' at the same time.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/04/v01</b>	Page 5 of 9
<b>Serology Manual</b>		

6. Scan reagent packs at the end of the day:
  - a. Remove all Reagent Packs.
  - b. Select F5- scan pack from Main Menu.
  
7. Probe Clean at the end of the day :
  - a. Select Maintenance from the Main Menu.
  - b. Select Probe Clean. Follow instructions on the screen.
  - c. Load two Reaction Cells at the designated locations on the Reaction Vessel Carousel.
  - d. Pipette 1 ml of TEAH(Probe Clean Solution) into both the buffer and predilution wells of both Reaction Vessels.
  - e. Select OK.
  - f. When finished, exit to Main Menu.
  
8. Archive new results of the week on Friday.

Weekly Maintenance :

1. Replace and cleaning Air Heater Inlet Filter.
2. Replace and cleaning Card Cage Air Filter.
3. Cleaning Sampling Probe.
4. Cleaning Processing Probe.
5. Cleaning Sampling Wash Station.
6. Cleaning Processing Wash Station.
7. Cleaning Dispenser Nozzles.
8. Flushing Pumps and Syringes.
9. Cleaning Processing Carousel.
10. Cleaning Matrix Cell Carousel.
11. MEIA Verification.
12. FPIA Verification.
13. Cleaning Segments and Sample Cup Adapters.

Remove Cell Hopper, and lift Processing Center Cover.

1. Replace and Cleaning Air Heater Inlet Filter:  
 The Air Heater Inlet Air Filter is located on the left hand side and near the back of the AXSYM.  
 Lift filter up and replace with a clean filter in the filter slot.  
 Clean the dirty filter under running tap water in the opposite direction of the airflow arrows. Shake off excess water and air-dried.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/04/v01</b>	Page 6 of 9
<b>Serology Manual</b>		

2. Replace and Cleaning Card Cage Air Filter  
 Open the Card Cage Access Panel located on the left side of the system.  
 Remove the Card Cage Filter.  
 Place the spare filter in the filter slot.  
 Install the Card Cage Access Panel.  
 Clean the dirty filter under running tap water in the opposite direction of the airflow arrows. Shake off excess water and air-dried.  
  
 Select Maintenance from Main Menu.  
 Select PRIME AND FLUSH.  
 Select F6- Raise probes.  
 Open the sampling Pipette Cover.
3. Cleaning Sampling Probe  
 Moisten a cotton swab with Deionized H<sub>2</sub>O.  
 Wipe the Sampling probe several times.  
 Moisten a cotton swab with 95 % Ethanol.  
 Wipe the Sampling probe several times.  
 Moisten a cotton swab with Deionized H<sub>2</sub>O.  
 Wipe the Sampling probe several times.  
 Visually check the probe for damage.
4. Cleaning Processing Probe:  
 Moisten a cotton swab with Deionized H<sub>2</sub>O.  
 Wipe the Processing probe several times.  
 Moisten a cotton swab with 95 % Ethanol.  
 Wipe the Processing probe several times.  
 Moisten a cotton swab with Deionized H<sub>2</sub>O.  
 Wipe the Processing probe several times.  
 Visually check the probe for damage.
5. Cleaning Sampling Wash Station:  
 Remove the Wash Station.  
 Rinse the Wash Station with Deionized H<sub>2</sub>O.  
 Rinse the Wash Station with 95 % Ethanol.  
 Rinse the Wash Station with Deionized H<sub>2</sub>O.  
 Clean the inside and outside of the Wash Station with a cotton swab moistened with Deionized H<sub>2</sub>O to remove any salt buildup.  
 Reinstall the Wash Station.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/04/v01</b>	Page 7 of 9
<b>Serology Manual</b>		

6. Cleaning Processing Wash Station:
  - Remove the Wash Station.
  - Rinse the Wash Station with Deionized H<sub>2</sub>O.
  - Rinse the Wash Station with 95 % Ethanol.
  - Rinse the Wash Station with Deionized H<sub>2</sub>O.
  - Clean the inside and outside of the Wash Station with a cotton swab moistened with Deionized H<sub>2</sub>O to remove any salt buildup.
  - Reinstall the Wash Station.
  - Home Probes.
  
7. Cleaning Dispenser Nozzles:
  - Remove the Bulk Solution 1 Dispenser by pulling olive-green lever toward you (↓).
  - Lift the Dispenser to inspect the nozzle for buffer deposits.
  - Moisten a cotton swab with Deionized H<sub>2</sub>O and clean the outside of the nozzle and the surrounding collar.
  - Inspect the nozzle for obstruction.
  - Reinstall the Dispenser by lifting the black clip to vertical position.( should hear ‘click’).
  - Check the Dispenser position. Make sure that the Dispenser sits properly, not on a raised position.
  
  - Remove the Bulk Solution 3 Dispenser by Dispenser by pulling olive-green lever toward you (↓).
  - Lift the Dispenser to inspect the nozzle for buffer deposits.
  - Moisten a cotton swab with Deionized H<sub>2</sub>O and clean the outside of the nozzle and the surrounding collar.
  - Inspect the nozzle for obstruction.
  - Reinstall the Dispenser by sliding the metal hinge pin on the right end of the Dispenser into the openings on the base. Lower the left end into the circular opening of the base plate.
  - Check the Dispenser position. Make sure that the Dispenser sits properly, not on a raised position.
  
8. Flushing Pumps and Syringes:
  - Select Prime and Flush
  - Enter 1 under sampling syringe flush and Processing syringe flush.
  - Select F2- Perform Maint.
  - Check for leaks or bubbles in the tubings.
  - Exit to Main Menu.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/04/v01</b>	Page 8 of 9
<b>Serology Manual</b>		

9. Cleaning Processing Carousel:
  - Remove the Processing Carousel Cover by unscrewing the two thumbscrews.
  - Grasp Processing Carousel Motor Handle, push back and hold open.
  - Remove by tilting Carousel and pulling up, careful not to hit the probe.
  - Clean the carousel and the surrounding area with lint-free tissue moistened with water.
  - Inspect the clips on the under side of the carousel for broken pieces/loose connections.
  - Dry the Carousel with Lint free tissue.
  - Reinstall the Carousel by grasping the Processing Carousel Motor Handle, push back and hold open.
  - Place the rim of the Carousel in the V-wheel located under the Carousel Motor Housing.
  - Tilt the Carousel downward and fit the rim into the two front V-wheels. Careful not to hit the probe.
  - Reinstall the Processing Carousel Cover by tightening the two thumbscrews.
  
10. Cleaning the Matrix Carousel:
  - Remove the Matrix Cell Carousel Access Panel.
  - Remove the Air Deflector.
  - Select F2- Shutdown from the Main Menu.
  - Verify that Shutdown was selected, select OK.
  - Turn the power switch to OFF. Once power is off, make sure that it is off for at least 5 minutes before turning AXSYM back 'ON'.
  - Rotate the Carousel so that the red dot is facing you.
  - Remove the Carousel by pushing back on the Carousel, tilting down and pulling out.
  - Clean the carousel and the surrounding area with lint-free tissue moistened with water.
  - Inspect the clips on the under side of the carousel for broken pieces/loose connections.
  - Dry the Carousel with Lint free tissue.
  - Reinstall the Carousel by holding the carousel with the red dot facing you, and fit the rim of the carousel into the rear V-wheel and push in towards the spring-loaded V-wheel.
  - Tilt up and fit the rim of the carousel into the front V-wheels.
  - Manually rotate Carousel to ensure proper positioning.
  - Reinstall Air Deflector. Reinstall the Matrix Cell Carousel Access Panel.
  - Close the Processing Center Cover.
  - Re-install Matrix Cell Hopper.
  - Turn the power ON.
  - Select Startup from the Main Menu.
  - If the power is off for less than 15 minutes, there is a 15-minute warm-up.**
  - If the power is off for more than 15 minutes, there is an hour warm-up.**

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/04/v01</b>	Page 9 of 9
<b>Serology Manual</b>		

11. MEIA Verification:

Select MAINTENANCE from the Main Menu.  
 Select CALS AND CHECKS.  
 Select OPTICS.  
 Select MEIA VERIFICATION.  
 Remove Matrix Cell Hopper.  
 Select CONTINUE. Follow instructions on screen.  
 Place the Standard Hopper into position.  
 Insert the Standard into the Standard Hopper.  
 Select CONTINUE to begin the MEIA Verification.  
 This will take ~ 10 minutes.  
 When finished, select OK to acknowledge completion of the verification.  
 Press PRINT on the keyboard.  
 Retrieve the Standard by pressing the white MEIA Standard Release lever.  
 Open the MEIA Optical Retrieval Door and remove the Standard.  
 Store it upside down in the black box.  
 Reinstall Matrix Cell Hopper.  
 Exit to the MAIN MENU/MAINTENANCE/OPTICS.

12. FPIA Verification:

Select FPIA VERIFICATION.  
 Follow instruction on the screen, select Continue.  
 Press down the back of the clip on top of the FPIA Optical Standard.  
 Load the Standard into the Reaction Vessel Carousel at the Load Station.  
 Press on the front of the clip to provide a flat surface.  
 Select Continue to begin the procedure.  
 This will take ~ 20 minutes.  
 When finished, select OK to acknowledge completion of the verification.  
 Press PRINT on the keyboard.  
 Press down on the back of the clip on top of the FPIA Optical Standard.  
 Lift and remove the Standard ,store in assigned place in the black box.  
 Exit to Main Menu.

13. Cleaning Segments and Sample Cup Adapters:

Use disinfectant wipes to clean segment and Sample Cup Adapters.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/05/v01</b>	Page 1 of 7
Section: <b>Serology Manual</b>	Subject Title: <b>BIO-RAD HBsAg</b>	
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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.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/05/v01</b>	Page 2 of 7
<b>Serology Manual</b>		

### 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

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/05/v01</b>	Page 4 of 7
<b>Serology Manual</b>		

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** '.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/05/v01</b>	Page 5 of 7
<b>Serology Manual</b>		

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.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/05/v01</b>	Page 6 of 7
<b>Serology Manual</b>		

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.  
TheHIV-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.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/05/v01</b>	Page 7 of 7
<b>Serology Manual</b>		

**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.

Bio-Rad Laboratories blood Virus division  
Redmond,WA 98052,U.S.A.  
U.S. License No.1109

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/06/v01</b>	Page 1 of 5
Section: <b>Serology Manual</b>	Subject Title: <b>Epstein Barr Virus Serology</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: March 14, 2001	
Approved by: Laboratory Director	Revision Date: June 6, 2003	

## 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.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/06/v01</b>	Page 2 of 5
<b>Serology Manual</b>		

**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'.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/06/v01</b>	Page 3 of 5
<b>Serology Manual</b>		

- 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’.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/06/v01</b>	Page 4 of 5
<b>Serology Manual</b>		

- 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.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/06/v01</b>	Page 5 of 5
<b>Serology Manual</b>		

**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.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/07/v01</b>	Page 1 of 5
Section: <b>Serology Manual</b>	Subject Title: <b>Human T-Lymphotropic Virus Type 1 (HTLV-1) EIA</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: March 14, 2001	
Approved by: Laboratory Director	Revision Date: May 26, 2003	

## **HUMAN T-LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1) EIA**

### **I. Introduction**

The Adaltis DETECT-HTLV™ EIA Test Kit is a solid phase enzyme immunoassay utilizing a mixture of synthetic peptides for the detection of antibodies to HTLV-I and II in human serum or plasma.

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

Blood is collected (5 mL for adults and 1 mL for neonates) in a serum separator tube and separated by centrifugation. The serum is stored at 4<sup>0</sup>C.

### **III. Procedure**

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

Positive Control  
 Negative control  
 Anti-Human IgG Peroxidase Conjugate  
 Conjugate Diluent  
 Tetramethylbenzidine (TMB) Reagent  
 Peroxide Reagent  
 Stop Solution  
 2x 96 well Microplate coated with HTLV-I & HTLVII peptide

#### **ii) Other Materials :**

P Lab Analyser System  
 5 ml graduated Pipettes  
 10 ml graduated Pipettes  
 1 ml graduated Pipettes  
 10-100 ul pipettor  
 Pipette tips  
 55x12 (3.5 ml) tubes  
 Automatic pipettor

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/07/v01</b>	Page 2 of 5
<b>Serology Manual</b>		

**iii) Method:**

**A. Using P-Lab (Personal Lab)**

**Before beginning EIA assay:**

1. Allow all components to reach room temperature.  
Prepare wash solution:  
Add 100 ml of 25x wash solution to 2400 ml of distilled water.  
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'.
2. Click once on third square from the left: '**open session**'.. Click on '**New**',  
Type in assay & date performed, e.g. HTLV 280499(HTLV tested April 28,99). Note session # e.g.'31'. Click on writer Name, enter initial.  
Click 'OK'
3. The screen will show e.g. **HTLV280499#31**.ssn highlighted , click 'OK'.
4. Click on 5 th square from the left: '**Profile Include**', click on ' DetectHTLV#0.  
prf',click 'OK'.
5. 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.  
Once finished, double click on 'Small Rack' icon in top left corner, "X's should appear next to all lab #s,click 'done'.
6. The screen will show plate format ( # of wells needed). Take out the required # of strips and wells. Load plate onto the **left** side in the P-Lab.  
Click on 'done'.
7. Click on 8 th square from the left : '**Setup Entry**'. Click on pulldown arrow under 'Lot".. If it is a new lot, Click on '**Change Date**' and specify kit expiration date if needed. Click 'OK'.
8. Click on last square '**Start Session**', 'Save document :using the new file...'will appear, click 'OK'.
9. **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.

**Position**      **Reagent & Controls**

R3	Sample Diluent
R4	Conjugate
R5	Substrate
R6	Stop Solution
1	Negative Control
2	Positive Control

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

Number of Strips	40X conjugate(ul)	Conjugate Diluent(ml)
1	30	1.2
2	50	2.0
3	80	2.9
4	100	3.9
5	130	4.9
6	150	5.9
7	180	6.8

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

Number of Strips	TMB Reagent(ml)	Peroxide Reagent(ml)
1	0.2	1.0
2	0.4	2.0
3	0.6	3.0
4	0.8	4.0
5	1.0	5.0
6	1.2	6.0
7	1.4	7.0

- Click on 2 nd 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.
- Click on '**Daily Maintenance**': click on '**Fill syringes**', check for bubbles in syringes, and if no bubbles present, click 'No'.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/07/v01</b>	Page 4 of 5
<b>Serology Manual</b>		

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 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 "**Black 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 you session and 'OK'.
20. Click on 3<sup>rd</sup> box from the left, '**report**', click on "DetectHTLV", 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**

9. Absorbance of the blank must be  $\leq$  or = 0.100.
10. The Positive Control mean must be  $\geq$  or = 0.800.  
**If the value is less than 0.80, the run must be repeated**
11. Cut off = NCx (negative control mean) + 0.150

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

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/07/v01</b>	Page 5 of 5
<b>Serology Manual</b>		

**v) Interpretation of Results:**

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

**vi) Quality Control:**

Two positive and three negative controls must be included with each run.

Run Virotrol® I as external control (Do not dilute) with each new lot.

All positive specimens are sent to PHL for confirmation.

Two negative specimens are sent to PHL every 6 months for verification. The PHL reports are filed in Reagent Lot binder.

**vii) Reference :**

Package insert from DETECT-HTLV™, EIA Test Kit for the Detection of Antibodies to Human T-Lymphotropic Viruses (HTLV) Types I and II. Adaltis, rev.06/02.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/08/v01</b>	Page 1 of 2
Section: <b>Serology Manual</b>	Subject Title: <b>Epstein Barr Virus Serology</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: March 14, 2001	
Approved by: Laboratory Director	Revision Date: May 27, 2003	

## **INFECTIOUS MONONUCLEOSIS HETEROPHILE ANTIBODIES**

### **I. Introduction**

The MONOSPOT LATEX slide test is a latex particle agglutination test for in vitro qualitative detection of infectious mononucleosis heterophile antibodies (IgM) in serum or plasma. These antibodies appear in the sera of 85 to 90% of patients with infectious mononucleosis within 2 to 3 weeks after onset of illness.

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

5 mL of blood is collected in a serum separator tube and separated by centrifugation. The tube is refrigerated until testing. Specimens are stored at -70<sup>0</sup>C after testing and discarded after 3 months.

### **III. Procedure**

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

MONOSPOT LATEX Kit:

Store refrigerated. Allow the reagent to warm up to RT. Mix well before use.

#### **ii) Other Materials:**

Supplied with kit:

Test slides

Paddle pipettes

#### **iii) Method:**

1. Dispense 1 drop of the latex reagent onto a labelled oval ring of the test card.
2. Add 1 drop (50 µL) of patients's serum or control to the same ring.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/08/v01</b>	Page 2 of 2
<b>Serology Manual</b>		

3. Mix the latex reagent and serum together and spread to cover the entire area of the ring with the blade end of the paddle pipette.
4. Immediately rotate the card on the serologic rotator at 100 rpm for 3 minutes.
5. Observe for agglutination using a light source to aid in visualization.

**iv) Interpretation of Results:**

Negative: No agglutination

Positive: Any degree of agglutination

**IV. Reporting**

Positive Report: "Infectious mononucleosis heterophile antibody: POSITIVE"

Negative Report: "Infectious mononucleosis heterophile antibody: NEGATIVE"

**V. Quality Control**

Negative and positive controls must be included with each run and results and kit lot number recorded on the tasklist. When opening a new kit, record the lot number in the reagent lot number binder. Refer to a senior technologist if control results are outside of limits or for any other problems with running or reporting the assay.

Run external control ( Accurrun 31) with each new lot. Result filed in Reagent Lot Binder. . If result is negative, the run is invalid. Inform Charge/senior technologist, and repeat testing.

CAP provides external proficiency testing.

**VI. References**

Manufacturer's package insert: Meridian Diagnostics, Inc., 3471 River Hills Dr., Cincinnati, Ohio 45244 U.S.A. 1-513-271-3700.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/09/v02</b>	Page 1 of 3
Section: <b>Serology Manual</b>	Subject Title: <b>Rapid Plasma Reagin Test for Syphilis Screening</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: March 14, 2001	
Approved by: Laboratory Director	Revision Date: May 27, 2003	

## **SYPHILIS SCREENING**

### **I. Introduction**

The NCS Rapid Plasma Reagin (RPR) Card test is a macroscopic non-treponemal flocculation test used to detect reagin antibodies.

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

Blood is collected (5 mL for adult and 1 mL for neonate) in a serum separator tube and separated by centrifugation. The serum is removed to a tube and refrigerated until testing. Specimens are stored in the refrigerator for 2 weeks after testing. A request for VDRL on spinal fluid (CSF) or neonate blood will be sent to PHL for testing.

### **III. Procedure**

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

RPR reagent kit (NCS Diagnostics Inc.)

#### **ii) Other Materials:**

3 mL dropper bottle  
 Dispensing needle (17 µL/drop)  
 RPR test card  
 0.05 mL disposable stirrer pipettes  
 Serological rotator at 100 rpm  
 dH<sub>2</sub>O

#### **iii) Precaution:**

Refrigerate reagents until required. Warm to RT and mix well before use. To ensure stability, return the antigen suspension to the original glass bottle after testing. The dispenser and needle assembly must be thoroughly washed in dH<sub>2</sub>O and air dried after use.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/09/v02</b>	Page 2 of 3
<b>Serology Manual</b>		

**iv) Needle Accuracy Check - When New Kit Opened:**

This procedure is performed to check the needle delivering antigen each time before testing. Using a pipette, deliver 0.5 mL antigen to the dropper bottle. Attach the needle and, holding in a vertical position, count the number of drops delivered in 0.5 mL. The needle is considered satisfactory if  $30 \pm 1$  drops are obtained. If the needle is unsatisfactory, repeat the check. Record the lot number of the newly opened kit in the reagent lot number binder.

**v) Method:**

1. Using the stirrer pipette held vertically, dispense one drop (50  $\mu$ L) of serum onto a circle on the test card. Use a fresh stirrer pipette for each sample. Repeat with the control sera.
2. Using the flat end of the stirrer pipette spread the sample over the entire area of the test circle.
3. Attach the needle to the dropper bottle. Mix the carbon antigen reagent well. Squeeze the dropper bottle and withdraw sufficient reagent into the bottle. Discard the first few drops into the reagent stock bottle and then dispense 1 drop into each circle in a vertical position. Do not mix the sample and the antigen. Rotate the card at 100 rpm for 8 minutes.
4. Observe for agglutination by two technologists independently.

**vi) Interpretation of Results:**

**Positive Result:** Any agglutination. Repeat test ---- see senior technologist for any discrepant results. Send all positive sera to PHL for VDRL and confirmatory tests. Write on PHL requisition: "RPR positive, please do confirmatory test"; DO NOT mark prenatal box for serum from prenatal patients.

**Negative Result:** No agglutination.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/09/v02</b>	Page 3 of 3
<b>Serology Manual</b>		

#### **IV. Reporting**

Positive Result: Enter in LIS as "TO PHL". VDRL send-out test is ordered reflexively. Send to PHL next day.

Negative Result: Negative

#### **V. Quality Control**

Strongly reactive, weakly reactive and non-reactive control sera are included in each run. If controls are not working or the antigen is not falling cleanly from the needle, perform a needle check as outlined in the method.

Record control results and kit lot number on the task list. Run external control (Accurun 156) with each new lot. When opening a new kit, record the lot number, needle check and external control results in the reagent lot binder.

Refer to a senior technologist if control results are outside of limits or for any other problems with running or reporting the assay.

CAP provides external proficiency testing.

#### **VI. Reference**

Manufacturer's package insert (RPR Card Test, NCS Diagnostics Inc., 130 Matheson Blvd. E. Mississauga, Ontario L4X 1Y6).

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/10/v02</b>	Page 1 of 4
Section: <b>Serology Manual</b>	Subject Title: <b>Varicella-Zoster Virus IgG VIDAS System</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: March 14, 2001	
Approved by: Laboratory Director	Revision Date: May 27, 2003	

## **VARICELLA-ZOSTER VIRUS IgG**

### **I. Introduction**

Varicella-Zoster IgG assays is used for evaluating patient's immune status to Varicella-Zoster viruses infection. The VIDAS is an enzyme-linked fluorescent immunoassay (ELFA) utilizing a virus coated Solid-Phase-Receptacle (SPR) to which antibody in serum binds. Anti-Human IgG conjugated with Alkaline Phosphatase reacts with substrate, 4-Methylumbelliferyl Phosphate to form a fluorescent product. All necessary reagents and test serum are contained in the VZG reagent strips.

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

Blood (5 mL) is collected in a serum separator tube and separated by centrifugation. The serum is removed to a vial and refrigerated until testing. Specimens are stored at -20°C after testing.

### **III. Procedure**

#### **Reagents**

VIDAS Varicella-Zoster IgG test kit:  
VZG Reagent Strips  
VZG SPRs  
Standard  
Positive Control  
Negative Control

Other materials:

Pipettor 100 uL

#### **Method**

1. Bring test kit and serum samples to room temperature. Check that lot number of kit matches lot currently in use (as posted on VIDAS instrument). If lot number does not match, ensure that all kits/test strips for the posted lot number have been used.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/10/v02</b>	Page 2 of 4
<b>Serology Manual</b>		

Enter new lot data by inserting bar code card found in kit into instrument using "7. Calibration Menu", "2. Read Master Lot Entry Card from Instrument", "Enter section to read data from (enter section letter)".

Post card of new lot currently in use on VIDAS along with the date.

2. Label MSG/VZG strips as follows:
  - s (Standard)
  - s (Standard)
  - C1 (Positive Control)
  - C2 (Negative Control)
  - 5
  - 6 etc. up to 30
  
3. Pipette 100 uL of Standard, Control or serum into the specimen well of the corresponding VZG strips. Check for adequate sample level and remove any bubbles.
  
4. At VIDAS instrument, start at Main Menu.
  - a) **Enter 1) "Create a Work List", "ENTER".**
  - b) **"Do you want to reserve sections now (y)?" "ENTER"**
  - c) **"Enter technologist name" (enter initials here)**
  - d) **"Enter assay code" (enter VZG here).**
  - e) **"Enter number of VZG tests" (enter number here).**  
Include standards and controls.
  - f) **"Enter sample ID":**  
Enter s (two positions automatically used)  
C1  
C2  
5  
6 etc.
  - g) When finished, hit **"ENTER"**

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/10/v02</b>	Page 3 of 4
<b>Serology Manual</b>		

- h) Recheck worklist entries.  
     Hit **"ENTER"** to accept worklist,  
     **"d"** to delete any entries,  
     OR  
     **"m"** to modify any entries.
- i) **"Do you want a worklist fill report"**,  
     hit **"n, ENTER"**.
- j) Load VZG strips according to assigned module  
     positions on the worklist.
- k) Load one VZG SPR for each VZG strip.
- l) **"Run worklist now"**, hit **"y, ENTER"**.
- m) Assay is complete when results are printed  
     and module lights are blinking.
- n) If instrument is beeping, exit program (11) to the vid prompt. Type  
     "message". To re-enter to the main menu type "vid", enter.
- o) If computer is not responding, log in: vid, password: enter (blank).

### **Interpretation of Results**

Specimens with test values greater than 0.9 (MEA/VZ) are considered positive. Test values ranging from 0.6-0.9 are equivocal.

Samples with test values less than 0.6 (MEA/VZ) are considered negative.

### **IV. Reporting**

Positive:                      Varicella-Zoster antibody:      Positive

Equivocal:                    Varicella-Zoster antibody:      Equivocal

Negative:                      Varicella-Zoster antibody:      Negative

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/10/v02</b>	Page 4 of 4
<b>Serology Manual</b>		

## V. **Quality Control**

Standard: RFV must be greater than or equal to RFV range posted on VIDAS instrument.

Positive and Negative Control: Test Values must be within ranges posted on VIDAS.

Quality Control ranges posted on VIDAS may change from lot to lot, therefore it is essential that the lot number of the test kit in use corresponds to that of the posted values.

Refer to a senior technologist if control results are outside of limits or for any other problems with running or reporting the assay.

Run external control (pooled positive sera) with each new lot. Results filed in Reagent Lot Binder. If result is negative, the run is invalid. Inform Charge/senior technologist, and repeat testing.

CAP provides external proficiency testing.

## VI. **Reference**

Manufacturer's Package Insert.  
VIDAS System Operational Manual.  
bioMerieux-Vitek, Inc.  
Missouri, USA 64042-2395

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/11/v03</b>	Page 1 of 5
Section: <b>Serology Manual</b>	Subject Title: <b>West Nile (Flavivirus) IgG</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: April 25, 2001	
Approved by: Laboratory Director	Revision Date: May 27, 2003	

## **WEST NILE ( FLAVIVIRUS) IgG**

### **I. Introduction**

The Focus Technologies Flavivirus ( West Nile) ELISA IgG is intended for qualitatively detecting IgG antibodies to flaviviruses( West Nile) in human serum. In conjunction with the Focus Technologies Flavivirus ( West Nile) ELISA IgM Capture ELISA, the test is indicated for testing persons having symptoms of arbovirus infection, as an aid in the presumptive diagnosis of flavivirus infection.

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

5 ml of blood is collected in a serum separator tube and separated by centrifugation. The serum is removed from the clot and refrigerated until testing. Specimens are stored at – 70<sup>0</sup>C after testing.

### **III. Procedure**

- i) Reagent Preparation:
  - a. Wash Buffer (100 ml) 10X  
Prepare working buffer (1X) by adding 100 ml of 10X Wash Buffer to 900 ml of Distilled H<sub>2</sub>O. Mix well. Labeled and dated
  - b. Controls: 1 vial of Positive Control  
1 vial of Negative Control
  - c. 1 vial of Cut-off Calibrator
  - d. IgG Conjugate
  - e. Substrate Reagent  
2 vials of Tetramethylbenzidine (TMB) and Hydrogen Peroxide in buffer
  - f. Stop Reagent  
1 vial of 1M sulfuric acid
- ii) Other Materials:
  - IgG Antigen Wells,96 wells
  - Sample Diluent

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/11/v03</b>	Page 2 of 5
<b>Serology Manual</b>		

iii) Method:

1. Label a set of 12x75 mm glass tubes as follows: Blk, Pos,Neg, Cal,7,8,9,10.....,and External Control (when required, e.g. a new lot #).
2. Add 1 ml of Sample Diluent to all tubes.
3. Leave 1<sup>st</sup> tube blank; add 10 ul of Controls, Calibrator and patient samples to corresponding tubes.
4. Take out the required # of strips (Open bag containing strips when strips reaches room temperature, ~10-15 minutes). Return the unused strips to the bag, and refrigerate immediately.
5. Change bottle from D H2O to Wash Buffer.
  - a. Turn Washer 'ON'.
  - b. Press button under 'Prime' (F2).
  - c. Press button under 'washing' (F1).
  - d. 'Select Program': Press memory (F1), press Number (F1).
  - e. **For dispensing 350 ul of buffer use Program '2'.**
  - f. 'Program No : press '2',enter.
  - g. '002 Name 1'should appear.
  - h. Press 'Set' at this point, press 'Change' (F2), then press 'Enter' until 'Number of strips to wash' appears-check that # of strip appears is what is needed. If it is the same, press 'Escape'. If is not the same, enter the right # of strip, and press 'enter 'until ' 002 Name 01' appears, press 'Enter'.
    - g. Press' Run' (F1), and press 'No' (F2) under 'Prime'.
- h. The Washer will dispense the 350 ml of buffer into each well, and soak for 5 minutes.
6. After 5 minutes. Aspirate Wash Buffer from wells.

**For aspiration of Wash Buffer, use Program ' 1 '.**

  - a. 'Select Program': Press memory (F1), press Number (F1).
  - b. 'Program No: press 1,enter.
  - c. '001 Name 01'should appear.
  - d. Press 'Set' at this point, press 'Change' (F2), then press 'Enter' until 'Number of strips to wash' appears-check that # of strip appears is what is needed. If it is the same, press 'enter'. If is not the same, enter the right # of strip, and press 'enter 'until ' 001 Name 01' appears, press 'Enter'.
  - e. Press' Run' (F1), and press 'No'(F2) under 'Prime'.
  - f. The Washer will aspirate the buffer.
  - g. 'End washing' will appear when washing cycle is finished, remove plate.
  - h. 'Repeat Washing?' will appear, press 'No' (F2).

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/11/v03</b>	Page 3 of 5
<b>Serology Manual</b>		

7. Add 100 ul of Blk to well # 1, 100 ul of Pos to well # 2, 100 ul of Neg to well # 3, 100 ul each of Cal to well # 4, 5, and 6. Add 100 ul of specimen to each well, starting from well # 7. Use last well as external Control well when needed.
8. Cover plate with sealing tape, and incubate for 60 minutes at Room Temperature.
9. Wash the wells three times using the Automatic Washer under Program '10'.
  - a. 'Select Program': Press memory (F1), press Number(F1).
  - b. For washing and soaking, use Program '10'.**
  - c. 'Program No : press 10,enter.
  - d. Program '10 Name IgG WN' should appear. Press 'Set' at this point, press 'Change' (F2), then press 'Enter' until 'number of strips to wash' appears-check that # of strip appears is what is needed. If it is the same, presses 'enter'. If is not the same, enter the right # of strip, and press 'enter 'until ' '11 Name IgG WN' appears, press 'Enter'.
  - e. Press 'Run' (F1), and press 'No' (F1) under 'Prime'. Will wash strip for 3 times, each time with working buffer.
  - f. 'End washing' will appear when washing cycle is finished.
  - g. 'Repeat Washing?' will appear, press 'No' (F2).
10. Remove plate from Plate Washer.
11. Add 100 ul of Conjugate into each well using an 8-channel pipette, cover plate with sealing tape and incubate for 30 minutes at room temperature.
12. Repeat wash steps -9a-g.
13. Add 100 ul of Substrate into each well using an 8-channel pipette, cover and incubate for 10 minutes at room temperature.
14. Add 100 ul of Stop Reagent into each well using an 8-channel pipette. In antibody-positive wells, color should change from blue to yellow.
15. Read the plate in spectrophotometer at dual wavelength 450 nm/630 nm within 1 hour of stopping the assay.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/11/v03</b>	Page 4 of 5
<b>Serology Manual</b>		

#### **IV. Calculation:**

Index value= Optical Density of Sample/the mean Optical Density of the Cut-Off Calibrators

Interpretation of Index Values:

Positive: > 1.10

Negative: < 0.9

Equivocal: >=0.9 and <= 1.1

All positive and equivocal specimens should be repeated the next day .

#### **V. Validation:**

The mean value for the Cut-Off Calibrator must be within 0.100 to 0.700 O.D. units minus the blank well.

The Positive Control Index value should be between 1.5 and 3.5.

The Negative Control Index value should be less than 0.8.

#### **VI. Reporting**

Positive result: Sent to PHL for confirmation. Report PHL result in LIS

Negative result: West Nile Virus(Flavivirus) IgG by EIA: Negative.  
This is a research test.

Equivocal result: repeat testing the next working day.

If result is negative,report as : West Nile Virus(Flavivirus) IgG  
by EIA: Negative.  
This is a research test.

If result is still equivocal /positive: Send to PHL for  
confirmation. Enter PHL for  
result in LIS.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/11/v03</b>	Page 5 of 5
<b>Serology Manual</b>		

**VII. Quality Control:**

Each plate must include 1 Positive Control, 1 Negative Control, and 3 Cut-off Calibrators.

An External Positive Control ( Accurun 165 West Nile virus IgM/IgG Positive Control Series 5000) must be included with each new lot. Result filed in Reagent Lot Binder.

**Reference**

Manufacture's insert, Focus Technologies, Cypress, California 90630.U.S.A. 2003.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/12/v03</b>	Page 1 of 5
Section: <b>Serology Manual</b>	Subject Title: <b>West Nile IgM Capture ELISA</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: September 30, 2003	
Approved by: Laboratory Director	Revision Date: October 27, 2003	

## **WEST NILE (FLAVIVIRUS) IgM**

### **I. Introduction**

The Focus Technologies Flavivirus ( West Nile) IgM capture ELISA is intended for qualitatively detecting IgM antibodies to flaviviruses( West Nile) in human serum. In conjunction with the Focus Technologies Flavivirus ( West Nile) ELISA IgG, the test is indicated for testing persons having symptoms of arbovirus infection, as an aid in the presumptive diagnosis of flavivirus infection.

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

5 ml of blood is collected in a serum separator tube and separated by centrifugation. The serum is removed from the clot and refrigerated until testing. Specimens are stored at – 70°C after testing.

### **III. Procedure**

i) Reagent Preparation:

- a. Wash Buffer (100 ml) 10X  
Prepare working buffer (1X) by adding 100 ml of 10X Wash Buffer to 900 ml of Distilled H<sub>2</sub>O. Mix well. Labeled and dated.
- b. West Nile Antigen (2 vials)  
Add 8 ml of the Sample Diluent to 1 vial of Antigen.( Do not use Distilled H<sub>2</sub>O). Keep re-constituted Antigen in fridge until use, and return it immediately to fridge after use. Each re-constituted Antigen can perform approximately 80 tests and expired in 14 days..
- c. Controls: 1 vial of Positive Control  
1 vial of Negative Control
- d. 1 vial of Cut-off Calibrator
- e. IgM Conjugate
- f. Substrate Reagent  
2 vials of Tetramethylbenzidine (TMB) and Hydrogen Peroxide in buffer
- g. Stop Reagent  
1 vial of 1M sulfuric acid

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/12/v03</b>	Page 2 of 5
<b>Serology Manual</b>		

ii) Other Materials:

IgM Capture Wells, 96 wells  
Sample Diluent

iii) Method:

1. Label a set of 12x75 mm glass tubes as follows: Blk, Pos,Neg, Cal,7,8,9,10.....,and External Control (when required, e.g. a new lot #).
2. Add 1 ml of Sample Diluent to all tubes.
3. Leave 1<sup>st</sup> tube blank; add 10 ul of Controls, Calibrator and patient samples to corresponding tubes.
4. Take out the required # of strips (Open bag containing strips when strips reaches room temperature, ~10-15 minutes). Return the unused strips to the bag, and refrigerate immediately.
5. Change bottle from D H2O to Wash Buffer.
  - a. Turn Washer 'ON'.
  - b. Press button under 'Prime' (F2).
  - c. Press button under 'washing' (F1).
  - d. 'Select Program': Press memory (F1), press Number (F1).
  - e. **For dispensing 350 ul of buffer use Program '2'**.
  - f. 'Program No : press '2',enter.
  - g. '002 Name 1' should appear.
  - h. Press 'Set' at this point, press 'Change' (F2), then press 'Enter' until 'Number of strips to wash' appears-check that # of strip appears is What is needed. If it is the same, press 'Escape'. If is not the same, enter the right # of strip, and press 'enter' until '002 Name 1' appears, press 'Enter'.
    - i. Press 'Run' (F1), and press 'No' (F2) under 'Prime'.
    - j. The Washer will dispense the 350 ml of buffer into each well, and soak for 5 minutes.
6. After 5 minutes. Aspirate Wash Buffer from wells.

For aspiration of Wash Buffer, use Program ' 1 ' .

  - a. 'Select Program': Press memory (F1), press Number (F1).
  - a. 'Program No: press 1,enter.
  - b. '001 Name 01' should appear.
  - c. Press 'Set' at this point, press 'Change' (F2), then press 'Enter' until 'Number of strips to wash' appears-check that # of strip appears is what is needed. If it is the same, press 'enter'. If is not the same, enter the right # of strip, and press 'enter 'until ' 001 Name 01' appears, press 'Enter'.
  - d. Press 'Run' (F1), and press 'No'(F2) under 'Prime'.
  - e. The Washer will aspirate the buffer.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/12/v03</b>	Page 3 of 5
<b>Serology Manual</b>		

- f. 'End washing' will appear when washing cycle is finished, remove plate.
  - g. 'Repeat Washing?' will appear, press 'No' (F2).
7. Add 100 ul of Blk to well # 1, 100 ul of Pos to well # 2, 100 ul of Neg to well # 3, 100 ul each of Cal to well # 4, 5, and 6. Add 100 ul of specimen to each well, starting from well # 7. Use last well as external Control well when needed.
  8. Cover plate with sealing tape, and incubate for 60 minutes at Room Temperature.
  9. Wash the wells three times using the Automatic Washer under Program '11'.
    - h. 'Select Program': Press memory (F1), press Number(F1).
    - i. For washing and soaking, use Program ' 11 '.**
    - j. 'Program No : press 11,enter.
    - k. Program '11 Name IgM WN' should appear. Press 'Set' at this point, press 'Change' (F2), then press 'Enter' until 'number of strips to wash' appears-check that # of strip appears is what is needed. If it is the same, presses 'enter'. If is not the same, enter the right # of strip, and press 'enter' until '11 Name IgM WN' appears, press 'Enter'.
    - m..Press 'Run' (F1), and press 'No'(F1) under 'Prime'. Will wash strip for 3 times, each time with working buffer.
    - n.'End washing' will appear when washing cycle is finished, soak strip for 5 minutes.
    - o. Aspirate Wash Buffer using Program '1',steps 6 a-i.
  10. Remove plate from Plate Washer.
  11. Add 100 ul of Antigen into each well using an 8-channel pipette, cover plate with sealing tape and incubate for 2 hours (120 minutes) at room temperature.
  12. Repeat wash steps 9a-h.
  13. Add 100 ul of Conjugate into each well using an 8-channel pipette, cover plate with sealing tape and incubate for 30 minutes at room temperature.
  14. Repeat wash steps.
  15. Add 100 ul of Substrate into each well using an 8-channel pipette, cover and incubate for 10 minutes at room temperature.
  16. Add 100 ul of Stop Reagent into each well using an 8-channel pipette.  
In antibody-positive wells, color should change from blue to yellow.
  17. Read the plate in spectrophotometer at dual wavelength 450 nm/630 nm within 1 hour of stopping the assay.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/12/v03</b>	Page 4 of 5
<b>Serology Manual</b>		

iv) Calculation:

Index value= Optical Density of Sample/the mean Optical Density of the Cut-Off Calibrators

v) Interpretation of Index Values:

Positive: > 1.10

Negative: < 0.9

Equivocal: >=0.9 and <= 1.1

All positive and equivocal specimens should be repeated the next day .

#### **IV. Validation**

The mean value for the Cut-Off Calibrator must be within 0.100 to 0.700 O.D. units minus the blank well.

The Positive Control Index value should be between 1.5 and 3.5.

The Negative Control Index value should be less than 0.8.

#### **V. Reporting**

Positive result: To PHL Report PHL result in LIS, and inform physician if result is confirmed Positive.

Negative result: West Nile Virus (Flavivirus) IgM by EIA: Negative.  
This is a research test.

Equivocal result: repeat testing,  
If result remains equivocal/ becomes Positive: Send to PHL.  
Inform physician if result from PHL is Positive.  
If result becomes negative : West Nile Virus(Flavivirus) IgM by  
EIA: Negative.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/12/v03</b>	Page 5 of 5
<b>Serology Manual</b>		

## **VI. Quality Control**

Each plate must include 1 Positive Control, 1 Negative Control, and 3 Cut-off Calibrators.

An External Positive Control ( Accurun 165 West Nile virus IgM/IgG Positive Control Series 5000) must be included with each new lot. Result filed in Reagent Lot Binder.

Inform charge/senior technologist of any QC failure.

## **VII. Reference**

Manufacture's insert, Focus Technologies, Cypress, California 90630.U.S.A. 2003.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/13/v02</b>	Page 1 of 6
Section: <b>Serology Manual</b>	Subject Title: <b>Molecular Testing - <i>Chlamydia Trachomatis &amp; Neisseria gonorrhoeae</i></b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: March 14, 2001	
Approved by: Laboratory Director	Revision Date: May 26, 2003	

## **CHLAMYDIA TRACHOMATIS AND NEISSERIA GONORRHOEAE**

### **I. Introduction**

Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC) testing are performed using BD ProbeTec™ ET System. The assay uses a Strand Displacement Amplification (SDA) technology for the direct, quantitative detection of CT and GC DNA in endocervical swabs, male urethral swabs, and urine specimens.

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

Use Culturette™ Direct Swab for collecting endocervical specimen, and Mini-Tip Culturette™ for urethral specimen. The swabs can be stored between 4 to 6 days at 2<sup>0</sup>C to 27<sup>0</sup>C before testing.

15-20 ml of the first voided urine can be collected in a preservative free, sterile container. The specimen should be forwarded to the Virology laboratory as soon as possible. Upon arrival in the laboratory, a UPP(Urine Processing pouch) is added to the specimen for at least 2 hours, which will remove inhibitors of SDA. Urine can be stored for 2 days at 15-27<sup>0</sup>C or 4 to 6 days at 2-8<sup>0</sup>C.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/13/v02</b>	Page 2 of 6
<b>Serology Manual</b>		

### III. Procedure

#### **Reagents:**

BD ProbeTec™ ET CT/GC reagent pouches containing:  
 CT priming and Amplification microwells  
 GC priming and Amplification microwells

Reagent pouches may be stored at 2 to 33<sup>0</sup>C. Once a pouch is opened, the microwells are stable for 4 weeks if sealed properly or until expiry date, whichever comes first.

BD ProbeTec™ ET control set  
 CT/GC Positive Controls  
 CT/GC Negative Controls

1. BD ProbeTec™ ET Sample Dilution Tubes
2. BD ProbeTec™ ET System supplies:  
 BD ProbeTec™ ET Instrument  
 Microcell plates  
 Lysing Heater and Lysing Rack  
 Primary and warming rack  
 Pipettor and Power Supply  
 Urine Processing Kit (UPP)  
 Sample tubes  
 Pipette tips

#### **ii) Other Materials:**

Centrifuge  
 Vortex mixer  
 Gloves (powder free)  
 Transfer Pipette  
 1%(V/V) Sodium hypochlorite  
 Timer  
 Absorbent Paper (roll)

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/13/v02</b>	Page 3 of 6
<b>Serology Manual</b>		

### iii) Sample Processing

#### 1. Swabs:

- a) Label and remove cap from sample diluent tube.
- b) Insert swab and swirl in diluent for 5-10 seconds.
- c) Express liquid from swab and discard.
- d) Tightly recap the tube and vortex for 5 seconds.

#### 2. Urine:

- a) UPP must be in contact with specimen for a minimum of two hours.
- b) Mix urine by swirling and pipetting 4 ml into empty sample tube.
- c) Cap tube and centrifuge at 2000xg for 30 minutes.
- d) Decant tubes. End decanting motion with a gentle “flick” of the wrist to remove the residual fluid from the tube.
- e) Add 2 ml of sample diluent.
- f) Recap tubes and vortex 5 seconds.

#### 3. Controls:

- a) Use 1 Positive Control and 1 Negative control for each run.
- b) Add 2 ml of sample diluent to each tube.
- c) Recap tubes and vortex 5 seconds.

Note: It is important to dispense liquid against the inside wall **of the microwells to insure complete specimen addition and to avoid cross-contamination.**

### iv) Sample Lysing:

- d) Use plate layout template to place samples and controls in correct position in lysing rack.
- e) Place rack in lysing heater for 30 minutes. Remove rack and let samples cool for at least 15 minutes.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/13/v02</b>	Page 4 of 6
<b>Serology Manual</b>		

**iv) Assay Procedure:**

1. Allow samples to cool at room temperature for at least 15 minutes (may be held up to 6 hours) after lysing is completed.
2. Remove and discard caps from tubes.
3. Change gloves to avoid contamination
4. Using the Plate Layout Report for the run, preparing the Priming Microwell Plate. Microwells must be in vertical correct order with CT, and follow by GC.
5. Using Program #3, expand pipettor by pulling the handle all the way out.
6. Aspirate samples from the first vertical column of tubes.

**Note: It is important to dispense liquid against the inside of the microwells to insure complete specimen addition and to avoid cross-contamination.**

7. Gently collapse the pipettor and dispense 150 ul into each of the corresponding column of Priming Microwell Plate.

**Note: Recap lysed specimen tubes; hold these until run is completed.**

8. Cover the Priming Microwell Plate with the priming cover and let the plate sit at room temperature for at least 20 minutes.

**Note: The plate may sit up to 6 hours before proceeding with the assay.**

9. Prepare the Amplification Microwell Plate by Configuring the Amplification Microwell Plate to match the Plate Layout Report.
10. Remove the cover from the Priming Microwell Plate.
11. Check the temperature of the Priming/Warming Heater:

Priming Heater: 72-73<sup>0</sup>C  
Warming Heater: 53.5-54.5<sup>0</sup>C

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/13/v02</b>	Page 5 of 6
<b>Serology Manual</b>		

12. Place the Priming Microwell Plate into the Priming Heater.
13. **Immediately** place the Amplification Microwell Plate into the Warming Heater
14. Set the timer for 10 minutes.
15. At the end of the 10 minutes incubation, select Program '5' on the pipettor.
16. Pick up new tips and transfer 100 ul from the column 1 of the Priming Microwell Plate to column 1 of the Amplification Microwell Plate.  
Allow pipette tips to touch sides of microwells  
After dispensing the liquid, allow pipettor to automatically mix the liquid in the wells, and discard tips.  
Continue transferring from column to column using new tips each time
17. When the last column has been transferred, seal the Amplification Microwell Plate using an Amplification Plate Sealer.
18. Place the Amplification Microwell Plate into the BD ProbeTec™ ET Instrument for analysis. The Amplification Microwell Plate must be loaded into the instrument within 30 seconds of transferring the last column of samples.  
**Timing is extremely critical. Do Not Delay entry of plate into the instrument.**
19. Test results will be printed out as soon as the testing is finished.
20. See Appendix VIII for Workflow Overview.

## II. Validation:

**MOTA Score** is a metric used to assess the magnitude of signal generated as a result of the reaction. The magnitude of the MOTA Score is not indicative of the level of organism in the specimen.

MOTA Score for CT or GC :

CT/NG Positive Control : > or = 2000

CT/NG Negative Control : < 2000

**If any of these two controls fails, the test is invalid. Should inform Senior/Charge technologists and repeat testing.**

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/13/v02</b>	Page 6 of 6
<b>Serology Manual</b>		

## V. Interpretation of Results:

MOTA score for CT/NG is  $\geq 2000$  is considered as Positive, reported as Positive for Chlamydia trachomatis / Neisseria gonorrhoeae by Nucleic Acid Amplification

MOTA Score for CT/NG is  $< 2000$  is considered as Negative, reported as Negative for Chlamydia trachomatis / Neisseria gonorrhoeae by Nucleic Acid Amplification

## VI. Reporting

### Positive report:

Positive for Chlamydia trachomatis by Nucleic Acid Amplification

Positive for Neisseria gonorrhoeae by Nucleic Acid Amplification

### Negative report:

Negative for Chlamydia trachomatis by Nucleic Acid Amplification

Negative for Neisseria gonorrhoeae by Nucleic Acid Amplification

## VII. Quality Control

One Positive Control and one Negative Control must be included in each run. Run external control (Accurun 341)) every second week. Result filed in Reagent Lot Binder.

External proficiency testing is provided by CAP and QMP-LS .

### Maintenance QC:

Enter temperature checks on lysing heater, priming heater, warming heater, and instrument in System Maintenance Log every time the test is performed.

Clean/replace Air Filter and verify BD ProbeTec ET temperature monthly.

Wipe checks for Analyser, Bench, Lysing rack, Pipette, Priming heater, Lysing heater are done every two weeks ( see appendix# ) .

## VIII. Reference

Package insert for BD ProbeTec™ ET CT/GC Amplified DNA Assay.

Becton, Dickinson and Company, 7 Loveton Circle, Sparks, MD 21152, USA.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/14/v02</b>	Page 1 of 5
Section: <b>Serology Manual</b>	Subject Title: <b>Molecular Testing - HBV DNA</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: March 14, 2001	
Approved by: Laboratory Director	Revision Date: October 10, 2003	

## **HEPATITIS B (HBV) DNA**

### **I. Introduction**

The COBAS Amplicor HBV Monitor™ Test is an in vitro nucleic acid amplification test for the quantitation of Hepatitis B virus DNA in human serum or plasma on the COBAS AMPLICOR™ Analyser.

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

10 ml of blood is collected in a sterile red-topped or lavender-topped(EDTA) tube and spun at 3000 rpm for 15 minutes. Serum/plasma is separated into two storage tubes and put in 'HBV DNA' box in -20<sup>0</sup>C freezer.

### **III. Procedure**

Pre-PCR: Specimen Preparation:

1. Use either pre-diluted specimens (See Specimen Dilution Protocol to follow) or if using frozen serum or plasma specimens, thaw the specimens at room temperature and vortex for at least 5 seconds.
2. Label one 1.5 ml screw cap tube for each specimen to be processed, including one tube for the Negative (-) Control, one tube for the Low (+) Control and one tube for the High (+) Control. Place a pellet orientation mark on each tube.
3. Vortex HBM LYS 1 for 5 seconds. Add 50ul of HBM LYS 1 to each of the tubes using a Repeater Pipette and 1.25 ml Combitip Reservoir or a micropipette with a plugged tip.
4. Vortex NHP for 5 seconds. To each of the three control tubes containing HBM LYS 1, add 100ul of NHP. Vortex for at least 10 seconds.
5. To each of the patient tubes containing HBM LYS 1, add 100uL of patient serum or plasma and vortex for at least 5-10 seconds.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/14/v02</b>	Page 2 of 5
<b>Serology Manual</b>		

6. Place tubes in the microcentrifuge with the orientation mark facing outward. Centrifuge tubes for 5 minutes at 12,500-16,000 x-g at room temperature.
7. Remove and discard the supernatant, using a new, fine-tip disposable transfer pipette or an aerosol resistant tip for each tube. Remove as much liquid as possible without disturbing the pellet. Withdraw the supernatant slowly, allowing the liquid to drain off the sides of the tube. Do not use vacuum aspiration.
8. Mix the HBV (-) C by vortexing for 5-10 seconds. Add 25uL of HBV(-) C to the tube labeled HBV(-) C.
9. Add 25uL of HBV (-) C to each of the patient tubes.
10. Mix the HBV L (+) C by vortexing for 5-10 seconds. Add 25uL of HBV L (+) C to the tube labeled HBV L(+) C.
11. Mix the HBV H (+) C by vortexing for 5-10 seconds. Add 25uL of HBV H (+) C to the tube labeled HBV H (+) C.
12. Prepare Working Lysis 2 Reagent as follows: vortex the HBM QS for 10 seconds. Add 50uL of HBM QS to one tube HBM LYS 2 and mix well by vortexing for at least 15 seconds.
13. Add 100uL of Working Lysis 2 Reagent to all the tubes and mix well by vortexing 15 seconds. The pellet does not always dissolve completely, but this will not interfere with assay performance.
14. Incubate all tubes for 60 minutes at 60° C ± 2° C. (Pulse spin tubes for 5 seconds to remove condensate from cap)
15. Add 100uL of HBM LYS 3 to each tube and mix well by vortexing for at least 10 seconds. Incubate all tubes for 10 minutes at 100° C ± 2° C.
16. Place tubes in the microcentrifuge with the orientation mark facing outward. Centrifuge tubes for 15 minutes at (12,500-16,000 x-g) at room temperature to collect insoluble particles. Amplify the processed specimens and controls within 1 hour of preparation or store frozen at -20 to -80° C for up to 1 month with no more than 1 freeze-thaw. More than one freeze-thaw cycle may result in loss of copy number.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/14/v02</b>	Page 3 of 5
<b>Serology Manual</b>		

If processed specimens and controls were stored frozen prior to amplification, thaw at room temperature, vortex for 5 seconds, centrifuge for 15 minutes before proceeding to step 17.

17. Preparation of Working Master Mix (to be done in clean room)

- a. Vortex HBM Mg 2+ for 5 seconds. Prepare Working Master Mix by adding 100uL HBM Mg 2+ to one vial HBM MMX. It is not necessary to measure the volume of Master Mix. Mix well by inverting the tube 10-15 times. Discard remaining HBM Mg 2+. The pink dye in HBM Mg 2+ is used for visual confirmation that HBM Mg 2+ has been added to HBM MMX.
  - b. Pipette 50 uL Working Master Mix into each A-tube using a pipette with an aerosol barrier tip. Do not close covers of the A-tubes at this time. Discard unused Working Master Mix.
  - c. Place A-rings containing Working Master Mix into a resealable plastic bag and seal plastic bag securely. Move A-rings into specimen preparation area and store at 2-8° C until ready for use. **Working Master Mix must be used within 4 hours of preparation.**
18. Add 50 ul of each processed specimen or control to the appropriate A-tubes containing working Master Mix using a micropipettor with a plugged tip. Do not transfer any precipitated material. Cap the tubes after each sample addition.
19. Transfer A-rings to COBAS AMPLICOR. Amplification on the COBAS AMPLICOR analyzer must be started within 1 hour of the time processed specimens and controls are added to the A-tubes containing Working Master Mix. The remainder of the processed specimens may be frozen at -20 to -80° C for up to one month.

Specimen Dilution Protocol:

If a quantitation value is required for specimens containing high levels of HBV DNA (>200,000 copies/ml) then a set of serial dilutions should be performed.

For this dilution you will need a new disposable microwell plate and Basematrix (this is stored in aliquots in freezer).

If using frozen serum or plasma specimens, thaw the specimens to be diluted at room temperature and vortex for at least 10 seconds.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/14/v02</b>	Page 4 of 5
<b>Serology Manual</b>		

1. Add 150 ul of each specimen to a well in the first row of a new disposable microwell plate (Row A).
2. Add 225 ul of Basematrix to the next 6 wells below each specimen to be diluted (Rows B through G).
3. Using a 25 ul pipet, remove 25 ul of the undiluted specimens located in Row A and dispense into the first row of Basematrix (Row B) to make a 1:10 dilution. Mix up and down 10 times using the pipet.
4. Remove 25 ul of the 1:10 dilution from row B and dispense into Row C to make a 1:100 dilution. Mix up and down 10 times using the pipet.
5. Repeat step 4 a total of four more times, to make 1:1,000, 1:10,000, 1:100,000 and 1:1,000,000 dilutions using Rows D, E, F, and G, respectively.
6. Use the 1:1,000,000 (Row G) dilution to process and run in the assay.
7. Date and seal used rows with a plastic seal and place lid over the plate, place in a zip lock bag and store at 2-8 C. The remaining specimen dilutions may be used, if necessary, on a subsequent run if the initial processed specimen falls outside of the reportable range.

**NOTE:** For specimens that have been pre-diluted, it will be necessary to multiply the copy number by the appropriate dilution factor to determine the final result.

#### **IV. Reporting**

Report results as the numeric value generated by the COBAS:  
# of copies/ml by Roche Assay. HBD DNA is reported for research purposes.

All 'Target OD-LO' and 'Result-LO' results should be reported as:  
<200 copies /ml by Roche Assay. HBD DNA is reported for research purposes.

All values over 200,000 copies /ml i.e. 'Result- HI' or ' QS-INVALID ' should Be diluted once according the dilution protocol, check with charge/senior regarding appropriate dilution to repeat testing. Then report the exact # of copies/ml plus dilution factor, unless it is 'Result-Hi' again.  
The 'Result-Hi' after dilution will be reported as >2xE10 copies/ml by Roche Assay. HBD DNA is reported for research purposes.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/14/v02</b>	Page 5 of 5
<b>Serology Manual</b>		

**V. Quality Control**

One HBV Monitor Negative control, One HBV Monitor Low Positive control, and one HBV Monitor High Positive control are included with each run. Each result must be within the set value for each Lot #, otherwise the run is invalid.

Run Accurun HBV DNA 300 and 600 on each run.

Plot results in chart provided, results has to be within  $\pm 3.S.D.$ , otherwise the run is invalid. Inform charge/senior technologist.

External proficiency testing is provided by LCDC.

**VI. Reference**

Package insert from COBAS AMPLICOR HBV MONITOR™, version 2.0.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/15/v04</b>	Page 1 of 7
Section: <b>Serology Manual</b>	Subject Title: <b>Molecular Testing - HCV RNA PCR</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: March 14, 2001	
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## HEPATITIS C RNA PCR

### **I. Introduction**

To test for the presence of HCV RNA in serum or plasma using The COBAS AMPLICOR Hepatitis C Virus Test.

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

Blood should be collected in sterile red –topped tube or in sterile tube using EDTA (Lavender-topped) and spun at 2000 rpm for 15 minutes. Serum or plasma is separated and stored in three serum tubes and placed in “HCV RNA” box in –20<sup>0</sup>C freezer. Separated specimen may be frozen and thawed up to two times without a loss in copy number.

Specimen collected in green-topped (Heparin) tube is unsuitable for this test.

### **III. Procedure**

#### **Pre-Amplification Area -Reagents Preparation:**

##### **Using the MagNA Pure:**

1. Make worklist in LIS lab using 8HCAP. Label as follows:

1---1A	13---2E
2---1B	14---2F
3---1C	15---2G
4---1D	16---2H
5---1E	17---3A
6---1F	18---3B
7---1G	19---3C
8---1H	20---3D
9---2A	21---3E
10---2B	22---3F
11---2C	23---3G
12---2D	24---3H

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/15/v04</b>	Page 2 of 7
<b>Serology Manual</b>		

2. Turn on MagNA Pure instrument. Now, turn computer on. Leave password blank.
3. Make 2 vials of Master Mix (24 samples) in clean room as per HCV protocol. Load Master Mix vials onto the MM position in the MagNa Pure. Put 2 new A-rings onto MagNA Pure into the correct A or B position. Remember to take off Master Mix caps later. Master Mix is only stable for 4 hours once made, and prepared A-ring(s) must be placed in Cobas before this time expired.
4. Make working lysis buffer in a 15 ml conical centrifuge tube. Vortex the HCV IC from HCV specimen prep kit for 5-10 s. For 24 samples including controls, add 162 ul HCV IC to 7800 ul Lysis/Binding Buffer (vial 4, green cap) from MagNA Pure kit (don't touch sides of tube when adding the HCV IC). Invert gently to mix.
5. For each sample and control, pipet 300 ul of working lysis reagent (with repeat pipettor) into 24 vials of the sample cartridge. Mark last two vials as negative and positive controls respectively.
6. Preparation of controls:
  - a. Prior to use, vortex the NHP, HCV (-) control and HCV (+) control for 5-10 s.
  - b. Add 200 ul NHP to each of the two corresponding control vials of the sample cartridge containing the working lysis reagent and mix up and down with pipette.
  - c. Add 20 ul HCV(-) control to the vial marked with "HCV (-)" and mix up and down with pipette.
  - d. Add 20 ul HCV (+) control to the vial marked with "HCV (+)" and mix up and down with pipette.
7. For each sample to be extracted, pipet 200 ul of sample material into the corresponding vial in the sample cartridge containing the working lysis reagent and mix up and down with pipette. Seal plate and place onto MagNA Pure. Remember to take off seal later.
8. At MagNA Pure computer screen:
  - pick "A-ring ordering".
  - Barcode order numbers or enter them manually.
  - Select "protocol" and choose "total NA" and then "total NA external lysis.blk".
  - Select "post elution protocol" and choose "HCV- Cobas setup.pep".
  - Change "lysed sample volume" to 350ul
  - Change "elution volume" to 65ul
  - Click on 'Liquid Waste Discard' square, (this will remove liquid at end of cycle before we discard them) from cartridges
  - Click on "stage setup"

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/15/v04</b>	Page 3 of 7
<b>Serology Manual</b>		

9. Load reagents according to MagNA Pure screen:
  - Wash buffer #1 – invert bottle to mix, pour amount noted on screen into large tub, put lid onto tub with points facing down, put into metal tray in correct position (color-coded), click image on MagNA Pure screen
  - Wash buffer #3 – as wash buffer #1
  - Proteinase K – reconstitute by first adding 3 ml elution buffer, mix by inversion, then add another 2 ml of elution buffer, mix by inversion
    - pour amount noted on screen into medium 20 tub
    - put lid onto tub with points facing down, put into metal tray in correct position (color-coded), click on image on screen
    - refrigerate unused portion immediately (put date on lid)
    - fresh aliquot must be used each day when test is run.
  - MGP – magnetic glass particles , TO BE MADE AFTER ALL OTHER REAGENTS AND DISPOSABLES ARE ON THE MAGNA PURE - this reagent needs to be mixed well by inversion, then pipet exact amount into medium 20 tub (letting it drain well into tub), put in reagent tray , click on image on screen
  - Elution buffer – invert bottle to mix, pour amount noted on screen into medium 20 tub, put lid onto tub with points facing down, put into metal tray in correct position ( colour-coded), click on image on screen
  - Buffer #2 – as wash buffer #1
  - Place reagent tray on MagNA Pure in designated position (left side in machine), lock bar needs to be opened in order to place reagent tray and tips in next step
10. Load tips, processing cartridges, tip stands and elution and storage cartridges as per screen and click on each image as you complete task.
11. Check and change liquid waste bottle. Click on image on screen.
12. Check and change waste bag if needed. Click on image on screen.
13. Click on image of drop catcher (this is changed weekly – see weekly maintenance for procedure).
14. Click on sample cartridge image and confirm barcode #.
15. MGP – magnetic glass particles , TO BE MADE NOW AND PLACED IN REAGENT TRAY ALREADY ON MAGNA PURE
16. **NOTE: LAST STEPS ARE TO PEEL OFF PLASTIC COVER FROM SAMPLE CARTRIDGE ,CLOSE LOCKING BAR OVER REAGENT TRAY AND TIPS, AND TAKE OFF LIDS TO BOTH MASTER MIX VIALS.**

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/15/v04</b>	Page 4 of 7
<b>Serology Manual</b>		

17. Check the 6 status bars at right side of screen. “Cover” should be closed, “cover lock” should be locked, “lock bar” should be locked, "heat unit" should have pass, “cool units 1 & 2 “ should have pass. An “OK” box will appear at bottom right hand corner of screen (which was not there before) once status of all are correct. Click on this “OK” and machine will start. If “OK” box doesn’t appear, check that everything on the screen is in place and that each item has been clicked.
18. Prepare COBAS as per HCV protocol.
19. After the MagNA Pure has finished the extraction, the “Results screen” will appear. Check to see if all passed. Cap the A-rings and transfer them to the COBAS. Continue as per HCV package insert.
20. Discard disposables (trays and tip stands) from the MagNA Pure into biohazard box. Wash buffers 1,2,3 and the elution buffers can be saved for one week. Cover with tub lid seals, date and put whole rack in fridge. Proteinase K and MGP tubs must be discarded.
21. Choose File and Exit, then file and save. When saving enter current date.
22. Exit to main screen.
23. Click the “Decontamination” button. Set decontamination time for 8 hours. On Friday set timer until end of workday. Click on Actions and choose “Start Decontamination). When the decontamination is complete (next day or end of day), turn off instrument. Click on “Start” at bottom left corner of screen and choose shut done.

### **Post Amplification Area- Amplification and Detection:**

Perform Daily Instrument Maintenance as outline in the Operator’s Manual of the COBAS AMLPIOCR Analyzer, including:

- Wipe initialization post with a lint-free moist cloth and dry
- Wipe D-cup handler tip with a lint-free moist cloth and dry
- Check Wash Buffer Reservoir and fill if necessary
- Prepare Working Wash Buffer( 1x) by adding 1 volume of WB(10x) to 9 volume of Deionized water. Mix well. Keep a minimum of 5-6 liters of Wash Buffer(1x) in the Wash Buffer Reservoir at all times.
- Empty waste container
- Prime the system : 1. Normal prime  
2.Extended prime (use if wash reservoir was refilled, or been idle for a long time).

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/15/v04</b>	Page 5 of 7
<b>Serology Manual</b>		

## Instrument Loading and System Operation using AmpliLink

### 1. Maintenance

- Click on 'Maintenance' window, 5<sup>th</sup> from the top at the side application bar.  
Click on 'Service actions' tab to get into 'Service Action folder'.  
Performing 'prime';
- Click on 'tools' from menu bar.
  - Highlight 'prime'
  - Choose either 'Normal' or 'Extended' prime. Record daily and weekly maintenance by clicking on 'action' to highlight it, and click 'Update' and 'OK'.

### 2. Reagent Check

- Click on 'Instrument Window', 2<sup>nd</sup> from the top at the side application bar.  
Click on 'Cassette' tab.  
To alter rack #: double click on 'Rack #', and change #, click 'OK'.  
To add cassette: double click on any of the cassette icons, and scan in barcode on new cassette.  
A single click on any cassette will show the # of tests left in that cassette.  
Color coding: green=reagent on rack and has >10 test left  
Yellow= reagent on rack expired and/or has <10 test left  
Red= zero test left or mismatched volumes

### 3. Creating orders

- Click on 'Order', 3<sup>rd</sup> window from the top at the side application bar.  
Click on '+' at top right corner to create a new order.  
Type in A-ring # at top left corner.  
Double click on 'Profile': HCV-11=12 specimens  
HCV-12=10 specimens, negative control, and  
Positive control  
Click on first space to enter specimen #(either type in or use barcode wand).  
Use '↓' to move down to the next position until finished.  
Click on 'save' to save order.  
Click on '+' again to create another order, repeat the above steps.

### 4. Loading the A-rings:

- Click on 'Analyzer' tab.  
Double clicks on thermocycler A, click on 'arrow', select A-ring #, click 'OK'.  
Do the same for thermocycler B.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/15/v04</b>	Page 6 of 7
<b>Serology Manual</b>		

5. Start the Run:

Click 'Start' to start the run, a 'Load check' will be performed.

Select 'Run Mode': basic.

Wait for 'Load check' to pass-see status at top right corner, print a copy by clicking on 'Print' Icon.

If 'Load Check' failed, click on 'Stop' which will stop the transferring step, but amplification will continue. Find out the reason for failing (e.g. not enough reagent in cassette, wrong rack#...). Ratify problem(s), and click on 'Start' again.

Wait for 'Load check' to pass-see status at top right corner, print a copy by clicking on 'Print' icon.

6. Printing the results:

When the run is finished, click on 'Result', 4<sup>th</sup> window from the top at the side application bar.

Scroll down to find 'A ring #' on the left side.

Double click on A-ring#, check results on screen, click on 'Accept All' at the bottom of the screen.

Click on 'View', 'List by tube', and 'print'.

#### IV. Validation

**Internal control** for each **negative** specimen must be '**positive**' to make sure the specimen does not contain inhibitory substance.

If internal control is negative or result is 'equivocal', repeat using another tube of the same specimen in next run.

If internal control is still negative after repeat testing, send out report in as 'No result available due to inhibitory substance present in specimen, please send another specimen'.

**Internal control** for each **positive** specimen can be '**positive**' or '**negative**'. Because specimen has already amplified, therefore giving a positive result.

Positive :  $\geq 0.15$  at  $A_{660}$

Negative :  $< 0.15$  at  $A_{660}$

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/15/v04</b>	Page 7 of 7
<b>Serology Manual</b>		

## V. Reporting

- Positive **All positive patients should be checked if they belong to the enlisted doctors. If not, the patients should be highlighted on the tasklist before submitting it to the charge technologist for verification.**
- Negative
- No result available due to inhibitory substance present in specimen, please send another specimen.

## VI. Quality Control

One AMPLICOR HCV(-) control and one AMPLICOR HCV (+) control are processed with each run.

The absorbance of HCV(-) should be less than 0.1 at 660 nm.

The absorbance of HCV(+) should be greater than or equal to 1.0 at 660 nm.

Run External Control (Accurun 305) monthly. If result is invalid, the run has to be repeated. Inform charge/senior, and repeat testing. Result filed in Reagent Lot Binder.

External proficiency testing is provided by LCDC.

## VII. Reference

COBAS Amplicor Operator's Manual and Insert for Hepatitis C Virus Test version 2.0, by Roche Diagnostics

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/16/v01</b>	Page 1 of 10
Section: <b>Serology Manual</b>	Subject Title: <b>Molecular Testing – West Nile Virus RT PCR</b>	
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## WEST NILE VIRUS RT PCR

### **I. Introduction**

West Nile virus (WNV) is a flavivirus belonging to the Japanese Encephalitis group. The primary host is wild birds and the virus is usually transmitted to humans via mosquitoes. Transplants and blood transfusions have also been implicated in its transmission. This assay involves isolating the viral RNA using the Qiagen Viral RNA Kit and amplifying it by RealArt™ WNV Reverse-Transcription (RT)-PCR Kit. The amplification and detection take place simultaneously, continually and in real time in the Roche LightCycler™.

In real-time PCR, fluorescent dyes are linked to oligonucleotide probes, which bind specifically to the amplified product. Monitoring the level of fluorescence allows the detection of the amplicon. In the Artus WNV assay, two fluorescent dyes are incorporated in the master mix to detect two amplified products: One for the 80 bp WNV amplicon; and the other for the Internal Control (IC). The IC is added to each specimen in the initial step to both control the isolation procedure and to check for PCR inhibition. The LightCycler™ simultaneously monitors the two different amplicons using two fluorimeter channels.

### **II. Collection and Transport**

Blood samples for WNV PCR should be collected in EDTA (purple top vacutainer tube). CSF specimens should be collected in a clean, sterile container and sent to the laboratory as soon as possible. If specimens cannot be transported immediately, they should be kept at 4°C. If a delay of more than 6 hours is anticipated, the CSF specimen should be frozen at –70°C. The EDTA samples should be processed within 6 hours of collection (plasma separated and refrigerated; frozen if delay is more than 6 hours). Allow only one freeze-thaw cycle.

NOTE: Repeated freezing-thawing will reduce test sensitivity and cryoprecipitates may accumulate in the plasma.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/16/v01</b>	Page 2 of 10
<b>Serology Manual</b>		

### **III. Procedure**

#### **A. Worklist Preparation:**

- a. Log on to **Softmic**,
- b. Select/type:
  - i. **Result**
  - ii. **Worklist**
  - iii. **Pagedown** to 86 (WNV PCR),
  - iv. **Enter**
  - v. **F12**
- c. Pending samples (plasma, serum or CSF) will appear on Worklist.
- d. Mark received samples using the **F5** key,
- e. Type "~" to print Worklist. The numbers on the left column of the Worklist will be used to label the 1.5 mL Eppendorf microcentrifuge tubes and the Qiagen Isolation Columns.

#### **B. LIS Label Printing (if needed for the RNA eluate):**

- a. While still in the Worklist, press **Enter** to go to the Test Screen
- b. Press **F1** to move cursor to Demographic field
- c. Press: / (to Order/Entry)
- d. Press: **No** (to confirm editing)
- e. Press: **F9**
- f. Press: \ (Print Labels)
- g. Press: **No** (modify record)
- h. Select label printer for the LIS label

#### **C. Specimens Processing:**

Specimens should be processed as soon as possible after arriving in virology laboratory.

- a. EDTA blood:  
The blood should be centrifuged and an aliquot of about 2 mL plasma frozen unless PCR can be performed immediately.
- b. CSF:  
An aliquot (0.2-2 mL) of the CSF should be frozen unless PCR can be performed immediately

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/16/v01</b>	Page 3 of 10
<b>Serology Manual</b>		

After processing, an aliquot of the left-over specimens should be stored frozen at -70°C.

D. Materials, Equipments and Facilities:

Clean Room with dedicated Biosafety Cabinet (MIBCT4), freezer (MIFTG), gowns and gloves

Specimen Preparation area with Biosafety Cabinet and microcentrifuge

Detection Room for LightCycler

Roche LightCycler programmed for Artus WNV LC PCR

Microcentrifuge (MICT14)

1.5 mL microcentrifuge tubes

Cooling Block with capillary adaptors pre-cooled to 4°C

Variable volume pipettes: 1 to 20 uL

10 to 200 uL

100 to 1000 uL

From Qiagen Viral Isolation Kit:

- Collection Tubes
- Lysis Buffer AVL (with RNA carrier) – ***thawed or heated to dissolve crystals formed during refrigeration***
- Spin Columns
- Wash Buffer 1 (Buffer AW1) – ***add 125 mL ethanol before use***
- Wash Buffer 2 (Buffer AW2) – ***add 160 mL ethanol before use***
- Elution Buffer (Buffer AVE)

From: RealArt™ WNV LC RT PCR Kit, Artus:

- Quantification Standard no. 3 (10<sup>2</sup> copies/uL)
- Internal Control

Ethanol, anhydrous, reagent grade, 96 - 100%

GENERAL PRECAUTIONS:

- Use only filtered pipette tips
- Store positive materials (specimens, controls and amplicons) separately from all other reagents and add to reaction mixes in a spatially separated facility (clean room)
- Clean and specimen pipettes and tips stay separated in their designated rooms
- Thaw components thoroughly at room temperature
- Mix components and centrifuge briefly
- Work quickly in the cooling block
- Put on fresh gloves and clean gown (colour-coded: blue) when entering the clean room

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/16/v01</b>	Page 4 of 10
<b>Serology Manual</b>		

E. Sample Preparation:

Use Specimen Preparation area, work in cabinet with gown and frequent glove change

Lysis and Purification Steps:

- a. For each specimen, Quantification Standard (QS), External Control and Negative Control (H<sub>2</sub>O) prepare and label two 1.5 mL microcentrifuge tubes (sterilized, RNase-free) with the corresponding number in the Worklist. Label one of them with the lab number (LIS label) also (this microcentrifuge tube will store the eluted RNA, the other will be used for processing and will be discarded). Also, label one QIAamp Spin Column (Qiagen) with the number on the Worklist. Arrange the 2 microcentrifuge tubes and Columns into 3 rows.
- b. If the Lysis Buffer is stored in the refrigerator and crystals are present, heat to dissolve the crystals. Add 560 uL Lysis Buffer (AVL with RNA carrier) into each of the microcentrifuge tubes in one row (these will contain the waste).
- c. Add 5 uL WNV Internal Control to each of the microcentrifuge tubes with Buffer AVL.
- d. Add 140 uL specimen to the Lysis Buffer (Buffer AVL with RNA carrier), vortex for 15 seconds.
- e. Incubate at Room Temperature (15-25°C) for 10 minutes.
- f. Centrifuge for 10 seconds at 2000 g to remove drops from the lids.
- g. Add 560 uL ethanol (96-100%) to the samples, vortex for 15 seconds. Centrifuge for 10 seconds at 2000 g to remove drops from the lids.
- h. Pipette 630 uL (half the volume) of the mixture (specimen-lysis buffer-ethanol) to the QIAamp spin column (in a 2 mL collection tube), close the cap and centrifuge at 6000g (8000 rpm) for 1 minute. Place the QIAamp spin column into a clean 2 mL collection tube and discard the collection tube containing the filtrate.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/16/v01</b>	Page 5 of 10
<b>Serology Manual</b>		

- i. Repeat Step 7 by pipeting the remaining 630 uL of the mixture to the QIAamp spin column (in a 2 mL collection tube), close the cap and centrifuge at 6000g (8000 rpm) for 1 minute. Place the QIAamp spin column into a clean 2 mL collection tube and discard the collection tube containing the filtrate.
- j. Open the spin column and add 500 uL of Buffer AW1. Close the cap and centrifuge at 6,000g (8,000 rpm) for 1 minute.
- k. Open the spin column and add 500 uL of Buffer AW2. Close the cap and centrifuge at full speed (20,000g; 14,000 rpm) for 3 minutes.
- l. Place the spin column into a clean 2 mL collection tube and discard the collection tube containing the filtrate. Centrifuge at 20,000g for 1 min.

#### Elution Steps:

- a. Place the spin column in the second clean 1.5 mL microcentrifuge tube with the LIS specimen label. Discard the collection tube containing the filtrate. Open the spin column and add 50 uL of Elution Buffer (Buffer AVE). Close the caps and incubate at room temperature for 1 minute.
- b. Centrifuge at 6000g (8000 rpm) for 1 min. Store the eluate at -20°C or -70°C if not able to proceed immediately.

#### PCR Amplification and Detection Procedures:

In the Clean Room: (Changed to dedicated clean room gown and gloves, work in Cabinet)

- a. Take out the necessary number of WNV Master Mix vials (12 tests per vial) from the freezer and thoroughly thaw inside the Biosafety Cabinet.
- b. Place a capillary for each purified sample, QS, external QC and H<sub>2</sub>O into the capillary adaptor of the Cooling Block (pre-cooled to 4°C).
- c. Pipette 15 uL Master Mix into the reservoir of each capillary.
- d. Changed out of dedicated clean room gown

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/16/v01</b>	Page 6 of 10
<b>Serology Manual</b>		

In the Specimen Processing Room: (Changed into specimen room gown)

- a. Using a separate filter tip each time, carefully pipette 5 uL each of purified sample, QS<sub>3</sub>, external QC and H<sub>2</sub>O (already spiked with 0.1 mL internal control per 1 mL H<sub>2</sub>O) directly into the capillary reservoir. Immediately close the capillary with a lid.
- b. Load capillary tubes into LightCycler (LC) adaptor and centrifuge at 3000 rpm for 15 seconds in LC centrifuge. Transfer the LC adaptor to the LC instrument.
- c. Alternatively, if the LC centrifuge is not available, centrifuge the capillary in the adaptors for 10 seconds at 2000 rpm (400g) to transfer the mixture from the reservoir to the capillary, making sure that the engraved numbers on the adaptors correspond to the correct sample.
- d. Place the adaptors and capillaries back in the Cooling Block and proceed to the LightCycler.

In the LightCycler room:

- a. Turn LightCycler and the computer on
- b. Click on the LightCycler icon and click "run"
- c. Select "selftest", skip if already done within 24 hr. or
- d. Press: OK
- e. Select:
  - "Run Experimental File"
  - " WNV protocol"
- f. Type in file name "*WNV-run number-year-month-date*"
- g. Select "run"
- h. Type in the number of samples to run including QS, ext. QC and H<sub>2</sub>O
- i. Click "Edit Sample Later"
- j. Type in the lab numbers; QS; ext. QC (if any) and H<sub>2</sub>O

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/16/v01</b>	Page 7 of 10
<b>Serology Manual</b>		

k. On the QS row, click the arrow-down button to change its description from “Unknown” to “STD” and enter the number of copies/uL (eg. QS3=100)

l. Press “done”

m. To Print Results, go to:

“FLUORESCENCE” and click

- F1
- Quantification
- Print Window

m. To Print Internal Control, go to:

“FLUORESCENCE” and click:

- F3/BACK F1
- Print Window

**IV. Reporting:**

Fluorimeter <b>Channel F1</b> (WNV amplicon)	Fluorimeter <b>Channel F3</b> <b>back F1</b> (IC amplicon)	<b>Interpretations</b>
-	-	Possible PCR inhibition- <b>Repeat assay.</b> If still -ve on both Fluorimeter Channels, report: <b>Indeterminate</b>
-	+	Report: <b>Negative</b>
+	-	Preliminary report: Positive Repeat assay to confirm. If still +ve on fluorimeter Channel F1 Report: <b>POSITIVE</b>
+	+	Preliminary report: Positive Repeat assay to confirm. If still +ve on fluorimeter Channel F1. Report: <b>POSITIVE</b>

Positive results for the WNV amplicon (Channel F1) will have the cycle number printed under the Cross Point Column in the chart and the Calculated values will be at least 10 copies/uL ( $\geq$ QS4). The curve will be rising exponentially and levels off to a plateau. The sample must be repeated for confirmation. Internal Control amplicon need not be positive.

Indeterminate results may be those with a positive signal for the WNV amplicon (Channel F1) cycle number printed under the Cross Point Column in the chart but the Calculated values are less than 10 copies/uL ( $<$ QS4). The curve will be rising at a high cycle number. The sample must be repeated.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/16/v01</b>	Page 9 of 10
<b>Serology Manual</b>		

Indeterminate results may also be those with no signal for the WNV amplicon (Channel F1) and also no signal for the IC amplicon (Channel F3 back F1), indicating possible PCR inhibition. The test must be repeated.

Negative results for the WNV amplicon (Channel F1) will be blank under the Cross Point Column in the chart and the curve will be rather flat. The IC amplicon (Channel F3 back F1) must be positive to be valid.

Negative Report:       Negative by RT-PCR.  
This is a research test performed by using RealArt WNV assay, Artus Biotech Inc.

Indeterminate Report: Indeterminate by RT-PCR  
This is a research test performed by using RealArt WNV assay, Artus Biotech Inc.

Positive Report:        **POSITIVE** by RT-PCR  
This is a research test performed by using RealArt WNV assay, Artus Biotech Inc.

Phone all Report positive results and inform senior/charge technologist. Record isolate in LIS by F7 as “77wnv”.

## V. **Quality Control**

### Equipment QC:

Every six months:

- Colour Compensation (to compensate interference between different fluorochromes) should be run to update the Colour Compensation File.

### Reagent QCs:

Before every lot change of isolation and/or master mix kit:

- An External Control (external to Artus Biotech) is used to monitor the isolation, amplification and detection procedures. The result must correspond to expected value supplied by the manufacturer.

Before every lot change of master mix kit:

- All four Quantification Standards (QS 1, 2, 3, and 4) must be run.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/16/v01</b>	Page 10 of 10
<b>Serology Manual</b>		

Daily QCs:

Every run:

- Each patient specimen must have an Internal Control (IC) added to monitor both isolation and PCR inhibition. The sample must show a positive reading in either fluorimeter Channel F1 (WNV amplicon) or fluorimeter Channel F3 back F1 (IC amplicon) to be valid (no PCR inhibition).
- A Positive Control (eg. Quantification Standard #3) is included and shows a positive reading in fluorimeter Channel F1 (WNV amplicon).
- A Negative Control (eg. H<sub>2</sub>O spiked with IC) is include and shows a negative reading in fluorimeter Channel F1 (WNV amplicon) and a positive reading in fluorimeter Channel F3 back F1 (IC amplicon).

Report all failed QCs to senior/charge technologist.

**VI. References:**

QIAamp Viral RNA Mini Kit Handbook 01/99, Qiagen  
 RealArt™ WNV LC RT PCR Kit User Manual March 2003, Artus  
 LightCycler, Roche Diagnostics

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/17/01/v02</b>	Page 1 of 2
Section: <b>Serology Manual</b>	Subject Title: <b>Appendix I Serology Test Schedule</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: March 14, 2001	
Approved by: Laboratory Director	Revision Date: March 19, 2002	

**APPENDIX I**

**SEROLOGY TEST SCHEDULE**

EBV Serology.....	Thursday
HBsAg .....	daily
HBsAg confirmation .....	when needed
HBsAb .....	daily
HBcAb-Total .....	daily
HBcAb-IgM.....	when needed
HAV-IgM .....	daily
HBeAg / HBeAb .....	daily
Monospot IM heterophile Ab .....	same day
Rubella Ab.....	daily
Varicella-Zoster Ab.....	STAT or Thursday
RPR for Syphilis.....	Wednesday
HCV Ab .....	daily
CMV IgG Ab.....	daily
HIV Ab .....	daily
HTLV Ab.....	Thursday
Chlamydia PCR.....	daily
HCV RNA PCR .....	once or twice per week
HBVDNA.....	Daily

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/17/01/v02</b>	Page 2 of 2
<b>Serology Manual</b>		

\* Stat testing may be required where clinically justified.

# All routine pre-Bone Marrow Transplant serology screening will be completed by Friday afternoon weekly (except RPR for Syphilis for samples received Thurs. pm or Fri.) Friday afternoon print an LIS report for 8TSC template. For all BMT patients/donors appearing on the 8TSC printout, check that they were faxed successfully. To maintain confidentiality, results for HIV testing will be reported using "Transplant Screen". These results will be printed in-lab and sent to the PMH BMT office daily by hospital mail in an envelope marked "CONFIDENTIAL".(FAX number 926-6585).

Occasionally a pre-BMT serology screen will be STAT. The microbiologist will inform the laboratory in addition to the requisition being clearly marked STAT. Such specimens will be processed within 24 hours of receipt and results will be FAXed back to the BMT office as soon as they are available.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/17/02/v01</b>	Page 1 of 2
Section: <b>Serology Manual</b>	Subject Title: <b>Appendix II - Send-Outs</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: March 14, 2001	
Approved by: Laboratory Director	Revision Date: September 20, 2001	

## APPENDIX II

### List of Tests Referred Out to Other Laboratories

TEST	REQUISITION	DESTINATION
1. Aspergillus precipitins	PHL General	Serology PHL
2. Chlamydial culture*	PHL General	Virology PHL
3. Mycoplasma culture*	PHL General	Bacteriology PHL
4. Mycoplasma pneumoniae Ab	PHL General	Virology PHL
5. Genital Mycoplasma/ Ureaplasma culture*	PHL General	Mycoplasma PHL
6. Viral antibodies	PHL General	Virology PHL
7. Legionella (tissue) Legionella urine antigen	PHL General PHL General	Bacteriology PHL Serology
8. Lyme disease Ab	PHL General	Serology PHL
9. Fungal antibodies	PHL General	Serology PHL
10. Diphtheria antitoxin toxin	Reference Bacteriology	Special Bacteriology PHL
11. Tetanus antitoxin titre	Reference Bacteriology	Special Bact. PHL
12. Electron microscopy	PHL General	Virology PHL
13. VDRL (serum or CSF)	PHL General	Serology PHL
14. Amoebic serology	PHL form 97-44(08/99)	Serology PHL

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/17/02/v01</b>	Page 2 of 2
<b>Serology Manual</b>		

<b>TEST</b>	<b>REQUISITION</b>	<b>DESTINATION</b>
15. Toxoplasma IgG Total Toxoplasma IgM	PHL form 97-44(08/99)	Serology PHL
16. Cystercosis Echinococcus Leishmania Schistosoma Strongyloides Toxocara Trichinella Trypanosoma Miscellaneous  Filaria	CDC 50.34 Rev.09/2002 (must be completed by ref .physician)  NIH Filaria form (must be completed by ref.physician)	Center for Disease Control and prevention 1600 Clifton Road,N.E. Atlanta, Georgia U.S.A. 30333  Laboratory of ParasiticDiseases National Institute of Allergy & Infectious Diseases National Institutes of Health Building 4,Rm 126 Bethesda,Maryland U.S.A. 20892
17 Avian precipitins	HICL label/requisition Specify bird in question	Hospital In-Common Lab 1 William Morgan Dr. Toronto, Ontario M4H 1N6 Tel. (416) 422-3000
18. Parvovirus Ab IgG, IgM	PHL General	Serology PHL
19. BK Virus (Urine)	PHL General	Virology PHL EM
20. Parvovirus PCR	Sick Kids	Virology, HSC 3 <sup>rd</sup> Floor, Elm Wing
21. JC/BK PCR	MSH Miscellaneous	St. Joseph's Hospital Virology Lab, Hamilton (Via PHL)

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/17/03/v01</b>	Page 1 of 1
Section: <b>Serology Manual</b>	Subject Title: <b>Appendix III Shipment of JC/BK Parvovirus PCR Samples</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: March 14, 2001	
Approved by: Laboratory Director	Revision Date: September 20, 2001	

### **APPENDIX III**

#### **SHIPMENT OF JC/BK PCR SAMPLES**

PHL requires that these samples be packaged in a standardized way before they will forward them to Hamilton for us:

1. Put sample tube inside plastic bag and place together with requisition into a plastic cylinder.
2. Place plastic cylinder into a cardboard box, padded with paper towels.
3. Seal the box with packing tape and label the box as follows:

**Public Health Laboratory, 81 Resources Rd.**

**DO NOT OPEN**

**Please forward to:**

**JC/BK PCR  
Dr. James Mahoney,  
REGIONAL VIROLOGY LABORATORY,  
ST. JOSEPH'S HOSPITAL,  
50 CHARLTON AVE., E.,  
HAMILTON, ONTARIO  
L8N2X6**

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/17/04/v01</b>	Page 1 of 5
Section: <b>Serology Manual</b>	Subject Title: <b>Appendix IV The Thermo-Cycler Gene Amp PCR System 9600</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: March 14, 2001	
Approved by: Laboratory Director	Revision Date: September 20, 2001	

## APPENDIX IV

### THE THERMO-CYCLER USED IN AMPLIFICATION IS GENE AMP PCR SYSTEM 9600

To use this machine for amplification, create the Method and the program for the experiment.

#### Creating a Method:

A method is a series of up to 17 programs linked together.  
For example, you can link a HOLD program to a CYCL program and conclude you  
experiment. Access the main menu.

Select option            9600 <u>R</u> N-CREATE-EDIT-UTIL
--------------------------------------------------------------

1. Press the **OPTION** key to select CREATE, then press **ENTER**, the following display appear

Create program <u>H</u> OLD-CYCL-AUTO-METH
-----------------------------------------------

2. Press the **OPTION** key to select METH, then press **ENTER**. The following display appears.

Link progs:     _ _ - - - - -
----------------------------------

3. Type the program numbers of the programs you want linked. After you type each number, press **ENTER**. For example, to link programs 1,3,and 5, your display would look like this:

Link progs:    1- 2- 3- 5- - -
-----------------------------------

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/17/04/v01</b>	Page 2 of 5
<b>Serology Manual</b>		

- Press **STEP** to move to the next display. If you are linking seven or more programs in a method, the following display appears:

```

      _ _ _ _
_ _ _ _ _

```

This display let you link up to ten additional programs in the method.

**NOTE:** You cannot link a method.

- Type the program numbers of the programs you want linked. After you type each number, press **ENTER**.
- Press **STEP** to move to the next display.

```

Meth # ???      25.6 °C
RN-STORE-PRINT-HOME

```

This display allows you to run, store, or print the method.

- Press **OPTION** key to move the cursor beneath **STORE**, then press **ENTER**. The following display appears:

```

Store
Enter program # x

```

X is the next available number.

- Press **ENTER** to store the method under the displayed number (1-150), the following display appears:

```

Store
Protect program? NO

```

**Now your method stored, so you need to program your programs which linked in your method To do this follow the steps as following:**



TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/17/04/v01</b>	Page 4 of 5
<b>Serology Manual</b>		

2. Press ENTER to store the method under the displayed number, OR  
 To use a different number, type the number (1-150), then press ENTER.  
 The following display appears:

Store Protect program? <u>NO</u>
-------------------------------------

3. The above display gives you the option of protecting the file under a user I.D.  
 By doing so, the file cannot be overwritten or deleted unless the user number is  
 entered. Select YES or NO by pressing the **OPTION** key, then press **ENTER**.  
 [Always selects NO unless if you want to keep it forever and will be under your I.D.  
 number].

### **CYCLE: The Cycle Program**

A CYCL program contains the thermal ramp and hold segment patterns for PCR cycling.  
 It contains up to nine set-points. Each set-point comprises a ramp segment and a hold  
 segment for the specific target temperature. The pattern can be repeated up to 99 times.  
 Typically there are two or three set-points in a CYCL program. A complete cycle  
 typically required less than two to four minutes.

The Displaying in a CYCL Program: example for displays CYCL program

<u>3</u> Temperature PCR
Setpt #1 Ramp 0:0 <u>0</u> 94.0C Hold 0:30
Setpt #2 Ramp 0:0 <u>0</u> 55.0C Hold 0:30
Setpt #3 Ramp 0:0 <u>0</u> 72.0C Hold 0:30
Total cycles = 35 Pause during run? NO

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/17/04/v01</b>	Page 5 of 5
<b>Serology Manual</b>		

Adjust your program temperature and number of cycles according your experiment protocol by following the table above. Store your programs as usual under the same number you create.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/17/05/v01</b>	Page 1 of 3
Section: <b>Serology Manual</b>	Subject Title: <b>Appendix V</b> <b>Entering Serology Refer-Out Results</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: March 14, 2001	
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## APPENDIX V

### ENTERING SEROLOGY REFER-OUT RESULTS

Refer-out test results for blood specimens can only be recorded in the LIS using the SCC **lab** module. When the reports come back from a reference lab, the reporting date, reference lab number and result are entered. A senior/charge technologist verifies the results in the LIS and the reference lab reports are photocopied and then forwarded to the appropriate clinic or doctor.

- a. Log on: '**lab**'
- b. Press **1-Order/Entry**
- c. Press **F3**, scan barcode on reference lab requisition, Press **F12**
- d. Check that it is the correct patient
- e. Press **F8** for result field
- f. Check that referred out tests have been ordered for all results coming back from the reference lab. If not, follow steps (i. to vi) for free text referred-out tests or proceed to step p for specific (non free text) refer-out tests:
  - i. **F1**
  - ii. Move cursor to next blank test field
  - iii. On the Microbiology Order/Entry screen press **W** for Serology tests
  - iv. Press **S** for Referred-out Test (8REF for MSH and 9MIS for UHN patients)
  - v. Press **F8** for result field
  - vi. Press **1** To PHL

Skip steps (vii – xi) if send-out tests results are complete (go to step g):

- vii. Press **4 @MOHR** on the 8REF or 9MIS result field
- viii. Press **\** to see the canned message "This sample has been referred to the Public Health Lab (PHL) 81 Resources Rd., Etobicoke, Ont., M9P 3T1 for the tests listed below..." will appear

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/17/05/v01</b>	Page 2 of 3
<b>Serology Manual</b>		

- ix. Type in the referred-out test(s)
- x. Press **F12, F12**
- xi. **Yes** to confirm

Continue from steps (g-o) below if referred-out tests are already ordered and results are ready to be entered:

- g. Press **F8** to enter refer-out results
- h. Select the appropriate test and enter the result from the keypad. If no keypad appears, type in "TO PHL" or "TO HSC" as appropriate in the result field (only 9 characters allowed)
- i. **Delete** the canned message **@MOHR** "This sample has been referred to..." but leave the referred out tests names
- j. Free text the results by opening the text window by pressing \ in the result field and type in the results after each referred out test name
- k. After the last result, enter the reporting date, reference lab number either by free text or by upressing **F5**-Canned message and select **31 "@9rep"**.
- l. Space, enter date that PHL reported the result. Arrow up, wand PHL bar code or enter PHL lab number. F12, F12, Confirm modification? "Y".
- m. Record the Clinic, Dr., MRN, and order number on the reference lab report if not already recorded.
- n. Give reports to senior/charge technologist to verify.
- o. Forward reports to Report Controllers for photocopying and mailing.

Alternate Method for non free-text specific refer-out tests, continued from steps (a-f) above:

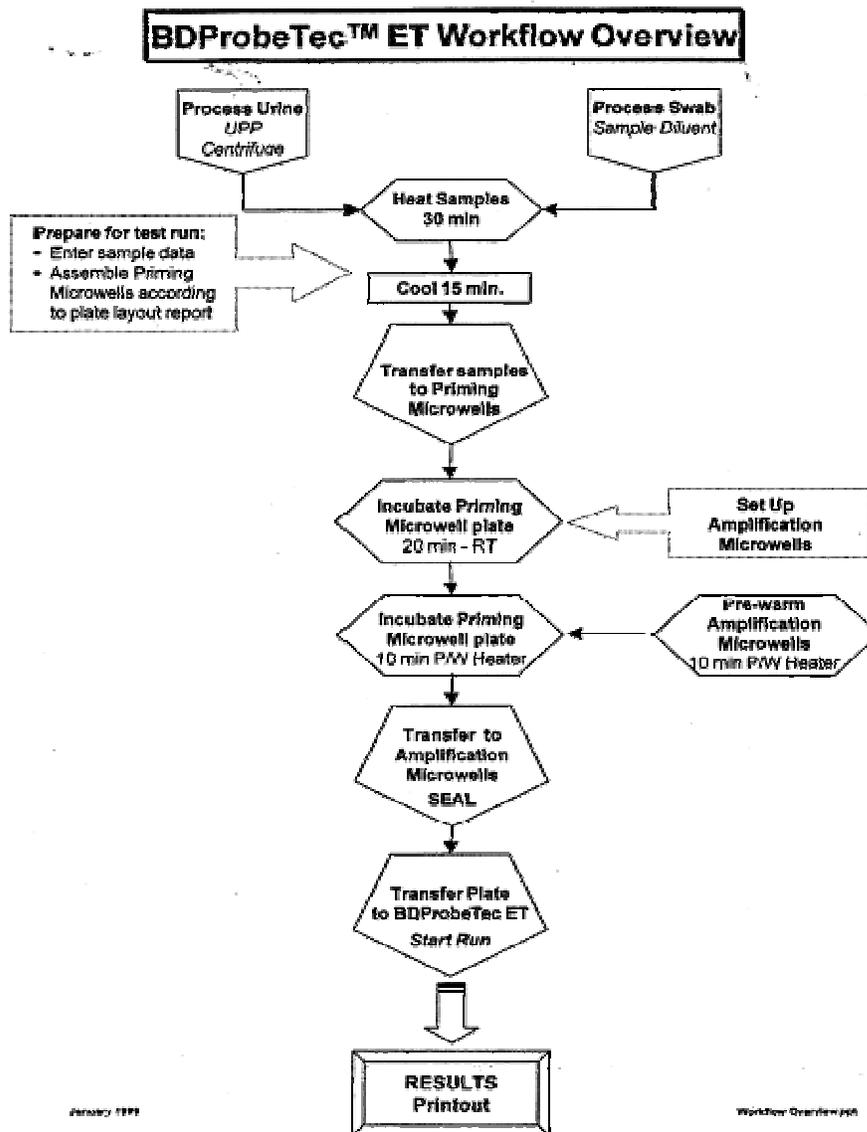
- p. Specific (non free text) refer-out tests as can be ordered as follows:  
-F1

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI/SER/17/05/v01</b>	Page 3 of 3
<b>Serology Manual</b>		

- Move cursor to next blank test area
- ^A-turn off keypad
- enter 9, F2, F12 to get a list of all send-out tests
- highlight the test to be ordered, ENTER
- repeat until all tests have been ordered
- F12, Confirm modification: Yes

- q. F8 to enter refer-out results
- r. Highlight the appropriate test and enter the result from the keypad. If no keypad appears, type in "SEE BELOW".
- s. After the last result is entered, highlight the last result: \ - free text, F5-Canned message: select 7. "@9rep".
- t. Space, enter date that PHL reported the result. Arrow up, wand PHL bar code or enter PHL lab number. F12, F12, Confirm modification? "Y".
- u. Record the Clinic, Dr., MRN, and order number on the reference lab report if not already recorded.
- v. Give reports to senior/charge technologist to verify.
- w. Forward reports to Report Controllers for photocopying and mailing.

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # MI/SER/17/06/v01	Page 1 of 1
Section: <b>Serology Manual</b>	Subject Title: <b>Appendix VI BDProbeTec ET Workflow</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: May 26, 2003	
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← Processed swabs good for:

- 6 hrs at RT
- Overnight at 4 C

← Cooled samples good for :

- 6 hrs at RT
- 5 days at 4 C, then re-lysed
- 98 days frozen (-20 C), then re-lysed

Good for 6 hrs at RT →

<b>TML\MSH Microbiology Department Policy &amp; Procedure Manual</b>	<b>Policy # MI\VIR\17\07\v02</b>	Page 1 of 3
Section: <b>Serology Manual</b>	Subject Title: <b>Appendix VII Autoverification Process</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: June 25, 2002	
Approved by: Laboratory Director	Revision Date:	

## APPENDIX VII

### AUTOVERIFICATION PROCESS

#### **I. Introduction**

To document the autoverification process.

*Autoverification is the process by which the computer performs the initial verification of test results. Any value, that falls outside of the defined criteria, must be assessed by a technologist before release of results.*

#### **II. Procedure**

1. The laboratory has a policy signed by the laboratory director approving the autoverification procedure.
  2. The results of autoverification is thoroughly tested, appropriately documented and signed by the section head/designee before implementation.
  3. If changes are made to the autoverification rules initially chosen and documented, the process is reverified as to its accuracy.
1. The autoverification process is checked yearly.

#### **AUTOVERIFICATION FOR THE AXSYM INSTRUMENT POLICY:**

The AxSYM instrument performs the following tests for the Serology department:

1. Hepatitis B Surface Antigen
2. Hepatitis B Surface Antibody
3. Hepatitis B Core Antibody

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI\VIR\17\07\v02</b>	Page 2 of 3
<b>Serology Manual</b>		

4. Hepatitis A IgM Antibody
5. Hepatitis B Core IgM Antibody
6. Hepatitis Be Antigen
7. Hepatitis Be Antibody
8. Hepatitis C Antibody
9. Rubella Antibody
10. Cytomegalovirus IgG Antibody
11. Transplant Screen

Autoverification will occur as follows:

TEST NAME	SOFT TEST CODE	RESULT	TRANSLATION	ACTION	REFLEX TESTS
Hep B Surface Ag	8HAG	NEGATIVE	Negative	Autoposted Autoverified	
Hep B Surface Ag	8HAG	REACTIVE	Review?	NOT Autoposted NOT Autoverified	8HBC Hep B Core Ab
Hep B Surface Ab	8HAB	REACTIVE	POSITIVE	Autoposted Autoverified	
Hep B Surface Ab	8HAB	NEGATIVE	Negative	Autoposted Autoverified	
Hep B Surface Ab	8HAB	GZ-NEGATIVE	Neg	Not Autoposted Not Autoverified	
Hep B Core Ab	8HBC	REACTIVE	REACTIVE	Not Autoposted Not Autoverified	8HBC2
Hep B Core Ab	8HBC	NEGATIVE	Negative	Autoposted Autoverified	
Hep A IgM Ab	8HAV	REACTIVE	REACTIVE	Not Autoposted Not Autoverified	

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI\VIR\17\07\v02</b>	Page 3 of 3
<b>Serology Manual</b>		

Hep B Core IgM Ab	8HBCM	REACTIVE	POSITIVE	Not Autoposted Not Autoverified	8HBC2
Hep Be Antibody	8HBEB	NONREACTIVE	NONREACTIVE	Autoposted Autoverified	
Hep Be Antibody	8HBEB	REACTIVE	REACTIVE	Autoposted Autoverified	
Hep Be Antigen	8HBEG	NONREACTIVE	NONREACTIVE	Autoposted Autoverified	
Hep Be Antigen	8HBEG	REACTIVE	REACTIVE	Autoposted Autoverified	
Rubella	8RUB	NEGATIVE	Negative	Autoposted Autoverified	
Rubella	8RUB	GRAYZONE	Negative	Autoposted Autoverified	

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI\VIR\17\08\v02</b>	Page 1 of 2
Section: <b>Serology Manual</b>	Subject Title: <b>Appendix VIII Autoverification for the Probetec</b>	
<b>Issued by:</b> LABORATORY MANAGER	Original Date: June 25, 2002	
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### APPENDIX VIII

#### AUTOVERIFICATION FOR THE PROBETEC

**POLICY:**

The Probetec performs the following tests for the Virology department:

1. Chlamydia trachomatis detection
2. Neisseria gonorrhoeae detection.

**Autoverification(Autofinalizing) will occur as follows:**

TEST NAME	SOFT TEST CODE	RESULT	TRANSLATION	ACTION	FINAL RESULT
Chlamydia Detection	CHLA	POSITIVE	?POSITIVE	No Autoverification	Canned Message: {CHLP
Chlamydia Detection	CHLA	NEGATIVE		Autoverifies/Autofinalizes	Canned Message: {CHLN
Neisseria gonorrhoeae	NGON	POSITIVE	?POSITIVE	No Autoverification	Canned Message: {GCPO
Neisseria gonorrhoeae	NGON	NEGATIVE	?NEGATIVE	Autoverifies/Autofinalizes	Canned Message: {GCNE

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI\VIR\17\08\v02</b>	Page 2 of 2
Section: <b>Serology Manual</b>		

**Canned Messages:**

}CHLP - POSITIVE for Chlamydia trachomatis by Nucleic Acid Amplification.

}CHLN - Negative for Chlamydia trachomatis by Nucleic Acid Amplification.

}GCPO - POSITIVE for Neisseria gonorrhoeae by Nucleic Acid Amplification.

}GCNE - Negative for Neisseria gonorrhoeae by Nucleic Acid Amplification.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI\VIR\17\09\v02</b>	Page 1 of 1
Section: <b>Serology Manual</b>	Subject Title: <b>Appendix IX Shipment of Parvovirus PCR to HSC</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: Dec 20,2003	
Approved by: Laboratory Director	Revision Date:	

## **APPENDIX IX**

### **SHIPMENT OF PARVOVIRUS PCR TO HSC**

1. Fill up a HSC requisition.
2. Put specimen and requisition in biohazard bag, and then in a brown paper bag.
3. Put 'Hospital for Sick Children, Microbiology Receiving, 3<sup>rd</sup> Floor Atrium, Rm3676' sticker on brown bag.
4. Put labeled brown bag into blue specimen container, and send to 'TG specimen management'.
5. Enter in LIS as 'specimen send to HSC' and finalize.
6. Also enter in serology send out binder.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI\VIR\17\10\v02</b>	Page 1 of 1
Section: <b>Serology Manual</b>	Subject Title: <b>Appendix X Posting of AxSYM results</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: Dec 20,2003	
Approved by: Laboratory Director	Revision Date:	

## APPENDIX X

### POSTING OF AxSYM RESULTS

1. Go to 'lab'
2. '8'-'Inerface'.
3. 'I'-'Interface Menu'.
4. '9'-'AXSYM'.
5. 'Open' will be highlighted, 'Enter'.
6. 'Choose **today's day**' and 'Enter'.
7. 'P'-Posting:  
'How to Post': change to 'By Order' using space bar.  
'starting From: enter LIS # to be posted'.  
'**F12**'.
8. Do you want to verify results with Posting (Y/N)?. Press 'N'.
9. Reflex window will appear, '**F12**'.
10. Press '[' to post result.  
Verify result with posting? Press 'N'.  
Under ST column 'O' will change to 'P'.
11. '**F12**' to go to the next reflex, repeat steps 10.
12. At the end , press '**F1**' to exit.

	<b>Test to be posted:</b>	<b>Report as:</b>
HBsAg+	HBC	HBsAg: Positive
HBC +	HBC 2: .x HBsAg +	
HBC+	HBC2	HBC: Positive
HBC2 +		
HCV +, S/CO >10.00	HCV 2	HCV : Positive
HCV 2 +, S/CO >10.00		
HCV +,S/CO <10.00	HCV2	Send to PHL
HCV 2		Not tested

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI\VIR\17\11\v02</b>	Page 1 of 1
Section: <b>Serology Manual</b>	Subject Title: <b>Appendix XI Looking Up Previous Hepatitis Results in Vista</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: Dec 20,2003	
Approved by: Laboratory Director	Revision Date:	

## APPENDIX XI

### LOOKING UP PREVIOUS HEPATITIS RESULTS IN VISTA

1. Log on 'Vista'.
2. Enter 'user ID' & 'Password'.
3. Click on 'All UHN Patients'.
4. Enter 'MRN #' in 'Patient ID', press 'Enter'.
5. Click on 'any visit', then click on 'Goto Selected visit'.
6. Click on 'Chart Review', 'Continue?', Click on ' (y) yes'.
7. Click on 'Hepatitis Profile', click on 'OK'.
8. All previous Hepatitis test results will be displayed, e.g. HBsAg, HBsAb, HBcAbIgM, HBeAg, HBeAb, HC Ab, and HAAbIgM.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI\VIR\17\12\v02</b>	Page 1 of 1
Section: <b>Serology Manual</b>	Subject Title: <b>Appendix XII Printing of Pending List</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: Dec 20,2003	
Approved by: Laboratory Director	Revision Date:	

## APPENDIX XII

### PRINTING OF PENDING LIST

1. Log on 'Lab'.
2. Enter 'ID' & 'Password'.
3. '3'-Results
4. 'View/Enter Results by Sel Tests'.
5. Select tests by 'Template', 'Enter'.
6. Enter '8SERO' under 'Template', 'Status- pend +nonver'.
7. From order( enter last month's lab #) to 'leave it blank'. 'F12'.
8. 'F9' to print. Choose either 'TC2RVIR' or 'TC3R MIC' printer to print.
9. Look up each record, and find out why the results are still pending.

TML\MSH Microbiology Department Policy & Procedure Manual	<b>Policy # MI\VIR\17\13\v02</b>	Page 1 of 1
Section: <b>Serology Manual</b>	Subject Title: <b>Appendix XIII Wipes Test for ProbeTec®</b>	
Issued by: <b>LABORATORY MANAGER</b>	Original Date: Dec 20,2003	
Approved by: Laboratory Director	Revision Date:	

### APPENDIX XIII

#### WIPES TEST FOR ProbeTec®

Wipes Test is done every two weeks.

1. Dip swab in a tube of specimen diluent labeled as ‘Analyser’, and swab surfaces of Analyser including the screen, keyboard, and all around the surface.
2. Put swab back in specimen diluent tube and twirl around and remove swab. Vortex well, and treat this as a specimen.
3. Do the same for :
  - a) Bench
  - b) Lysing rack
  - c) Pipette
  - d) Priming heater
  - e) Lysing heater
4. Run all 6 swabs with specimen run.
5. Record results in BDProbeTec<sup>TM</sup>ET Binder.