

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # MI/SER/14/v02	Page 1 of 5
Section: Serology Manual	Subject Title: Molecular Testing - HBV DNA	
Issued by: LABORATORY MANAGER	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 -20⁰C 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	Policy # MI/SER/14/v02	Page 2 of 5
Serology Manual		

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	Policy # MI/SER/14/v02	Page 3 of 5
Serology Manual		

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	Policy # MI/SER/14/v02	Page 4 of 5
Serology Manual		

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	Policy # MI/SER/14/v02	Page 5 of 5
Serology Manual		

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.