Laboratory Procedure Manual

Analyte: **Hepatitis B Surface Antigen Antibody (anti-HBs)**

Matrix: **Serum**

Method: **Auszyme Monoclonal**

Method No.:

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as performed by: *Hepatitis Branch*
Division of Viral Hepatitis
National Center for Infectious Diseases

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Important Information for Users

The National Center for Infectious Diseases periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the item listed in the following table:

File Name	Variable Name	SAS Label
HEPB_S_d	LBXHBS	Hepatitis B surface antibody

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The AUSAB EIA for anti-HBs uses the "sandwich principle" a solid phase enzyme-linked immunoassay technique (1,2) to detect anti-HBs levels in serum or plasma. Polystyrene beads coated with human Hepatitis B Surface Antigen (HBsAg) are incubated with either the patient specimen or the appropriate controls.

During incubation, antibody, if present, is immunologically coupled to the solid phase antigen. After removal of the unbound material and washing of the bead, human HBsAg tagged with biotin (B-HBsAg) and rabbit anti-biotin, conjugated with horseradish peroxidase (anti-H-HRPO), are incubated with the antibody-antigen complex on the beads. The biotinylated surface antigen binds to this complex crating an antigen-antibody-antigen "sandwich". The anti-biotin horseradish peroxidase binds to the biotin component of the "sandwich", forming a sold phase network. Unbound conjugates are removed and the beads are washed. Next, o-Phenylenediamine (OPD) solution containing hydrogen peroxide is added to the bead, and after incubation, a yellow color develops in proportion to the amount of anti-HBs which is bound to the bead. Within limits, the greater the amount of antibody in the sample, the higher the absorbance. The enzyme reaction is stopped by the addition of acid. The absorbance of controls and specimens is determined using a spectrophotometer with wavelength set at 492 nm. Testing for anti-HBs can be useful for: a) evaluating the recovery and prognosis of patents infected with HBV, b) screening for potential vaccine recipients, and c) epidemiologic factors associated with transmission of HBV. The detection of anti-HBs is indicative of a prior immunologic exposure to the antigen or vaccine.

The anti-HBs standards contained in the AUSAB quantitation panel kit are assayed with the AUSAB EIA for the quantitative determination of anti-HBs in human serum or plasma. The concentration of anti-HBs expressed in milli-international units per mL (mIU/mL) is determined by comparison with a standard curve generated from measurement of the standards run in duplicate with the AUSAB EIA kit. A curve is obtained by plotting the anti-HBs concentration of the standard vs. the absorbance. The anti-HBs concentration of specimens run concurrently with the standards can then be read from the curve. Specimens with values above the highest standard can be diluted with the specimen dilution buffer and retested. For the purposes of this study, samples with an absorbance above the highest standard curve will not be diluted, but reported out as > 150 mIU/mL. Samples with a result of < 10 mIU/mL will be reported out as < 10 mIU/mL.

2. SPECIAL SAFETY PRECAUTIONS

Test kits for anti-HBs contain human sourced components. No known test method can offer complete assurance that products derived from human sources will not transmit infection. Therefore, all human sourced material should be considered potentially infectious. It is recommended that all specimens and kit reagents be handled in accordance with biosafety level 2 practices as described in the CDC NIH publication, Biosafety in Microbiological and Biomedical Laboratories (3), or equivalent guidelines. (4,5)

Material Safety Data Sheets (MSDS) for sodium azide and sodium hypochlorite are available through the National Center for Infectious Diseases (NCID) local area network (LAN). Risk is minimal because of the limited quantity of chemicals, packaging, and limited handling by the operators using the test kits.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

a. Raw data are transcribed manually from an instrument readout sheet into a computerized database. This database was custom-designed for the management of CDC Hepatitis Reference Laboratory (HRL) test results, and functions within SQL Server software (Microsoft, Redmond, WA) with a Visual Basic (Microsoft, Redmond, WA) user interface. Test values are compared with a cutoff value calculated from the controls. Results are expressed as "positive" or "negative" Other information in the database may typically include the HRL identification

number, the specimen number, the date collected, the date tested and results of testing for other hepatitis markers. Reporting is done directly from the database in printed form or by electronic transfer. Electronically stored data are backed up routinely.

b. Finished data are reviewed by the lab supervisor. After each NHANES container is completed (i.e., when all clinical evaluations and analyses from each mobile survey site are complete), the supervisor will transmit the results to the SQL Server along with other NHANES IV data.

c. Files stored on the CDC Local Area Network (LAN) are automatically backed up nightly to tape by CDC Data Center staff.

d. Documentation for data system maintenance is maintained with printed copies of data records for 2 years.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

a. Specimens submitted for testing are handled according to the HRL SOP entitled "Sample Handling" (W. Kuhnert, 10/02).

b. No special instructions such as fasting or special diets are required. Diurnal variation is not a major consideration.

c. Specimens may be serum, recalcified plasma, or plasma. Serum specimens may be collected using regular red-top or serum-separator Vacutainers.

d. Required sample volume is 200 µL for the assay; 1.0 mL will permit repeat analyses as well as other testing.

e. Samples should be stored in labeled 2 mL Nalgene cryovials or equivalent and sealed tightly to prevent desiccation of the sample.

f. Serum or plasma samples are collected aseptically to minimize hemolysis and bacterial contamination.

g. Serum is best stored frozen. Store samples at 4-8°C for no more than 5 days.

h. For storage >5 days, samples are held at -20°C. Samples held in long-term storage at -20°C are indexed in the database for easy retrieval.

i. Specimens are rejected if contaminated, hemolyzed, or stored improperly. However, rejection is done only after consultation with NCHS.

j. Avoid multiple freeze/thaw cycles.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

a. **Instrumentation**

(1) Abbott QWIKWASH, model 6258-27 (Abbott Laboratories, North Chicago, IL).

(2) Abbott COMMANDER dynamic incubator, model 6210-01, set at 40 °C for incubations (Abbott Laboratories).

(3) Abbott QUANTAMATIC spectrophotometer, model 7553, set for reading at 492 nm (Abbott Laboratories).

(4) Gilson Pipetman micropipettors, 10- and 200-µL sizes (Rainin Instrument Co., Woburn MA).

b. **Other Materials**

(1) Abbott AUSAB Enzyme Immunoassay kit, cat. no. 755424 (Abbott Laboratories). Each kit contains the following.

(a) Antibody to Hepatitis B surface antigen (guinea pig)-1 bottle (100 beads) or 2 bottles (500 beads each).

(b) Antibody to hepatitis B surface antigen (human), peroxidase-conjugated -- 2 vials (10 mL each) or 5 vials (40 mL each). Potency adjusted by the manufacturer with Tris buffer containing protein stabilizers.

(c) Negative control -- 1 vial (5 mL) or 1 vial (35 mL). Recalcified human plasma nonreactive for HBsAg and anti-HBs. Preservative: 0.1% sodium azide.

(d) Positive control -- 1 vial (3 mL) or 1 vial (16 mL). Human plasma positive for HBsAg. Potency: 20±5 ng/mL adjusted with Tris Buffer containing protein stabilizers. Preservative: 0.1% sodium azide.

(2) Abbott AUSAB Quantitation Panel, cat no. 695218 (Abbott Laboratories). Each kit contains the following:

(a) 1 vial (2mL) anti-HBs (human) standard 0 mIU/mL; non reactive for HBsAg, HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2 Ag and anti-HBs. Preservative: 0.1% Sodium Azide.

(b) 4 vials (2 mL each) anti-HBs (human) standards 15, 40, 75, 150 mIU/mL ; non reactive for HBsAg, HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2 Ag and reactive for anti-HBs. Preservative: 0.1% Sodium Azide.

(c) 1 vial (36mL) Specimen dilution buffer is human plasma, nonreactive for HBsAg, HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2 Ag. Preservative: 0.1% Sodium Azide.

(3) Reaction trays, Costar cat. no. 4870 (VWR Scientific, Bridgeport, CT).

(4) Cover seals provided as part of the anti-HAV test kit (Abbott Laboratories).

(5) Deionized water (Continental Water Systems, Inc., San Antonio, TX).

(6) 1.0 N sulfuric acid, cat. no. 7212 (Baxter).

(7) Pipet tips, cat. nos. RT20 & RT200 (Rainin Instrument Co.).

(8) Protective gloves, small/medium/large (any vendor).

(9) 2-mL cryovials, cat. no. 5000-0020 (Nalgene Company, Inc., Rochester, NY).

(10) Cryovial boxes, cat. no. 5026-0909 (Nalgene Company, Inc., Rochester, NY).

(11) 1.5-mL microtubes (Marsh Biomedical Products, Rochester, NY).

(12) 50-mL polypropylene tubes (Corning Glass Works, Corning, NY)

(13) 5.25% sodium hypochlorite, household bleach (any vendor).

c. **Reagent Preparation**

Reagents for these procedures are prepared by the manufacturer of the test kits.

d. **Standards Preparation**

This method does not employ conventional calibrators or standards. Calibration is based on the results of defined "positive" and "negative" controls.

(1) Positive and negative control reagents are supplied with each test kit. Calibrators for the standard curve are provided in the Quantitative kit.

e. **Preparation of Quality Control Material**

(1) Gently invert all standards 3 – 4 times prior to use

(2) Pipette standards (200 µl) in duplicate in order of increasing concentrations (0, 15, 40, 75, 150 mIU/mL)

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. **Calibration Curve**

No calibration curve is generated by the user as part of these assay methods except in preparation of the in-house control reagent. Calibration of instruments is either automatic or is carried out periodically by contracted service personnel.

b. **Verification**

The instrument used to read assay results (see section 6.e.3.) is equipped to analyze the positive and negative controls for each test series. If, within a testing series, positive or negative controls do not conform to specifications as defined in the product literature, the entire series is invalidated by the instrument.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. **Preliminaries**

(1) Remove the test kit from 4-8 ^{o}C storage. Allow 30-40 min for the reagents to warm to 20-25 ^{o}C. Swirl gently before use.

(2) Reagents are used per kit of 100 tests. Kit components are occasionally interchanged within a manufacturer's lot but never interchanged between lots.

(3) 5 standards are run in duplicate for each run

(4) Ensure that all reaction trays are subjected to the process and incubation times that are specified in the manufacturer's instructional literature.

(5) Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.

b. **Sample preparation**

(1) Bring the serum specimens to 20-25 ^{o}C. Serum and plasma samples may stratify when frozen or stored at 4-8 ^{o}C for extended periods. Mix gently before testing.

(2) Identify the reaction tray wells for each specimen or control.

c. **Instrument setup**

(1) Operation of the Abbott QWIKWASH
The Abbott QWIKWASH is a semi-automated instrument that is used to wash the beads of the Abbott immunoassays between reagent steps. The wash solution is deionized water.

(a) Turn on the QWIKWASH using the toggle switch on the back of the instrument. The vacuum pump will come on, as will the "Power" indicator on the instrument.

(b) Ensure that the "Low Pressure" and "Low Water Level" indicators are NOT illuminated before washing beads. See note below.

(c) Place the bead tray on the QWIKWASH with the first row of beads aligned with the washing heads.

(d) Push down on the handle on the top of the instrument. The beads will automatically go through one wash cycle, which will take about 4 sec.

(e) Raise the handle and slide the tray over until the second row of beads is aligned with the washing heads.

(f) Repeat until all of the beads have been washed, and then proceed directly to the next step of the assay procedure.

NOTE: The wash water is held in a stainless-steel pressure tank near the instrument. Waste water is collected in a plastic container, also nearby. When the "Low Water Level" light on the instrument comes on, fill the tank with deionized water AND empty the waste tank. Never fill the water tank without also emptying the waste container! Add approximately 200 mL of bleach to the waste container prior to reconnecting it to the system so that waste water can be discarded down the sink as "decontaminated liquid waste." Never put any solution other than deionized water into the water tank. If the "Low Pressure" light on the instrument comes on, check the connections and seals on the stainless steel pressure tank.

(2) Operation of the QUANTAMATIC plate reader

(a) After the beads have been transferred to tube rack(s), place the rack(s) into the appropriate QUANTAMATIC carrier tray(s).

(b) Place the carrier tray(s) onto the QUANTAMATIC to be automatically fed into the tube pick-up area.

(c) On the instrument keypad choose RUN ASSAYS. Answer the prompts as shown in Table 2.

Table 2
QUANTAMATIC Run Settings

Prompt	Response
Run which assay?	Assay # from TABLE 1
Lot #/Tech	Kit lot # & initials
Positive ID?	NO
Number of patients?	Number of patients
Tray 1 size - 20?	YES if tray size is 20; NO if tray size is 60
Is tray in back track?	YES if tray is in the back track; NO if tray is in the front track
How many tubes in tray?	Total number of tubes
Enter pat no. ID	NO
List operator entries?	NO
Are trays ready?	YES if trays are ready to be read

d. **Operation of Assay Procedure**

(1) Pipette 200 µL of each control or test specimen into the wells of a reaction tray.

(2) Carefully add one bead to each well containing a specimen or control.

(3) Apply cover seal. Gently tap the tray to cover the beads and remove any trapped air.

(4) Incubate at 20-25 ^{o}C overnight (20 ± 2 hours).

(5) Remove and discard the cover seal. Wash the beads in the tray using the QWIKWASH bead washer.

(6) Add 200 µL of peroxidase-HBsAg conjugate to each well containing a bead.

(7) Apply the cover seal. Gently tap the tray to cover the beads and remove any trapped air.

(8) Incubate the specimens at 20-25 ^{o}C for 4 hours.

(9) Remove and discard the cover seal. Wash the beads again as in step 5.

(10) Transfer the beads to 10- x 75-mm tubes.

(11) Add 300 uL of prepared substrate solution to each tube containing a bead.

(12) Cover and incubate the tubes for 30 min at 20-25 ^{o}C.

(13) Stop the reaction by adding 1 mL of 1 N sulfuric acid per tube.

(14) Read the reaction using the QUANTAMATIC.

e. **Recording of Data**

(1) Quality Control Data
Raw data are transcribed manually from the instrument readout sheets into a computerized database. Quality control of individual control values is maintained by the QUANTAMATIC, which will reject the test run if control values do not conform to specifications.

(2) Analytical Results
Raw data are transcribed manually from the instrument readout sheets into a computerized database, and are expressed as absorbance units at 492 nm. Data reduction is done by the laboratory management software that is part of the computerized database.

f. **Replacement and Periodic Maintenance of Key Components**

(1) Instruments are on a routine service contract and, except for the most basic daily maintenance, are serviced by an Abbott technical representative.

(a) Abbott QUANTAMATIC is self-monitoring and requires only routine service per contract.

(b) Quality of water used in QWIKWASH, refrigerator temperature, freezer temperature, and room temperature are monitored and documented on a weekly basis.

(c) All micropipettors used in testing clinical specimens are checked for calibration every six months. Pipettors that do not conform to specifications are autoclaved and sent out for recalibration in accordance with the manufacturer's recommendations. Calibration records should be kept for each pipettor by serial number.

g. **Calculations**

Calculations are done by the reading instrument.

h. **Special Procedure Notes**

(1) When dispensing beads, remove the cap from the bead bottle, attach the bead dispenser and dispense the beads into the wells of the reaction tray.

(2) Do not splash liquid while tapping trays.

(3) When washing the beads, follow the directions provided with the washing apparatus.

9. REPORTABLE RANGE OF RESULTS

Final results are expressed as positive or negative. A positive result is has a quantitative interpretation of > 10 mIU and a negative result has an interpretation of < 10 mIU.

10. QUALITY CONTROL (QC) PROCEDURES

The method described in this protocol has been used extensively in the HRL for epidemiological health studies. This method has proven to be accurate, precise, and reliable. The instrumentation used is state-of-art.

For the run to be valid the following values and ratios for the standards must be met:

	EIA Quantum II
0 mIU/mL	0.000 – 0.080
15 mIU/mL – 0 mIU/mL	0.050 – 0.175
Ratios:	
$\frac{\text{40 mIU/mL}}{\text{15 mIU/mL}}$	2.0 – 3.3
$\frac{\text{75 mIU/mL}}{\text{40 mIU/mL}}$	1.3 – 2.2

The precision of these procedures is as claimed for licensure and is maintained by the manufacturer under the authority of the FDA.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

By definition, if controls do not conform to specifications, the test results are rejected. All samples are tested again. Data from nonqualifying test runs are not used.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

(a) The sample is restricted to human serum or plasma.

(b) No interfering substances have been identified.

13. REFERENCE RANGES (NORMAL VALUES)

Results are reported out as positive or negative.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

A value greater than 10 mIU of anti-HBs is considered protective against HBV infection (6-8). However, this is a generic guideline since there is a considerable degree of error associated with this determination for reasons that may be technical or biologic in nature (9, 10).

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may remain at 20-25 ^{o}C during preparation and testing for 4 hours.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

Other FDA-licensed tests for total anti-HBs may be substituted but must be accompanied by validation data to show substantial equivalence with these assays. Substitution of test methods may not be done without approval from NCHS.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Test results are documented through the lab management database (see Section 3). Generally, studies conducted in the HRL are sponsored by a CDC epidemiologist who communicates the findings to

other participants in the study. Final reports may be electronic or in printed form. All electronically held data are backed up routinely.

Specimens in long-term storage are arranged by study group. The storage location of each sample is listed with the test data.

19. SUMMARY STATISTCS AND QC GRAPHS

Qualitative assays are qualitative assays with a positive, negative or borderline/indeterminate result. The absorbance or reactivity values of specimens are compared with a cutoff value that is a ratio of the negative control mean and the positive control mean. Since the controls are read as cutoff values, plots of these values are not generated for quality control purposes.

REFERENCES

1. Peters, R.L., Collins, M.J., O'Beirne, A.J., Howton, P.A., Hourihan, S.L., and Thomas, S.F., Enzyme –linked immunosorbent assays for detection of antibodies to murine hepatitis virus. J. Clin. Mircorbiol. 10:595-597, 1979.

2. Hildebrand, R.L., Elisa (Enzyme-Linked Immunosorbent Assay) p. 71-78. In rapid Diagnosis in Infectious Disease, Rytel, M.D., Ed. CRC Press, New York, 1979.

3. U.S. Department of Health and Human Services. Biosafety in microbiological and biomedical laboratories.. HHS Publication (NIH) 88-8395. Washington: US Government Printing Office, May 1988.

4. World Health Organization. Laboratory biosafety manual. Geneva. World Health Organization, 1983.

5. National Committee for Clinical Laboratory Standards. Protection of laboratory workers from infectious disease transmitted by blood, body fluids, and tissue: Tentative guideline. NCCLS Document M29-T. Villanova, PA.: NCCLS, 1989.

6. Centers for Disease Control. Hepatitis B Virus: A comprehensive strategy for eliminating transmission in the United States through universal childhood. MMWR 40:RR-13. 1991.

7. Centers for Disease Control. Recommendations for the Immunization Practices Advisory Committee (ACIP). Inactivated Hepatitis B Virus Vaccine. MMWR 31:317-28. 1982.

8. Centers for Disease Control. Hepatitis surveillance report no.42. June, 1978.

9. Ling CM, Decker RH, Foemmel RS, Overby LR. Comparison of confirmation methods for hepatitis B antigen and the nature of false positives detected by ^{125}I-immunoglobulins. J Lab Clin Med 1975;86:690-99.

10. Grangeot-Keros L, Lambert T, Dubreuil P, et al. False reactions in radioimmunoassay for viral hepatitis B markers in patients suffering from coagulation disorders. Vox Sang 1982;42:160-3.