ANTIBODY TO HEPATITIS B SURFACE ANTIGEN (MOUSE MONOCLONAL): PEROXIDASE (HORSERADISH) CONJUGATE

AUSZYME® MONOCLONAL

NOTE CHANGES HIGHLIGHTED

NAME AND INTENDED USE
AUSZYME MONOCLONAL IS A QUALITATIVE THIRD GENERATION ENZYME IMMUNOASSAY FOR THE DETECTION OF HEPATITIS B SURFACE ANTIGEN (HBsAg) IN HUMAN SERUM, PLASMA OR CADAVERIC SERUM SPECIMENS.

69-4878/R11

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CAUTION: Handle as if capable of transmitting infection.

SUMMARY AND EXPLANATION OF THE TEST

Sensitive enzyme immunoassays used to detect the presence of HBsAg were first described by Engvall and Perlmann[1-3] and VanWeemen and Schuurs[4] in 1971. In 1976 and 1977, solid phase "sandwich" enzyme immunoassays for the detection of HBsAg were described by Wisdom,[5] Wolters, *et al*,[6] and Wei, *et al*.[7] The production, characterization and application of monoclonal antibodies for the detection of HBsAg has previously been reported.[8-13]

Specimens nonreactive by the AUSZYME Monoclonal tests are considered negative for HBsAg and need not be tested further. All specimens considered reactive initially should be repeat tested in duplicate using the same procedure as that used in the initial test. If neither of the repeat tests are reactive, the specimen should be considered negative for HBsAg. If the specimen is reactive in either of the repeat tests, the sample should be considered repeatedly reactive. Repeatedly reactive specimens should be tested by the HBsAg Confirmatory Assay (List No. 1012), a licensed, neutralizing confirmatory test. Only those specimens which the HBsAg can be neutralized by the confirmatory test procedure may be designated as positive for HBsAg.

BIOLOGICAL PRINCIPLES OF THE PROCEDURES

One Step Assay. In the AUSZYME Monoclonal enzyme immunoassay procedure, beads coated with mouse monoclonal antibody to Hepatitis B Surface Antigen (Anti-HBs) are incubated with serum or plasma, appropriate Controls, and mouse monoclonal Anti-HBs Peroxidase (Horseradish) Conjugate (Anti-HBs:HRPO). During the incubation period, any HBsAg present is bound to the solid phase antibody and simultaneously bound by the Anti-HBs:HRPO. Unbound material is then aspirated and the beads washed. See COLOR DEVELOPMENT.

Two Step Assay. In the AUSZYME Monoclonal enzyme immunoassay procedure, beads coated with mouse monoclonal Antibody to Hepatitis B Surface Antigen (Anti-HBs) are incubated with serum, plasma or cadaveric serum and Positive and Negative Controls. Any HBsAg present is bound to the solid phase antibody. After aspiration of the unbound material and washing of the bead, mouse monoclonal Anti-HBs conjugated with horseradish peroxidase (Anti-HBs:HRPO) is allowed to react with the antibody-antigen complex on the bead. Unbound enzyme conjugate is then aspirated and the beads washed. See COLOR DEVELOPMENT.

COLOR DEVELOPMENT

Next, o-Phenylenediamine (OPD) Solution containing hydrogen peroxide is added to the bead and, after incubation, a yellow-orange color develops in proportion to the amount of HBsAg which is bound to the bead.

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. Specimens giving absorbance values equal to or greater than the absorbance value of the Negative Control Mean plus a factor are considered initially reactive for HBsAg.

One Step

)– Anti-HBs $\xrightarrow[\text{Anti-HBs:HRPO}]{\text{HBsAg}}$

)– Anti-HBs · HBsAg · Anti-HBs:HRPO $\xrightarrow[H_2O_2]{\text{OPD}}$

yellow-orange color
absorbing at 492 nm

Two Step

)– Anti-HBs $\xrightarrow{\text{HBsAg}}$

)– Anti-HBs · HBsAg $\xrightarrow{\text{Anti-HBs:HRPO}}$

)– Anti-HBs · HBsAg · Anti-HBs:HRPO $\xrightarrow[H_2O_2]{\text{OPD}}$

yellow-orange color
absorbing at 492 nm

REAGENTS

AUSZYME Monoclonal Diagnostic Kit

100 Tests/1000 Tests/5000 Tests

NOTE: The number of tests per kit is based on Procedures A, B and C. Fewer tests per kit will be obtained when using Procedure D due to the increased conjugate volume requirement.

① 100/1000/5000 Anti-HBs (Mouse) Monoclonal Coated Beads. Antibody to Hepatitis B Surface Antigen (Mouse Monoclonal).

② 1 Vial (5 mL)/2 Vials (25 mL each)/10 Vials (25 mL each) AUSZYME (Mouse) Monoclonal Conjugate. Antibody to Hepatitis B Surface Antigen (Mouse Monoclonal): Peroxidase (Horseradish). Minimum Concentration: 0.2 µg/mL in TRIS Buffer with Protein Stabilizers. Preservatives: 0.01% Gentamicin Sulfate and 0.01% Thimerosal. Dye: Red No. 33.

③ 1 Vial (6 mL)/1 Vial (12 mL)/5 Vials (12 mL each) Positive Control (inactivated). Recalcified Human Plasma in TRIS Buffer, reactive for HBsAg, and nonreactive for HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2. HBsAg concentration 9 ± 2 ng/mL. Preservatives: 0.01% Gentamicin Sulfate and 0.01% Thimerosal. Dye: Bromophenol Blue.

④ 1 Vial (9 mL)/1 Vial (18 mL)/5 Vials (18 mL each) Negative Control. Recalcified Human Plasma, nonreactive for HBsAg, HIV-1Ag, anti-HCV, anti-HIV-1/HIV-2, and anti-HBs. Preservatives: 0.01% Gentamicin Sulfate and 0.01% Thimerosal.

⑨ 1 Bottle (10 Tablets)/2 Bottles (40 Tablets each)/10 Bottles (40 Tablets each) OPD (o-Phenylenediamine • 2HCl) Tablets. OPD/Tablet: 12.8 mg.

⑩ 1 Bottle (55 mL)/2 Bottles (220 mL each)/10 Bottles (220 mL each) Diluent for OPD (o-Phenylenediamine • 2HCl). Citrate-Phosphate Buffer containing 0.02% Hydrogen Peroxide.

The stopping reagent is provided as an accessory to the AUSZYME Monoclonal Kit and consists of:

⑪ 1 *N* Sulfuric Acid, No. 7212 (Most U.S. and International Locations).

Additional Reagents available for most International Locations:

⑫ 1 *N* Sulfuric Acid, No. 7212-01 (110 mL)

⑬ 6 *N* Sulfuric Acid, No. 7212-03 (110 mL)

Use of acid other than that supplied by ABBOTT may result in instability of the developed color. To be suitable as a stopping reagent, Sulfuric Acid must pass the following test each time it is prepared. The following test cannot be performed on the COMMANDER® system. Use a Quantum™ II to perform this test.

1. Pipette 300 µL of OPD Substrate Solution into 5 EIA assay tubes or acid washed/distilled or deionized water-rinsed tubes.
2. Add 1.0 mL of the 1 *N* Sulfuric Acid under test to each of the five tubes.
3. Measure the A_{492} of the OPD/Acid Solution against distilled or deionized water at "0 TIME" and "120 MIN".
4. Calculate the Mean Absorbance at "0 TIME" and "120 MIN".
5. To be acceptable, acid must exhibit:
   a. an A_{492} of less than 0.04 at "0 TIME" *and*
   b. difference of less than 0.03 units in the values obtained at "0 TIME" and "120 MIN".

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE

Safety Precautions

CAUTION:

This product contains human sourced and/or potentially infectious components. Some components sourced from human blood have been tested and found to be reactive for HBsAg by FDA licensed tests. Refer to the **REAGENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[14] Biosafety Level 2[15] or other appropriate biosafety practices[16,17] should be used for materials that contain or are suspected of containing infectious agents. These precautions include, but are not limited to the following:

1. Wear gloves when handling specimens or reagents.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
4. Clean and disinfect all spills of specimens or reagents using a tuberculocidal disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.[18,19]
5. Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with local, state and federal regulations.[20,21]

The OPD tablets listed in the **REAGENTS** section of this package insert contain o-Phenylenediamine • 2 HCl and Sodium Carbonate. The OPD tablets are classified per applicable European Community (EC) Directives as: Harmful (Xn) and Dangerous for the Environment (N). The following are the appropriate Risk (R) and Safety (S) phrases.

R36	Irritating to eyes.
R40/22	Possible risks of irreversible effects if swallowed.
R43	May cause sensitization by skin contact.
R51/53	Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

S35	This material and its container must be disposed of in a safe way.
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection.
S46	If swallowed, seek medical advice immediately and show this container or label.

The 6*N* Sulfuric Acid listed in the **REAGENTS** section of this package insert is classified per applicable European Community (EC) Directives as: Corrosive (C). The following are the appropriate Risk (R) and Safety (S) phrases.

R35	Causes severe burns.
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S35	This material and its container must be disposed of in a safe way.
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection.
S45	In case of accident or if you feel unwell, seek medical advice immediately.

Handling Precautions

1. Do not use kit beyond the expiration date.
2. Do not mix reagents from different master lots.
   Any OPD or Sulfuric Acid lot may be used with any AUSZYME Monoclonal kit lot.
3. Do not expose OPD reagents to strong light during incubation or storage.
4. Avoid contact of the OPD Substrate Solution and 1 *N* Sulfuric Acid with any oxidizing agent. Do not allow OPD Substrate Solution or 1 *N* Sulfuric Acid to come into contact with any metal parts. Prior to use, thoroughly rinse glassware used for OPD Substrate Solution with 1 *N* Sulfuric Acid using approximately 10% of the container volume. Follow with three washes of distilled water at the same volume.
5. Avoid microbial contamination of reagents when removing aliquots from the reagent vials. Use of disposable pipette tips is recommended.
6. If the desiccant obstructs the flow of beads, remove from bead bottle prior to dispensing beads. Replace desiccant in bottle and tightly cap bottle for storage. Do not store beads with dispenser attached to bottle. Any unused beads remaining in the dispensers should be returned to the original container (see Bead Dispenser inserts).
7. Cadaveric serum specimens and specimens containing sodium azide (which inactivates horseradish peroxidase) require the use of the two step (Procedure D) assay. Procedure D is not intended for routine testing, as this procedure requires four times the conjugate volume as that required in Procedures A, B, and C. Procedure D should be used only for cadaveric serum specimens and specimens containing sodium azide.

INSTRUCTIONS FOR PREPARATION OF OPD SUBSTRATE SOLUTION

Bring OPD Reagents to room temperature (15 to 30°C).

CAUTION: Do not open OPD Tablet bottle until it is at room temperature.

At least 5 minutes, but not more than 60 minutes, prior to dispensing for Color Development, prepare the OPD Substrate Solution by dissolving the OPD (o-Phenylenediamine • 2HCl) Tablet(s) in Diluent for OPD. DO NOT USE A TABLET THAT IS NOT INTACT.

Using clean pipettes and metal-free containers (such as plastic ware or acid-washed and distilled/deionized water-rinsed glassware), follow the procedure below:

1. Transfer into a suitable container 5 mL of Diluent for OPD for each tablet to be dissolved.
2. Transfer appropriate number of OPD Tablets (see OPD Preparation Chart) into measured amount of Diluent for OPD using a nonmetallic forceps or equivalent. Return desiccant to bottle immediately, if removed to obtain a tablet, and close bottle tightly. Allow tablet(s) to dissolve. Do not cap or stopper the OPD Substrate Solution bottle while the tablets are dissolving.
   NOTE: The OPD Substrate Solution MUST be dispensed within 60 minutes of preparation and MUST NOT be exposed to strong light. (Record the preparation time and expiration time of the OPD Substrate Solution on the container.)
3. Just prior to dispensing for Color Development, swirl container gently to obtain a homogeneous solution. Remove air bubbles from dispenser tubing, and prime dispenser prior to use.

OPD PREPARATION CHART

No. Tests	Tablets	Diluent
13	1	5 mL
28	2	10 mL
43	3	15 mL
58	4	20 mL
73	5	25 mL
88	6	30 mL
103	7	35 mL
118	8	40 mL
133	9	45 mL
148	10	50 mL

NOTE: 300 µL of OPD Substrate Solution is required for each specimen or Control as well as for each substrate blank. Laboratories using the COMMANDER Parallel Processing Center (PPC™) will require approximately an additional 3 mL of OPD Substrate Solution for instrument priming.

STORAGE INSTRUCTIONS

1. Store kit reagents at 2 to 8°C. OPD Tablets and 1 *N* Sulfuric Acid may be stored at 2 to 30°C.
2. Kit reagents must be dispensed at room temperature. Return to 2 to 8°C storage immediately after use.
   CAUTION: Do not open the OPD Tablet bottle until it is at room temperature (15 to 30°C).
3. Retain desiccant in bead bottle and in OPD Tablet bottle at all times during storage.
4. Reconstituted OPD Substrate Solution MUST be stored at room temperature and MUST be dispensed within 60 minutes of preparation. Do not expose to strong light.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

The OPD Substrate Solution (OPD plus Diluent for OPD) should be colorless to pale yellow. A yellow-orange color of the solution indicates that the reagent has been contaminated and must be discarded.

A value of less than 0.400 absorbance units (Procedures A, B, and D) and less than 0.200 absorbance units (Procedure C) for the difference between the Positive Control and Negative Control Means (P-N) may indicate deterioration of the kit or OPD reagents. Such runs must be repeated.

Precipitates in the reagent solutions may indicate reagent instability or deterioration.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Serum, plasma or cadaveric serum may be used in the test.

NOTE: Cadaveric serum specimens and specimens containing sodium azide must be tested using Procedure D.

Anticoagulants typically used for blood collection do not interfere with this test. Remove the serum or plasma from the clot or red cells, respectively, as soon as possible to avoid hemolysis. Lipemic, icteric, or hemolyzed serum or plasma specimens generally give consistent test results.

For cadaveric serum specimens, no performance differences were observed in 10 negative and 10 low-level reactive specimens tested with and without elevated levels of bilirubin (≤20 mg/dL) and triglycerides (≤3000 mg/dL). In addition, equivalent performance was observed in 40 negative and 37 low-level reactive cadaveric specimens tested with endogenous hemoglobin concentrations ≤500 mg/dL.

Serum, plasma or cadaveric serum specimens containing particulate matter may give inconsistent results and should be clarified prior to testing. Do not use heat-treated specimens.

If serum or plasma specimens are to be stored, they should be refrigerated at 2 to 8°C. For long-term storage, the specimens should be frozen (-15°C or colder). Do not freeze the specimens on the clot or red blood cells.

Cadaveric serum specimens may be stored for up to seven days at 2 to 8°C. If storage periods greater than seven days are anticipated, the specimens should be stored frozen at -20°C or colder. Do not freeze the specimens on the clot.

For cadaveric serum specimens, no performance differences were observed in ten low-level reactive specimens subjected to three freeze-thaw cycles. However, multiple freeze-thaw cycles should be avoided. Specimens must be mixed thoroughly after thawing prior to testing.

If serum, plasma, or cadaveric serum specimens are to be shipped, they should be packaged and labeled in compliance with state, federal and international regulations covering the transportation of clinical specimens and etiologic agents.[25]

Since serum from heparinized patients may be incompletely coagulated, false reactive results could occur due to fibrin deposition on the bead and subsequent trapping of the HRPO conjugated antibody. To prevent this phenomenon, draw serum specimen prior to heparin therapy or draw specimen into a plasma collection tube. Serum specimens drawn after heparin therapy may be treated with thrombin or protamine sulfate to assure complete clotting.[22,23]

PROCEDURE

Materials Provided

No. 1980, AUSZYME Monoclonal Diagnostic Kit

100/1000/5000 Tests

The list of accessories required for the COMMANDER Flexible Pipetting Center (FPC™) and Parallel Processing Center (PPC) are found in the appropriate COMMANDER Operations Manual(s). A combination of accessories is included with the COMMANDER FPC and PPC. AUSZYME Monoclonal is designed to be compatible with the COMMANDER FPC and PPC. This product may be used with a Quantum II.

An optimum combination of the following accessories is provided for performance of the tests ordered:

- Reaction Trays
- Cover Seals (tear along perforation for use with 20 well trays)
- Assay Tubes with identifying cartons (for transfer of beads from Reaction Trays)
- 1 *N* Sulfuric Acid, No. 7212 (Most U.S. and International locations)

Materials Required but not Provided

- Precision pipettes with disposable tips, EIA Pipetting Package, or similar equipment to deliver 50 µL (tolerance is ± 10%), 100 µL, 200 µL, 300 µL (tolerance is ± 5%), and 1 mL (tolerance is ± 10%).
- QwikWash®; or device for washing beads with a vacuum source and a double trap for retaining the aspirate and maintaining minimum vacuum of 21 inches of mercury to deliver a total wash volume of 11-18 mL per well.
- COMMANDER Dynamic Incubator or water bath capable of maintaining temperature ± 1°C around a setpoint of 39 to 40°C.
- Disposable, graduated pipettes or dispenser for measuring Diluent for OPD.
- Metal-free containers for the OPD Substrate Solution, can be plastic ware or acid washed, distilled/deionized water-rinsed glassware.
- Disposable protective gloves.
- Disinfectant as described in Safety Precautions.
- *• Nonmetallic forceps.
- COMMANDER PPC, Quantum II, or spectrophotometer capable of reading absorbances at 492 nm.
- *• Bead Dispenser.
- Test tubes and rack for dilution of specimens.
- *• Membrane Seal Puncture Tool for acid bottles.

- OPD Tray Covers (for COMMANDER testing).
- Reagent Blanking Beads (for COMMANDER testing).
- Distilled or deionized water.
- HBsAg Confirmatory Assay.

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* Included in EIA Pipetting Package

Additional Reagents Available

1. Abbott OPD (o-Phenylenediamine • 2 HCl) Reagent, No. 6172.
2. 1 *N* Sulfuric Acid, No. 7212. (Most U.S. and International Locations).
3. 6 *N* Sulfuric Acid No. 7212–03 (110 mL).

AUSZYME Monoclonal TEST PROCEDURE

PRELIMINARY COMMENTS

Laboratories using the COMMANDER Dynamic Incubator (DI), Flexible Pipetting Center (FPC), or Parallel Processing Center (PPC) should refer to the appropriate instrument manual for detailed operation instructions, and note special COMMANDER instructions below.

1. Three (3) Negative Controls and two (2) Positive Controls should be assayed with each run of specimens. Ensure that all reaction trays containing Controls and/or specimens are subjected to the same processing and incubation times. This may require maintenance of specific time intervals between processing trays. Once the assay has been started, complete all subsequent steps without interruption.
2. Prior to beginning the assay procedure, bring all reagents to room temperature (15 to 30°C) and mix gently.
3. Follow the exact order of specimen and reagent additions as instructed in the steps of the Test Procedure.
4. Set the Dynamic Incubator to 40°C or adjust water bath 38 to 41°C.
   CAUTION: Use a separate disposable pipette tip for each Control and specimen in order to avoid cross-contamination.
5. Identify the reaction tray wells for each specimen or Control.
6. After each step, visually verify the presence of solution and bead in each well.
7. When using other automated instrumentation to deliver Controls and specimens, ensure the instrumentation is compatible with this assay. Follow the manufacturer's directions to achieve the appropriate volumes required within the recommended time limits. Precise timing of enzyme immunoassays is critical.

PROCEDURAL NOTES

SAMPLE PIPETTING AND DILUTION

1. When using a manual method for sample pipetting, follow the steps in the **ASSAY PROCEDURE** section, **PROCEDURES A, B, AND C**, or **PROCEDURE D** sub-sections, as appropriate.
2. When using the COMMANDER Flexible Pipetting Center (FPC), use system software Versions 2.5 or higher. Use the appropriate assay protocol 1980, AUSZYME MONO PPC for PPC processing, or AUSZYME MONO QT for Quantum II processing.

ASSAY SELECTION ON THE PPC

1. Insert tray and select the appropriate assay number for either AUSZYME MC A, B, C, or D. An operator-edited version may be used if the edited lines are consistent with the assay package insert specifications and are supported by documentation at the time of edit. Follow the instructions on the instrument display board.
   When using an automated pipetting device, such as a COMMANDER Flexible Pipetting Center, verify that the correct PPC Assay Protocol has been selected for processing.
2. Verify the reagent dispenser assignment on the PPC:

Station	Reagent	Dispenser Volume
1	Conjugate	50 µL (Proc. A, B, C) 200 µL (Proc. D)
4	OPD Solution	300 µL
5	Acid	300 µL

   NOTE: Always prime the Tri-Continent Dispensers to assure the appropriate volume of reagent is dispensed. Verify dispenser accuracy per your Standard Operating Procedures.
3. If using a water bath, carefully wipe or blot the bottom of the tray dry before inserting it into the PPC.

BLANKING (PPC only)

NOTE: Use ABBOTT COMMANDER Reagent Blanking Beads only.

1. During the conjugate incubation step, prepare a "blanks" tray using a separate tray. Place one reagent blanking bead into each of the five wells, A1 through A5.
2. At the conclusion of the conjugate incubation step, press the Blank key and insert the "blanks" tray, followed immediately by the first assay tray.
3. At the conclusion of the OPD incubation step, insert the "blanks" tray prior to the first tray of the final read pass.

General Notes

1. The Negative and Positive Controls should be treated as specimens.
2. Verify that dispensing equipment delivers specified volume for each procedure.
   NOTE: Conjugate and OPD Substrate dispensers must be rinsed with distilled or deionized water after each use. Refer to dispenser inserts for cleaning procedure.
3. When dispensing beads, remove cap from bead bottle, attach Single Bead Dispenser for 100 test kit or Multi-Bead Dispenser for 1000/5000 test kit and dispense beads into wells of the reaction tray as directed in the Single Bead Dispenser and Multi-Bead Dispenser inserts.
4. Do not splash liquid while tapping trays.
5. Make sure cover seals adhere tightly to all wells. Proper mixing of samples is required for accurate results. Tap trays to thoroughly mix contents and beads. The beads should move within the well during the tapping process.
6. When washing beads, follow the directions provided with your washing apparatus to provide a total wash volume of 11 to 18 mL for each bead. Use distilled or deionized water.

COMMANDER Dynamic Incubator

When using the COMMANDER Dynamic Incubator in connection with the PPC, use the MIX incubation method. When reading the assay on the Quantum II, select the STATIC or MIX incubation method. Select the incubation temperature and time as designated in the **ASSAY PROCEDURE** section.

CAUTION: Failure to use the Dynamic Incubator in the manner described in the Dynamic Incubator Operator's Manual may result in incorrect assay results.

COLOR DEVELOPMENT (QUANTUM II AND SPECTROPHOTOMETER)

1. When transferring beads from wells to assay tubes, align inverted rack of oriented tubes over the reaction tray. Press the tubes tightly over the wells and invert tray and tubes together so that beads fall into corresponding tubes. Blot excess water from top of tube rack.
2. Avoid strong light during Color Development.
3. Dispense acid in same tube sequence as OPD Substrate Solution.
4. Do not allow acid solution to contact metal.

READING (QUANTUM II AND SPECTROPHOTOMETER)

1. Remove air bubbles prior to reading absorbance.
2. Visually inspect both substrate blanks and discard those that are contaminated (indicated by yellow-orange color). If both substrate blanks are contaminated, the run must be repeated.
3. A determination of the absorbance of the substrate blank must be made. The absorbance of the substrate blank relative to that of the water tube must be greater than or equal to -0.020 and less than or equal to 0.040 in order for the assay to be valid. In Mode 0, blank the instrument with the water tube and read the substrate blank as a sample. (Mode 0 refers to Mode 0 on the Quantum II.) Check the blank absorbance for assay validity. Stop the Mode 0 assay.
4. If the substrate blank is valid, use it to blank the instrument. Read Negative and Positive Controls, then specimens. If the substrate blank is not valid, repeat steps 3 and 4 using the alternate substrate blank.
5. If there is an interruption during the reading of samples, re-blank the instrument with the substrate blank using the second substrate tube if necessary. Continue reading specimens.

ASSAY PROCEDURE

Laboratories using the COMMANDER Flexible Pipetting Center or Parallel Processing Center should follow procedures in the appropriate COMMANDER Operations Manual(s). When using other automated instrumentation to deliver Controls and specimens, ensure the instrumentation is compatible with this assay. Follow the manufacturer's directions to achieve the appropriate volumes required.

The following **PROCEDURE A, B, AND C** and **PROCEDURE D** instructions should be used for PPC, Quantum II, and spectrophotometer processing. The following **COLOR DEVELOPMENT, PREPARATION OF WATER TUBE**, and **READING** procedures should be used for Quantum II and spectrophotometer processing. For PPC color development and reading, refer to the PPC Operations Manual.

CAUTION: Verify that dispensing equipment delivers specified sample and/or reagent volumes and does not introduce cross-contamination.

Four procedures for the detection of HBsAg in serum or plasma are described below:

	Incubation		Color Development	
Procedure	Temp.	Time	Temp.	Time
A	38 to 41°C	3 HRS.±10 MIN.	15 to 30°C	30 to 35 MIN.
B	15 to 30°C	12 to 20 HRS.	15 to 30°C	30 to 35 MIN.
C	38 to 41°C	75 to 80 MIN.	15 to 30°C	30 to 35 MIN.

	First Incubation		Second Incubation		Color Development	
Procedure	Temp.	Time	Temp.	Time	Temp.	Time
D	38 to 41°C	2 HRS. ± 10 MIN.	38 to 41°C	1 HR. ± 5 MIN.	15 to 30°C	30 to 35 MIN.

PROCEDURES A, B, AND C

1. Dispense 200 µL of each Control or specimen* into appropriate wells of the reaction tray (3 Negative Controls and 2 Positive Controls [blue]). Be careful not to touch the sides of the well with the pipette tip.
2. Add 50 µL of Conjugate [red] to each well containing a specimen or Control. Gently tap the tray to enhance mixing of Conjugate with Controls or specimens.
3. Carefully add one bead to each well containing a specimen or Control.
4. Apply cover seal. Gently tap the tray to assure beads are completely covered with liquid and remove any trapped air bubbles.
5. Procedure A: Incubate at 40°C for 3 hours.
   Procedure B: Incubate at room temperature (15-30°C) for 16 hours.
   Procedure C: Incubate at 40°C for 75 minutes.

6. At the end of the incubation period, remove and discard cover seal. Aspirate the liquid and wash each bead with 11-18 mL of distilled or deionized water.
7. Proceed to Color Development.

PROCEDURE D
(only for cadaveric serum specimens and specimens containing sodium azide)

FIRST INCUBATION
1. Dispense 200 µL of each Control or specimen into appropriate wells of the reaction tray (3 Negative Controls and 2 Positive Controls [blue]). Be careful not to touch the sides of the well with the pipette tip.
2. Carefully add one bead to each well containing a specimen or Control.
3. Apply cover seal. Gently tap the tray to assure beads are completely covered with liquid and remove any trapped air bubbles.
4. Incubate at 40°C for 2 hours.
5. At the end of the incubation period, remove and discard cover seal. Aspirate the liquid and wash each bead with 11-18 mL of distilled or deionized water.

SECOND INCUBATION
6. Pipette 200 µL of Conjugate [red] into each well containing a bead. Be careful not to touch the sides of the well or the bead with the pipette tip.
7. Apply new cover seal. Gently tap the tray to assure beads are completely covered with liquid and remove any trapped air bubbles.
8. Incubate at 40°C for 1 hour.
9. At the end of the incubation period, remove and discard cover seal. Aspirate the liquid and wash each bead with 11-18 mL of distilled or deionized water.

COLOR DEVELOPMENT (QUANTUM II AND SPECTROPHOTOMETER)
10. Immediately transfer beads to properly identified assay tubes.
11. Prime dispenser immediately prior to dispensing OPD Substrate Solution.
12. Pipette 300 µL of freshly prepared OPD Substrate Solution into two empty tubes (substrate blanks), and then into each tube containing a bead.
13. Cover and incubate at room temperature (15-30°C) for 30 minutes.
14. Add 1 mL of 1 *N* Sulfuric Acid to each tube. Agitate to mix.

PREPARATON OF WATER TUBE (QUANTUM II AND SPECTROPHOTOMETER)
15. Pipette approximately 2 mL of distilled or deionized water into an empty tube.

READING (QUANTUM II AND SPECTROPHOTOMETER)
NOTE: Reading of the assay must be done within 2 hours after acid addition.
16. In Mode 0, blank the instrument with the water tube (see the Quantum II Operator's Manual for running in Mode 0).
17. Determine the absorbance of the substrate blank at 492 nm. The substrate blank must be greater than or equal to -0.020 and less than or equal to 0.040. Stop the assay.
18. Select the mode for processing AUSZYME Monoclonal.
19. Blank the instrument with a valid substrate blank.
20. Determine the absorbance of Controls and test specimens at 492 nm.

*Specimen volume may vary from 200 µL to 400 µL without affecting the performance of the test.

READING RESULTS
INSTRUMENTS

Performance of the AUSZYME Monoclonal assay requires the use of a precision spectrophotometer (i.e., COMMANDER Parallel Processing Center (PPC), or Quantum II). REFER TO THE APPROPRIATE INSTRUMENT MANUAL FOR PROPER OPERATION AND CALIBRATION. SHOULD SOFTWARE NEED TO BE INSTALLED OR RELOADED, ANY EDITED ASSAY PROTOCOLS MUST BE RECREATED.

IMPORTANT: Assure that the appropriate Cutoff Calculation is performed.

Procedures A, B, and D:	**Use NC$\bar{x}$ + 0.050**
Procedure C:	**Use NC$\bar{x}$ + 0.025**

COMMANDER PPC and Quantum II perform all calculations automatically. Refer to the appropriate instrument manual for calculations performed.

1. Laboratories using the COMMANDER Parallel Processing Center (PPC) must use software Versions 8.01/8.11 or greater. Use the assay protocol AUSZYME MC A, B, C, or D as provided in the software without editing. When using the PPC with the COMMANDER Flexible Pipetting Center (FPC), the assay list number and the assay procedure code in the PPC assay protocol should match the assay list number and the assay procedure code configured on the FPC.
2. Laboratories using the Quantum II must use Module A, List Number 4045-96/4045-97 or greater. Use the assay protocol AUSZYME A, B, & D (Mode 1.1) or AUSZYME C (Mode 1.2) as provided in the software without editing.
3. Laboratories using an instrument other than a Quantum II Analyzer or COMMANDER Parallel Processing Center (PPC) should read this assay as follows:
   - Read the absorbance for each Standard, Control, and specimen.
   - Rinse cuvettes thoroughly with distilled or deionized water between the reading of each specimen.
   - Refer to **QUALITY CONTROL PROCEDURES** and **RESULTS** sections for calculations.

QUALITY CONTROL PROCEDURES

For the run to be valid, the difference between the Means of the Positive Control and Negative Control (P-N) should be 0.400 or greater for Procedures A, B, and D, and 0.200 or greater for Procedure C. If not, technique may be suspect and the run must be repeated. If the P-N Value is consistently low, deterioration of reagents may be suspect.

1. Substrate Blank Acceptance Criteria
   a. QUANTUM II users: An assay run is considered valid with respect to the substrate blank if the blank has an absorbance value that is greater than or equal to -0.020 and less than or equal to 0.040. This determination of assay validity due to substrate blank must be done by the user. The substrate blank value is an indication of the integrity of the OPD Substrate Solution. If the substrate blank absorbance falls outside the acceptance range, the preparation of the substrate blank is in question and the alternate substrate blank may be used. If the alternate substrate blank is unacceptable, the assay is invalid and the run must be repeated.
   b. PPC users: Quality control with respect to the substrate blank is determined automatically by the PPC instrument according to the procedure described in the PPC Operations Manual. If the run is invalid, technique errors in the preparation of the OPD Substrate Solution are suspect and the run must be repeated.

NOTE: When a Quantum II Analyzer or a Parallel Processing Center is used, refer to the appropriate instrument manual to determine which calculations are performed automatically. If one of these instruments is not used, perform the following calculations on the assay data.

2. Calculation of Negative Control Mean Absorbance (NC$\bar{x}$)
   a. Determine the Mean of the Negative Control values.
   Example:
   Negative Control

Sample No.	Absorbance
1	0.010
2	0.011
3	0.009
TOTAL	0.030

$$\frac{\text{Total Absorbance}}{3} = \frac{0.030}{3} = 0.010(\text{NC}\bar{x})$$

   b. Individual Negative Control values should be less than or equal to 0.100 and greater than or equal to -0.010.

Negative Control values should also be greater than or equal to 0.5 times the NC$\bar{x}$ and less than or equal to 1.5 times the NC$\bar{x}$. Where the NC$\bar{x}$ is below 0.012, the calculation of 0.5 to 1.5 times the Mean may be disregarded.

In such cases, all Negative Control values should fall within the range Mean ± 0.006. If one value is outside the acceptable range, discard this value and recalculate the Mean. If two values are outside the range, the test should be repeated. If more than an occasional value falls outside the range, technique problems must be corrected.

Example:

0.010 + 0.006 = 0.016 and

0.010 - 0.006 = 0.004 Range: 0.004 and 0.016

In the example, no Negative Control sample is rejected as aberrant and the NC$\bar{x}$ need not be revised.

3. Calculation of the Positive Control Mean Absorbance (PC$\bar{x}$)
   Determine the Mean of the Positive Control values.
   Example:
   Positive Control

Sample No.	Absorbance
1	1.024
2	1.030
TOTAL	2.054

$$\frac{\text{Total Absorbance}}{2} = \frac{2.054}{2} = 1.027(\text{PC}\bar{x})$$

4. Calculation of the P-N Value
   Determine the P-N Value by subtracting the NC$\bar{x}$ from the PC$\bar{x}$.
   Example:

PC$\bar{x}$	1.027
NC$\bar{x}$	-0.010
P-N Value	1.017

   For the run to be valid, the P-N Value must be 0.400 or greater for Procedures A, B, and D, and 0.200 or greater for Procedure C. If not, technique or deterioration of reagents may be suspect and the run must be repeated.

RESULTS

1. Calculation of the Cutoff Value

   Determine the Cutoff Value by following the Examples below:

   a. Procedures A, B, and D: Add the factor 0.050 to the $\overline{NCx}$.

      Example:

$\overline{NCx}$	0.010
	+0.050
Cutoff Value	0.060

   b. Procedure C: Add the factor 0.025 to the $\overline{NCx}$.

      Example:

$\overline{NCx}$	0.010
	+0.025
Cutoff Value	0.035

2. Calculation of the Unknown

   The presence or absence of HBsAg is determined by relating the absorbance of the unknown specimen to the Cutoff Value. The Cutoff Value is the absorbance of the Negative Control Mean plus the factor 0.050 for Procedures A, B, and D, or the factor 0.025 for Procedure C. If the absorbance of the unknown specimen is greater than or equal to the Cutoff Value, it is considered reactive by the criteria of the ABBOTT AUSZYME Monoclonal assay.

INTERPRETATION OF RESULTS

Specimens with absorbance values below the Cutoff are considered nonreactive by the AUSZYME Monoclonal assay and need not be tested further. Specimens with absorbance values greater than or equal to the Cutoff are considered reactive for HBsAg. Specimens considered initially reactive should be retested in duplicate using the same procedure that was used in the initial test. If neither of the retests are reactive, the specimen is considered negative for HBsAg. If the specimen is reactive in either of the retests, the sample is considered repeatedly reactive. Repeatedly reactive specimens should be tested by the HBsAg Confirmatory Assay (List No. 1012), a licensed, neutralizing confirmatory test. Only those specimens in which the HBsAg can be neutralized by the confirmatory test procedure may be designated as positive for HBsAg.

False reactive results may be obtained with any diagnostic test. Two types of false reactive results may occur with the AUSZYME Monoclonal test:

Nonrepeatedly Reactives: Some specimens which are reactive in the AUSZYME Monoclonal screening procedure may not be reactive on repeat testing. This phenomenon is highly dependent on technique used in running the test. The most common sources of such nonrepeatedly reactives are:

- Improper bead washing
- Cross-contamination of nonreactive specimens caused by transfer of high titer antigen specimen[24]
- Contamination of the OPD Substrate Solution by oxidizing agents
- Contamination of the acid used as stopping reagent
- Contamination of reaction tray well rim with Anti-HBs Conjugate reagent, or specimen

Nonspecific Reactives: All highly sensitive immunoassay systems have a potential for nonspecific reactions, but specificity of repeatedly reactive specimens can be confirmed by neutralization tests.

It is desirable to perform a specificity analysis with an FDA-licensed neutralization confirmatory test (e.g., the ABBOTT HBsAg Confirmatory Assay) prior to informing a donor/patient that he is an HBsAg carrier.

A repeatedly reactive specimen, confirmed by neutralization with human Anti-HBs must be considered positive for HBsAg.

LIMITATION OF THE PROCEDURE

Although the association of infectivity and the presence of HBsAg is strong, it is recognized that presently available methods for HBsAg detection are not sensitive enough to detect all potentially infectious units of blood or possible cases of hepatitis.

EXPECTED RESULTS

In random blood donor populations, the number of specimens found repeatedly reactive for HBsAg by the AUSZYME Monoclonal test has typically been less than 0.5%.

SPECIFIC PERFORMANCE CHARACTERISTICS

Accuracy

This product meets the requirements for a third generation test when tested against the FDA Reference Panel.

Detectability

The ability of Procedures A, B, and C to detect HBsAg in blood bank donor specimens is shown in Table I. The data include 6,683 serum and plasma samples obtained from consecutive blood donors from four blood banks.

TABLE I
Detection of HBsAg in Serum and Plasma Samples from Blood Donors

Procedure	Number Tested	No. (%) Confirmed Positive
A	4,839	1(0.02%)
B	513	0
C	4,834	0

In these studies, the one reactive specimen tested only by AUSZYME Monoclonal Procedure A was also reactive by AUSZYME II (Procedure A). The specimen was confirmed as positive by the HBsAg Confirmatory Assay.

In addition, out of 528 HBsAg positive specimens, AUSZYME Monoclonal detected as positive all known subtypes that were also reactive by AUSZYME II.

Specificity

The percentage of specimens found reactive with Procedures A, B, or C and the proportion of these specimens found to be repeatedly reactive were determined by testing 6,683 serum and plasma samples in a clinical investigation performed at four blood banks and at Abbott Laboratories.

The presence of HBsAg in the repeatedly reactive specimens was confirmed by neutralization with human Anti-HBs using the HBsAg Confirmatory Assay. The results of these tests are shown in Table II.

TABLE II
Number (%) of Reactive Specimens Detected by Procedures A, B, and C and Confirmed as Positive for HBsAg

Procedure	Negative Screen	Reactive Screen	Repeatedly Reactive	Confirmed Positive
A	4,813 (99.46%)	26 (0.54%)	1 (0.02%)	1 (0.02%)
B	510 (99.42%)	3 (0.58%)	1 (0.19%)	0
C	4,806 (99.42%)	28 (0.58%)	0	0

In these studies:

1. Procedure A detected 0.52%, Procedure B detected 0.39%, and Procedure C detected 0.58% screening procedure reactive specimens which were not reactive by retesting.
2. In this random donor population, 1 of the repeatedly reactive specimens detected by Procedure A was confirmed as positive by neutralization with human Anti-HBs using the HBsAg Confirmatory Assay. These data indicate no significant differences between AUSZYME Monoclonal and AUSZYME II.

Sensitivity

The relative sensitivity of AUSZYME Monoclonal Procedures A, B, and C was compared using a 25 member proficiency panel of purified HBsAg (*ad* and *ay*) prepared at Abbott Laboratories. Each panel member was assayed in replicates of 3 for at least 3 days by two procedures at each of 4 blood banks using the same lot of material. The mean sensitivity detected for each procedure is shown in Tables III and IV.

TABLE III
Detection of Purified HBsAg/*ad* by Each Procedure of AUSZYME Monoclonal

Concentration (ng/mL)	Procedure A (A_{492}-$\overline{NCx}$)	Procedure B (A_{492}-$\overline{NCx}$)	Procedure C (A_{492}-$\overline{NCx}$)
8.00	1.082	1.323	0.634
4.00	0.598	0.812	0.332
1.60	0.256	0.404	0.136
1.28	0.202	0.316	0.107
1.04	0.166	0.277	0.085
0.80	0.127	0.199	0.065
0.64	0.102	0.169	0.052
0.48	0.076	0.121	0.039
0.32	0.055	0.096	0.025
0.24	0.036	0.062	0.021
0.16	0.024	0.037	0.012
0.08	0.012	0.021	0.006

TABLE IV
Detection of Purified HBsAg/*ay* by Each Procedure of AUSZYME Monoclonal

Concentration (ng/mL)	Procedure A (A_{492}-$\overline{NCx}$)	Procedure B (A_{492}-$\overline{NCx}$)	Procedure C (A_{492}-$\overline{NCx}$)
10.31	1.450	1.610	0.905
5.15	0.828	1.003	0.470
2.06	0.389	0.637	0.226
1.65	0.302	0.500	0.161
1.34	0.250	0.401	0.132
1.03	0.191	0.319	0.100
0.82	0.152	0.263	0.083
0.62	0.116	0.191	0.058
0.41	0.058	0.101	0.029
0.31	0.056	0.098	0.028
0.21	0.037	0.077	0.018
0.10	0.019	0.030	0.008

Typically the sensitivity results from clinicals and studies done at Abbott Laboratories have ranged from 0.3 to 0.7 ng/mL for Procedures A and C and from 0.2 to 0.4 ng/mL for Procedure B. Sensitivity testing demonstrated that AUSZYME Monoclonal was as sensitive or more sensitive than AUSZYME II.

PERFORMANCE CHARACTERISTICS OF CADAVERIC SERUM TESTING

Reproducibility

Inter-assay reproducibility of AUSZYME Monoclonal was assessed using 20 postmortem and 20 normal donor sera. These sera specimens were spiked with human plasma positive for Hepatitis B Surface Antigen (HBsAg) to create low-level reactive specimens. Each of the specimens was tested once on six different days on each of three lots of AUSZYME Monoclonal at one site. For inter-assay reproducibility over all lots, the percent coefficient of variation (%CV) ranged from 6.0% to 10.4% for the low-level reactive postmortem specimens, and from 6.3% to 10.5% for the low-level reactive normal donor specimens. For inter-lot reproducibility, the %CV ranged from 12.3% to 18.7% for the low-level reactive postmortem specimens, and from 12.1% to 19.2% for the low-level reactive normal donor specimens.

Specificity

Specificity was evaluated using 62 postmortem and 65 normal donor specimens. Each of the specimens was tested once on each of three lots of AUSZYME Monoclonal. The mean sample to cutoff (S/CO) ratio for 186 postmortem replicates (62 specimens with three reagent lots) was 0.362, and the mean S/CO ratio for 195 normal donor replicates (65 specimens with three reagent lots) was 0.255. Results are presented in Table V.

TABLE V

Reactivity with AUSZYME Monoclonal

Population	No. of Specimens	No. of Replicates	Mean S/CO	Nonreactive	Repeat Reactive, Non-Confirming
Postmortem	62	186	0.362	183 (98.39%)	3 (1.61%)
Normal Donor	65	195	0.255	195 (100.0%)	0 (0.0%)

The reactivity of AUSZYME Monoclonal has an estimated specificity of 98.39% (binomial confidence interval[26] = [95.36%, 99.67%]) for postmortem specimens.

Sensitivity

Sensitivity was evaluated using 50 postmortem and 50 normal donor specimens that were pre-screened for HBsAg and found to be negative. The 50 specimens were spiked with human plasma positive for HBsAg to create low-level reactive specimens. Each of the specimens was tested once on each of three lots of AUSZYME Monoclonal. The mean sample to cutoff (S/CO) for the 150 postmortem replicates (50 specimens with three reagent lots) was 3.820, and the mean S/CO ratio for the 150 normal donor replicates (50 specimens with three reagent lots) was 3.628. The calculated difference between the postmortem specimens and the normal donor specimens was 0.192 S/CO, which was determined not to be statistically significant by the F-test analysis (p-value = 0.354). Results are presented in Table VI.

TABLE VI

Reactivity with AUSZYME Monoclonal

Population	No. of Specimens	No. of Replicates	Mean S/CO	Nonreactive	Initially Reactive
Postmortem spiked HBsAg	50	150	3.820	0 (0.00%)	150 (100%)
Normal Donor spiked HBsAg	50	150	3.628	0 (0.00%)	150 (100%)

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