ANTIBODY TO HEPATITIS B SURFACE ANTIGEN (HUMAN)

HBsAg CONFIRMATORY ASSAY

NOTE CHANGES HIGHLIGHTED

Name and Intended Use
HBsAg Confirmatory Assay
For Confirmatory Neutralization of AUSZYME® Monoclonal Repeatedly Reactive Specimens.

69-5486/R11

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SUMMARY AND EXPLANATION OF THE TEST

The HBsAg Confirmatory Assay uses the principle of specific antibody neutralization to confirm the presence of HBsAg in specimens found to be repeatedly reactive by AUSZYME Monoclonal. A specimen which is found to be repeatedly reactive should be confirmed by neutralization procedures using human Anti-HBs (HBsAg Confirmatory Assay). If the sample is neutralizable in the confirmatory test, the specimen is considered positive for HBsAg and need not be tested further.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The HBsAg Confirmatory Assay uses the principle of specific antibody neutralization to confirm the presence of HBsAg. The Confirmatory Reagent (human antibody to Hepatitis B Surface Antigen) is incubated with the specimen in solution. If HBsAg is present in the specimen, it will be bound by the Confirmatory Reagent. The neutralized HBsAg is subsequently blocked from binding to the antibody coated bead. This results in a reduction of signal when compared to the non-neutralized specimen in which Negative Control is used in place of Confirmatory Reagent.

By definition, a specimen is confirmed as positive if the reduction in signal of the neutralized specimen is at least 50% **and** the non-neutralized control generates a signal greater than or equal to the assay cutoff.

REAGENTS

HBsAg Confirmatory Assay

① 1 Vial (1.2 mL) Antibody to Hepatitis B Surface Antigen (Human) Confirmatory Reagent (Solution A) contains human plasma reactive for anti-HBs and nonreactive for HBsAg, HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2. Preservatives: 0.01% Gentamicin Sulfate and 0.01% Thimerosal.

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

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 anti-HBs 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.[1] Biosafety Level 2[2] or other appropriate biosafety practices[3,4] 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.[5,6]
5. Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with local, state and federal regulations.[7,8]

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 3 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.

STORAGE INSTRUCTIONS

1. Store reagents at 2 to 8°C.
2. All reagents should be brought to room temperature before use and returned to 2 to 8°C storage after use.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

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

The specimens to be tested by the confirmatory neutralization test are those found to be repeatedly reactive by the AUSZYME Monoclonal screening assay. See the AUSZYME Monoclonal package insert for specific instructions on specimen collection and preparation.

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).

Cadaveric serum specimens may be stored for up to seven days at 2 to 8°C. However, if storage periods greater than seven days are anticipated, the specimens should be stored frozen at -20°C or colder.

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.[9]

PROCEDURE

Materials Provided

List No. 1012 HBsAg Confirmatory Assay

Materials required but not provided:

- AUSZYME Monoclonal Kit, No. 1980
- See MATERIALS REQUIRED BUT NOT PROVIDED in AUSZYME Monoclonal package insert.

HBsAg Confirmatory Test Procedure

Preliminary Comments

DETERMINATION OF PROCEDURE TO USE

1. The HBsAg Confirmatory Assay should be used with AUSZYME Monoclonal to confirm specimens found to be repeatedly reactive by AUSZYME Monoclonal.
2. Depending on the procedure used to screen the specimens, the confirmatory assay may be performed by one of three procedures: A, B, or D.

Screening Procedure Used	Confirmatory Procedure to Use
Procedure A	Procedure A or B
Procedure B	Procedure B
Procedure C	Procedure A or B
Procedure D	Procedure D

SPECIMEN DILUTION AND REPEAT TESTING

1. If the specimen to be confirmed had an A_{492} greater than or equal to 2.000 in the HBsAg screening assay, the specimen should be diluted 1:25 in Negative Control prior to testing by the Confirmatory Assay. However, if a 1:25 dilution of the specimen is tested and the A_{492} of the non-neutralized specimen control (B-N) is less than 0.025, the test must be repeated using the specimen undiluted.

   NOTE: In some instances high titer HBsAg specimens will not be neutralized at least 50% by the addition of Confirmatory Reagent when assayed undiluted or diluted 1:25. These specimens should be diluted 1:500 or greater in Negative Control and assayed again by the HBsAg Confirmatory Assay.
2. If the specimen had an A_{492} less than 2.000 in the screening assay, the specimen should be tested undiluted. However, if the undiluted specimen is not neutralized 50% or greater and the A_{492} of the non-neutralized specimen control (B-N) is greater than 0.025, the test must be repeated using the specimen diluted 1:500 or greater.

   NOTE: In some instances very high titer HBsAg specimens will yield paradoxical results of A_{492} less than 2.000 and less than 50% neutralization in the 1-step procedures, A and B. In such instances no conclusion can be drawn until repeat testing of the specimen, diluted 1:500 or greater, has occurred (See **EVALUATION OF RESULTS**).

Procedural Notes

1. Three Negative and four Positive Controls (two to be neutralized, two as non-neutralized controls) must be assayed with each run of unknowns. For each unknown specimen to be assayed, four samples are run (two to be neutralized, two as non-neutralized controls).
2. Ensure that all reaction trays containing Controls and/or unknowns are subjected to the same process and incubation times.

   CAUTION: Use a separate disposable tip for each transfer to avoid cross-contamination.

   NOTE: Once the assay has been started, all steps should be completed without interruption.
3. Prior to beginning the assay procedure, bring all reagents to room temperature (15 to 30°C). Swirl gently before using. Set water bath or incubator to 40°C (38 to 41°C; Procedures A and D).
4. Identify the reaction tray wells for each specimen or Control.
5. When dispensing beads, remove cap from bead bottle, attach single Bead Dispenser for 100 test kit or Multi-Bead Dispenser for 1000 test kit and dispense beads into wells of the reaction tray as directed in the single Bead Dispenser or Multi-Bead Dispenser insert.
6. Tap trays to thoroughly mix contents and beads without splashing liquid. Proper mixing of samples is required for accurate results.
7. 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.
8. 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.
9. Avoid strong light during Color Development.
10. Dispense acid in same tube sequence as OPD Substrate Solution.

    NOTE: Conjugate and OPD Substrate dispensers must be rinsed with distilled or deionized water after each use. Refer to dispenser inserts for cleaning procedure.
11. Do not allow acid solution to contact metal. If necessary, agitate tubes to ensure thorough mixing.

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

Three procedures for the confirmation of HBsAg in serum or plasma are described below. Each of the three procedures uses the same Step 1 and Step 3.

		Incubation	
Step 1.	Specimen Neutralization:	15 to 20 minutes;	15 to 30°C
Step 2.	Confirmatory Procedure	Time	Temperature
	A	3 hrs. ± 10 min.	38 to 41°C
	B	16 hrs. ± 4 hrs.	15 to 30°C
	D	1st Incubation	
		2 hrs. ± 10 min.	38 to 41°C
		2nd Incubation	
		1 hr. ± 5 min.	38 to 41°C
Step 3.	Color Development:	30 to 35 minutes;	15 to 30°C

NEUTRALIZATION PROCEDURES A, B, AND D

NOTES:
- Make appropriate dilution of specimens, if necessary.
- For AUSZYME Monoclonal use 200 µL of Controls and specimens (Steps 1 and 2).

1. Pipette the appropriate volume of each Control into the bottom of the appropriate wells of the reaction tray (three Negative Controls and four Positive Controls).

2. Pipette the appropriate volume of serum, plasma or cadaveric serum into each of four (4) wells for each specimen being tested for confirmation.
3. Dispense 50 µL of Confirmatory Reagent, Solution A, into two of the four wells containing Positive Control. Into the other two Positive Control wells and the three Negative Control wells dispense 50 µL Negative Control, Solution B.
4. For each unknown specimen, dispense 50 µL Confirmatory Reagent, Solution A, into two of the four wells. Into the other two wells dispense 50 µL Negative Control, Solution B.
5. Tap tray gently to facilitate mixing; allow neutralization reaction to proceed for 15 minutes at room temperature.

INCUBATION: PROCEDURES A AND B

1. Add 50 µL of conjugate to each well containing a specimen or Control. Gently tap tray to enhance mixing.
2. Carefully add one bead to each well containing a specimen or Control.
3. Apply cover seal. Gently tap tray to cover beads and remove any trapped air bubbles.
4. Procedure A: Incubate at 40°C for 3 hours.

   Procedure B: Incubate at room temperature for 16 hours on a level surface.
5. Remove and discard cover seal. Aspirate the liquid and wash each bead with 11-18 mL of distilled or deionized water.
6. Proceed to "Color Development".

PROCEDURE D

First Incubation

1. Carefully add one bead to each well containing a specimen or Control.
2. Apply cover seal. Gently tap the tray to cover beads and remove any trapped air bubbles.
3. Incubate at 40°C for 2 hours.
4. Remove and discard cover seal. Aspirate the liquid and wash each bead with 11-18 mL of distilled or deionized water.

Second Incubation

5. Pipette 200 µL of conjugate into each well containing a bead.
6. Apply a new cover seal. Gently tap the tray to cover beads and remove any trapped air bubbles.
7. Incubate at 40°C for 1 hour.
8. Remove and discard cover seal. Aspirate the liquid and wash each bead as in Step 4.
9. Proceed to "Color Development".

COLOR DEVELOPMENT

(See AUSZYME Monoclonal package insert for preparation of OPD.)

PROCEDURES A, B, AND D

1. Immediately transfer beads to properly identified assay tubes.
2. Pipette 300 µL of freshly prepared OPD Substrate Solution into two empty tubes (substrate blanks) and then into each tube containing a bead.

   NOTE: Prime dispenser immediately prior to dispensing OPD Substrate Solution. Verify dispenser accuracy per your Standard Operating Procedures.
3. Cover and incubate at room temperature for 30 minutes.
4. Add 1 mL of 1 *N* Sulfuric Acid to each tube. Agitate to mix.

PREPARATION OF WATER TUBE (QUANTUM II AND SPECTROPHOTOMETER)

5. 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.

6. In Mode 0, blank the instrument with the water tube (see the Quantum II Operator's Manual for running in Mode 0).
7. 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.
8. Select the mode for processing HBsAg Confirmatory Assay.
9. Blank the instrument with a valid substrate blank.
10. Determine the absorbance of Controls and specimens at 492 nm.

READING RESULTS

QUANTUM™ II

Laboratories using a Quantum II: Use Module L, List No. 4045-17 or above containing Mode 1.1, AUSZYME CONF protocol. When using a Quantum II Analyzer, refer to the Operator's Manual to determine which calculations are performed automatically.

Laboratories using instruments other than a Quantum II Analyzer: Following the reading of any specimen, thoroughly rinse cuvettes with distilled or deionized water prior to reading the next tube.

QUALITY CONTROL PROCEDURES

1. Substrate Blank Acceptance Criteria

   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.

RESULTS

NOTE: For Automatic Data Reduction on the Quantum II, the non-neutralized (Solution B) duplicates (for Positive Control and unknowns) must be read immediately before the respective neutralized (Solution A) duplicates.

If the Quantum II Analyzer is not used, perform the following calculations on the assay data.

1. Calculate the Mean A_{492} for the three Negative Control Values. Eliminate aberrant values as described in the AUSZYME Monoclonal Package Insert.
2. Determine the Mean A_{492} for both the neutralized and non-neutralized Positive Control and each test specimen.
3. Determine the percent reduction for the Positive Control and for each specimen using the following equation:

$$\% \text{ Reduction} = \frac{A_{492} \text{ Solution B} - A_{492} \text{ Solution A}}{A_{492} \text{ Solution B} - A_{492} \text{ NC}\bar{x}} \times 100$$

Example:	Mean Absorbance
Negative Control (NC$\bar{x}$)	0.027
Positive Control + Solution B (Negative Control)	1.011
Positive Control + Solution A (Confirmatory Reagent)	0.019
Specimen + Solution B (Negative Control)	0.649
Specimen + Solution A (Confirmatory Reagent)	0.017

$$\text{Positive Control: } \frac{1.011 - 0.019}{1.011 - 0.027} = \frac{0.992}{0.984} \times 100 = 100.9\% \text{ Reduction}$$

$$\text{Specimen: } \frac{0.649 - 0.017}{0.649 - 0.027} = \frac{0.632}{0.622} \times 100 = 101.6\% \text{ Reduction}$$

4. Determine the B - N Value for the Positive Control and for each specimen using the following equation:

   B - N = A_{492} Solution B - A_{492} NC$\overline{x}$

   Example:

   Positive Control: 1.011 - 0.027 = 0.984

   Specimen: 0.649 - 0.027 = 0.622

   NOTE: If a Quantum II Analyzer is used for reading and an absorbance reading of greater than or equal to 2.000 is obtained for sample plus Negative Control, use the absorbance value of 2.000 for calculation purposes.

EVALUATION OF RESULTS

The test is valid if the B - N Value for the Positive Control is greater than or equal to 0.400 and the Positive Control absorbance is reduced by at least 50% with the addition of Confirmatory Reagent.

A specimen is considered to be positive if both of the following criteria are met:

1. The B - N Value (net absorbance on Quantum II) for the specimen (or the diluted specimen) is equal to or greater than 0.025 (the Assay Cutoff).
2. The AUSZYME Monoclonal reactivity of the specimen (or diluted specimen) is reduced by at least 50% by the addition of Confirmatory Reagent (Solution A).

	Results			
	(A_{492}) Solution A	(A_{492}) B-N	% Neutraliz.	Assay Interpretation
1.	Any Value	<Cutoff	Any Value	HBsAg Repeat Reactive; Non-Confirmable
2.	Any Value	≥Cutoff	≥ 50	Confirmed HBsAg Positive
3.	Any Value	≥Cutoff	< 50	Repeat with Dilution

If specimen dilution of 1:25 yielded B-N result less than assay cutoff, repeat the test using the specimen undiluted.

NOTE: Any specimen yielding less than 50% neutralization with B - N greater than 0.025 should be diluted 1:500 or greater and reassayed.

A specimen which is repeatedly reactive by the AUSZYME Monoclonal test and is confirmed by neutralization with Confirmatory Reagent must be considered positive for HBsAg.

LIMITATION OF THE PROCEDURE

The association of infectivity and HBsAg is strong, although 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.

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