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
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 bead. This results in a reduction of signal when compared to the non-neutralized specimen in which Negative Control bead is used in place of Confirmatory Reagent. By definition, a specimen is confirmed as positive if the strength of the signal generated in the non-neutralized control generates a signal greater than or equal to the assay cutoff.

REAGENTS

HBsAg Confirmatory Assay

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. Vial (18 mL) Negative Control (Solution B). Recalified Human Plasma nonreactive for HBsAg, HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2. 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, anti-HIV-1, anti-HIV-2, and anti-HCV. All 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. (Biosafety level 2 practices) should be used for reagents 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.

PROCEDURE

Materials Provided

List No. 1012 HBsAg Confirmatory Assay

Materials required but not provided

1. AUSZYME Monoclonal Kit, No. 1390

2. See MATERIALS REQUIRED BUT NOT PROVIDED in AUSZYME Monoclonal package insert.

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. Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with local, state and federal regulations.

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 transporting 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 caps off.

7. Cadaveric serum specimens and specimens containing sodium azide (which inactivates horseradish peroxidase) reagents use the two step (Procedure D) assay. Procedure D is not intended for routine testing. The 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.

8. Avoid microbial contamination of reagents when transporting aliquots from the reagent vials. Use of disposable pipette tips is recommended.

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HBsAg Confirmatory Test Procedure

**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 specimen, the confirmatory assay may be performed by one of three procedures: A, B, or D.

**Specimen Dilution and Repeat Testing**

1. If the specimen to be confirmed had an A492 greater than 0.025, the test must be repeated using the specimen undiluted.

2. If the specimen had an A492 less than 2.000 in the HBsAg screening assay, the specimen should be diluted 1:500 or greater in Negative Control and assayed by the HBsAg Confirmatory Assay.

**Procedural Notes**

1. Three Negative and four Positive Controls (two to be neutralized, two as non-neutralized controls) must be included with each set of unknowns.

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 tray of specimen to be assayed, two as neutralized Controls and/or unknowns, and four Positive Controls.

**Procedure Time Temperature**

- Neutralization: 15 to 20 minutes; 15 to 30°C
- 1st Incubation: 2 hrs. ± 10 min; 38 to 41°C
- 2nd Incubation: 1 hr. ± 5 min; 38 to 41°C
- 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).
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 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. Incubate at 40°C for 2 hours.

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 tray to cover beads and remove any trapped air bubbles.

3. Incubate at 40°C for 3 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 with 11-18 mL of distilled or deionized water.

9. Neutralize each bead. Rinse cuvettes with distilled or deionized water prior to aspirating the liquid.

10. Determine the absorbance of Controls and specimens at 492 nm.

NOTE: Reading of the assay must be done within 2 hours after acid addition.

5. In Mode B, blank the instrument with the water tube (see the Quantum II Operator’s Manual for running in Mode II).

6. 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.

7. Select the mode for processing HBsAg Confirmatory Assay.

8. Blank the instrument with a valid substrate blank.

9. Determine the absorbance of Controls and specimens at 492 nm.

REASUMADO DE PROCEDIMENTO AUSZYME MONOCLONAL

1. Carefully add one bead to each well containing a specimen or Control.

2. Apply cover seal. Gently tap 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.

5. Proceed to "Color Development".

COLOR DEVELOPMENT

1. Prime dispenser immediately prior to dispensing OPD Substrate Solution. Verify dispenser accuracy per your Standard Operating Procedures.

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

\[ B - N = \frac{A_{492 \text{ Solution B} - A_{492 \text{ NCx}}} \times 100}{A_{492 \text{ NCx}}} \]

**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**

<table>
<thead>
<tr>
<th>(A492) Solution A</th>
<th>(A492) B-N</th>
<th>% Neutral.</th>
<th>Assay Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any Value &lt; Cutoff</td>
<td>Any Value &lt; 0.025</td>
<td>HBsAg Repeat Reactive; Non-Confirmable</td>
<td></td>
</tr>
<tr>
<td>Any Value &gt; Cutoff</td>
<td>Any Value &gt; 0.025</td>
<td>Confirmed HBsAg Positive</td>
<td></td>
</tr>
<tr>
<td>Any Value &gt; Cutoff</td>
<td>&lt; 0.025</td>
<td>Repeat with Dilution</td>
<td></td>
</tr>
</tbody>
</table>

If specimen dilution of 1:25 yields 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 1:50 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.

**BIBLIOGRAPHY**


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