Confirmation of the presence of HBsAg is determined by comparing the mean absorbance value (Xmean) of the non-neutralized samples to the mean absorbance value of the positive control. This comparison is expressed as a % reduction. The % reduction for all controls and samples is determined by applying the following equation:

\[
\% \text{ reduction} = \left( \frac{X_{\text{mean, non-neutralized sample} - X_{\text{mean, neutralized sample}}} \times 100}{X_{\text{mean, non-neutralized sample}}} \right)
\]

Example:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control, non-neutralized</td>
<td>0.025</td>
</tr>
<tr>
<td>Positive Control, non-neutralized</td>
<td>1.720</td>
</tr>
<tr>
<td>Positive Control, neutralized</td>
<td>0.016</td>
</tr>
</tbody>
</table>

% reduction of HBsAg Positive Control (PC) = 1.720 - 0.025 = 1.695

% reduction of Lows Positive Control (LP) = 0.024

% reduction of % reduction of HBsAg Positive Control and Low Positive Control is ≥ 50%.

A run is valid if the following criteria are met:

- The absorbance values of the Negative Controls are greater than 0.000 AU and less than or equal to 0.100 AU. One Negative Control value may be discarded. If two or more Negative Controls are out of limit, the run must be repeated.

- The mean absorbance value of the non-neutralized Positive Controls (PC) must be greater than or equal to 1.000 AU and the individual absorbance values must be within range of 0.65 to 1.35 times the PCX. No Positive Control value may be discarded.

- The mean absorbance value of the non-neutralized Low Positive Controls (LP) must be greater (than or equal to) than the upper linearity limits of the reader, use the reader.

Note: If the absorbance value of patient samples is greater than 0.000 AU and less than or equal to 0.100 AU, and there is still not a 50% reduction in signal by the addition of HBsAg Negative Control (Human), and assay again by the GS HBsAg Confirmatory Assay 3.0.

A specimen is considered to be positive for HBsAg if the following criteria are met:

- The specimen is repeatedly reactive by the GS HBsAg EIA 3.0.

- The absorbance value of the non-neutralized specimen is greater than or equal to the calculated cutoff value of the GS HBsAg Confirmatory Assay 3.0.

- The % reduction of the specimen Xmean is ≥ 50%.

LIMITATIONS OF THE PROCEDURE

1. The GS HBsAg EIA 3.0 Procedure and GS HBsAg Confirmatory Assay 3.0 Procedure package insert recommendations must be followed when testing serum, plasma, or cadaveric serum specimens for the presence of HBsAg. The user of this kit is advised to read the package insert carefully prior to conducting the test. In particular, the test procedure must be carefully followed for specimen and reagent pipetting, plate washing and timing of the incubation steps.

2. False negative results can occur if the quantity of marker present in the sample is too low for the detection limits of the assay, or if the marker which is detected is not present during the stage of disease in which a sample is collected.

3. Failure to add specimen or reagent as instructed in the procedure could result in a falsely negative test. Repeat testing should be considered where there is clinical suspicion of infection or procedural error.

4. An absorbance value of 0.000 AU may indicate a procedural or instrument error which should be evaluated. That result is invalid and that specimen must be re-run.

5. Factors that can affect the validity of results include false positive results and that reagent to the wash, inadequate washing of microplate wells, failure to follow the stated incubation times and temperatures, usage of wrong reagents to wells, the presence of metals, or spilling of bleach into wells.

BIBLIOGRAPHY


For Confirmatory Neutralization of GS HBsAg EIA 3.0 Reactive Specimens

For In Vitro Diagnostic Use

Store the kit at 2-8°C. Bring all reagents to room temperature (15-30°C) before use. Return to 2-8°C immediately after use.

WARRIORS FOR USERS

1. In Vitro Diagnostic Use

2. Wear protective clothing, including lab coat, eye/face protection and disposable gloves (synthetic, non-latex gloves are recommended) while handling kit reagents. Wash hands thoroughly after performing the test.

3. Do not smoke, eat or drink in areas where specimens or kit reagents are being handled.

4. Do not pipette by mouth.

5. This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with the potential hazards associated with HBV; proper protective clothing, gloves and eye/face protection and handle appropriately with the good Laboratory Practices.

The following is a list of potential chemical hazards contained in kit components:

- 0.16% ProClin™ 950 (0.16%), a biocidal preservative that is irritating to eyes and skin, may be detrimental if exposed to eyes, skin, may be detrimental if not ingested, and may cause sensitization to skin; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

- 0.005% Gencisul Sulfate, a biocidal preservative which is a known reproductive toxin, photosensitizer and sensitizer; prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals.

6. The GS HBsAg Confirmatory Assay 3.0 contains human blood components. No known test method can offer complete assurance that infectious agents are absent. Therefore, all human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious diseases, following recommended Universal Precautions for bloodborne pathogen as defined by OSHA, the guidelines from the current CDC/NHI Biosafety in Microbiological and Biomedical Laboratories and/or local, regional and national regulations.

7. Biological spills: Human source material spills should be treated as potentially infectious.

Spills not containing acid should be immediately washed with an appropriate disinfectant. Acid spills not containing acid should be immediately washed with a biocidal preservative that is irritating to eyes and skin, may be detrimental if exposed to eyes, skin, may be detrimental if not ingested, and may cause sensitization to skin; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

Spills containing acid should be appropriately absorbed (wiped up) or neutralized, the area flushed with water and wiped dry; materials used to absorb the spill should be discarded as biohazard waste. The area should be decontaminated with one of the chemical disinfectants.
For Reference Use Only

specimens that are weakly reactive may become nonreactive increased levels of protein, lipids, bilirubin, or microbiological serum samples with increased levels of hemolysis have been detected in assay results of serum or plasma samples...11. The GS HBsAg Confirmatory Assay 3.0 performance is 9. Use only adequately calibrated equipment with this assay. 8. Inadequate adherence to package insert instructions may result in erroneous results.

PRECAUTIONS FOR USERS

1. Do not use the kit beyond the stated expiration date. 2. The cutoff calculations for the GS HBsAg EIA 3.0 and GS HBsAg Confirmatory Assay 3.0 are performed computerized. Do not use the cutoff calculations in the GS HBsAg EIA 3.0 package insert for confirmatory testing. 3. Exercise care when opening vials and removing aliquots to avoid microorganism contamination of the reagents. 4. Bring all reagents except the GS HBsAg EIA 3.0 Conjugate Concentrate to room temperature before use. 5. Clinical samples may contain very high levels of HBsAg. Therefore, care must be exercised when handling specimens to avoid cross contamination through aerosols or carryover. For manual pipetting of controls and specimens, an individual pipette tip for each sample and do not allow other parts of the pipetting device to touch the rim or interior of the specimen container. Consider using new stoppers/caps to seal specimen tubes after use, to avoid errors or contamination of the work area while recapping tubes. 6. Handle negative and positive controls in the same manner as patient specimens. 7. If a specimen or reagent is inadvertently not added to a well, the assay results will read negative. 8. Inadequate adherence to package insert instructions may result in erroneous results. 9. Use only adequately calibrated equipment with this assay. 10. Use of dedicated equipment is recommended if equipment performance validations have not precluded the possibility of crosstalk or carryover. 11. The GS HBsAg Confirmatory Assay 3.0 performance is highly dependent upon incubation times and temperatures. Temperatures outside of the validated ranges may result in invalid assay results. 12. Components of this kit meet FDA potency requirements.

SPECIMEN COLLECTION, PREPARATION, AND STORAGE

The specimens to be tested for the GS HBsAg EIA 3.0 screening assay. Serum, plasma, or cadaveric serum specimens may be used. The following anticoagulants in glass tubes have all been evaluated and found to be acceptable: EDTA, sodium heparin, sodium citrate, CPDA-1, and ACD. In addition, plas- tic tubes with serum, plasma, and the following anticoagulants have also been evaluated and found to be acceptable: EDTA, lithium heparin, and sodium citrate. Samples which are collected into anticoagulant tubes should be filled as labeling indicates to avoid improper dilution. Specimens with observable particulate matter should be clarified by cen- trifugation prior to testing or storage. Reagents and controls used in this assay may contain increased levels of protein, lipids, bilirubin, or hemolysis, or after heat inactivation of whole blood, EDTA plasma samples with increased levels of hemolysis have been tested, and no clinically significant effect has been detected in assay results. Note: Cadaveric serum or plasma specimens with increased levels of protein, lipids, bilirubin, or microbiological contaminants may not be available to evaluate with this assay. Serum, plasma, or cadaveric serum specimens may be stored at 2-8°C for up to seven days. Samples should not be used if they have incurred more than 5 freeze/thaw cycles. Mix samples thoroughly before thawing. NOTE: Cadaveric specimens which are weakly reactive may become nonreactive after freeze/thaw cycles.

If specimens are shipped, they should be packed in com- pliance with Federal Regulations covering the transportation of infectious agents. Studies have demonstrated that specimens may be shipped refrigerated (2-8°C) or at ambient temperatures for up to 7 days. For shipments that are in transit for more than 7 days, specimens should be kept frozen (-20°C or lower). Refrigerate samples at 2-8°C at receipt, or freeze for longer storage.

This kit is not intended for use with specimens other than serum, plasma, and cadaveric specimens. This kit is not intended for use on saliva/oral fluids or urine samples.

GS HBsAg CONFIRMATORY ASSAY 3.0 PROCEDURE

Materials Provided

See Reagents Section on page 2.

Materials Required but not Provided

1. GS HBsAg EIA 3.0. 2. Plastic test tubes. 3. Precision pipettes to deliver volumes from 10 µL to 200 µL, 1 mL, 5 mL, and 10 mL (accuracy within ± 10%). 4. Multichannel pipette capable of delivering 100 µL or 200 µL is optional. 5. Pipette tips. 6. Dry-heat static or shaker incubator capable of maintaining 37°C ± 1°C using a dry-heat static incubator. 7. Unmarked brown plastic centrifuge tubes. 8. 273 x 12 mm polypropylene microtiter plates or other appropriate means to minimize evaporation.

G. Mix each tube by gentle vortex mixing or tapping. Avoid excessive foaming.

6. Avoid exposure of the plates to light during the final incubation step (following the addition of the Working Conjugate Solution). 7. Adhere to the recommended time constraints for the use of the Working TMB Solution (8 hours), Working Conjugate Solution (8 hours) and Wash Solution (4 weeks). 8. Avoid the formation of air bubbles in each microwell.

G. Procedure

The following procedures for the confirmation of HBsAg in human serum or plasma, procedures A and B. For the confirmation of HBsAg in cadaveric serum specimens, only procedure A can be used. The following procedures for the confirmation of HBsAg are described below:

Procedure

Specimen incubation Conjugate incubation Color development

A neutralized dry heat, 36°C, 15 to 30 minutes

A neutralized dry heat, 36°C, 15 to 30 minutes

A neutralized 30 to 60 minutes

B shaker incubation, 36°C, 60 min. B shaker incubation, 36°C, 60 min.

B shaker incubation, 36°C, 60 min. B shaker incubation, 36°C, 60 min.

Note: Specimens found to be repeatedly reactive by the GS HBsAg EIA 3.0 using procedure A should be confirmed with GS HBsAg Confirmatory Assay 3.0 using static incubation (Procedure A). Specimens found to be repeatedly reactive by the GS HBsAg EIA 3.0 using shaker incubations (Procedure B) should be confirmed with GS HBsAg Confirmatory Assay 3.0 using shaker incubations (Procedure B).

1. Prepare equipment maintenance and calibration, where necessary, as required by the manufacturer.

2. Prepare Working Wash Solution, Working Conjugate Solution, and Working TMB Solution as described in the GS HBsAg EIA 3.0 package insert. See Reagents Section on page 2.

3. Prepare Working Wash Solution, Working Conjugate Solution, and Working TMB Solution as described in the GS HBsAg EIA 3.0 package insert. See Reagents Section on page 2.

4. For manual pipetting of controls and specimens, an individual pipette tip for each sample should be used. Pipette tips should be labeled as either Neutralized or Non-neutralized. Avoid cross contamination.

5. For manual pipetting of controls and specimens, an individual pipette tip for each sample should be used. Pipette tips should be labeled as either Neutralized or Non-neutralized. Avoid cross contamination.

6. Mix each tube by gentle vortex mixing or tapping. Avoid excessive foaming.

7. Add 100 µL of the Working TMB Solution to each well containing a specimen or control.

8. Cover the microwell plate with a plate cover or use other means to minimize evaporation.

Procedure A: Incubate the plate for 60 to 65 minutes at 37°C using a dry-heat static incubator.

Procedure B: Incubate the plate for 60 to 65 minutes at 37°C using a shaker incubator.

9. At the end of the incubation period, carefully remove the plate cover, and aspirate the fluid from each well into a biohazard container. Wash the microwell plate or strip of microtiter plates with the Wash Solution (at least 400 µL/well/wash). Soak for 30 to 60 sec- onds between each wash. Aspirate the Wash Solution after each wash. If the last wash, if excess liquid remains, blot the inverted plate on clean, absorbent paper towels. Note: Grasp the plate holder firmly at the center of the long sides before inverting to blot.

10. Add 100 µL of Working Conjugate Solution to each well containing a specimen or control.

11. Cover the microwell plate with a plate cover or use other means to minimize evaporation.

Procedure A: Incubate the plate for 60 to 65 minutes at 37°C using a dry-heat static incubator.

Procedure B: Incubate the plate for 60 to 65 minutes at 37°C using a shaker incubator.

12. At the end of the incubation period, carefully remove the plate cover and aspirate the fluid from each well into a biohazard container. Wash the microwell plate or strip of microtiter plates with the Wash Solution (at least 400 µL/well/wash). Soak for 30 to 60 sec- onds between washes. Aspirate the Wash Solution after each wash. If excess liquid remains, blot the inverted plate on clean, absorbent paper towels. Note: Grasp plate firmly at the center of the long sides before inverting to blot.

13. Add 100 µL of the Working TMB Solution to each well. Cover the microwell plate with a fresh plate cover or use other means to minimize evaporation, and incubate in the dark for 30 to 33 minutes at room temperature. (For example, cover the plates with black plastic or place them in a drawer.)

14. Carefully remove the plate cover, and add 100 µL of EIA Stop Solution to each well. Tap the plate to remove the reaction. Top plate gently or use other means to assure complete mixing. Complete mixing is required for acceptable results.

15. Read absorbance within 30 minutes after adding the EIA Stopping Solution, using the 450 nm filter with 615 nm to 630 nm as the reference. Ensure that all strips have been pressed firmly into place before reading.

Decontamination Disinfect instruments and materials used to perform the test as though they contain an infectious agent. Disposal should comply with applicable waste disposal requirements.

QUALITY CONTROL

Determine the mean absorbance for the Negative, Positive, and Low Positive Controls both neutralized and non-neutralized, by dividing the sum of their absorbance values by the number of acceptable controls. One Negative Control may be discarded if it is outside the acceptable validation range. No Positive Controls or Low Positive Controls may be discarded. For the procedure to calculate mean absorbance values, follow the criteria in the Quality Control section of the GS HBsAg EIA 3.0 package insert.