ANTIBODY TO HEPATITIS B SURFACE ANTIGEN (Mouse Monoclonal)

GS HBsAg EIA 3.0

Enzyme Immunoassay (EIA) for the Detection of Hepatitis B Surface Antigen (HBsAg) in Human Serum, Plasma, and Cadaveric Serum Specimens

For *In Vitro* Diagnostic Use

32591	• 480 Tests
32592	• 960 Tests
25258	• 4800 Tests

LEXICON	
IVD	For In Vitro Diagnostic Use
	Number of Tests
	European Conformity
REF	Catalog Number
	Consult Instructions for Use
	Temperature Limit
	Manufactured by
EC REP	Authorized Representative in the European Community
WASH	Wash Solution Concentrate (30X)
TMB SOLUTION	Chromogen: TMB Solution
SUB BUF	Substrate Buffer
STOP	Stopping Solution
	Biohazard
	WARNING
	Corrosive

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1 - NAME AND INTENDED USE

The GS HBsAg EIA 3.0 is a qualitative enzyme immunoassay for detection of Hepatitis B Surface Antigen (HBsAg) in human serum and plasma. It is indicated as a screening test for specimens from individual human donors, including donors of whole blood, blood components, and source plasma, and from other living donors. It is also intended for use in testing plasma and serum specimens to screen organ donors when specimens are obtained while the donor's heart is still beating, and in testing blood specimens from cadaveric (non-heart-beating) donors. The assay is not intended for use on cord blood specimens.

The GS HBsAg EIA 3.0 is intended for manual use and use with the ORTHO® Summit System (OSS) in the screening of blood donors.

2 - SUMMARY AND EXPLANATION OF THE TEST

Hepatitis B virus (HBV) is a major public health problem worldwide, with significant transmission of the virus occurring through the use of contaminated donor blood and plasma. Also of concern is the transmission of HBV and other infectious diseases through tissue transplantation.[1] Because the presence of circulating Hepatitis B Surface Antigen (HBsAg) closely follows the course of infection, screening for HBsAg is used to detect potentially infectious blood and plasma.[2] Enzyme immunoassays to detect HBsAg have replaced relatively insensitive gel diffusion methods and have been reported to have equivalent sensitivity to radioimmunoassay methods.[3] The application of monoclonal antibodies for the detection of HBsAg has previously been reported.[4,5] The GS HBsAg EIA 3.0 is a third generation enzyme immunoassay, which uses mouse monoclonal antibodies to detect HBsAg in human serum, plasma, or cadaveric specimens.

Specimens that are non-reactive when tested with the GS HBsAg EIA 3.0 are considered negative for HBsAg and need not be tested further. Reactive specimens should be retested, in duplicate, using the GS HBsAg EIA 3.0 to determine whether they are repeatedly reactive. A repeatedly reactive specimen

should be confirmed by a licensed neutralization procedure utilizing human anti-HBs (GS HBsAg Confirmatory Assay 3.0). If the HBsAg in the specimen can be neutralized by the confirmatory procedure, the specimen is considered positive for HBsAg and need not be tested further.

3 - BIOLOGICAL PRINCIPLES OF THE PROCEDURE

Wells of the microwell strip plates are coated with mouse monoclonal antibody to HBsAg (anti-HBs). Serum or plasma and appropriate controls are added to the wells, and incubated with the bound antibody. If HBsAg is present, it will bind to the antibody and not be removed by washing. The strips are washed to remove any unbound material. Washing is followed by the addition of Conjugate Solution (peroxidase-conjugated mouse monoclonal antibodies directed against HBsAg). The Conjugate Solution will bind to the antibody-HBsAg complex, if present. Unbound conjugate is removed by a wash step. Next, Working TMB Solution is added to the plate and allowed to incubate. A blue or blue-green color develops in proportion to the amount of HBsAg present in the sample. The enzyme reaction is stopped by the addition of acid, which changes the blue-green color to yellow. The optical absorbance of specimens and controls is determined with a spectrophotometer set at 450 nm wavelength.

4 - REAGENTS

GS HBsAg EIA 3.0 Product Description

Product No: 32591 • 480 Tests, 32592 • 960 Tests, 25258 • 4800 Tests

Component	Contents	Preparation
R3 • HBsAg Conjugate Concentrate 1, 1, or 5 vials (1.2 ml)	• Anti-HBsAg (mouse monoclonal): horseradish peroxidase conjugate • Buffer with protein stabilizers (bovine) • 0.005% Gentamicin Sulfate • 0.5% ProClin 300 • Green dye	Dilute in HBsAg Conjugate Diluent as described.
R1 • Strip Plates Anti-HBsAg Microwell 5, 10 or 50	• Microwell strips in holder, coated with antibody to HBsAg (mouse monoclonal) • Potential Sodium Azide and ProClin 150 residue • Tabs are labeled “CC”	Use as supplied. Return unused strips to the pouch and reseal. Do not remove desiccant.

GS HBsAg EIA 3.0 Product Description (*cont.*)

Component	Contents	Preparation
R2 • Wash Solution Concentrate (30X) 2, 3 or ** bottles (120 ml)	• Sodium Chloride • Tween 20	Dilute to working dilution with deionized water. Clinical laboratory reagent water is acceptable.
C0 • HBsAg Negative Control (Human) 1, 1, or 5 vials (12 ml)	• Normal Human Serum • Non-reactive for HBsAg, Anti-HBsAg • Non-reactive for Antibody to HIV and HCV • 0.005% Gentamicin Sulfate • 0.16% ProClin 950	Ready to use as supplied.
C1 • HBsAg Positive Control (Human) 1, 1, or 5 vials (8 ml)	• Purified HBsAg (human *ad* and *ay* subtypes) in synthetic diluent with protein stabilizers (bovine) • 0.5% ProClin 300 • 0.005% Gentamicin Sulfate	Ready to use as supplied.
C2 • HBsAg Low Positive Control (Human) 1, 1, or 5 vials (8 ml)	• Purified HBsAg (human *ad* and *ay* subtypes) in synthetic diluent with protein stabilizers (bovine) • 0.5% ProClin 300 • 0.005% Gentamicin Sulfate	Ready to use as supplied.
R4 • HBsAg Conjugate Diluent 1, 1, or 5 bottles (120 ml)	• Buffer with protein stabilizers (murine and bovine) • 0.005% Gentamicin Sulfate • 0.5% ProClin 300	Ready to use as described under Working Conjugate Solution.
R8 • Substrate Buffer 1, 1, or 5 bottles (120 ml)	• Hydrogen Peroxide • Citric Acid/Sodium Acetate buffer • Dimethylsulfoxide (DMSO)	Ready to use as supplied.
R9 • Chromogen 1, 1, or 5 bottles (12 ml)	• Tetramethylbenzidine (TMB)*	Dilute with Substrate Buffer as described.
R10 • Stopping Solution 1, 1, or ** bottles (120 ml)	• 1N H_2SO_4 (Sulfuric Acid)	Ready to use as supplied.

*NOTE: Tetramethylbenzidine is a non-carcinogenic and non-mutagenic chromogen for peroxidase.[6,7]

**Wash Solution Concentrate and Stopping Solution must be purchased separately for the 50 plate (4800 test) kit. Refer to catalog number 25261 for the Wash Solution Concentrate and catalog number 25260 for the Stopping Solution. These reagents are included in the 5 plate (480 test) and 10 plate (960 test) kits.

Store the kit at 2–8°C. Bring all reagents, except Conjugate Concentrate, to room temperature (15–30°C) before use. Return to 2–8°C immediately after use. Store all unused strips/plates with desiccant at 2–8°C.

5 - WARNINGS FOR USERS

1. **For *In Vitro* Diagnostic Use.**
2. This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Handle appropriately with the requisite Good Laboratory Practices. Wear protective clothing, including lab coat, eye/face protection, and disposable gloves (synthetic, non-latex gloves are recommended) while handling kit reagents and patient samples. Wash hands thoroughly after performing the test.
3. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
4. Do not pipette by mouth.
5. The following is a list of potential chemical hazards contained in some kit components (refer to Product Description chart):

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

   b. **WARNING:** Components C1, C2, R3, and R4 contain **0.5% ProClin 300**.

H317: **May cause allergic skin reaction.**

P280: Wear protective gloves/protective clothing/ eye protection/face protection.

P302 + P352: IF ON SKIN: Wash with plenty of soap and water.

P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.

P501: Dispose of contents and container in accordance with local, regional, national, and international regulations.

ProClin 300 (0.5%) is a biocidal preservative that is irritating to eyes and skin, may be detrimental if enough is ingested, and may cause sensitization by skin contact;

prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

DANGER! The Stopping Solution (R10) contains **1N Sulfuric Acid**.

H314: **Causes severe skin burns and eye damage.**

H290: **May be corrosive to metals.**

P280: Wear protective gloves/protective clothing/ eye protection/face protection.

P301 + P330 + P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.

P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P501: Dispose of contents and container in accordance with local, regional, national, and international regulations.

The 1N Sulfuric Acid (H_2SO_4) Stopping Solution is severely irritating or corrosive to eyes and skin, depending on the amount and length of exposure; greater exposures can cause eye damage, including permanent impairment of vision. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Keep away from strong bases, reducing agents, and metals; do not pour water into this component. Waste from this material is considered hazardous acidic waste, however if permitted by local, regional, and national regulations, it might be neutralized to pH 6-8 for non-hazardous disposal.

6. The GS HBsAg EIA 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 disease, following recommended *Standard and Universal Precautions* for bloodborne pathogens as defined by OSHA, Biosafety Level 2 guidelines from the current CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*[8], WHO *Laboratory Biosafety Manual*[9], and/or local, regional, and national regulations. The following human blood derivatives are found in this kit:

   a. Human source material used in the preparation of the Negative Control (C0) has been tested and found non-reactive for Hepatitis B surface antigen (HBsAg), anti-HBsAg, and antibodies to Hepatitis C virus (HCV) and human immunodeficiency virus (HIV-1 and HIV-2).

   b. The human plasma derived viral antigen HBsAg subtypes *ad* and *ay* used in the preparation of the Positive Control (C1) and Low Positive Control (C2) are highly purified and heat treated.

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

   Spills not containing acid should be immediately decontaminated, including the spill area, materials, and any contaminated surfaces or equipment with an appropriate chemical disinfectant that is effective for the potential biohazards relative to the samples involved (commonly a 1:10 dilution of bleach, 70-80% ethanol or isopropanol, an iodophor (such as 0.5% Wescodyne™ Plus), or a phenolic, etc.) and wiped dry.[10-12]

   Spills containing acid should be appropriately absorbed (wiped up) or neutralized, and then the area wiped with one of the chemical disinfectants. Material used to absorb the spill may require biohazardous waste disposal.

NOTE: DO NOT PLACE SOLUTIONS CONTAINING BLEACH INTO THE AUTOCLAVE.

8. Dispose of all specimens and material used to perform the test as though they contain an infectious agent. Laboratory chemical and biohazardous wastes must be handled and discarded in accordance with all local, regional, and national regulations.

9. Complete hazard information and precautions are located in the Safety Data Sheet (SDS) available at bio-rad.com or upon request.

6 - PRECAUTIONS FOR USERS

1. Do not use any kit components beyond their stated expiration date.

2. The reagents that may be used with different lots of the GS HBsAg EIA 3.0 kit are the Chromogen (R9), Substrate Buffer (R8), Wash Solution Concentrate (R2), and Stopping Solution (R10). Do not mix any other reagents from different lots. Any lot number of the following reagents may be used with this assay provided they have the correct catalog number and are not used beyond their labeled expiration date:

   - Chromogen (R9)—Catalog #26182
   - Substrate Buffer (R8)—Catalog #26181
   - Wash Solution Concentrate (R2)—Catalog #25261
   - Stopping Solution (R10)—Catalog #25260

3. Exercise care when opening vials and removing aliquots to avoid microbial contamination of the reagents.

4. Use a clean, disposable container for the Conjugate. Exposure of the Conjugate to sodium azide will result in its inactivation.

5. Avoid exposing Chromogen or Working TMB Solution to strong light during storage or incubation. Do not allow the

Working TMB Solution to come into contact with any oxidizing agents, including metals.

6. Use clean, polypropylene containers to prepare and store the Working TMB Solution. If glassware must be used, pre-rinse thoroughly with 1N sulfuric or hydrochloric acid followed by at least three washes of deionized water. Be sure that no acid residue remains on the glassware.
7. Bring all reagents except Conjugate Concentrate to room temperature before use.
8. Clinical samples may contain very high levels of HBsAg. Therefore, care must be exercised when dispensing samples to avoid cross contamination through aerosols or carryover. For manual pipetting of controls and specimens, use 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.
9. Handle the Negative and Positive Controls in the same manner as patient specimens.
10. If a specimen or reagent is inadvertently not added to a well, the assay results will read negative.
11. Inadequate adherence to package insert instructions may result in erroneous results.
12. Use only adequately calibrated equipment with this assay.
13. Use of dedicated equipment is recommended if equipment performance validations have not precluded the possibility of cross-contamination.
14. The GS HBsAg EIA 3.0 performance is highly dependent upon incubation times and temperatures and effective washing. Temperatures outside of the validated ranges may result in invalid assays. Incubation temperatures should be carefully monitored using calibrated thermometers, or equivalent.

15. **Caution: Certain washer conditions such as partially blocked cannulae can lead to sub-optimal washing and false reactive test results. It is recommended that users of ORTHO® Summit System or any other microplate washer carefully verify that the washing system is clear and operating properly before performing an assay.**

16. Components of this kit meet FDA potency requirements.

7 - REAGENT PREPARATION AND STORAGE

Working Conjugate Solution

Bring Conjugate Diluent (R4) to room temperature. Invert Diluent and Conjugate Concentrate (R3) to mix before using. **Use only the matched lot of Conjugate Concentrate provided with the kit master lot being used (See "PRECAUTIONS FOR USERS", item 2, page 10.)** Prepare a 1:101 dilution for each strip to be tested by mixing 10 µl of Conjugate Concentrate with 1.0 ml of Conjugate Diluent in a clean, polypropylene container. Note Concentrate lot number, date and time of preparation, and date and time of expiration (8 hours from preparation) on container. Mix Working Solution thoroughly when combined and again just prior to use. Working Solution should be used within 8 hours.

Return Conjugate Concentrate to the refrigerator immediately after use. To avoid contamination of Conjugate, wear clean gloves and do not touch tips of pipettes. Store Working Conjugate Solution at room temperature until use.

Prepare only the amount of reagent to be used within 8 hours, ensuring that the volume of diluted reagent will be adequate for the entire run. Use the following table as a guide:

Preparation of Working Conjugate Solution by Strip

Number of Strips to be used	1	2	3	4	5	6	7	8	9	10	11	12*
Amount of Conjugate Concentrate (µl)	10	20	30	40	50	60	70	80	90	100	110	120
Amount of Conjugate Diluent (ml)	1	2	3	4	5	6	7	8	9	10	11	12

* Complete Plate

Preparation of Working Conjugate Solution by Plate

Number of Complete Plates to be used	1	2	3	4	5	6	7	8	9	10
Amount of Conjugate Concentrate (µl)	120	240	360	480	600	720	840	960	1080	1200
Amount of Conjugate Diluent (ml)	12	24	36	48	60	72	84	96	108	120

Working TMB Solution

Note: 1:11 Dilution. Bring Chromogen (R9) and Substrate Buffer (R8) to room temperature. Invert the Chromogen and Substrate Buffer to mix before using. Prepare a 1:11 dilution for each strip to be tested by mixing 100 µl of Chromogen with 1.0 ml of Substrate Buffer in a clean, polypropylene container. Note Chromogen lot number, date and time of preparation, and date and time of expiration (8 hours from preparation) on container. Mix Working TMB Solution gently prior to use. Working TMB Solution should be kept in the dark at room temperature and used within 8 hours.

Chromogen should be colorless to slightly yellow. Any other color indicates that the reagent is contaminated. Do not use this reagent.

The Working TMB Solution should be colorless. A distinct blue color indicates that the reagent is contaminated. Discard the Working TMB Solution and prepare fresh reagent in a clean polypropylene container.

Prepare only the amount of the reagent to be used within 8 hours, ensuring that the volume of diluted reagent will be adequate for the entire run. Extra Chromogen is provided. Use the following table as a guide:

Preparation of Working TMB Solution by Strip

Number of Strips to be used	1	2	3	4	5	6	7	8	9	10	11	12*
Amount of Chromogen (µl)	100	200	300	400	500	600	700	800	900	1000	1100	1200
Amount of Substrate Buffer (ml)	1	2	3	4	5	6	7	8	9	10	11	12

* Complete Plate

Preparation of Working TMB Solution by Plate

Number of Plates to be used	1	2	3	4	5	6	7	8	9	10
Amount of Chromogen (ml)	1.2	2.4	3.6	4.8	6.0	7.2	8.4	9.6	10.8	12.0
Amount of Substrate Buffer (ml)	12	24	36	48	60	72	84	96	108	120

Wash Solution

Prepare Wash Solution by adding one part Wash Solution Concentrate (R2) (30X) to 29 parts of deionized or distilled water (e.g., 120 ml of Wash Solution Concentrate to 3480 ml of deionized water). Any lot of Wash Solution Concentrate, provided it is catalog number 25261 and within its labeled shelf life, may be used with this assay. Clinical laboratory reagent water is acceptable. The diluted Wash Solution can be stored at room temperature for up to four weeks in a plastic container. Note the lot number, date prepared, and expiration date on the container. Discard if no suds are evident in the Wash Solution. Prepare a sufficient quantity of Wash Solution to complete a full run.

8 - SPECIMEN COLLECTION, PREPARATION, AND STORAGE

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, plastic tubes with serum, serum separators, 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 centrifugation prior to testing. No clinically significant effect has been detected in assay results in serum and plasma samples with increased levels of protein, lipids, bilirubin, or hemolysis, or after heat inactivation of patient samples. Cadaveric serum samples with increased levels of hemolysis have been tested, and no clinically significant effect has been detected in assay results. Note: Cadaveric serum

samples with increased levels of protein, lipids, bilirubin, or microbiological contaminants have not been available to evaluate with this assay.

Serum, plasma, or cadaveric serum specimens may be stored at 2–8°C for up to seven days, including the time that samples are in transit. Sera/plasma should be removed from the clot, red blood cells, or separator gel before storage. Samples should not be used if they have incurred more than 5 freeze/thaw cycles. Mix samples thoroughly after thawing. Note: Cadaveric specimens that are weakly reactive may become nonreactive after freeze/thaw cycles.

Note: If specimens are to be shipped, they should be packed in compliance with Federal Regulations covering the transportation of etiologic 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), after removal from the clot, red blood cells, or separator gel. 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, or cadaveric serum specimens. This kit is not intended for use on saliva/oral fluids or urine samples.

9 - GS HBsAg EIA 3.0 PROCEDURE

Materials Provided

See Reagents Section on pages 5-6.

Materials Required but Not Provided

1. Precision pipettes to deliver volumes from 10 µl to 200 µl, 1 ml, 10 ml, 25 ml, and 50 ml (accurate within ±10%). A multichannel pipettor capable of delivering 100 µl is optional.
2. Pipette tips.
3. Dry-heat static or shaker incubator capable of maintaining 37 ±1°C. If a shaker incubator is used, it should have the following specifications:

Amplitude: 0.75 to 3.00 mm

Frequency: 500 to 2300 R.P.M.

4. Microwell plate or strip washer qualified for use with this assay. The washer must be capable of dispensing 400 µl per well, cycling 5 times, and soaking for 30–60 seconds between each wash. Use validated equipment which does not cause carryover.

5. Microwell plate or strip reader qualified for use with this assay. The spectrophotometer should have the following specifications at wavelength 450 nm:

   Bandwidth: 10 nm HBW (Half Band Width), or equivalent
   Absorbance Range: 0 to 2 AU (Absorbance Units)
   Repeatability: ± (0.5% + 0.005) AU
   Linearity or Accuracy: 1% from 0 to 2.0 AU

   The instrument should contain a reference filter for reading at 615 to 630 nm.

6. The GS HBsAg EIA 3.0 is approved for use with the ORTHO® Summit System as a screening test for human serum and plasma specimens. Within the OSS the operator can process by three different modes: manually (manual pipetting + manual processing), semi-automated (automated pipetting + manual processing), or automated (automated pipetting + automated processing). The automated mode can consist of the stand-alone ORTHO VERSEIA® Pipetter with the stand-alone ORTHO® Summit Processor (OSP), or the ORTHO VERSEIA® Pipetter integrated with the OSP as the ORTHO VERSEIA® Integrated Processor (VIP). The GS HBsAg EIA 3.0 ORTHO® Assay Protocol Disk is available from ORTHO® Clinical Diagnostics.

7. Household bleach (5% to 8% sodium hypochlorite) which may be diluted to a minimum concentration of 10% bleach (or 0.5% sodium hypochlorite). Alternative disinfectants include: 70% ethanol or 0.5% Wescodyne Plus™ (West Chemical Products, Inc.).

8. Paper towels or absorbent pads for blotting.
9. Labeled null strips, for testing partial plates.
10. Clean polypropylene containers, with caps, for preparation of TMB and Conjugate Working Solutions, 15 or 50 ml.
11. Deionized or distilled water. Clinical laboratory reagent water is acceptable.
12. Gloves.
13. Laboratory timer.
14. EIA reagent reservoirs (optional).
15. Plate Sealers (Catalog #0210-00, or equivalent) are required, except on an approved microplate processor.

Preliminary Statements

1. The expected run time for this procedure is approximately 3-3.5 hours from initiation of the first incubation step. Each run of this assay must proceed to completion without interruption after it has been started. The maximum allowable time from start of pipetting to start of incubation is ≤1 hour.
2. Positive and Negative Controls supplied in the kit must be run on each plate. The cutoff for patient samples is determined by the controls on each individual plate.
3. The number of controls to be included in each run of this assay are two Positive Controls, two Low Positive Controls, and three Negative Controls.
4. Do not splash controls, specimens, or reagents between microwells of the plate.
5. Cover plates for each incubation step using plate sealers or other appropriate means to minimize evaporation.
6. Avoid exposure of the plates to light during the final incubation step (following the addition of the Working TMB 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.
9. Dry residue from the plate blocking process may be visible in the microwells. Assay results will not be affected by this material. Before reading the plates, carefully wipe the bottom of the plates to remove any material that remains on the outside of the wells, and ensure that all strips have been pressed firmly into place.
10. For additional procedural instructions when running this assay with the ORTHO® Summit System, refer to the following documents:
    - ORTHO® Summit System User's Guide
    - ORTHO® Summit Processor User's Guide
    - ORTHO VERSEIA® User's Guide
    - ORTHO VERSEIA® Integrated Processor User's Guide
11. When testing with the ORTHO® Summit System, remove strips not needed for the assay and replace them with labeled null strips, as necessary. A minimum of 4 strips, including both assay strips and null strips, should be included on each plate in order to avoid excessive evaporation in the incubator. Working Wash Solution may be used as barcoded samples on the plate map, in conjunction with null strips, so there are a minimum of 4 strips that contain liquid. At the end of processing, results from the Wash Solution samples should be invalidated.

There are two procedures for the detection of HBsAg in serum or plasma, procedures A and B. **For the detection of HBsAg in cadaveric serum specimens, only procedure A can be used.**

The two procedures for the detection of HBsAg are described below:

Procedure	Specimen incubation	Conjugate incubation	Color development
A	Dry heat, 36-38°C, static incubation, 60 min.	Dry heat, 36-38°C, static incubation, 60 min.	15 to 30°C; 30 min. in the dark.
B	Shaker incubation, 36-38°C, 60 min.	Shaker incubation, 36-38°C, 60 min.	15 to 30°C; 30 min. in the dark.

For samples that are originally tested on either procedure A or B, any repeat testing or confirmation must be tested using the same procedure.

EIA Procedures A and B

1. Perform equipment maintenance and calibration, where necessary, as required by the manufacturer.
2. **Bring all of the reagents, except Conjugate Concentrate, to room temperature before beginning the assay procedure.**
3. Prepare working concentrations of Wash Solution, Conjugate Solution, and TMB Solution. Mix gently, by inversion. **Be sure that Conjugate Solution is completely mixed.** Mix again just before use.
4. Remove strips not needed for the assay and replace them with labeled Null Strips, if necessary. Take care when assembling partial plates with coated and uncoated (null) strips, as automated systems cannot distinguish between the strips and will report results for all wells that are assigned a sample ID number (even if a null strip is inadvertently placed where sample IDs have been assigned).
5. Microwell strips not needed for the assay may be returned to the plate pouch and sealed, and then used at a later time. Strips from different plates can only be mixed to assemble full or partial plates if they are from the same plate lot and have come from plates that have previously been tested with kit controls and yielded valid runs. When assembling a plate

that contains strips from a newly opened, previously untested plate, one of these strips should be placed at the beginning of the plate and tested with the kit controls.

6. If sample identity is not maintained by an automatic procedure, identify the individual wells for each specimen or control on a data sheet.

7. **Add 100 µl of the controls or specimens to the appropriate wells** of the microwell plate. Two Positive Controls, two Low Positive Controls, and three Negative Controls should be assayed on each plate or partial plate of specimens.

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

   Procedure A: Incubate the plate for 60 to 65 minutes at 37 ±1°C using a dry-heat static incubator.

   Procedure B: Incubate the plate for 60 to 65 minutes at 37 ±1°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 a minimum of five times** with the Wash Solution (at least 400 µl/well/wash), or as otherwise validated. **Soak for 30 to 60 seconds between each wash.** Aspirate the Wash Solution after each wash. After 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 sealer or use other means to minimize evaporation. Incubate the plate for 60 to 65 minutes at 37 ±1°C using either a dry-heat static incubator or shaker incubator as was utilized in Step 8.

12. At the end of the incubation period, carefully remove the plate cover and aspirate the fluid in each well into a biohazard container. **Wash the plates a minimum of five times** with Wash Solution (at least 400 µl/well/wash), or as otherwise validated. **Soak for 30 to 60 seconds between each wash.** Aspirate the Wash Solution after each wash. After the last wash, if excess liquid remains, blot the inverted plate on a clean, absorbent paper towel. NOTE: Grasp the plate holder firmly at the center of the long sides before inverting to blot.

13. **Add 100 µl of the Working TMB Solution to each well containing a specimen or control.** Cover the microwell plate with a fresh plate sealer or use other means to minimize evaporation. **Incubate plates in the dark for 30 to 33 minutes at room temperature (15–30°C).** (For example, cover the plates with black plastic or place in a drawer.)

14. Carefully remove the plate cover and **add 100 µl of Stopping Solution to each well** to terminate the reaction. **Tap the plate gently, or use other means to ensure complete mixing. Complete mixing is required for acceptable results.**

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

Decontamination

Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Disposal should comply with all applicable waste disposal requirements.

10 -QUALITY CONTROL

Determine the mean absorbance for the Negative Controls, Positive Controls and Low Positive Controls by dividing the summation of the values by the number of acceptable controls. One Negative Control may be discarded if it is outside of the

acceptable validation range. No Positive Controls may be discarded.

Mean Absorbance of the Negative Controls (NCX)

Determine the NCX as shown in the example below:

Negative Control Sample Number	Absorbance	Total Absorbance	=	0.099 / 3 =	0.033 (NCX)
1	0.032				
2	0.034				
3	0.033				
	0.099				

The individual absorbance values of the Negative Controls must be greater than 0.000 AU and less than or equal to 0.100 AU. One Negative Control absorbance value may be discarded if it is outside this range. The NCX may be calculated from the two remaining absorbance values.

Mean Absorbance of the Positive Controls (PCX)

Determine the PCX as shown in the example below:

Positive Control Sample Number	Absorbance	Total Absorbance	=	3.440 / 2 =	1.720 (PCX)
1	1.683				
2	1.757				
	3.440				

The PCX must be greater than or equal to 1.000 AU, and each Positive Control absorbance value must be within the range of 0.65 to 1.35 times the PCX. No Positive Control absorbance value may be discarded.

Both Positive Control absorbance values above are within the range of 0.65 to 1.35 times the PCX as shown by the calculation below.

$$0.65 \times PCX = 0.65 \times 1.720 = 1.118$$
$$1.35 \times PCX = 1.35 \times 1.720 = 2.322$$

Therefore, the acceptable range is 1.118 to 2.322.

Mean Absorbance of the Low Positive Controls (LPCX)

Determine the LPCX as shown in the example below:

Low Positive Control Sample Number	Absorbance	Total Absorbance	=	0.762	= 0.381 (LPCX)
1	0.360	2		2	
2	0.402				
	0.762				

The LPCX must be positive (i.e. greater than or equal to the assay cutoff value).

Cutoff Value

Determine the cutoff value by adding the NCX to 0.070 as shown in the example below:

NCX = 0.033
Cutoff Value = 0.033 + 0.070 = 0.103

Validation

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 average of the absorbance values of the Positive Control must be greater than or equal to 1.000 and the individual absorbance values must be within range of 0.65 to 1.35 times the PCX. No Positive Control values may be discarded.
- The average absorbance of the Low Positive Controls must be positive (≥ assay cutoff). No Low Positive Control absorbance values may be discarded.

11 -INTERPRETATION OF RESULTS

The presence or absence of HBsAg is determined by relating the absorbance value of the specimen to the cutoff values. The cutoff value is determined by addition of 0.070 to the mean absorbance value of the Negative Controls (NCX). An example of

values obtained from an assay run and the interpretation are as follows:

Example:

Negative Control OD values	0.032	Individual Negative Control OD Values	
	0.034		Valid
	0.033	Negative Control mean	0.033
Positive Control OD values	**1.683**	Positive Control mean	**1.720**
	1.757		Valid
Low Positive Control OD values	**0.360**	Low Positive Control mean	**0.381**
	0.402		Valid
Cutoff Value = 0.033 + 0.070 =	0.103		
Patient OD values	**1.910**	Interpretation	Reactive
	0.295		Reactive
	0.011		Nonreactive
	0.726		Reactive
	0.100		Nonreactive

Specimens with absorbance values that are <0.000 must be repeated. Those with values greater than the upper linearity limits of the reader should be reported as reactive.

Specimens with absorbance values less than the cutoff value are considered non-reactive by the GS HBsAg EIA 3.0 and may be considered negative for HBsAg. Further testing is not required.

Specimens with absorbance values greater than or equal to the cutoff value are considered initially reactive by the GS HBsAg EIA 3.0. Initially reactive specimens should be retested in duplicate to validate the initial test results. If, after repeat testing, the absorbance values of both duplicate specimens are less than the cutoff value, the original specimen may be considered non-repeatedly reactive and negative for HBsAg. Reasons for non-repeatedly reactive specimens include:

- Improper washing of microwell plates
- Cross-contamination of non-reactive specimens with HBsAg from a high titered specimen
- Contamination of the TMB Reagent solution by oxidizing agents (sodium hypochlorite, hydrogen peroxide, etc.)
- Contamination of the Stopping Solution

If, after repeat testing, the absorbance value of either of the duplicates is greater than or equal to the cutoff value, the specimen must be considered repeatedly reactive. If a confirmation is performed, repeatedly reactive specimens must be confirmed by the GS HBsAg Confirmatory Assay 3.0, a licensed neutralization procedure utilizing human anti-HBs. The specimen can be considered positive for HBsAg only if the HBsAg can be neutralized by the confirmatory procedure.

12 -LIMITATIONS OF THE PROCEDURE

1. The GS HBsAg EIA 3.0 Procedure and the Interpretation of Results 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 sample and reagent pipetting, plate washing and timing of the incubation steps.
2. A designation of reactive for HBsAg must not be based on a single reactive test result. Additional testing, such as confirmatory testing, is required to establish the specificity of any specimen reactive by the screening procedure.
3. False positive results can be expected with a kit of this nature. The proportion of reactives that are false will depend on the sensitivity and specificity of the test kit and upon the prevalence of hepatitis B virus in the population being screened.
4. 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.
5. 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.

6. An absorbance value of less than 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.

7. Factors that can affect the validity of results include failure to add the specimen to the well, inadequate washing of microplate wells, failure to follow stated incubation times and temperatures, addition of wrong reagents to wells, the presence of metals, or splashing of bleach into wells.

8. A person who is positive for HBsAg is assumed to be infected with the virus, except that a person who has been vaccinated for hepatitis B may have transient reactivity for up to 18 days, and this is clinically insignificant.[13]

13 -PERFORMANCE CHARACTERISTICS OF SERUM AND PLASMA TESTING

Reproducibility

Intra-assay and inter-assay reproducibility of the GS HBsAg EIA were assessed for Procedure A (static mode) and Procedure B (shaker mode) using a nine member precision panel. Each member of the precision panel was tested four times on five different days on each of three lots of the GS HBsAg EIA at six sites. The mean, standard deviation, and coefficient of variation of the absorbance values are shown in Table 1A (Procedure A) and Table 1B (Procedure B) below:

Table 1A: Reproducibility of GS HBsAg EIA Procedure A (Static)

Intra-assay Reproducibility					Inter-assay Reproducibility				
Panel Member	N	Mean OD	SD[1]	%CV	Panel Member	N	Mean OD	SD[2]	%CV
1	360	1.305	0.092	7.0%	1	360	1.305	0.142	10.9%
2	360	0.390	0.033	8.5%	2	360	0.390	0.050	12.8%
3	360	0.217	0.015	6.9%	3	360	0.217	0.026	12.0%
4	359*	0.125	0.013	10.4%	4	359*	0.125	0.018	14.4%
5	360	1.606	0.069	4.3%	5	360	1.606	0.148	9.2%
6	360	0.612	0.041	6.7%	6	360	0.612	0.066	10.8%
7	359*	0.323	0.022	6.8%	7	359*	0.323	0.035	10.8%
8	360	0.165	0.012	7.3%	8	360	0.165	0.018	10.9%
9	358*	0.030	0.006	20.0%	9	358*	0.030	0.010	33.3%

*Outliers not included in statistical calculations.
[1]NCCLS Vol. 12 No.4, p.32, Eq 11. [2]NCCLS Vol. 12 No.4. p.33, Eqs 12 and 13.

Table 1B: Reproducibility of GS HBsAg EIA Procedure B (Shaker)

Intra-assay Reproducibility					Inter-assay Reproducibility				
Panel Member	N	Mean OD	SD[1]	%CV	Panel Member	N	Mean OD	SD[2]	%CV
1	359*	1.901	0.082	4.3%	1	359*	1.901	0.191	10.0%
2	360	0.783	0.057	7.3%	2	360	0.783	0.089	11.4%
3	359*	0.453	0.026	5.7%	3	359*	0.453	0.051	11.3%
4	360	0.255	0.018	7.1%	4	360	0.255	0.029	11.4%
5	360	2.069	0.061	2.9%	5	360	2.069	0.202	9.8%
6	360	1.109	0.059	5.3%	6	360	1.109	0.101	9.1%
7	360	0.645	0.037	5.7%	7	360	0.645	0.060	9.3%
8	360	0.330	0.022	6.7%	8	360	0.330	0.032	9.7%
9	355*	0.033	0.006	18.2%	9	355*	0.033	0.008	24.2%

*Outliers not included in statistical calculations.
[1]NCCLS Vol. 12 No.4, p.32, Eq 11. [2]NCCLS Vol. 12 No.4. p.33, Eq's 12 and 13.

Specificity

Reactivity in Random Donor Populations

In clinical investigations performed at five blood centers and a plasmapheresis center, 19,319 specimens from random blood donors were tested for HBsAg. The proportions of these specimens found initially and repeatedly reactive by the GS HBsAg EIA for both Procedure A (static mode) and Procedure B (shaker mode) are shown in Table 2. The presence of HBsAg in repeatedly reactive specimens was confirmed by neutralization with human anti-HBs using the GS HBsAg Confirmatory Assay.

Table 2A: Detection of HBsAg in Serum from Blood Donors

		Procedure A (Static)			Procedure B (Shaker)			
Group	Total Tested	Non-Reactive	Initially Reactive	Repeatedly Reactive	Non-Reactive	Initially Reactive	Repeatedly Reactive	Confirmed Positive
Random Donors, Site A	2000	1993 (99.65%)	7 (0.35%)	2 (0.10%)	1991 (99.55%)	9 (0.45%)	2 (0.10%)	0
Random Donors, Site B	2000	1980 (99.00%)	20 (1.00%)	17 (0.85%)	1996 (99.80%)	4 (0.20%)	3 (0.15%)	0
Random Donors, Site E	4369	ND*	ND*	ND*	4353 (99.63%)	16 (0.37%)	3 (0.07%)	0
Random Donors, Site F	6914	ND*	ND*	ND*	6848 (99.05%)	66 (0.95%)	9 (0.13%)	0
Total Serum Donors	15,283	3973 (99.33%)	27 (0.67%)	19 (0.47%)	15,188 (99.38%)	95 (0.62%)	17 (0.11%)	0 (0.00%)

ND* = Not Done

Table 2B: Detection of HBsAg in Plasma from Blood Donors

Group	Total Tested	Procedure A (Static) Non-Reactive	Procedure A (Static) Initially Reactive	Procedure A (Static) Repeatedly Reactive	Procedure B (Shaker) Non-Reactive	Procedure B (Shaker) Initially Reactive	Procedure B (Shaker) Repeatedly Reactive	Confirmed Positive
Random Donors, Site C	1520	1504 (98.95%)	16 (1.05%)	15 (0.99%)	1498 (98.55%)	22 (1.45%)	20 (1.32%)	0
Random Donors, Site D	2516	2511 (99.80%)	5 (0.20%)	2 (0.08%)	2514 (99.92%)	2 (0.08%)	2 (0.08%)	1
Total Plasma Donors	4036	4015 (99.48%)	21 (0.52%)	17 (0.42%)	4012 (99.41%)	24 (0.59%)	22 (0.55%)	1 (0.02%)

Table 2C: Detection of HBsAg in Serum and Plasma from Blood Donors

Group	Total Tested	Procedure A (Static) Non-Reactive	Procedure A (Static) Initially Reactive	Procedure A (Static) Repeatedly Reactive	Procedure B (Shaker) Non-Reactive	Procedure B (Shaker) Initially Reactive	Procedure B (Shaker) Repeatedly Reactive	Confirmed Positive
Total Serum and Plasma Donors	19,319	7988 (99.40%)	48 (0.60%)	36 (0.45%)	19,200 (99.38%)	119 (0.62%)	39 (0.20%)	1 (0.01%)

Specificity of the GS HBsAg EIA was estimated from the results of screening tests in random U.S. blood and plasma donors. Specificity was estimated by the following formula:

$$\frac{\text{(\# normal donor specimens - \# repeatedly reactive specimens)}}{\text{(\# normal donor specimens - repeatedly reactive specimens confirmed positive for HBsAg)}} \times 100$$

A total of 8036 donor specimens were tested with procedure A and 19,319 donor specimens were tested with procedure B of the GS HBsAg EIA; 36 of these specimens were repeatedly reactive by procedure A; 39 specimens were repeatedly reactive by procedure B; one (1) specimen was confirmed to be positive for HBsAg with procedures A and B. Thus the GS HBsAg EIA has an estimated specificity of 99.55% (95%; binomial confidence interval = (0.9940, 0.9970)) for procedure A; and of 99.80% (95%; binomial confidence interval = (0.9973, 0.9987)) for procedure B.

Random blood donors tested with the ORTHO® Summit System

Additional specificity studies have been performed with the GS HBsAg EIA 3.0 on the ORTHO® Summit System with the ORTHO® Summit Sample Handling System.[1] A total of 24,244

1. The ORTHO® Summit Sample Handling System is now a legacy device and no longer available for marketing.

normal donors (including a combination of serum and plasma specimens) were tested at 3 U.S. blood centers. A total of 42 samples were repeat reactive and either negative (9), non-neutralized (30), or QNS (3) for confirmatory testing. An additional sample was initially reactive and QNS for further testing. Therefore, the GS HBsAg EIA 3.0 had an estimated specificity in this study of ≥99.82% (24,244-43/24,244; 95% confidence interval: 99.77–99.88).

Specificity studies have also been performed with unlinked random donor serum and plasma specimens on the ORTHO® Summit System using the ORTHO VERSEIA® Pipetter. The combined testing at 3 testing sites showed a specificity of 99.81% (3225/3231; 95% confidence interval: 99.60–99.91%).

Additional studies have also been performed with unlinked random donor serum and plasma specimens with the stand-alone ORTHO VERSEIA® Pipetter and a stand-alone ORTHO® Summit Processor (OSP) in comparison to the ORTHO VERSEIA® Integrated Processor (VIP). Both the VIP system and the stand-alone system showed a specificity of 99.75% (2015/2020; 95% confidence interval: 99.42 – 99.92%). The VIP S/CO percent difference increased from the stand-alone VERSEIA®/stand-alone OSP by 0.01%.

Sensitivity

The sensitivity of the GS HBsAg EIA 3.0 was determined in three different product lots by testing dilutions of purified antigens (*ad* and *ay*) in human serum. In Table 3, the mean absorbance to cutoff ratio for each antigen concentration (ng/ml) is presented for each incubation procedure (static and shaker) for each of the lots. The antigen concentrations are expressed as both ng/ml (calibrated against Boston Biomedics, Inc. panel PHA805) and PEI units/ml (calibrated against the Paul Ehrlich Institute Standard).

Table 3: Detection of Purified HBsAg *ad* and *ay* subtypes

HBsAg Subtype	ng/ml	PEI units/ml	Procedure A (Static) Mean Absorbance/cutoff Lot 1	Lot 2	Lot 3	Procedure B (Shaker) Mean Absorbance/cutoff Lot 1	Lot 2	Lot 3
ad-01	2.5	0.31	**17.95**	**15.87**	**13.86**	**25.61**	**25.24**	**22.20**
ad-02	1.5	0.17	**10.44**	**9.51**	**8.38**	**18.20**	**17.71**	**15.95**
ad-03	1.0	0.10	**7.30**	**6.48**	**5.71**	**13.61**	**12.96**	**11.47**
ad-04	0.8	0.09	**6.32**	**5.57**	**5.04**	**11.90**	**11.52**	**10.35**
ad-05	0.6	0.07	**4.80**	**4.29**	**3.81**	**9.55**	**9.14**	**8.11**
ad-06	0.5	0.06	**4.02**	**3.78**	**3.29**	**8.46**	**7.88**	**7.07**
ad-07	0.4	0.05	**3.42**	**3.08**	**2.77**	**6.69**	**6.48**	**5.77**
ad-08	0.3	0.03	**2.73**	**2.46**	**2.29**	**5.48**	**5.20**	**4.55**
ad-09	0.2	0.02	**1.95**	**1.91**	**1.58**	**4.12**	**3.63**	**3.43**
ad-10	0.1	0.01	**1.10**	**1.05**	**1.00**	**2.19**	**2.01**	**1.90**
ay-01	2.4	0.21	**14.35**	**13.23**	**10.95**	**23.45**	**22.01**	**19.79**
ay-02	1.3	0.11	**9.08**	**7.75**	**6.73**	**17.14**	**15.98**	**13.80**
ay-03	0.9	0.07	**6.22**	**5.56**	**4.65**	**12.58**	**11.70**	**10.18**
ay-04	0.8	0.06	**5.36**	**4.70**	**4.04**	**11.17**	**10.23**	**8.72**
ay-05	0.6	0.05	**4.06**	**3.58**	**3.10**	**8.76**	**8.03**	**6.81**
ay-06	0.5	0.04	**3.76**	**3.35**	**2.90**	**8.18**	**7.37**	**6.22**
ay-07	0.4	0.03	**2.97**	**2.86**	**2.38**	**6.80**	**6.35**	**5.47**
ay-08	0.3	0.03	**2.48**	**2.28**	**1.91**	**5.49**	**4.95**	**4.36**
ay-09	0.2	0.02	**1.80**	**1.66**	**1.46**	**3.89**	**3.64**	**3.18**
ay-10	0.1	0.01	**1.05**	0.97	0.92	**2.18**	**2.03**	**1.72**
Negative	0.0	0.00	0.37	0.36	0.46	0.43	0.43	0.44

Reactivity in Patients Diagnosed with Hepatitis B

One hundred (100) specimens from individuals diagnosed with acute hepatitis were tested with procedures A and B of the GS HBsAg EIA. Of the 100 specimens, 94 were reactive with a licensed HBsAg EIA, all 94 specimens were reactive with the GS HBsAg EIA, procedure B; 93 specimens were reactive with the GS HBsAg EIA, procedure A.

Of 106 specimens from individuals diagnosed with chronic hepatitis B, all were reactive with the GS HBsAg EIA, procedures A and B, and in 100% agreement with a licensed HBsAg EIA.

Of 25 anti-Delta positive specimens, all were reactive with the GS HBsAg EIA, procedures A and B, and in 100% agreement with a licensed HBsAg EIA.

Reactivity in Seroconversion Panels

The sensitivity of the GS HBsAg EIA 3.0, procedures A and B, was assessed in 20 seroconversion panels purchased from Boston Biomedical, Inc. (West Bridgewater, MA) and 1 seroconversion panel purchased from Serologicals, Inc. (Clarkston, GA). The number of the bleed at which HBsAg was detected (S/CO ≥1.0) by the GS HBsAg EIA 3.0, procedures A and B, is compared to other licensed HBsAg assays (as recorded in the certificates of analysis) in Table 4 below.

Table 4: GS HBsAg EIA 3.0 Reactivity in HBsAg Seroconversion Panels

Panel I.D.#	GS HBsAg EIA 3.0 Static	GS HBsAg EIA 3.0 Shaker	HBsAg EIA #1 (overnight)	HBsAg EIA #2
902	7*	7*	9	9
903	3	2*	5	5
904	2	1*	3	3
905	1*	1*	1*	1*
906	2*	2*	2*	3
907	6	5*	6	6
908	6	4*	7	7
909	4	3*	4	5
910	3*	3*	3*	4
911	20*	20*	21	22
912	7	6*	8	8
914	2*	2*	5	5
915	1*	1*	9	11
917	2*	2*	3	3
918	2*	2*	3	3
919	5	4*	6	7
920	3*	3*	3*	3*
924	3	2*	3	4
931	5	3*	NT	7
932	10	8*	NT	10
40565L	2	1*	3	NT

*Earliest detection

As can be seen from Table 4, the GS HBsAg EIA 3.0 detected the presence of HBsAg as early as, or earlier than, the licensed HBsAg assays in all 21 seroconversion panels using Procedure A (static) and Procedure B (shaker). The shaker procedure detected 11 panels earlier than the static procedure.

Sensitivity testing with the ORTHO® Summit System

Additional sensitivity studies have been completed with the GS HBsAg EIA 3.0 on the ORTHO® Summit System with both the ORTHO® Summit Sample Handling System[1] and the ORTHO VERSEIA® Pipetter, and demonstrate equivalent performance with Bio-Rad manual equipment.

Additional studies were performed with the stand-alone ORTHO VERSEIA® Pipetter and a stand-alone ORTHO® Summit Processor (OSP) in comparison to the ORTHO VERSEIA® Integrated Processor (VIP). These studies used dilution panels and known positive samples. With the HBsAg dilution panels the VIP showed an overall S/CO percent difference increase from the stand-alone system of 12.5%. For the HBsAg known positive samples the VIP showed a percent difference increase in S/CO of 12.5%.

14 -PERFORMANCE CHARACTERISTICS OF CADAVERIC SPECIMEN TESTING

Reproducibility

Inter-assay reproducibility of the GS HBsAg EIA was assessed for Procedure A (static mode) using twenty post-mortem sera and twenty normal donor sera, spiked with HBsAg positive serum to give reactivity near the cutoff. Each of the samples was tested once on six different days on each of three lots of the GS HBsAg EIA at one site. For inter-assay reproducibility over all lots, percent coefficient of variation (%CV) ranged from 6.3% to 11.8% for the spiked post-mortem samples and from 5.9% to 12.6% for the spiked normal donor samples.

Specificity

In a clinical investigation at one site, sixty-three post-mortem samples and sixty-three normal donor samples were tested concurrently with procedure A (static mode) of the GS HBsAg EIA. The mean signal to cutoff (S/CO) ratio for 63 post-mortem samples was 0.347 and the mean S/CO ratio for the 63 normal

1. The ORTHO® Summit Sample Handling System is now a legacy device and no longer available for marketing.

donors was 0.185. The presence of HBsAg in repeatedly reactive specimens was confirmed by neutralization with human anti-HBs using procedure A (static mode) of the GS HBsAg Confirmatory Assay. Results are presented in Table 5 below.

Table 5: Reactivity with GS HBsAg EIA Procedure A

Population	Number Tested	Nonreactive	Initially Reactive	Repeatedly Reactive	Confirmed Positive
Post-mortem	63	62 (98.41%)	1 (1.59%)	1 (1.59%)	0 (0.0%)
Normal Donor	63	63 (100.0%)	0 (0.0%)	NA	NA

NA = Not Applicable

Specificity of the GS HBsAg EIA (Procedure A) was estimated by the following formula:

$$\frac{(\#\text{ specimens} - \#\text{ repeatedly reactive specimens})}{(\#\text{ specimens} - \#\text{ repeatedly reactive specimens confirmed positive for HBsAg})} \times 100$$

A total of sixty-three post-mortem specimens were tested with procedure A of the GS HBsAg EIA; one (1.59%) of these specimens was repeatedly reactive but did not confirm positive for HBsAg. Thus, the GS HBsAg EIA (Procedure A) has an estimated specificity of 98.4% [95%; binomial confidence interval = (94.39%, 100%)] for post-mortem specimens.

Sensitivity

Sixty-six post-mortem samples and sixty-six normal donor samples were pre-screened for HBsAg and antibody to HBsAg and found to be nonreactive. Each sample was divided into two portions. One portion of each post-mortem and normal donor sample was spiked at a potency near cutoff with a positive serum containing HBsAg *ad/ay* and the remaining portion was left unspiked. The sixty-six spiked and unspiked post-mortem samples were tested concurrently with sixty-six spiked and unspiked normal donor specimens on the same run of the GS HBsAg EIA (Procedure A). Spiked specimens were expected to be reactive and therefore were not retested in duplicate. The presence of HBsAg in initially reactive specimens was confirmed by neutralization with human anti-HBs using procedure A (static

mode) of the GS HBsAg Confirmatory Assay. Results are presented in Table 6 below.

Table 6: Reactivity with GS HBsAg EIA Procedure A

Population	Number Tested	Non-reactive	Initially Reactive	Repeatedly Reactive	Confirmed Positive
Spiked Post-mortem	66	0 (0.00%)	66 (100.0%)	NT	66 (100.0%)
Unspiked Post-mortem	66	65 (98.5%)	1 (1.5%)	QNS	0 (0.0%)
Spiked Normal Donor	66	0 (0.00%)	66 (100.0%)	NT	66 (100.0%)
Unspiked Normal Donor	66	66 (100.0%)	0 (0.00%)	NA	NA

NT = Not Tested NA = Not Applicable QNS = Quantity Not Sufficient

As can be seen in Table 6, of sixty-six post-mortem samples and sixty-six normal donor samples, spiked at a potency near cutoff and tested concurrently, all (100.00%) were reactive with the GS HBsAg EIA (Procedure A) and all confirmed positive with the GS HBsAg Confirmatory Assay (Procedure A).

Furthermore, according to the Student's t-test, there is no significant statistical difference between the spiked post-mortem mean optical density signal and that of the spiked normal donor mean optical density signal (two sample assuming unequal variances). These results demonstrate that the detection of HBsAg in post-mortem samples is comparable to the detection in normal donors.

15 -BIBLIOGRAPHY

1. Delmonico FL, Snydman DR: Organ donor screening for infectious diseases. **Transplantation 65 (5):** 603-610, 1998.
2. Zuckerman AJ: Viral hepatitis with special reference to hepatitis B. **Immunoassays for the 80's.** eds Voller A, Bartlett A, and Bidwell D, University Park Press. 361-373, 1981.
3. Wolters G, Kuijpers L, Kacaki J, Schuurs AHWM: Solid-phase enzyme-immunoassay for detection of hepatitis B surface antigen. **J Clin Pathol 29 (10):** 873-879, 1976.
4. David GS, Present W, Martinis J, Wang R, Bartholomew R, Desmond W, Sevier ED: Monoclonal antibodies in the detection of hepatitis infection. **Med Lab Sci 38:** 341-348, 1981.
5. Kennedy RC, Ionescu-Matiu I, Adler-Storthz K, Henkel RD, Sanchez Y, Dreesman, GR: Characterization of anti-hepatitis B surface antigen monoclonal antibodies. **Intervirology 19:** 176-180, 1983.
6. Bos ES, van der Doelen AA, van Rooy N, Schuurs AHWM: 3,3',5,5' - tetramethylbenzidine as an ames test negative chromogen for horseradish peroxidase in enzyme immunoassay. **J Immunoassay 2:** 187-204, 1981.
7. Garner RC, Walpole AL, Rose FL: Testing of some benzidine analogues for microsomal activation to bacterial mutagens. **Cancer Letters 1:** 39-42, 1975.
8. US Department of Health and Human Services. **Biosafety in Microbiological and Biomedical Laboratories**. 5th ed. Washington, DC: US Government Printing Office, HHS Publication No. (CDC) 21-1112. December 2009.
9. World Health Organization. **Laboratory Biosafety Manual**. 3rd ed. Geneva: World Health Organization, 2004.

10. Bond WW, Favero MS, Petersen NJ, Ebert JW: Inactivation of hepatitis B virus by intermediate-to-high-level disinfectant chemicals. **J Clin Microbiol 18:** 535-538, 1983.

11. U.S. Environmental Protection Agency, Office of Solid Waste: **EPA Guide for Infectious Waste Management,** Washington D.C., 1986. (USG PO 530-SW-86-014).

12. Sehulster LM, Hollinger FB, Dreesman GR, Melnick JL: Immunological and biophysical alteration of hepatitis B virus antigens by sodium hypochlorite disinfection. **Appl and Envi Microbiol 42:** 762-767, 1981.

13. Mast E, et al. A Comprehensive Immunization Strategy to Eliminate Transmission of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices (ACIP) Part II: Immunization of Adults. **MMWR** 55/RR-16:1-25. 1996.

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