Caution - Federal law restricts this device to sale by or on the order of a physician.

1. INTENDED USE

ETI-AB-AUK PLUS is an *in vitro* enzyme immunoassay (EIA) intended for the qualitative detection of antibodies to hepatitis B surface antigen (anti-HBs) in human serum or plasma (EDTA, citrate or heparin). The ETI-AB-AUK PLUS is intended for manual use only.

The detection of anti-HBs is indicative of laboratory diagnosis for seroconversion from hepatitis B virus (HBV) infection. Anti-HBs is also used in the laboratory diagnosis of past exposure to hepatitis B in potential hepatitis B vaccine recipients and to determine the presence of an immune response in vaccine recipients. The ETI-AB-AUK PLUS assay's performance has not been established for the monitoring of HBV disease or therapy.

Assay performance characteristics have not been established when the ETI-AB-AUK PLUS anti-HBs assay is used in conjunction with the other manufacturers' assays for specific HBV serological markers or automated microplate instruments. Under these conditions, users are responsible for establishing their own performance characteristics.

Assay performance characteristics have not been established for use of the ETI-AB-AUK PLUS anti-HBs assay as an aid in determining susceptibility to HBV infection prior to or following vaccination in infants, children, or adolescents.

The performance characteristics of this assay have not been established for newborn testing.

Caution: Performance characteristics for the DiaSorin ETI-AB-AUK PLUS were largely determined using archival specimens which may not be representative of test results obtained from fresh specimens. Laboratories are advised that they should monitor patient results using other appropriate HBV serological markers or retest questionable specimens with another legally-marketed anti-HBs assay.

2. SUMMARY AND EXPLANATION OF THE TEST

Hepatitis is an inflammatory disease of the liver that can severely damage the organ. The disease can result from non-infectious causes—such as biliary obstruction, biliary cirrhosis, Wilson's disease, drug toxicity, and drug hypersensitivity reactions—or from infectious viral and bacterial agents (1). Viral hepatitis is commonly caused by one of several viruses: hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis non-A, non-B virus(es) (known as NANB, of which hepatitis C virus [HCV] is one), or hepatitis D (delta) virus (HDV) in conjunction with HBV (1, 2), hepatitis E virus (HEV), and other as yet uncharacterized or partially characterized hepatitis viruses (non-A-E). Other viruses, including yellow fever virus, human cytomegalovirus, Epstein-Barr virus, rubella virus, herpes simplex virus, varicella-zoster virus, and some enteroviruses, can cause forms of hepatitis (1, 2).

Hepatitis B, also known as serum hepatitis, is endemic throughout the world (3, 4). The infection is spread primarily through percutaneous contact with infected blood products, e.g., blood transfusion, sharing of needles by drug addicts (1, 3, 5, 6). The virus is also found in virtually every type of human body fluid and has been known to be spread through oral and genital contact (1, 3, 5). HBV can be transmitted from mother to child during delivery through contact with blood and vaginal secretions; it is not commonly transmitted transplacentally (5).

The incubation period for hepatitis B averages 90 days (range 40-180 days). Common symptoms include malaise, fever, gastroenteritis, and jaundice (icterus) (3, 7). HBV infection can lead to a)icteric hepatitis, b)subclinical anicteric hepatitis, c)fulminant hepatitis, or d)chronic active or persistent hepatitis (1, 3). Over 90% of adults with hepatitis B completely recover from acute illness,

approximately 1% die of fulminant hepatitis, and approximately 3 to 5% become chronic or persistent carriers (1-3).

The complete hepatitis B virus, also called the Dane particle, is composed of an outer surface or envelope that carries the hepatitis B surface antigen (HBsAg) (8, 9). The envelope surrounds an inner core that contains the hepatitis B core antigen (HBcAg) (10-12). Inside the core is the HBV deoxyribonucleic acid (DNA) genome. Another antigen, the hepatitis B *e* antigen (HBeAg), is a viral core protein found in the bloodstream during active replication of HBV (1, 13).

Because HBV is very difficult to isolate in cell culture, hepatitis B diagnosis has been based on detection of serologic markers. Early methods used to detect serologic markers were immunodiffusion and counterimmunoelectrophoresis (3). Methods commonly used now include hemagglutination, immune adherence, latex agglutination, radioimmunoassay (RIA), and enzyme immunoassay (EIA) (1, 3). The EIA and RIA methods are the most widely utilized because of their high analytical sensitivity, analytical specificity, and ease of use.

When determining the stage of disease caused by HBV, the HBV serologic markers commonly tested for are HBsAg, antibody to HBsAg (anti-HBs), total antibody to HBcAg (total anti-HBc), immunoglobulin M antibody to HBcAg (IgM anti-HBc), HBeAg, and antibody to HBeAg (anti-HBe). Testing for these markers helps determine the presence of past or ongoing HBV infection, the acute or chronic stage of the disease, response to therapy, and/or the immune status of the patient (1, 14).

Before the onset of clinical illness, HBsAg is detectable in the serum, and its presence persists through the symptomatic phase of illness. Following clinical illness, the titer of HBsAg begins to decline and eventually falls below a detectable level. After HBsAg disappears, anti-HBs appears in the serum, although there is often a gap called the window period between the disappearance of HBsAg and the appearance of anti-HBs (3, 15). In approximately 10% of patients, HBsAg persists indefinitely in the serum, indicating a chronic carrier state, and anti-HBs does not appear (3, 16).

The presence of anti-HBs in serum indicates previous exposure to HBV (3). Detection of anti-HBs is therefore critical in establishing whether complete resolution of the infection has occurred as well as in establishing the acquisition of immunity, whether acquired as a result of natural HBV infection or vaccination. Vaccines based on either human plasma-derived or recombinant HBsAg are in widespread use (17), and measurement of anti-HBs in vaccinees is essential to assessing the duration of protection after primary immunization and the need of booster doses (18-21). Anti-HBs testing is also crucial in identifying HBV-susceptible individuals in prevaccination screening programs and in diagnosing inapparent hepatitis B infection (diagnosis of past exposure to HBV).

The determination of anti-HBs levels has been standardized by use of the WHO Anti-HBs Reference Preparation (22), expressed in milli-International Units per milliliter (mIU/mL). A level of ≥ 10 mIU/mL is generally considered as the standard for demonstrating post-vaccination protection against HBV (21, 23, 24). The verification of at least a minimum anti-HBs titer of 10 mIU/mL, i.e., an immunity threshold titer, is crucial to the appropriate management of vaccinated individuals who are subsequently exposed to an HBsAg-positive source (20, 25). The ETI-AB-AUK PLUS kit includes a WHO-referenced calibrator that is used to assess whether a positive test sample contains greater than 10 mIU/mL anti-HBs.

3. PRINCIPLE OF THE PROCEDURE

The assay is a direct, non-competitive enzyme immunoassay based on the use of polystyrene microwells coated with recombinant HBsAg (subtypes *ad* and *ay*). An enzyme tracer containing horseradish peroxidase-labeled HBsAg (human, subtypes *ad* and *ay*) detects any captured anti-HBs from the patient's specimen.

In the assay procedure, patient specimens and controls are incubated with incubation buffer in hepatitis B surface antigen-coated microwells. If anti-HBs is present in the sample, it binds to the

antigen. Excess sample is removed by a wash step, and the enzyme tracer is then added to the microwells and allowed to incubate. The enzyme tracer binds to any antigen-antibody complexes present in the microwells. Excess enzyme tracer is removed by a wash step, and a chromogen/substrate solution is added to the microwells and allowed to incubate. If a sample contains anti-HBs, the bound enzyme (horseradish peroxidase) chemically reduces the substrate peroxide, which concurrently oxidizes the chromogen tetramethylbenzidine (TMB) to a blue color (650 nm). The blue color turns to yellow (450 nm) after addition of the stop solution. If a sample does not contain anti-HBs, the microwell will be colorless after the chromogen/substrate solution is added and will remain colorless after the stop solution is added. Color intensity, which is measured spectrophotometrically, is indicative of the concentration of anti-HBs. Absorbance value readings for patient specimens are compared to a cutoff value determined from the mean absorbance of the calibrator.

4. REAGENTS AND OTHER MATERIALS PROVIDED

Catalog Number	Product Description	Quantity/ Volume
P001931	**ETI-AB-AUK PLUS**	192 tests
	Coated Strips Microwells coated with recombinant HBsAg (subtypes *ad* and *ay*, molecular weights 23 Kd, 46 Kd and 69 Kd, expressed in Hansenula polymorpha).	Twenty-four 8-well strips (contained in 2 pouches)
	Enzyme Tracer Horseradish peroxidase-labeled human HBsAg (subtypes *ad* and *ay*), buffer, protein stabilizers. Preservative: 0.2% ProClin 300.	0.7 mL
	Tracer Diluent Human serum/plasma, buffer. Preservative: 0.2% ProClin 300.	Two 14.7-mL vials
	Immunity Calibrator Human serum/plasma containing 15 mIU/mL anti-HBs referenced to WHO Anti-Hepatitis B Immunoglobulin International Reference Preparation, protein stabilizers. Preservative: 0.2% ProClin 300.	2.5 mL
	Negative Control (Human) Human serum/plasma non-reactive for all known HBV markers. Preservative: 0.2% ProClin 300.	3.3 mL
	Positive Control (Human) Human serum/plasma reactive for anti-HBs, protein stabilizers. Preservative: 0.2% ProClin 300.	2.5 mL
	Incubation Buffer Buffer, protein stabilizers, an inert blue dye. Preservative: 0.2% ProClin 300.	16 mL
	Wash Buffer (concentrate)* Buffer, detergents, preservatives.	Two 40-mL vials
	Chromogen/Substrate* Tetramethylbenzidine/hydrogen peroxide system.	Two 16-mL vials
	Stop Solution* 1N sulfuric acid. Caution: corrosive.	30 mL
	Strip Sealers	48

Catalog Number	Product Description	Quantity/ Volume
	Plate Sealers	4
	Pouch Sealer	1

** All lots of wash buffer concentrate, chromogen/substrate and stop solution are interchangeable between assay kits.*

5. WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- The human blood source material used to prepare this product (e.g. Enzyme Tracer, Tracer Diluent, Immunity Calibrator, and controls) has been tested and found non-reactive for HBsAg, antibodies to HCV, and antibodies to HIV-1 and HIV-2 by licensed methods. Because no known test method can offer complete assurance that products derived from human blood are pathogen-free, handle all materials of human origin as though they were potentially infectious as discussed in OSHA's Final Rule on Occupational Exposure to Bloodborne Pathogens (26, 27).
- All specimens, reagents, and controls should be handled as if capable of transmitting disease. Follow standard precautions for handling infectious agents during all procedures:
  - Do not pipette by mouth.
  - Do not eat, drink, smoke, or apply cosmetics in areas where specimens are handled.
  - Wear protective clothing such as lab coats, protective glasses, and disposable gloves when handling specimens and assay reagents. Wash hands thoroughly afterwards.
  - Perform all work with infectious materials in a designated area.
- Dispose of all specimens and used assay materials as if capable of transmitting disease:
  - Decontaminate liquid wastes, including those containing neutralized acid, either:

    (a) by autoclaving for 60 minutes at 121°C; or

    (b) by treating with a 1:10 or 1:100 dilution of household bleach (sodium hypochlorite concentration approximately 5%). The wastes should remain in contact with the sodium hypochlorite solution for 30 minutes for effective decontamination, after which they can be disposed of in the sink (26, 28). Do not autoclave solutions containing sodium hypochlorite.
  - Autoclave non-ignitable solids for 60 minutes at 121°C.
  - Incinerate disposable ignitable materials.
- Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
- Use only dispensing equipment that has been calibrated to deliver accurate volumes, per the laboratory's standard procedures.
- **WARNING - Chromogen/substrate and the stop solution contain ingredients that can irritate skin and cause eye damage. Handle them with care. Avoid getting them in eyes or on skin or clothing. In case of contact with skin or eyes, immediately flush the affected area with water for 15 minutes. For eyes, obtain medical attention.**

  Reagents containing ProClin 300 may cause allergic reactions. Avoid prolonged contact with skin. Wash thoroughly after handling.

6. REAGENT PREPARATION

- Bring reagents to room temperature (20-25°C).
- The coated strips, calibrator, negative and positive controls, incubation buffer, chromogen/substrate and stop solution are provided ready to use.
  ***Note** - Use clean, plastic containers or acid-washed glassware for preparing the following solutions. A clean, dedicated dispenser is recommended for the working enzyme tracer to avoid contamination.*
- **Working enzyme tracer.** Bring reagents to room temperature (20-25°C). To prepare the working enzyme tracer, dilute the enzyme tracer 1:50 with tracer diluent (see chart below). After dilution, the working enzyme tracer can be used for one week if stored at 2-8°C.
  ***Caution** - Verify that the total volume prepared is sufficient for the number of tests included in the run. Use a clean container for each dilution and label the container with the reagent name, lot number of kit, lot number of reagent, plus the date of preparation and date of expiration of the working enzyme tracer.*

Number of Strips	Enzyme Tracer (µL)	Tracer Diluent (µL)	Total Volume (mL)
2	48	2352	2.4
4	80	3920	4.0
6	112	5488	5.6
8	144	7056	7.2
10	176	8624	8.8
12	208	10192	10.4

***Note** - Sufficient reagents are provided to allow for six runs per kit.*

- **Wash solution.** To prepare the working wash buffer, dilute the wash buffer concentrate (40 mL) to 1000 mL (1.0 L) with distilled or deionized water. If crystallization has occurred at 2-8°C, warm the wash buffer concentrate to 37°C and mix well before diluting. Water used for wash buffer dilution should be stored in a clean, non-metallic container to prevent contamination with peroxidase-inactivating substances. Record on the storage vial the expiration date and date of preparation of the working wash buffer. The working wash buffer can be stored for one week at 2-8°C.
  Smaller volume users may prepare less than 1 L of working wash buffer at a time if desired. If diluting only a portion of the wash buffer concentrate, check concentrate for crystallization. If crystallization has occurred during storage, warm the wash buffer concentrate to 37°C and mix well to eliminate crystals before removing aliquot for dilution.
  ***Note** - All lots of wash buffer concentrate are interchangeable.*
- Working wash buffer containers should be thoroughly cleaned with 70% ethanol and thoroughly rinsed with distilled or deionized water before preparation of the next batch of working wash buffer.

7. REAGENT STORAGE AND HANDLING INSTRUCTIONS

- Store the test components in the refrigerator at 2-8°C away from intense light. Allow them to reach room temperature (20-25°C) before use. Return the test components to the refrigerator after use.
- Do not expose the test components to intense light, direct sunlight, or temperatures above 25°C. Do not freeze the kit.

- When stored as directed, test components will remain stable until expiration dates printed on their labels.
- Keep unused coated strips sealed in their pouches until time for use. Allow the pouch to reach room temperature (20-25°C) before opening it. Return any unused strips to the pouch as soon as possible; seal the pouch with the pouch sealer and refrigerate pouch at 2-8°C.
- After dilution, the working enzyme tracer can be stored for one week at 2-8°C.
- After dilution, the working wash buffer can be stored for one week at 2-8°C.

8. REAGENT INSTABILITY OR DETERIORATION

- The chromogen/substrate may have a slightly blue tinge. If the chromogen/substrate turns a darker blue, it may have become contaminated and should be discarded.
- Any reagent that contains visible particulate matter should be discarded.

9. SPECIMEN COLLECTION AND PREPARATION

- **This assay is not designed to test body fluids other than human serum or plasma.**
- Specimens containing precipitate may give inconsistent test results. Do not test specimens containing particulate material, or grossly hemolyzed or lipemic specimens.
- There is a specimen dilutional effect with citrated plasma due to the liquid nature of this anticoagulant. Borderline or high-negative results obtained from citrated specimens should be retested using serum as the matrix.
- Each assay requires 100 µL human serum or plasma. EDTA, citrate or heparin anticoagulants have been tested and may be used with this assay. Follow manufacturer's instructions carefully when using plasma collection containers with anticoagulants.
- The testing of heat inactivated samples is not recommended.
- Samples that are to be used fresh may be stored for up to two hours at 2-8°C in the presence of clots. Serum separated from the clot may be stored at 2-8°C up to 48 hours, but then must be frozen and stored deep-frozen (at –20°C or below) in sterile containers until use (29). If sample is stored frozen, mix thawed sample well before testing. It has been shown that up to three freeze-thaw cycles do not interfere with the assay.
- For shipping, specimens should be frozen at –20°C or below and shipped with dry ice. Temperature level during entire shipment should be no greater (warmer) than –20 °C. Pack specimens in compliance with government regulations covering the transportation of etiologic agents (30).

10. MANUAL ASSAY PROCEDURE

Materials Provided

ETI-AB-AUK PLUS

Coated Strips
Enzyme Tracer
Tracer Diluent
Immunity Calibrator
Negative Control (Human)
Positive Control (Human)
Incubation Buffer
Wash Buffer (Concentrate)
Chromogen/Substrate
Stop Solution
Strip Sealers
Plate Sealers
Pouch Sealer.

Materials Required But Not Provided

Microwell plate washer - The following instrument specifications are recommended for the kit performance:

Volume dispensed: 350-370 μL
Number of wash cycles: 5
Soak time: 30 seconds
Aspirate the last aliquot of dispensed liquid: yes.
***Note** - The volume of each microwell is approximately 400 μL. Make sure the volume of working wash buffer dispensed into each well does not cause the wells to overflow. If the wells overflow, set the washer to dispense less working wash buffer.*

Microwell plate reader - The following instrument specifications are recommended for the kit performance:

Wavelength: dual wavelength, 450 nm and 600-650 nm
Bandwidth: ≤ 10 nm
Absorbance range: 0 absorbance units to ≥ 3 absorbance units
Repeatability: better than or equal to 0.005 absorbance units, or 1%, whichever is greater
Linearity or accuracy: better than or equal to 0.010 absorbance units, or 2%, whichever is greater
Drift: less than 0.005 absorbance units per hour.

Incubator, 37°C ± 1°C.
***Note** - Gravity convection incubators are recommended. Forced-air incubators may cause edge effects. Do not use water baths as incubators.*

Micropipettes with disposable clean tips (50 μL and 100 μL).
***Note** - Suggested specifications for micropipetters (based on gravimetric testing) are:*
50 μL: accuracy ± 3%, precision 2%
100 μL: accuracy ± 2%, precision 1%.

Miscellaneous clean glass or plastic containers
Hazardous waste disposal materials
Disposable gloves
Distilled or deionized water
Pipetter-diluter (optional)
Multichannel pipetter (optional)
Pipette tips for multichannel pipetter (if multichannel pipetter is used)
Disposable reagent reservoirs (if multichannel pipetter is used)
Printer compatible with microwell reader.

Assay Procedure

Perform all assay steps in the order given and without any delays between the steps. A cutoff value is calculated for each plate based on the absorbance values of the calibrators run on that plate. A maximum of one plate should be set up (completed through the first incubation step) at a time. If multiple plates are being run as a batch, each plate must be treated as a single entity; i.e., the calibrators, controls and patient specimens for the plate must be added and the incubation time started before moving on to the next plate. Proper instrument maintenance is critical for good assay performance. Follow the manufacturer's instructions for performing instrument warm-up, quality control, calibration and maintenance procedures on all equipment used in this assay.

NOTE: **All steps must be completed within four hours. Calibrator, positive and negative controls must be run with each plate of patient specimens.**

1. **Prepare assay reagents.** Allow all test components to reach room temperature (20-25°C). Prepare working wash buffer and working enzyme tracer according to the directions given in Section 6, Reagent Preparation. Refer to the chart in Section 6 to ensure preparation of sufficient reagent volumes for the number of tests included in the run.

2. **Prepare coated plate.** Prepare enough microwells for the calibrators, controls and samples to be tested. Allow one blank well containing only chromogen/substrate and stop solution in well A1. Allow one well for each patient sample. The calibrator must be tested in triplicate and the negative and positive controls tested in singlet. Calibrators are to be placed in wells B1, C1 and D1; negative control is to be placed in well E1; positive control is to be placed in well F1 (for details, refer to the recommended plate map at the end of this section). Test calibrator and controls as you would patient specimens.

Coated strips may be separated. Avoid handling the bottoms of the microwells because scratches or marks could affect the reading of test results. Store unused strips in their original pouch, seal the pouch carefully, and refrigerate at 2-8°C.

3. **Add incubation buffer.** Add **50 µL** incubation buffer to all microwells (except for the blank well).

4. **Add samples and controls.** If sample was stored frozen, mix thawed sample well (vortex) before proceeding. Add **100 µL** of each calibrator, control or sample to its respective microwell. To avoid cross-contamination, use a clean micropipette tip to dispense each calibrator, control or specimen. Record the microwell position of each calibrator, control or patient specimen on a laboratory data sheet.

Incubation buffer is light blue in color. On addition of calibrators, controls or samples, the color will turn to green or dark blue. This color change may vary from sample to sample, but it will always be visible.

5. **Incubate.** Cover the microwells with a plate or the strip sealer provided with this kit. Use a roller to affix the sealer or press firmly by hand around microwell and plate edges to ensure that the sealer is firmly attached over the entire strip or plate. Tap the coated plate gently to release any air bubbles trapped in the liquid making sure samples do not splash onto the sealer. Ensure that all microwells are filled equally. Incubate the microwells **for 2 hours ± 10 minutes at 37°C ± 1°C.**

6. **Wash coated plate.** Remove and discard the sealer. Aspirate the liquid from the microwells and wash each well five times as follows: Deliver 350-370 µL of working wash buffer to each microwell, let the wells soak for 30 seconds, and then aspirate the working wash buffer completely from each microwell. Microwell plate washers vary by manufacturer. Make sure the volume of working wash buffer dispensed into each well completely fills the well but does not cause the well to overflow.

7. **Remove excess working wash buffer.** Ensure that all microwells are aspirated completely before proceeding. With some washers it may be necessary to invert the microplate and tap it forcefully on a paper towel to effectively remove residual working wash buffer.

8. **Add working enzyme tracer.** Immediately add **100 µL** working enzyme tracer to each well (except for the blank well).

9. **Incubate.** Cover the microwells with a plate or strip sealer using the sealers provided for this kit. Ensure that sealer is applied correctly (see Step 5). Tap the coated plate gently to release any air bubbles trapped in the liquid. Ensure that all microwells are filled equally. Incubate the microwells **for 60 ± 5 minutes at 37°C ± 1°C.**

Warning - Timing of this incubation step is critical.

10. **Wash coated plate.** Remove and discard the sealer. Aspirate the working enzyme tracer from the microwells and wash them as described in Steps 6 and 7.

11. Add chromogen/substrate. Immediately add **100 µL** chromogen/substrate to all microwells, including the blank well.

***Note** - The chromogen/substrate may have a slightly blue tinge. However, if it turns a darker blue, it may have become contaminated and should be discarded.*

12. Incubate. Incubate the microwells **for 30 ± 2 minutes at room temperature** (20-25°C). Avoid exposing the microwells to direct or intense light. Do not exceed the time limits of this incubation.

13. Add stop solution. Add **100 µL** stop solution to each microwell in the same order as chromogen/substrate was added.

14. Read results. Within one hour after addition of stop solution, read the absorbance values of the calibrators, negative control, positive control, and samples with the microwell reader set at 450/630 nm in the bichromatic mode. Check for and remove air bubbles before reading results. Record the absorbance value for each calibrator, control and sample.
***Note** - Blank the instrument on the blank well. The absorbance of the blank well containing only chromogen/substrate and stop solution (see Step 2 in Section 10, Procedure) is evaluated as described in the QC section. The value for the blank well should be recorded and subtracted from each calibrator, control and sample value before calculating mean values and cutoff, and before interpreting results.*

15. Perform assay quality control procedures. Before evaluating results, perform quality control procedures (see Section 11, Quality Control).

16. Perform equipment quality control and maintenance procedures. Proper instrument maintenance including calibration is critical for good assay performance. Follow the manufacturer's instructions for performing quality control and maintenance procedures on all equipment used in this assay.

Recommended Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S3										
B	CAL1	S4										
C	CAL2	S5										
D	CAL3	S6										
E	NC	S7										
F	PC	S8										
G	S1	etc.										S89
H	S2											S90

11. QUALITY CONTROL

The negative and positive controls are intended to monitor for substantial reagent failure. The positive control will not ensure precision at the assay cutoff.

The quality control material furnished is in a serum matrix. It may not adequately control the assay for plasma specimens. The user should provide alternate control material for testing of plasma matrices.

Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Use the following steps to validate quality control. References 31 and 32 provide guidance on quality control recommendations. Record the results on the QC Verification Worksheet provided for the assay.

Compute the mean absorbance value for the calibrator.

> **Always evaluate mean calibrator value and negative and positive control values for each plate when running more than one plate in a batch. Be sure to compare the absorbance value of each patient sample with the cutoff value computed for the plate containing that sample.**

1. Evaluate the absorbance value of the substrate blank.

Blank the instrument on the blank well containing only chromogen/substrate and stop solution (see Step 14 in Section 10, Procedure). The absorbance value for the blank well must be between 0.000 and 0.150 for the assay to be valid. If the absorbance value of the substrate blank is less than 0.000 or greater than 0.150, the run must be repeated.
Note - Subtract the substrate blank absorbance value from each absorbance value before performing the following evaluations.

2. Evaluate the mean calibrator absorbance value (Cal $\bar{x}$).

Each calibrator absorbance value (after subtraction of the blank) must be greater than 0.040 and less than 0.450.

$$0.040 < \text{Cal} < 0.450$$

If one of the calibrator absorbance values does not meet this criterion, it should be discarded and the mean value recalculated using the remaining two values. If more than one calibrator absorbance values does not meet this criterion, the run is invalid and must be repeated.

Example 1: Calculation of mean of calibrators

Calibrator Well	Absorbance	minus blank abs.	final calibrator abs.
B1	0.263	0.075	0.188
C1	0.247	0.075	0.172
D1	0.285	0.075	0.210
Total absorbance			0.570

$$\text{Mean of calibrators (Cal } \bar{x}) = \frac{\text{Total absorbance}}{3} = \frac{0.570}{3} = 0.190.$$

The mean calibrator absorbance value must be greater than 0.040 and less than 0.450.

$$0.040 < \text{Cal } \bar{x} < 0.450$$

If the mean calibrator absorbance value does not meet this criterion, the run is invalid and must be repeated.

3. Evaluate the negative control absorbance value (NC).

After subtracting the substrate blank absorbance, the negative control absorbance value must be greater than –0.020 and less than 0.120.

$$-0.020 < NC < 0.120$$

If the negative control absorbance value does not meet this criterion, the run is invalid and must be repeated.

4. Evaluate the positive control absorbance value (PC).

After subtracting the substrate blank absorbance, the positive control absorbance value must be greater than 0.500 and less than 2.500.

$$0.500 < PC < 2.500$$

If the mean positive control absorbance value does not meet this criterion, the run is invalid and must be repeated.

5. Evaluate the difference between the positive control absorbance value and the negative control absorbance value.

The difference between the positive control absorbance value and the negative control absorbance value must be greater than 0.450.

$$PC - NC > 0.450$$

If the difference between the positive control absorbance value and the negative control absorbance value does not meet this criterion, the run is invalid and must be repeated.

Example 2: Calculation of difference between PC and NC

Positive control absorbance (PC)	= 1.004
Negative control absorbance (NC)	= 0.016
Difference (PC – NC) = 1.004 – 0.016	= 0.988

6. Evaluate the ratio of the mean calibrator absorbance value to the negative control absorbance value.

The ratio of the mean calibrator absorbance value to the negative control absorbance value must be greater than 4.

$$\frac{Cal\,\bar{x}}{NC} > 4$$

If the ratio of the mean calibrator absorbance value to the negative control absorbance value does not meet this criterion, the run is invalid and must be repeated.

Example 3: Calculation of Cal $\bar{x}$ to NC ratio

Calibrator mean absorbance (Cal $\bar{x}$)	= 0.190
Negative control absorbance (NC)	= 0.016
Ratio (Cal $\bar{x}$ / NC) = 0.190 / 0.016	= 11.87

12. QUALITY CONTROL PROBLEM SOLVING

It is important to follow the assay procedure precisely. If calibrator or control values are not within acceptable limits (see Section 11, Quality Control) or results differ markedly from those expected, check these assay variables:

- Check incubator, incubation times, and temperatures.

- A properly functioning washer is critical to the assay. Ensure that the washer is filling and aspirating all wells, that no probes are plugged, and that the probes are placed correctly in the microwells. No fluid should be left in the wells at the end of the wash step.
- Be sure that wells do not dry out between washing and addition of the next reagent. Add reagent within a few minutes of removal of the plate from the washer. If a probe (or probes) on the washer becomes plugged during washing, identify the affected microwell(s) but continue with the assay procedure. Retest the affected specimen(s). To unplug probes, refer to the washer operator's manual.
- Check that all reagents and specimens are at room temperature (20-25°C) before starting the assay.
- Check that all reagents are within the expiration date, that appropriate assay kit components and ancillaries are used, and that there are no visible signs of contamination such as cloudiness or precipitates.
- Avoid cross-contamination of reagents and wells. If multichannel pipette tips have been contaminated, replace the tips.

13. INTERPRETATION OF RESULTS

The presence or absence of anti-HBs above the immunity threshold is determined by comparing the absorbance values of patient samples with an immunity cutoff value. The immunity cutoff value is determined for each plate based on the absorbance values of the calibrators run on that plate. Be sure to compare the absorbance value of each patient sample with the immunity cutoff value computed for the plate containing that sample.

Calculation of Immunity Cutoff Value

The immunity cutoff value is determined by the mean absorbance of the calibrator values after subtraction of the substrate blank.

$$\text{CUTOFF} = \text{Cal } \bar{x}$$

Example 4: Calculation of immunity cutoff value

Mean absorbance of calibrator values	0.190
Immunity Cutoff value for this run	0.190

The cutoff was established by testing 348 samples (174 volunteer blood donors and 174 hospitalized patients) with three lots of ETI-AB-AUK PLUS. The results were examined as the ratio between single sample absorbance and calibrator absorbance. In the apparently healthy adult (volunteer donor) population, 95% had ratios less than 0.299 and 99% had ratios less than 0.668; in the hospitalized patient population, 95% had ratios less than 0.138 and 99% had ratios less than 0.413.

Interpretation of Results

Absorbance Values	Result	Interpretation
absorbance < 90% x Immunity Cutoff	Negative	Anti-HBs not detected by ETI-AB-AUK PLUS; level is less than 10 mIU/mL, generally considered as the standard for indicating protective immunity. When determining disease state, this result should not be used alone but in conjunction with other hepatitis B serological markers.
absorbance within 90-110% of Immunity	Equivocal	Presence of anti-HBs indeterminate by ETI-AB-AUK PLUS. Specimen should be retested using ETI-AB-AUK PLUS kit to establish

Cutoff		presence or absence of antibody. If a specimen is found repeatedly equivocal, the pattern of other hepatitis B serological markers should be used to identify status of disease, or another sample should be collected and tested at a later date.
absorbance > 110% x Immunity Cutoff	Positive	Anti-HBs detected by ETI-AB-AUK PLUS; level is greater than 10 mIU/mL, generally considered as the standard for indicating protective immunity. When determining disease state, this result should not be used alone but in conjunction with other hepatitis B markers.

***Note** - The magnitude of the measured result, above the immunity cutoff, is not indicative of the total amount of antibody present. Due to the expression of the recombinant HBsAg in H. polymorpha, samples containing antibodies to H. polymorpha may cause false negative results.*

Example 5: Interpretation of results

Cutoff = 0.190
Equivocal Zone = 0.171 – 0.209
Sample No. 1 absorbance = 0.038
Sample No. 2 absorbance = 0.912
Sample No. 1 should be considered negative for anti-HBs; sample No. 2 should be considered positive for anti-HBs.

14. LIMITATIONS OF THE PROCEDURE

- Results obtained from immunosuppressed patients should be interpreted with caution.
- This assay is not designed to test body fluids other than human serum or plasma.
- Any laboratory test result should be interpreted in conjunction with the patient's clinical presentation and the results of other diagnostic tests. A negative result on a given laboratory assay does not by itself rule out the possibility of infection.
- The prevalence of the analyte will affect the assay's predictive value.
- Assay performance characteristics have not been established when the ETI-AB-AUK PLUS anti-HBs assay is used in conjunction with the other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.
- Assay performance characteristics have not been established for use of the ETI-AB-AUK PLUS anti-HBs assay as an aid in determining susceptibility to HBV infection prior to or following vaccination in infants, children, or adolescents.
- This assay does not differentiate between a vaccine-induced immune response and an immune response induced by infection with HBV. To determine if the anti-HBs response is due to vaccine or HBV infection, a total anti-HBc assay may be performed.
- Individuals that have received blood component therapy (e.g., whole blood or clotting factors) or immunoglobulin administration, or in neonates of mothers with current or past hepatitis B may have a positive anti-HBs reaction for three to six months due to passive transfer of anti-HBs.
- The affinity and/or avidity of IgG or IgM antibody to HBsAg have not been determined for this assay.
- The analytical sensitivity of the DiaSorin ETI-AB-AUK PLUS assay has been determined to be approximately 15 mIU/mL.

15. EXPECTED VALUES

The 236 prospective samples used in the expected values study for the DiaSorin ETI-AB-AUK PLUS assay were from patients who were sent to the laboratory for HBV testing. Of those samples, 100 (42%) were frozen and 136 (58%) were fresh. The patients represented Florida, Georgia, Pennsylvania, California, Utah, and the southeastern US. The group was 69% (162/236) female, 29% (68/236) male and 2% (6/236) unspecified; the ethnicity of the patients was unspecified. The ages ranged from 5 to 88 years old, with 6 samples unspecified. The percent DiaSorin ETI-AB-AUK PLUS positive results in these samples was 25%.

The table below summarizes the percent DiaSorin ETI-AB-AUK PLUS positive and negative results by gender and age range. There were 6 samples for which gender and age were not reported; they were all negative. There were 6 other samples for which age was not reported, 2 were from females and 4 were from males; 2 were positive and 4 were negative. These 12 results were not included in the table. DiaSorin equivocal results were not repeated in this study.

		DiaSorin ETI-AB-AUK PLUS						
		+		-		E*		TOTAL
Age Range	Gender	n	%	n	%	n	%	
0-9	F	0	0%	2	100%	0	0%	2
	M	0	0%	0	0%	0	0%	0
10-19	F	3	18%	14	82%	0	0%	17
	M	1	50%	1	50%	0	0%	2
20-29	F	8	16%	43	84%	0	0%	51
	M	4	31%	9	69%	0	0%	13
30-39	F	8	16%	40	82%	1	2%	49
	M	5	29%	11	65%	1	6%	17
40-49	F	7	35%	13	65%	0	0%	20
	M	5	36%	9	64%	0	0%	14
50-59	F	4	80%	1	20%	0	0%	5
	M	4	50%	4	50%	0	0%	8
60-69	F	0	0%	3	100%	0	0%	3
	M	0	0%	2	100%	0	0%	2
70-79	F	3	30%	7	70%	0	0%	10
	M	3	60%	2	40%	0	0%	5
80-89	F	0	0%	3	100%	0	0%	3
	M	0	0%	3	67%	0	0%	3
TOTAL		55	25%	166	74%	2	1%	224

* E = equivocal result

High Risk Population

Single repository samples belonging to high-risk populations (66 hemodialyzed patients, 148 hemophiliacs, 150 IV drug users) were tested with the DiaSorin ETI-AB-AUK PLUS assay to determine frequency of positive results in that population. The group was 12% (42/364) female, 69% (252/364) male, and 19% (70/364) unspecified, with ages ranging from 19 to 87 years old. No geographical locations were specified. The table below summarizes the ETI-AB-AUK PLUS results. The data in the table represent the number of specimens in each category. DiaSorin equivocal results were not repeated in this study.

High Risk Population

Population	Frequency of Positive Results (# Positive/Total # Samples)

IV drug users	36/150 = 24.0% (3 equivocal)
Hemophiliacs	37/148 = 25.0% (1 equivocal)
Hemodialysis patients	21/66 = 31.8% (2 equivocal)
TOTAL	94/364 = 25.8% (6 equivocal)

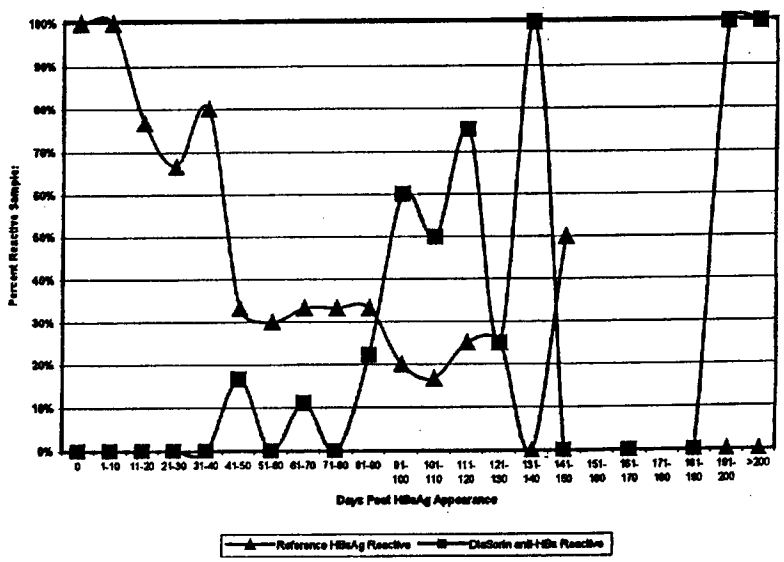
Acute Serial Panels

One hundred twenty-four (124) archived serial samples from 9 individuals were analyzed. Most (8/9) of these individuals were defined as being acutely infected by the appearance of HBsAg and HBeAg with the subsequence appearance of IgM anti-HBc, total anti-HBc, anti-HBe, and anti-HBs. One individual had detectable HBsAg but did not have detectable HBeAg in any specimen. However, this individual did seroconvert for anti-HBe.

The specimens were collected from individuals undergoing plasmaphereses for further manufacturing purposes. Three individuals were found to be infected with HBV during the first plasmaphereses and others became infected with HBV during subsequent plasmaphereses. It is unknown how long these three initially HBsAg reactives were infected prior to the first plasmaphereses. All nine individuals underwent sequential plasmaphereses after becoming HBV infected. However, the timing of subsequent plasmaphereses varied from individual to individual. The specimens draw times were normalized to represent the day that HBsAg was first detected by an FDA licensed assay as Day 0. For the remaining specimens, draw days ranged from day 0 (HBsAg first detected) through day 355 post day 0. Since all panels did not contain the same draw day, sample results were grouped within day intervals (e.g. days 0, 1-10, 11-20, etc., representing days since first detection of HBsAg).

The results are summarized in the following table and graph. In the graph below, the graph for the reference HBsAg percent reactive has been overlaid for reference.

Day Range	Number Speci-mens	DiaSorin anti-HBs Reactive	% Positive
0	9	0	0.0%
1-10	10	0	0.0%
11-20	13	0	0.0%
21-30	9	0	0.0%
31-40	10	0	0.0%
41-50	6	0	0.0%
51-60	10	0	0.0%
61-70	9	0	0.0%
71-80	6	0	0.0%
81-90	9	2	22.2%
91-100	10	4	40.0%
101-110	6	1	16.7%
111-120	4	0	0.0%
121-130	4	0	0.0%
131-140	3	3	100.0%
141-150	2	0	0.0%
151-160	0	0	NA
161-170	1	0	0.0%
171-180	0	0	NA
181-190	1	0	0.0%
191-200	1	0	0.0%
355	1	1	100.0%



40

16. PERFORMANCE DATA

Clinical Samples

Since the majority of studies were performed on pre-selected retrospective specimens, no calculations for the assay's positive and negative predictive values may be performed or inferred.

Prospective Samples: A study of 136 prospective specimens was conducted. These specimens represented individuals who were sent to the laboratory for hepatitis testing. Specimens were collected at a reference laboratory and assayed at the California clinical trial site. The patients were 86% (117/136) female and 14% (19/136) male. The ages ranged from 5 to 77 years old, with 3 specimens not specified.

The study (testing) sites were blinded to the previous specimen categorization. All testing was performed by the manual ETI-AB-AUK PLUS procedure. Specimens were characterized by testing with six HBV serological markers (HBsAg, HBeAg, IgM anti-HBc, total anti-HBc, anti-HBe, anti-HBs) using FDA-licensed or approved assays. Testing with these assays followed the FDA-licensed or approved procedure, including confirmation by neutralization of repeatably reactive HBsAg samples.

Results by Specimen Classification:

After study completion all samples were assigned a specimen classification based on the patterns of the six HBV serological markers established by the reference assays. Based on these serological marker patterns, the samples were categorized into the HBV classifications described in the table below. There were 6 unique HBV marker patterns observed in the DiaSorin ETI-AB-AUK PLUS prospective clinical studies.

Characterization Based On Single Point Specimen	HBsAg	HBeAg	IgM anti-HBc	Total anti-HBc	anti-HBe	anti-HBs	n
Chronic Infection	+	-	-	+	+	-	1
Recovery	-	-	-	+	+	+	2
Past Infection	-	-	-	+	-	+	4
	-	-	-	+	-	-	4
HBV Vaccine Response	-	-	-	-	-	+	38
Not Previously Infected with HBV	-	-	-	-	-	-	87

Based on the above classifications the ETI-AB-AUK PLUS anti-HBs results for the prospective samples were compared to a reference assay's anti-HBs results. The reference assay was not calibrated to the WHO standard of 10 mIU/mL established for immunity. The following table shows this comparison and percent agreement with 95% exact confidence intervals with the reference anti-HBs results. DiaSorin equivocal results were not repeated in this study.

Prospective Samples Comparison

Reference Serology Classification	Reference Assay - ETI-AB-AUK PLUS	Reference Assay + ETI-AB-AUK PLUS			TOTAL
	-	-	+	E*	
Chronic Infection	1	0	0	0	1
Recovery	0	0	2	0	2
Past Infection	4	0	4	0	8

HBV vaccine response	0	2	34	2	38
Not previously infected	87	0	0	0	87
TOTAL	92	2	40	2	136

* E = equivocal result

Prospective Samples:

Chronic Infection

Positive agreement with reference assay results = N/A (0/0)
95% CI = N/A

Negative agreement with reference assay results = 100% (1/1)
95% CI = 2.5 to 100%

Recovery

Positive agreement with reference assay results = 100.0% (2/2)
95% CI = 15.8 to 100.0%

Negative agreement with reference assay results = N/A (0/0)
95% CI = N/A

HBV Vaccine Response

Positive agreement with reference assay results = 89.5% (34/38)
95% CI = 75.2 to 97.1%

Negative agreement with reference assay results = NA (0/0)
95% CI = NA

Past Infection

Positive agreement with reference assay results = 100% (4/4)
95% CI = 39.8 to 100.0%

Negative agreement with reference assay results = 100% (4/4)
95% CI = 39.8 to 100.0%

Not Previously Infected

Positive agreement with reference assay results = NA (0/0)
95% CI = NA

Negative agreement with reference assay results = 100% (87/87)
95% CI = 98.5 to 100%

Retrospective Samples: Retrospective studies were carried out at three clinical laboratories in the United States (California, Missouri, and Minnesota) and at DiaSorin (Italy) to assess the performance of the ETI-AB-AUK PLUS assay in detecting anti-HBs. The study set included 650 frozen repository samples (the majority of which were purchased from commercial vendors) from the following populations:

- patients with chronic hepatitis B infection (HBsAg positive for greater than 6 months) – 111 frozen repository samples;
- patients with serologically diagnosed hepatitis B infection (acute, chronic, asymptomatic, convalescent, etc.) – 82 frozen repository samples;
- patients sent to the laboratory for hepatitis B testing – 100 frozen repository samples;
- a general hospital patient population – 357 frozen repository samples.

The specimens represented Midwestern (2%), Southeastern (25%), Western (13%), and Northeastern US (2%), outside of the US (1%), and unspecified (57%). The group was 44% (287/650) female, 42% (270/650) male, and 14% (93/650) unspecified. Approximately 13% (84/650) were Caucasian, 4% (27/650) were African American, <1% (5/650) were Hispanic, <1% (3/650) were Asian, and 82% (531/650) were unspecified. The ages ranged from 5 to 98 years old, with 131 specimens not specified.

The study (testing) sites were blinded to the previous specimen categorization. All testing was performed by the manual ETI-AB-AUK PLUS procedure. Specimens were characterized by testing with six HBV serological markers (HBsAg, HBeAg, IgM anti-HBc, total anti-HBc, anti-HBe, anti-HBs) using FDA-licensed or approved assays. Testing with these assays followed the FDA-licensed or approved procedure with the exception of the HBsAg assay at two of the three sites. At these sites, the majority of specimens that were initially HBsAg-positive were repeated in duplicate, however the repeatedly reactive specimens were not confirmed by the licensed HBsAg confirmation assay at the

two sites. Therefore, true HBsAg result was determined in one of three ways: 1) confirmed by reference assay neutralization during clinical trials, 2) based on a statement by the attending physician that HBsAg was positive for greater than 6 months, or 3) information provided by the vendor regarding confirmatory testing performed at their location or by the material source facility.

Results by Specimen Classification:

After study completion all samples were assigned a specimen classification based on the patterns of the six HBV serological markers established by the reference assays. Based on these serological marker patterns, the samples were categorized into the HBV classifications described in the table below. There were 35 unique HBV marker patterns observed in the DiaSorin ETI-AB-AUK PLUS retrospective clinical studies.

Characterization Based On Single Point Specimen	HBsAg	HBeAg	IgM anti-HBc	Total anti-HBc	anti-HBe	anti-HBs	n
Acute Infection	+	+	+ or I*	+	-	-	52
	+	-	+ or I	+	+	-	4
	+	-	-	-	-	-	2
	+	+	-	-	-	-	2
Chronic Infection	+	-	-	+	+	-	82
	+	+	-	+	-	-	21
	+	-	-	+	- or I	-	23
	+	+	+	+	-	+	4
	+	+	- or I	+	-	+	2
	+	-	-	+	+	+	2
	+	+	-	+	+ or I	+	2
	+	+	+	+	+	+	1
	+	+	-	+	+	-	1
	+	-	-	+	-	+	1
Recovery	-	-	-	+	+ or I	+	40
	-	-	-	+	+	-	6
	-	-	+	+	+	-	2
	-	-	+ or I	+	+	+	2
Past Infection	-	- or I	-	+	-	+	12
	-	-	-	+	-	-	9
HBV Vaccine Response	-	-	-	-	-	+	20
Not Previously Infected with HBV	-	-	-	-	-	-	343
Uninterpretable	-	+ or I	-	-	-	-	13
	-	+	-	+	-	+	2
	-	+	-	+	+	+	1
	-	I	-	+	-	-	1

* I = indeterminate result

Based on the above classifications the ETI-AB-AUK PLUS anti-HBs results for the retrospective samples were compared to a reference assay's anti-HBs results. The reference assay was not calibrated to the WHO standard of 10 mIU/mL established for immunity. The following table shows this comparison and percent agreement with 95% confidence intervals with the reference anti-HBs results. DiaSorin equivocal results were not repeated in this study.

Retrospective Samples Comparison

Reference Serology Classification	Reference Assay - ETI-AB-AUK PLUS		Reference Assay + ETI-AB-AUK PLUS			TOTAL
	-	+	-	+	E*	
Acute infection	60	0	0	0	0	60
Chronic infection	127	0	8	3	1	139
Recovery	7	1	6	35	1	50
Past infection	9	0	4	8	0	21
HBV vaccine response	0	0	5	15	0	20
Not previously infected	341	2	0	0	0	343
Uninterpretable	14	0	2	1	0	17
TOTAL	558	3	25	62	2	650

* E = equivocal result

Acute Infection

Positive agreement with reference assay results = NA (0/0)
95% CI = NA
Negative agreement with reference assay results = 100% (60/60)
95% CI = 90.0 – 100.0%

Chronic Infection

Positive agreement with reference assay results = 25.0% (3/12)
95% CI = 5.5 – 57.2%
Negative agreement with reference assay results = 100% (127/127)
95% CI = 97.1 – 100.0%

Recovery

Positive agreement with reference assay results = 83.3% (35/42)
95% CI = 68.6% – 93.0%
Negative agreement with reference assay results = 87.5% (7/8)
95% CI = 47.3 – 99.7%

Past Infection

Positive agreement with reference assay results = 66.7% (8/12)
95% CI = 34.9 – 90.1%
Negative agreement with reference assay results = 100% (9/9)
95% CI = 66.4 – 100.0%

HBV Vaccine Response

Positive agreement with reference assay results = 75.0% (15/20)
95% CI = 50.9 to 91.3%
Negative agreement with reference assay results = NA (0/0)
95% CI = NA

Not Previously Infected

Positive agreement with reference assay results = NA (0/0)
95% CI = NA
Negative agreement with reference assay results = 99.4% (341/343)
95% CI = 97.9 to 99.9%

Uninterpretable

Positive agreement with reference assay results = 33.3% (1/3)
95% CI = 0.8 to 90.6%
Negative agreement with reference assay results = 100% (14/14)
95% CI = 76.8% - 100.0%

Clinical Performance with Individuals Who Have Received Hepatitis B Vaccine

A retrospective study was conducted to evaluate a total of 59 serum samples from subjects who had received a full course of injections (3) from either *SmithKline-Beecham Biologicals Engerix-B®* HBV vaccine or *Merck & Co., Inc. Recombivax* HB® vaccine (one 90 or one 180 day vaccination regiment). These subjects had no pre-vaccination sample tested to indicate immunity status before vaccination. Results were expressed as "Immune" or "Not Immune" corresponding to a reference assay quantitative result of > 10 mIU/mL or a DiaSorin result ≥ mean absorbance value of the calibrator.

Individuals Who Had Received One 90 Or One 180 Day Vaccination Regiment

DiaSorin Anti-HBs Result	Reference Anti-HBs Result		
	I	NI	Total
I	44	3	47
NI	0	12	12
Grand Total	44	15	59

	% (n)	95% Exact Confidence Interval
Immune % Agreement with Reference Method	100.0 (44/44)	92.0 –100.0%
Not Immune % Agreement with Reference Method	80.0 (12/15)	51.9 – 95.7%

Clinical Performance with Matched Pre- and Post-Vaccination Samples

Pre- and post-vaccination samples from 31 subjects who had received a full course of vaccinations were tested with the DiaSorin ETI-AB-AUK PLUS assay and a reference method. The results are shown in the table below. Results were expressed as "Immune" or "Not Immune" corresponding to a reference assay quantitative result of > 10 mIU/mL for post-vaccination samples or a DiaSorin result ≥ mean absorbance value of the calibrator. Reference assay results for pre-vaccination samples were not quantitated. However, all samples except 1 had absorbance values near the negative control, a sample that is negative for anti-HBs.

Pre-Vaccination Panel

DiaSorin Anti-HBs Result	Reference Anti-HBs Result[a]		
	I[b]	NI[b]	Total
I	0	0	0
NI	1	30	31
Grand Total	1	30	31

[a] Not quantitated [b] I = Immune; NI = Not Immune

	% (n)	95% Exact Confidence Interval
Immune % Agreement with Reference Method	0.0% (0/1)	0.0 –97.5%
Not Immune % Agreement with Reference Method	100.0% (30/30)	88.4 – 100.0%

Post-Vaccination Panel

DiaSorin Anti-HBs Result	Reference Anti-HBs Result		
	I	NI	Total
I	28	0	28
NI	0	2	2
E*	0	1	1
Grand Total	28	3	31

*E = Equivocal, not retested in this study

	% (n)	95% Exact Confidence Interval
Immune % Agreement with Reference Method	100.0% (28/28)	87.7 –100.0%
Not Immune % Agreement with Reference Method	66.7% (2/3)	9.4 – 99.0%

Combined Pre- and Post-Vaccination Panels

DiaSorin Anti-HBs Result	Reference Anti-HBs Result		
	I	NI	Total
I	28	0	28
NI	1	32	33
E*	0	1	1
Grand Total	29	33	62

	% (n)	95% Exact Confidence Interval
Overall Immune % Agreement with Reference Method	96.6% (28/29)	82.2 – 99.9%
Overall Not Immune % Agreement with Reference Method	97.0 (32/33)	84.2 – 99.9%

*E = Equivocal, not retested in this study

Reproducibility

Manual Assay: Intra-assay, inter-assay, inter-lot, and inter-site variability studies were carried out on the ETI-AB-AUK PLUS kit to test the variability within runs, between runs, between days, between kit lots, and between test sites. Variability was measured on a panel of ten sera that included negative, borderline, and positive samples. Three ETI-AB-AUK PLUS kit lots were tested at three independent test sites. Due to the requirement that assay cutoff be established for each plate, reproducibility was evaluated based on specimen absorbance to cutoff ratios (S/CO) rather than absolute absorbance values. The results of that study are tabulated below.

Clinical Site Serum Reproducibility

ID#		# of Tests per Sample	Mean S/CO's	Within-run %CV*	Between-run %CV	Between-lot %CV	Between-day %CV	Between-site %CV	Total
S01	High	108	7.55	7.35	7.62	5.76	1.81	26.33	10.04
S02	High	108	4.03	3.69	6.22	5.08	3.64	21.60	12.67
S03	Low	108	2.26	4.80	6.59	7.57	3.76	44.20	13.29
S04	Equiv	108	1.11	7.98	5.28	7.96	3.95	22.29	13.45
S05	Equiv	108	1.22	7.23	7.20	8.58	3.29	17.56	14.49
S06	Equiv	108	0.81	3.55	7.99	6.56	3.09	26.25	16.94
S07	Equiv	108	0.54	5.01	6.66	11.54	1.68	31.66	11.38
S08	Neg	108	0.31	10.63	9.87	10.87	4.78	16.26	18.21
S09	Neg	108	0.24	9.69	8.57	14.18	7.87	15.51	24.79
S10	Neg	108	0.03	12.86	25.65	54.94	55.23	112.01	125.64

* %CVs were calculated using specimen absorbance-to-cutoff ratios (S/CO) which normalized the data plate-to-plate

Assay reproducibility using plasma has not been established with samples near the cutoff. If plasma is used, the user should establish appropriate assay reproducibility in accordance with NCCLS EP5-A, Evaluation of Precision Performance of Clinical Chemistry Devices.

Cross-Reactivity

Of the 535 potentially interfering samples, 450 (84%) were negative and 85 (16%) were positive by ETI-AB-AUK PLUS. Among the 85 positive samples, 82 were positive by reference testing. Percent positive was 96% (82/85). Due to the expression of the recombinant HBsAg in yeast, samples containing antibodies to yeast may cause false positive results. In this study, two out of ten (20%) patients with yeast infections gave false positive results. Disease was determined by serological testing, there is no guarantee that the associated antibody was present in the tested material. Interference testing with the described specimens was not performed.

Cross-Reactivity Study Results

GROUP	n	ETI-AB-AUK PLUS Negative or Equivocal Samples	ETI-AB-AUK PLUS Positive Samples	% Positive By Additional Testing
Acute EBV infection	16	10	6	83% (5/6)
Acute CMV infection	20	19	1	100% (1/1)
Acute HSV infection	10	10	0	–
Acute toxoplasmosis	18	16[a]	2	100% (2/2)
Acute parvovirus B19 infection	5	4	1	100% (1/1)
HTLV-I/II infection	50	43	7	100% (7/7)
Syphilis	26	22[b]	4	75% (3/4)
HCV Infection	50	45	5	100% (5/5)
HDV infection	20	20	0	–
HIV infection	50	42	8	100% (8/8)
Acute HAV infection	50	45	5	100% (5/5)
Past HAV infection	50	38	12	92% (11/12)[c]

GROUP	n	ETI-AB-AUK PLUS Negative or Equivocal Samples	ETI-AB-AUK PLUS Positive Samples	% Positive By Additional Testing
Rheumatoid factor (RF) +	40	37	3	100% (3/3)
Autoimmune disease, including SLE	30	29	1	100% (1/1)
Autoimmune hepatitis	5	5	0	–
Myeloma	20	14	6	100% (6/6)
Hypergammaglobulinemia	20	17	3	100% (3/3)
Influenza vaccine	5	4	1	100% (1/1)
Elevated liver enzymes	10	7	3	100% (3/3)
Cirrhosis	20	16	4	100% (4/4)
Alcoholic hepatitis	10	5	5	100% (5/5)
Yeast Infection	10	2	8	75% (6/8)
TOTAL	535	450 (84%)	85 (16%)	96% (82/85)

[a] 1 sample was DiaSorin repeatedly equivocal.
[b] 1 sample was DiaSorin equivocal, not repeated.
[c] 1 sample was total anti-HBc and anti-HBe positive, indicating recovery with Abbott anti-HBs result false negative.

Substances That Do Not Interfere

As recommended by NCCLS Protocol EP7 (33), the ETI-ABAUK PLUS assay was evaluated for interference by testing the following substances. Testing was performed using matched pairs of negative donor serum and negative donor serum spiked with a high-titer anti-HBs sample to obtain a result near the cutoff. None of the compounds at the levels indicated were found to interfere with the clinical interpretation of the assay in serum. No interference was found with bilirubin in plasma (EDTA, heparin or citrate), testing for interference with hemoglobin and triolein was not performed in plasma.

Compound	Concentration	
Bilirubin	0.35 mmol/L	20 mg/dL
Hemoglobin	0.06 mmol/L	100 mg/dL
Triolein	33.9 mmol/L	3000 mg/dL

17. ABBREVIATED TEST PROCEDURE

1. DISPENSE 50 µL INCUBATION BUFFER.
2. DISPENSE 100 µL CALIBRATOR, CONTROLS AND SAMPLES INTO WELLS, LEAVING AN EMPTY WELL FOR THE BLANK.
3. INCUBATE FOR TWO HOURS AT 37°C.
4. ASPIRATE THE LIQUID. WASH THE WELLS REPEATEDLY WITH WORKING WASH BUFFER.

5. DISPENSE 100 µL ENZYME TRACER TO EACH WELL.
6. INCUBATE FOR 60 MINUTES AT 37°C.
7. ASPIRATE THE LIQUID. WASH THE WELLS REPEATEDLY WITH WORKING WASH BUFFER.
8. DISPENSE 100 µL CHROMOGEN/SUBSTRATE TO EACH WELL.
9. INCUBATE FOR 30 MINUTES AT ROOM TEMPERATURE.
10. DISPENSE 100 µL STOP SOLUTION TO EACH WELL.
11. READ THE ABSORBANCE VALUES WITH A PHOTOMETER AT 450/630 nm WITHIN 60 MINUTES.

18. REFERENCES

1. Escobar MR: Chronic viral hepatitis, in Specter S, Lancz GJ (eds): Clinical Virology Manual. New York, Elsevier, 1986, pp 329-348.
2. Lee HS, Vyas GN: Diagnosis of viral hepatitis. Clin Lab Med 1987; 7: 741-757.
3. Hirschman SZ: Hepatitis viruses *and* Viral hepatitis, in Braude AI, Davis CE, Fierer J (eds): Infectious Diseases and Medical Microbiology, ed 2. Philadelphia, WB Saunders, 1986, pp 557-564; 989-995.
4. Szmuness W: Recent advances in the study of the epidemiology of hepatitis B. Am J Pathol 1975; 81 (3): 629-650.
5. Christie AB: Infectious Diseases: epidemiology and clinical practice. London, Churchill Livingstone, 1980, pp 447-518.
6. Morbidity and Mortality Weekly Report. Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, 1995, pp. 43-53.
7. Feitelson MA: Biology of hepatitis B virus variants. Lab Invest 1994; 71 (3): 324.
8. Heermann KH et al: Large surface proteins of hepatitis B virus containing the pre-S sequence. J Virol 1984; 52 (2): 396-402.
9. Norder H et al: Comparison of the amino acid sequences of nine different serotypes of hepatitis B surface antigen and genomic classification of the corresponding hepatitis B virus strain. J Gen Virol 1992; 73: 1201-1208.
10. Gerlich WH et al: Specificity and localization of the hepatitis B virus-associated protein kinase. J Virol 1982; 42 (3): 761-766.
11. Cohen BJ, Richmond JE: Electron microscopy of hepatitis B core antigen syn-thesized in E. coli. Nature 1982; 296 (5858): 677-679.
12. Milich DR, McLachlan A: The nucleocapsid of hepatitis B virus is both a T-cell-independent and a T-cell-dependent antigen. Science 1986; 234 (4782): 1398-1401.
13. Schlicht HJ, Salfeld J, Schaller H: The duck hepatitis B virus pre-C region encodes a signal sequence which is essential for synthesis and secretion of processed core proteins but not for virus formation. J Virol 1987; 61 (12): 3701-3709.
14. Gerlich W, Thomssen R: Terminology, structure and laboratory diagnosis of hepatitis viruses, in McIntyre N et al (eds): Oxford Textbook of Clinical Hepatology. Oxford University Press, 1991, pp. 543-560.

15. Dusheiko G, Hoofnagle JH: Hepatitis B, in McIntyre N et al (eds): Oxford Textbook of Clinical Hepatology. Oxford University Press, 1991, pp. 571-577.

16. Rizzetto M, Lanfranco G, Pagni R, Verme G: The diagnostic approach to chronic hepatitis, in Dobrilla G, Felder M, de Pretis G (eds): Advances in Hepatobiliary and Pancreatic Disease - special clinical topics. Kluwer Academic Publishers, 1995, pp. 3-13.

17. Galanti LM et al: Assay of anti-HBs antibodies using a recombinant antigen and latex particle counting: comparison with five commercial kits. J Virol Meth 1991; 32: 221-231.

18. Jilg W, Schmidt M, Deinhardt F: Vaccination against hepatitis B: comparison of three different vaccination schedules. J Infect Dis 1989; 160: 766-769.

19. Jilg W, Schmidt M, Deinhardt F: Decline of anti-HBs after hepatitis B vaccination and timing of revaccination. Lancet 1990; 335: 173-174.

20. Jilg W, Schmidt M, Deinhardt F: Hepatitis B vaccination: strategy for booster doses in high risk population groups. Prog Hepatitis B Immun 1990; 194: 419-427.

21. Hepatitis B virus: a comprehensive strategy for eliminating transmission in the United States through universal childhood vaccination. Recommendations of the Immunization Practices Advisory Commitee (ACIP) 1991; 40 (RR-13): 1-25.

22. Player VA, White D: Comparison of an ELISA system for the quantification of hepatitis B antibody with an automated and semi-automated system. J Virol Meth 1993; 45: 67-72.

23. Jilg W, Schmidt M, Deinhardt F: Persistence of specific antibodies after hepatitis B vaccination. J Hepatol 1988; 6: 201-207.

24. Bornhak H, Jilg W, Hüdig H, Kaufmann J: Quantitation of anti-HBs in solid phase immunoassays. What influences the results? International Symposium on Viral Hepatitis and Liver Disease, Tokyo, May 10-14, 1993.

25. Bonanni P et al: A new approach of assaying anti-HBs in subjects immunized with B vaccine. J Prev Med Hyg 1991; 32: 51-55.

26. Biosafety in Microbiological and Biomedical Laboratories, Richardson JH, Barkley WE (eds). Atlanta, GA, US Dept of Health and Human Services, Public Health Service, Centers for Disease Control; Bethesda, MD, National Institutes of Health, 3rd ed., 1993. HHS Publication No. (CDC) 93-8395.

27. Occupational Exposure to Bloodborne Pathogens; Final Rule. Federal Register. Part II; Department of Labor, Occupational Safety and Health Administration (OSHA); 29 CFR Part 1910.1030; Friday, December 6, 1991.

28. National Committee for Clinical Laboratory Standards. Protection of laboratory workers from infectious disease transmitted by blood and tissue; Approved Guideline. NCCLS Document M29-A. Villanova, PA; NCCLS: 1997.

29. National Committee for Clinical Laboratory Standards. Procedure for the Handling and Processing of Blood Specimens; Approved Guideline, 2nd ed. NCCLS Document H18-A2. Villanova, PA; NCCLS: 1999.

30. U.S. Public Health Services, HHS 1996. Code of Federal Regulations Title 42 Part 72 - Interstate shipment of etiologic agents. U.S. Government Printing Office, Washington, D.C.

31. Westgard JO, Barry PL: Cost-effective Quality Control: managing the quality and productivity of analytical processes. Washington, D.C., AACC Press, 1986.

32. National Committee for Clinical Laboratory Standards. Internal Quality Control Testing: principles and definition; Approved Guideline. NCCLS Document C24-A. Villanova, PA; NCCLS: 1991.

33. National Committee for Clinical Laboratory Standards. Interference Testing in Clinical Chemistry; Proposed Guideline. NCCLS Document EP7-A. Villanova, PA; NCCLS: 1986.