



# METHODOLOGY SHEET

Vitros Immunodiagnostic Products HBsAg Confirmatory Kit

## HBCon

HBsAg Confirmatory Kit

**CAUTION:** Federal law restricts this device to sale by or on the order of a physician.

### Intended Use

For the qualitative confirmation of hepatitis B surface antigen (HBsAg) in human serum and plasma specimens (heparin, EDTA, and sodium citrate) that have been found to be repeatedly reactive using the Vitros Immunodiagnostic Products HBsAg Reagent Pack and the Vitros Immunodiagnostic Products HBsAg Calibrator with the Vitros ECI Immunodiagnostic System.

- Assay performance characteristics have not been established when the VITROS HBsAg Confirmatory Kit is used in conjunction with 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 testing of newborns.

### WARNING:

- This assay has not been FDA cleared or approved for the screening of blood or plasma donors.

### Summary and Explanation of the Assay

The presence of HBsAg in samples found to be repeatedly reactive in the Vitros HBsAg assay may be confirmed by a neutralization reaction using human antibody to HBsAg (anti-HBs).

A sample that can be neutralized is considered positive for HBsAg.

### Principles of the Procedure

The Vitros HBsAg Confirmatory Kit uses the principle of specific antibody neutralization to confirm the presence of HBsAg. The sample is tested twice: one aliquot is incubated with a neutralizing reagent containing high titer anti-HBs (the Confirmatory Antibody); the second aliquot is incubated with a non-neutralizing control reagent (the Sample Diluent). The Confirmatory Antibody binds to HBsAg in the sample inhibiting its reaction in the Vitros HBsAg assay. This leads to a reduced result compared to that for the non-neutralized control sample. Refer to the Vitros

HBsAg Reagent Pack instructions for use for a description of the principles of that assay.

#### Assay Type

Specific antibody neutralization

#### Assay Time and Temperature

Incubation time: 29 minutes

Time to first result: 37 minutes

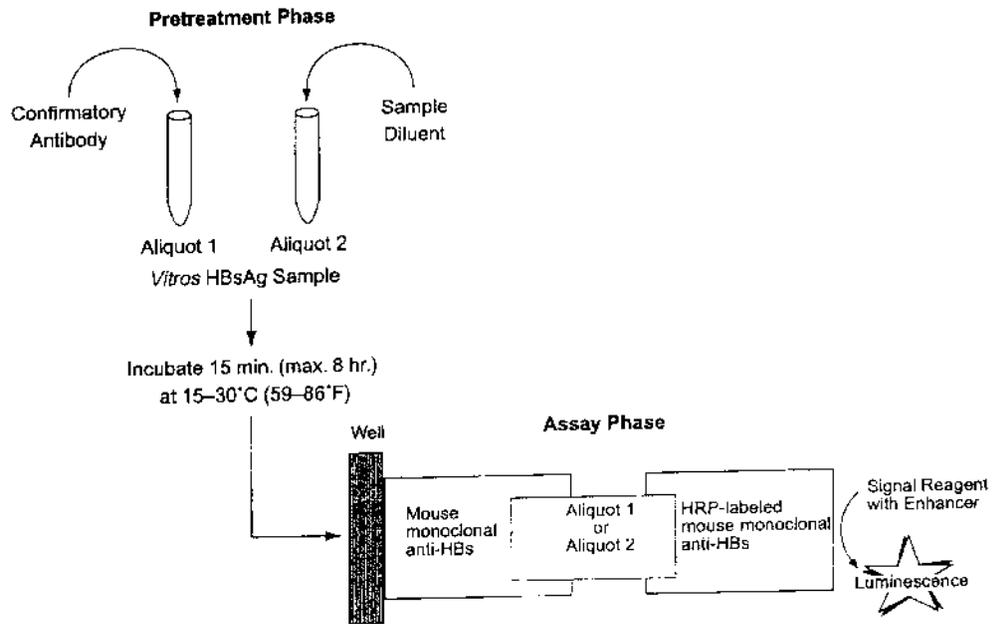
Temperature: 37° C

# HBCon

HBsAg Confirmatory Kit

## Principles of the Procedure (continued)

### Reaction Scheme



## Warnings and Precautions

For in vitro diagnostic use only.

Human blood products provided as components of this pack have been obtained from donors who were tested individually and found to be negative for HBsAg, and for antibodies to human immunodeficiency virus (HIV 1+2) and hepatitis C virus (HCV), using FDA approved methods (enzyme immunoassays, EIA). Treat as if capable of transmitting infection.

Care should be taken when handling material of human origin. All samples should be considered potentially infectious. No test method can offer complete assurance that hepatitis B virus, HCV, HIV 1+2 or other infectious agents are absent. Handling of samples and assay components, their use, storage and disposal should be done at a biological safety level 2 and be in accordance with the procedures defined by the appropriate national biohazard safety guideline or regulation.<sup>1,2</sup>



# HBCon

## HBsAg Confirmatory Kit

### Reagents

#### Kit Contents

- One *Vitros* HBsAg Confirmatory Kit (CAT No. 680 1324) contains:
- 2 vials of freeze-dried Confirmatory Antibody reagent (anti-HBs positive human serum) with antimicrobial agent (1% Kathon w/v). Each binds  $\geq 1 \mu\text{g}$  HBsAg. Reconstitution volume is 1.5 mL.
  - 2 bottles (each 27 mL) of Sample Diluent (human serum, non-reactive for HBsAg, anti-HBs negative) with antimicrobial agent (0.5% bronidox w/v).
  - Protocol card for the *Vitros* HBsAg Confirmatory Kit.

#### Kit Handling

- The Confirmatory Antibody reagent is supplied freeze-dried.
- The Sample Diluent is supplied ready for use.

#### Kit Stability

When stored and handled as specified in the package labeling, the *Vitros* HBsAg Confirmatory Kit is suitable for use until the expiration date printed on the outside of the carton.

#### Kit Storage and Preparation

- Store the unopened kit components refrigerated at 2°–8°C (36°–46°F). Do not freeze.
- Reconstitute the Confirmatory Antibody with 1.5 mL distilled water and mix by inversion. After reconstitution, store refrigerated for up to 8 weeks at 2°–8°C (36°–46°F), or frozen for up to 12 weeks at -20°C (-4°F), with no more than one freeze-thaw cycle.
- After opening the Sample Diluent, store refrigerated for up to 12 weeks at 2°–8°C (36°–46°F), or frozen for up to 12 weeks at -20°C (-4°F), with no more than one freeze-thaw cycle.
- Mix the reconstituted Confirmatory Antibody and Sample Diluent by inversion and bring to 15°–30°C (59°–86°F) before use.

### Specimen Collection and Preparation

#### Patient Preparation

No special patient preparation is necessary.

#### Recommended Specimen Types

Serum, EDTA, heparin, or citrated plasma.

#### Specimens Not Recommended

Turbidity in samples may affect assay results.

#### Special Precautions

Some sample collection devices have been reported to be detrimental to the integrity of certain analytes, and could interfere with some method technologies.<sup>3</sup> Because of the variety of sample collection devices available, it is not possible to issue a definitive statement on the performance of *Vitros* Immunodiagnostic Products when used with these devices. Each user should confirm that the chosen device is used according to the manufacturer's instructions and is compatible with this assay.

#### Specimen Collection and Preparation

- Collect specimens using standard procedures.<sup>4</sup>
- The *Vitros* HBsAg Confirmatory Kit uses a minimum of 400  $\mu\text{L}$  of sample for each determination (200  $\mu\text{L}$  each for the neutralized and non-neutralized test).
- For details on minimum fill volume of sample cups or containers, refer to the *Vitros* ECI Immunodiagnostic System Operator's Guide.
- Samples should be thoroughly separated from all cellular material.

# HBCon

HBsAg Confirmatory Kit

## Specimen Collection and Preparation (continued)

### Handling and Storage Conditions

- Handle specimens in stoppered containers to avoid cross-contamination and evaporation. Use a separate disposable tip if samples are manually pipetted. Avoid splashing, forming an aerosol, or cross-contaminating sample tube stoppers.
- The amount of time samples are on board the system prior to analysis should be limited to avoid evaporation. This time should not exceed two hours. Refer to the *Vitros* ECI System Operator's Guide for further information.

- The National Committee for Clinical Laboratory Standards (NCCLS) provides the following recommendations for storing blood specimens:<sup>5</sup>
  - Store samples at 22°C (72°F) for no longer than 8 hours.
  - If the assay will not be completed within 8 hours, refrigerate the sample at 2°–8°C (36°–46°F).
  - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at or below -20 °C (-4°F).
- Samples are not to be repeatedly frozen and thawed because this can cause analyte deterioration. Samples are to be thawed only once.

## Neutralization Procedure

The neutralization assay is carried out manually, off-board the system.

Samples with a result >500 signal/cutoff (s/c) in the *Vitros* HBsAg assay should be diluted 1 in 151 in Sample Diluent. To dilute sample, add 1.5 mL Sample Diluent to 10µL of sample. Samples with a result ≤500 s/c in the *Vitros* HBsAg assay should be tested without dilution.

The volume of the sample neutralized may be varied. A minimum volume of 200 µL should be used and the ratio of sample: Confirmatory Antibody (or Sample Diluent) must remain constant at 4:1.

1. Take two 200 µL aliquots of HBsAg positive control (C1), or sample (or diluted sample) which is repeatedly reactive for HBsAg and which requires confirmation, and add one aliquot to each of two sample containers, e.g. *Vitros* sample cups.
2. Add 50 µL (i.e. 0.25 x sample volume) of Confirmatory Antibody to one aliquot. Add 50 µL (i.e. 0.25 x sample volume) of Sample Diluent to the other aliquot.
3. The *Vitros* HBsAg Confirmatory Kit requires a minimum of 400 µL sample or control for each determination (200 µL each for the neutralized and non-neutralized test). This does not take into account the minimum fill volume of the chosen sample container.

4. Incubate for at least 15 minutes at 15°–30°C (59°–86°F), up to a maximum of 8 hours (including 2 hours on board the *Vitros* ECI Immunodiagnostic System). If the total incubation time (including on board storage) is to exceed 2 hours, sample containers should be capped to minimize evaporation. If necessary, transfer to a sample cup or container compatible with the *Vitros* ECI System. Refer to the Operators Guide, Section 6, Preparing Samples for details of preparation and treatment of samples.
5. Process samples selecting the **HBCon** assay button from the Sample Programming screen on the *Vitros* ECI System. See *Vitros* HBsAg Reagent Pack instructions for use for details of *Vitros* HBsAg assay calibration.
6. In sample programming, give unique ID's to the neutralized and non-neutralized samples submitted for confirmation to allow clear differentiation on laboratory reports from the previously reactive HBsAg test results.



# HBCon

HBsAg Confirmatory Kit

## Assay Procedure

### Materials Required But Not Provided

The following items are required for use with the *Vitros* HBsAg Confirmatory Kit:

- *Vitros* ECi System
- *Vitros* HBsAg Reagent Pack
- *Vitros* HBsAg Calibrator
- *Vitros* Immunodiagnostic Products Signal Reagent
- *Vitros* Immunodiagnostic Products Universal Wash Reagent
- *Vitros* Immunodiagnostic Products HBsAg Controls
- *Vitros* Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant
- Pipettes
- Distilled water

### Operating Instructions

Refer to the *Vitros* ECi System Operator's Guide for complete instructions on the operation of your *Vitros* ECi System.

## Calibration

### Required Calibrators

*Vitros* HBsAg Calibrator

### Calibrator Preparation, Handling, and Storage

Refer to the calibrator instructions for use for information on the use of *Vitros* HBsAg Calibrator.

### Calibration Procedure

The *Vitros* HBsAg Confirmatory Kit requires that a valid calibration for the *Vitros* HBsAg assay be available on the *Vitros* ECi System prior to performing this assay.

Refer to the *Vitros* HBsAg Reagent Pack and Calibrator instructions for use for information on establishing a valid calibration.

### When to Calibrate

- Calibrate when the lot of *Vitros* HBsAg Reagent Pack and Calibrator changes
- Calibrate every 28 days

The *Vitros* HBsAg assay may also need to be calibrated:

- After specified service procedures have been performed (see the *Vitros* ECi System Operator's Guide)
- If quality control results are consistently outside of the manufacturer's or your acceptable range

For additional information on when to calibrate, refer to the *Vitros* ECi System Operator's Guide.

# HBCon

HBsAg Confirmatory Kit



## Quality Control

### Procedure Recommendations

- Choose control levels that check performance at clinically relevant points. The recommendation is to run a positive control close to the HBsAg decision point (signal/cutoff  $\geq 1.00$ ).
- The *Vitros* HBsAg positive control or equivalent control material should be tested with the *Vitros* HBsAg Confirmatory Kit as neutralized and non-neutralized sample in the same manner as patient specimens.
- The *Vitros* HBsAg positive control should show  $\geq 80\%$  neutralization.
- Controls should be processed with each HBsAg Confirmatory run.
- Refer to the *Vitros* HBsAg Reagent Pack and Calibrator instructions for use for details of quality control of the *Vitros* HBsAg assay.
- If control results fall outside the stated range or outside your established acceptable range, patient results should not be reported. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run.
- For more detailed information on quality control procedures, refer to the *Vitros* ECi System Operator's Guide.
- Refer to *Internal Quality Control Testing: Principles and Definitions* or other published guidelines for general quality control recommendations.<sup>6</sup>
- Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

### Quality Control Material Selection

Choose control material that has a composition similar to or identical with the patient sample matrix being analyzed.<sup>7</sup>

*Vitros* HBsAg Controls are recommended for use with the *Vitros* ECi System. The performance of other commercial control fluids should be evaluated for compatibility with this assay before they are used for quality control.

Appropriate quality control value ranges should be established for all commercially available quality control materials used with the *Vitros* HBsAg assay.

### Quality Control Material Preparation and Storage

Refer to the manufacturer's product literature for preparation, storage, and stability information.



# HBCon

## HBsAg Confirmatory Kit

### Interpretation of Results and Expected Results

#### Interpretation of Results

Results will be generated for the non-neutralized and neutralized samples.

To determine the % neutralization, make the following calculation:

$$\% \text{ Neutralization} = \frac{(\text{result A} - \text{result B}) \times 100\%}{\text{result A}}$$

Where: A = non-neutralized result (s/c)  
B = neutralized result (s/c)

NOTE: In the *Vitros* HBsAg Confirmatory Kit, the neutralized and non-neutralized results are used for the calculation of the % neutralization.

The cutoff value for positive results in the *Vitros* HBsAg Confirmatory Kit is set at 0.80 s/c. Samples are diluted by 20% in the Confirmatory Kit by the addition of the Confirmatory Antibody (neutralized) or Sample Diluent (non-neutralized). A sample which has an initial result in the *Vitros* HBsAg assay at the cutoff value of 1.00 s/c (result classification "reactive") would therefore have a result of 0.80 s/c when re-assayed in the *Vitros* HBsAg Confirmatory Kit.

A sample is considered neutralized if the result of the non-neutralized sample is  $\geq 0.80$  s/c and the % neutralization is  $\geq 50\%$ . A sample that is repeatedly reactive in the *Vitros* HBsAg assay, and which is neutralized in the *Vitros* HBsAg Confirmatory Kit, is considered positive for HBsAg.

If the result of the non-neutralized sample is  $< 0.80$  s/c the sample should be retested in the *Vitros* HBsAg Confirmatory Kit.

Samples for which the result of the non-neutralized sample is  $\geq 0.80$  s/c and the % neutralization is  $< 50\%$  should be diluted in sample diluent to 1 in 151 and retested.

For samples which have been diluted and for which the % neutralization is  $< 50\%$  the following interpretations are made:

- If the result of the non-neutralized sample is  $> 100$  s/c, even when assayed diluted 1 in 151, the sample is likely to contain high levels of HBsAg and should be further diluted in sample diluent to 1 in 1500 or more and retested.
- If the result of the non-neutralized sample is  $\leq 100$  s/c, the sample is considered negative for HBsAg.

The following tables show a summary of the interpretation of results and actions to be taken:

All samples		
Non-neutralized result (s/c)	% Neutralization	Confirmatory Test Result Classification or Action to be taken
$\geq 0.80$	$\geq 50\%$	Confirmed (HBsAg positive)
$\geq 0.80$	$< 50\%$	Dilute further and retest
$< 0.80$	Any value	Retest

Samples after dilution and retest		
Non-neutralized result (s/c)	% Neutralization	Confirmatory Test Result Classification or Action to be taken
$\geq 0.80$	$\geq 50\%$	Confirmed (HBsAg positive)
$> 100$	$< 50\%$	Dilute further and retest
$\leq 100$	$< 50\%$	Not confirmed (HBsAg negative)

# HBCon

## HBsAg Confirmatory Kit

### Limitations of the Procedure

- Heterophilic, e.g. human anti-mouse, antibodies in the serum or plasma of certain individuals are known to cause interference with immunoassays.<sup>8</sup> These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products.
- Individuals recently vaccinated for hepatitis B may give a transient positive result for HBsAg because of its presence in the vaccine.<sup>9</sup>
- HBsAg results should only be used and interpreted in the context of the overall clinical picture. A negative or false reactive test result does not exclude the possibility of exposure to or infection with hepatitis B virus. Levels of HBsAg may be undetectable both in early infection and late after infection. In rare cases HBsAg tests do not detect certain HBV mutant strains.<sup>10</sup>

### Performance Characteristics

#### Vitros HBsAg Confirmatory Kit Performance Among Known HBsAg Reference Assay Positive Samples from Various Sources

The *Vitros* HBsAg Confirmatory Kit was evaluated using reference HBsAg positive samples from various sources. All samples were reactive by the reference HBsAg assay and confirmed positive by neutralization using the reference HBsAg confirmatory kit.

The performance of the *Vitros* HBsAg Confirmatory Kit was evaluated among three groups of samples:

- Prospectively collected samples from individuals with signs or symptoms of hepatitis or who are at high risk for HBV infection.
- Archived commercial samples historically HBsAg reactive by the reference HBsAg assay and confirmed by neutralization using the reference HBsAg confirmatory kit.
- HBsAg positive samples from pregnant women, archived samples from dialysis patients, and archived samples from acute or chronic HBV infections.

The following two tables summarize the performance of the *Vitros* HBsAg Confirmatory Kit on samples from the prospective population where *Vitros* HBsAg results were repeatedly reactive ( $s/c \geq 1.00$  and  $\leq 5.00$ ) or HBsAg positive ( $s/c > 5.00$ ).

#### Vitros HBsAg Confirmatory Testing in Repeatedly Reactive Samples with Two of Three Initial and Repeat *Vitros* HBsAg S/C Results $\geq 1.00$ and $\leq 5.00$

Disease Classification	Final Reference HBsAg Assay Result*				Total
	+		-†		
	Vitros HBsAg Confirmatory Result				
	+	NT‡	+	-	
Acute Infection	0	1	0	0	1
Chronic Infection	1	0	0	0	1
Early Recovery	0	0	0	1	1
Not Previously Infected with HBV	0	0	2	1	3
<b>Grand Total</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>2</b>	<b>6</b>

\* Final reference HBsAg assay result is based on the initial test result, and confirmatory testing of repeatedly reactive samples.

† Reference HBsAg initial screening was negative; confirmatory testing was not performed.

‡ Specimen was repeatedly reactive with the *Vitros* HBsAg assay; *Vitros* HBsAg confirmatory testing was not performed.



# HBCon

HBsAg Confirmatory Kit

## Performance Characteristics (continued)

*Vitros* HBsAg Confirmatory Testing in Samples with an Initial or Two of Three Repeat *Vitros* HBsAg S/C Results > 5.00

Disease Classification	Final Reference HBsAg Assay Result*			Total
	+	-†		
	Vitros HBsAg Confirmatory Result			
	+	+	-	
Acute Infection	3	0	0	3
Chronic Infection	2	0	0	2
Uninterpretable	1	0	0	1
Not Previously Infected with HBV	0	1	1	2
<b>Grand Total</b>	<b>6</b>	<b>1</b>	<b>1</b>	<b>8</b>

\* Final reference HBsAg assay result is based on the initial test result, and confirmatory testing of repeatedly reactive samples.

† Reference HBsAg initial screening was negative; confirmatory testing was not performed.

The table below summarizes the samples evaluated from all sources (prospective and archived) using the *Vitros* HBsAg Confirmatory Kit. An HBsAg positive result by neutralization was obtained in 82 of 82 (100%) samples using the *Vitros* HBsAg Confirmatory Kit on HBsAg reference assay reactive and confirmed positive samples.

Sample Sources	N	Reference HBsAg Reactive and Confirmed Positive by Neutralization	<i>Vitros</i> HBsAg Confirmatory Kit Confirmed Positive by Neutralization
Individuals with signs or symptoms of hepatitis or at high risk for HBV infection	7	7	7
Known HBsAg positive archived samples	48	48	48
Individuals with early acute infection	21	21	21
Individuals with clinically diagnosed chronic infection	1	1	1
Pregnant women	5	5	5
<b>Total</b>	<b>82</b>	<b>82</b>	<b>82 (100%)</b>

# HBCon

## HBsAg Confirmatory Kit

### Performance Characteristics (continued)

The table below summarizes the distribution of *Vitros* HBsAg results and corresponding *Vitros* HBsAg Confirmatory Kit results among the combined 82 samples obtained from the various sources listed above.

<i>Vitros</i> HBsAg Assay Result (s/c)	N	Number (%) Confirmed in the <i>Vitros</i> HBsAg Confirmatory Kit
≥ 0.90 and < 5.00	11	11 (100)
≥ 5.00 and < 10.0	6	6 (100)
≥ 10.0 and < 20.0	10	10 (100)
≥ 20.0 and < 100	14	14 (100)
≥ 100 and < 500	9	9 (100)
≥ 500	32	32 (100)
<b>Total</b>	<b>82</b>	<b>82 (100)</b>

### Substances that do not Interfere

As recommended by NCCLS Protocol EP7<sup>11</sup>, the *Vitros* HBsAg Confirmatory Kit was evaluated for interference by testing the following substances. Testing was performed using two lots of reagent. None of the levels tested were found to interfere with the clinical interpretation of the assay.

Compound	Compound Concentration	
Bilirubin	0.35 mmol/L	20 mg/dL
Hemoglobin	0.31 mmol/L	500 mg/dL
Triglycerin	33.9 mmol/L	3000 mg/dL

**HBCon****HBsAg Confirmatory Kit****References**

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Intended for Use in the United States

# HBCon

HBsAg Confirmatory Kit



When this Methodology Sheet is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

Signature

Obsolete Date



Manufactured by  
Ortho-Clinical Diagnostics, Amersham, UK.

Distributed in the US by  
Ortho-Clinical Diagnostics, Inc.  
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# Vitros Immunodiagnostic Products

## Anti-HBs Calibrators

JFC206/50.0

**CAUTION:** Federal law restricts this device to sale by or on the order of a physician.

### Intended Use

For use in the calibration of the *Vitros* Immunodiagnostic System for the qualitative *in vitro* determination of total antibody to hepatitis B surface antigen (anti-HBs) in human serum using *Vitros* Anti-HBs Reagent Packs. The *Vitros* Anti-HBs Calibrators have been validated for use only on the *Vitros* System with the *Vitros* Immunodiagnostic Products Anti-HBs Reagent Pack. Refer to the *Vitros* Anti-HBs Reagent Pack instructions for use for further details. The *Vitros* Anti-HBs Calibrators are traceable to the WHO First International Reference Preparation for Antibody to HBsAg (1977).

### Principles of Procedure

Calibration is lot specific; reagent packs and calibrators are linked by lot number. A Master Calibration is established for each new reagent lot by performing multiple assays on a number of *Vitros* Systems. Values for the linked lot of calibrators are determined from the Master Calibration. These values, and the data which enables a *Vitros* System to reconstruct the Master Calibration are encoded on the lot calibration card.

Scanning the lot calibration card loads the encoded data onto the *Vitros* System. When the calibrators are processed the signal expected for each calibrator is compared against the actual signal obtained. The Master Calibration is then rescaled to reflect the differences between the actual and expected signals. The validity of this calibration is assessed against a range of quality parameters, if acceptable it is stored for use with any reagent pack of that lot. The quality of calibration cannot be completely described by a single parameter. The calibration report must be used in conjunction with verifier<sup>2</sup> values or control ranges to determine the validity of the calibration. Recalibration is required after a pre-determined calibration interval (refer to the *Vitros* Anti-HBs Reagent Pack calibration instructions) or when a different reagent lot is loaded.

### Warnings and Precautions

For *In Vitro* Diagnostic Use Only

#### Warning - Potentially Infectious Material

Human blood products provided as components of this pack have been obtained from donors who were tested individually and found to be negative for hepatitis B surface antigen, and for antibodies to human immunodeficiency virus (HIV 1+2) and hepatitis C virus (HCV), using FDA approved methods (enzyme immunoassays).

Care should be taken when handling material of human origin. All samples should be considered potentially infectious. No test method can offer complete assurance that hepatitis B virus, HCV, HIV 1+2 or other infectious agents are absent. Handling of samples and assay components, their use, storage and disposal should be done at a biological safety level 2 and be in accordance with the procedures defined by the appropriate biohazard safety guideline or regulation.<sup>2, 3</sup>

### Materials Provided

- 1 set of Anti-HBs Calibrators 1, 2 and 3 (2 mL, recalcified human plasma with antimicrobial agent, Brunidox 1.0%, nominal results of 0, 3, 25, values encoded on the lot calibration card).
- Lot calibration card.
- Protocol card.
- 24 calibrator bar code labels (8 for each calibrator)

### Reagent Preparation and Storage

Anti-HBs Calibrators are supplied ready for use. Store unopened at 2-8 °C (36-46 °F). Do not use beyond the expiration date. After opening, store for up to 13 weeks at 2-8 °C (36-46 °F) or 13 weeks at -20 °C (-4 °F) (with no more than 1 freeze-thaw cycle).

### Quality Control and Procedural Notes

- Use only with reagent packs of the same lot number. Mix thoroughly by inversion and bring to 15-30 °C (59-86 °F) before use. Each pack contains sufficient volume for a minimum of 6 determinations of each calibrator.
- Evaporation will occur when calibrators are stored open on board the *Vitros* System, refer to the Operators Guide, Chapter 6, Preparing Samples Return to 2-8 °C (36-46 °F) as soon as possible after use, or load only sufficient volume for a single determination. Calibrators may be aliquoted into appropriate alternative stoppered containers, which may be bar coded with the labels provided.

### Procedure

For further information refer to the *Vitros* Anti-HBs Reagent Pack instructions for use. For detailed instructions on calibration refer to the *Vitros* Immunodiagnostic System Operator's Guide, Chapter 5, Performing Calibration.

### References

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Made by:

Ortho-Clinical Diagnostics Amersham UK

Distributors include:

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CFM.C206/Cat No. 680 1320



# METHODOLOGY SHEET

## HBsAg

Vitros Immunodiagnostic Products HBsAg Reagent Pack

**CAUTION:** Federal law restricts this device to sale by or on the order of a physician.

### Intended Use

For the in vitro qualitative detection of hepatitis B surface antigen (HBsAg) in human serum and plasma (heparin, EDTA, and sodium citrate) using the Vitros ECI Immunodiagnostic System.

Assay results, in conjunction with other serological and clinical information, may be used for the laboratory diagnosis of individuals with acute or chronic hepatitis B. In addition, this assay may be used to screen for hepatitis B infection in pregnant women to identify neonates who are at high risk of acquiring HBV during the perinatal period.

### WARNING:

- *This assay has not been FDA cleared or approved for the screening of blood or plasma donors.*
- *Assay performance characteristics have not been established when the VITROS HBsAg assay is used in conjunction with 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 testing of newborns.*

### Summary and Explanation of the Assay

Viral hepatitis is a major public health problem of global importance with an estimated 300 million persistent carriers of hepatitis B virus (HBV) worldwide.<sup>1</sup> Infection with HBV results in a wide spectrum of acute and chronic liver diseases that may lead to cirrhosis and hepatocellular carcinoma.<sup>2</sup>

Viral hepatitis is a disease of the liver that is caused by a number of well-characterized viruses including HBV.<sup>3</sup> Transmission of HBV occurs by percutaneous exposure to blood products and contaminated instruments, sexual contact and perinatally from HBV-infected mothers to their unborn child.<sup>4</sup>

HBV infection produces an array of unique antigens and antibody responses that, in general,

follow distinct serological patterns. Hepatitis B surface antigen (HBsAg), derived from the viral envelope, is the first antigen to appear following infection and can be detected serologically as an aid in the laboratory diagnosis of acute HBV infection.

Detection of HBsAg by sensitive enzyme immunoassays was described by Engvall and Perlmann,<sup>5</sup> Engvall, Jonsson and Perlmann,<sup>6</sup> and VanWeemen and Schuur<sup>7</sup> in 1971. Subsequently, solid-phase sandwich enzyme immunoassays for the detection of HBsAg were described by Wisdom,<sup>8</sup> Wolters et al,<sup>9</sup> and Wei et al.<sup>10</sup> Production, characterization and application of monoclonal antibodies<sup>11,12</sup> for the detection of HBsAg have also been described.

### Principles of the Procedure

The Vitros HBsAg assay is performed using the Vitros HBsAg Reagent Pack and Vitros Immunodiagnostic Products HBsAg Calibrator on the Vitros ECI Immunodiagnostic System.

An immunometric technique is used. This involves the simultaneous reaction of HBsAg in the sample with mouse monoclonal anti-HBs antibody coated onto the wells and a horseradish peroxidase (HRP)-labeled mouse monoclonal anti-HBs antibody in the conjugate. Unbound conjugate is removed by washing.

A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells.<sup>13</sup> The HRP in the bound conjugate catalyzes the

oxidation of the luminol derivative, producing light. The electron transfer agent increases the level and duration of the light produced. The light signals are read by the Vitros ECI System. The amount of HRP conjugate bound is indicative of the level of HBsAg present in the sample.

#### Assay Type

Immunometric assay

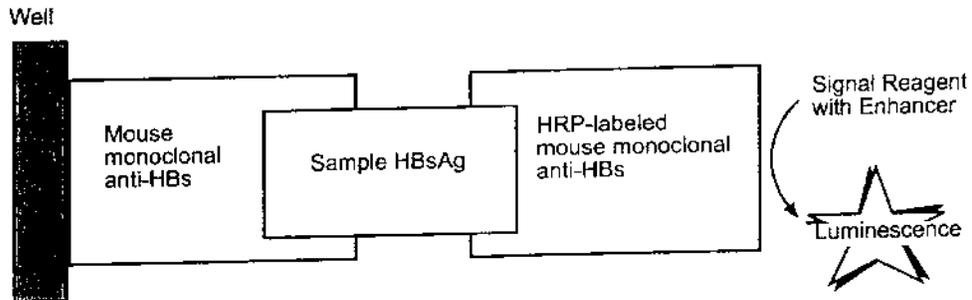
#### Assay Time and Temperature

Incubation time: 29 minutes  
Time to first result: 37 minutes  
Temperature: 37° C

# HBsAg

## Principles of the Procedure (continued)

### Reaction Scheme



## Warnings and Precautions

For in vitro diagnostic use only.

Human blood products provided as components of this pack have been obtained from donors who were tested individually and found to be negative for HBsAg, and for antibodies to human immunodeficiency virus (HIV 1+2) and hepatitis C virus (HCV), using FDA approved methods (enzyme immunoassays, EIA). The *Vitros* HBsAg Calibrator contains human HBsAg purified from donors who were tested individually and found to be negative for antibodies to HIV 1+2 and HCV (using EIA). The purified HBsAg has been heat

inactivated (10 hours at 60 °C). Treat as if capable of transmitting infection.

Care should be taken when handling material of human origin. All samples should be considered potentially infectious. No test method can offer complete assurance that hepatitis B virus, HCV, HIV 1+2 or other infectious agents are absent. Handling of samples and assay components, their use, storage and disposal should be done at a biological safety level 2 and be in accordance with the procedures defined by the appropriate biohazard safety guideline or regulation.<sup>14,15</sup>

## Reagents

### Reagent Pack Contents

One *Vitros* HBsAg Reagent Pack; 100 tests (CAT No. 680 1322) contains:

- 100 coated wells (mouse monoclonal anti-HBs (directed to the "a" region determinant), coated at 1 µg/well)
- 9.0 mL conjugate reagent (HRP- mouse monoclonal anti-HBs, 0.9 µg/mL) in buffer with bovine serum albumin, goat serum, and antimicrobial agent (Kathon 1% w/v)
- 11.5 mL assay reagent with human serum, newborn calf serum, mouse serum, and antimicrobial agent (Kathon 1% w/v)

### Reagent Pack Handling

- The reagent pack is supplied ready for use.
- Reagent packs do not need mixing.
- Avoid agitation, which may cause foaming or the formation of bubbles.

### Reagent Pack Stability

When stored and handled as specified in the package labeling, the *Vitros* HBsAg Reagent Pack is suitable for use until the expiration date printed on the outside of the carton.

### Reagent Pack Storage and Preparation

- Store the unopened reagent pack refrigerated at 2°–8°C (36°–46°F). Do not freeze.
- Load reagent packs directly from refrigerated storage to minimize condensation.
- Use opened reagent packs within 8 weeks.
- Store opened reagent packs in the *Vitros* ECI System reagent supply, or refrigerated at 2°–8°C (36°–46°F) in a sealed reagent pack storage box that contains dry desiccant.
- Exposure of Reagent Pack and Calibrator to temperatures > 30°C (86°F) for extended periods of time may affect assay performance.



# HBsAg

## Specimen Collection and Preparation

### Patient Preparation

No special patient preparation is necessary.

### Recommended Specimen Types

Serum, EDTA, heparin, or citrated plasma.

Heparin and citrate have been shown to lower the signal/cutoff (s/c) values in some HBsAg reactive samples. High negative results (0.80–0.99 s/c) obtained on samples collected with these anticoagulants should be interpreted accordingly. Supplemental tests may be required. Follow manufacturer's instructions for using plasma collection containers with anticoagulants.

### Specimens Not Recommended

Turbidity in samples may affect assay results.

### Special Precautions

Some sample collection devices have been reported to be detrimental to the integrity of certain analytes, and could interfere with some method technologies.<sup>16</sup> Because of the variety of sample collection devices available, it is not possible to issue a definitive statement on the performance of *Vitros* Immunodiagnostic Products when used with these devices. Each user should confirm that the chosen device is used according to the manufacturer's instructions and is compatible with this assay.

### Specimen Collection and Preparation

- Collect specimens using standard procedures.<sup>17</sup>
- The *Vitros* HBsAg assay uses 80  $\mu$ L of sample for each determination.
- For details on minimum fill volume of sample cups or containers, refer to the *Vitros* ECI Immunodiagnostic System Operator's Guide.
- Mix samples, calibrators, and controls by inversion and bring to 15°–30°C (59°–86°F) before use.
- Samples should be thoroughly separated from all cellular material. Failure to do so may lead to a falsely elevated result.

### Handling and Storage Conditions

- Handle specimens in stoppered containers to avoid cross-contamination and evaporation. Use a separate disposable tip if samples are manually pipetted. Avoid splashing, forming an aerosol, or cross-contaminating sample tube stoppers.
- The amount of time samples are on board the system prior to analysis should be limited to avoid evaporation. This time should not exceed two hours. Refer to the *Vitros* ECI System Operator's Guide for further information.
- The National Committee for Clinical Laboratory Standards (NCCLS) provides the following recommendations for storing blood specimens:<sup>18</sup>
  - Store samples at 22°C (72°F) for no longer than 8 hours.
  - If the assay will not be completed within 8 hours, refrigerate the sample at 2°–8°C (36°–46°F).
  - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at or below -20°C (-4°F).
- Samples are not to be repeatedly frozen and thawed because this can cause analyte deterioration. Samples are to be thawed only once.

# HBsAg

## Assay Procedure

### Materials Required But Not Provided

The following items are required to perform the *Vitros* HBsAg assay:

- *Vitros* ECi System
- *Vitros* HBsAg Calibrator
- *Vitros* Immunodiagnostic Products Signal Reagent
- *Vitros* Immunodiagnostic Products Universal Wash Reagent
- Quality control materials, such as *Vitros* Immunodiagnostic Products HBsAg Controls
- *Vitros* Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant

### Operating Instructions

Refer to the *Vitros* ECi System Operator's Guide for complete instructions on the operation of your *Vitros* ECi System.

## Calibration

### Required Calibrators

*Vitros* HBsAg Calibrator

### Calibrator Preparation, Handling, and Storage

Refer to the calibrator instructions for use for information on the use of *Vitros* HBsAg Calibrator.

### Calibration Procedure

- Calibration must be performed using a calibrator of the same lot number as the reagent pack.
- Refer to the *Vitros* ECi System Operator's Guide for detailed instructions on how to calibrate.

### When to Calibrate

- Calibrate when the lot of reagent pack and calibrator changes
- Calibrate every 28 days

The *Vitros* HBsAg assay may also need to be calibrated:

- After specified service procedures have been performed (see the *Vitros* ECi System Operator's Guide)
- If quality control results are consistently outside of the manufacturer's or your acceptable range

For additional information on when to calibrate, refer to the *Vitros* ECi System Operator's Guide.



# HBsAg

## Quality Control

### Procedure Recommendations

- Choose control levels that check performance at clinically relevant points. The recommendation is to run a negative control and a positive control close to the HBsAg decision point (signal/cutoff  $\geq 1.00$ ).
- To verify system performance, analyze control materials:
  - After calibration
  - At least once every 24 hours
  - After specified service procedures or maintenance to critical parts or subsystems that might influence performance of the assay (see the *Vitros* ECI System Operator's Guide)
- Analyze quality control materials in the same manner as patient specimens.
- If control results fall outside the stated range or outside your established acceptable range, patient results should not be reported. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run.
- For more detailed information on quality control procedures, refer to the *Vitros* ECI System Operator's Guide.
- Refer to *Internal Quality Control Testing: Principles and Definitions* or other published guidelines for general quality control recommendations.<sup>19</sup>
- Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

### Quality Control Material Selection

Choose control material that has a composition similar to or identical with the patient sample matrix being analyzed.<sup>20</sup>

*Vitros* HBsAg Controls are recommended for use with the *Vitros* ECI System. The performance of other commercial control fluids should be evaluated for compatibility with this assay before they are used for quality control.

Appropriate quality control value ranges should be established for all commercially available quality control materials used with the *Vitros* HBsAg assay.

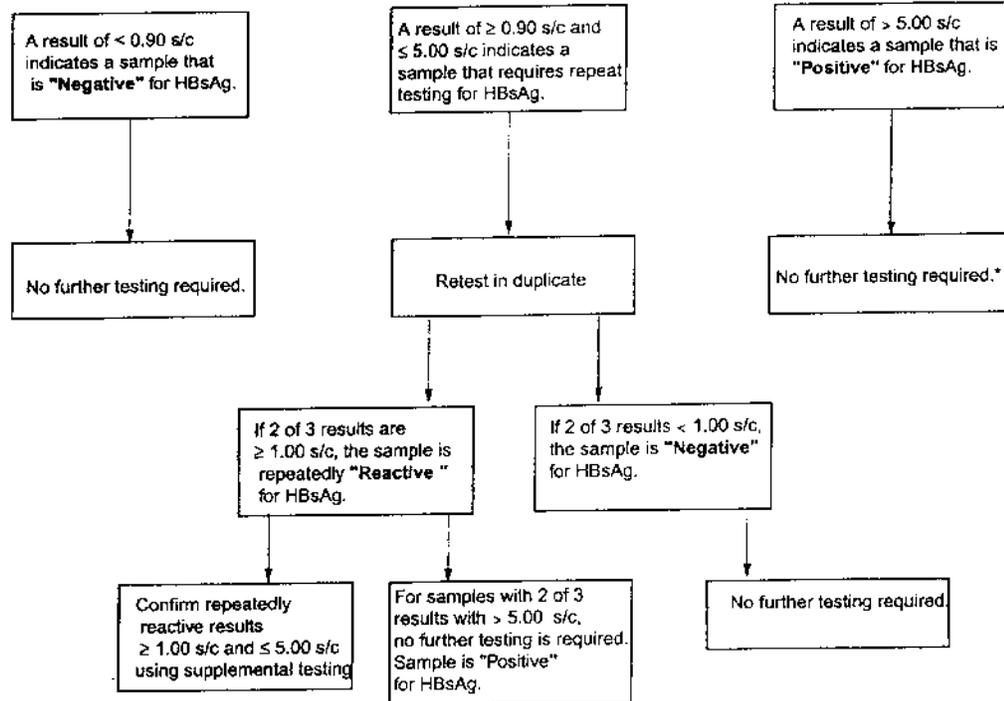
### Quality Control Material Preparation and Storage

Refer to the manufacturer's product literature for preparation, storage, and stability information.

# HBsAg

## Interpretation of Results and Expected Results

### Testing Algorithm



### Interpretation of Results

Vitros HBsAg Assay Result (s/c)	Conclusion from Testing Algorithm	Interpretation
< 1.00	Negative	Specimen is presumed to be negative for HBsAg.
$\ge 1.00$ and $\le 5.00$	Reactive	Specimen is reactive for HBsAg. If a repeat reactive result is confirmed by supplemental tests, such as the Vitros Immunodiagnostic Products HBsAg Confirmatory Kit, the specimen is positive for HBsAg.
> 5.00	Positive	Specimen is positive for HBsAg. *

\* In instances where HBsAg is used as a stand alone assay (for example in pregnant women being screened to identify neonates who are at risk for acquiring HBV during the perinatal period), it is suggested that supplemental testing such as the Vitros HBsAg Confirmatory Kit be used to confirm the result.



# HBsAg

## Interpretation of Results and Expected Results (continued)

- Results obtained with the *Vitros* HBsAg assay may not be used interchangeably with values obtained with different manufacturers' assay methods.
- The magnitude of a *Vitros* HBsAg assay result cannot be correlated to an endpoint titer.
- The ability of the *Vitros* HBsAg assay to detect HBV mutants has not been determined. Testing using alternative methodologies may be warranted if signs, symptoms, and risk factors are indicative of viral hepatitis and other laboratory tests are nonreactive for the diagnosis of viral hepatitis.
- Heparin and citrate have been shown to lower the signal/cutoff (s/c) values in some HBsAg reactive samples. High negative results (0.80-0.99 s/c) obtained on samples collected with these anticoagulants should be interpreted accordingly. Supplemental tests may be required.

## Expected Results

Approximately 66.1% (1439/2177) of the prospective subjects participating in the *Vitros* HBsAg clinical study were asymptomatic and reported no recent or current signs or symptoms of hepatitis. Of these individuals, 20.9% were enrolled in Miami, FL, 46.1% were enrolled in Dallas, TX, 32.6% were enrolled in Chicago, IL, and 0.4% were enrolled in New York, NY. The group was Caucasian (28%), African American (46%) Hispanic (18%), and Asian (4%) with the remaining 4% represented by three or more ethnic groups. The group was 54% male and 46% female and ranged in age from 5 to 96 years. All were at risk for viral hepatitis due to lifestyle, behavior, occupation or known exposure event. The *Vitros* HBsAg assay was positive in 3.3% of the individuals in this group. The percent *Vitros* HBsAg positive results observed in the asymptomatic population at each site was 4.3% at Miami, FL, 3.5% at Dallas, TX, 2.0% at Chicago, IL, and 20% at New York, NY.

The table below summarizes the percent *Vitros* HBsAg positive and negative results by gender and age range.

Age Range	Gender	Vitros HBsAg Result				Total
		+		-		
		n	Percent	n	Percent	
0-9	F	0	NA	0	NA	0
	M	0	NA	1	100	1
10-19	F	2	11	16	89	18
	M	0	NA	11	100	11
20-29	F	1	1	122	99	123
	M	2	2	110	98	112
30-39	F	1	1	149	99	150
	M	18	8	214	92	232
40-49	F	3	2	154	98	157
	M	10	4	235	96	245
50-59	F	3	3	106	97	109
	M	4	4	101	96	105
60-69	F	0	NA	67	100	67
	M	3	7	42	93	45
70-79	F	0	NA	27	100	27
	M	0	NA	26	100	26
80-89	F	0	NA	5	100	5
	M	0	NA	2	100	2
90-100	F	0	NA	0	100	0
	M	0	NA	1	100	1
<b>Total</b>		47		1389		1436*

\* Age was not reported for three subjects.

# HBsAg

## Limitations of the Procedure

- Heterophilic, e.g. human anti-mouse, antibodies in the serum or plasma of certain individuals are known to cause interference with immunoassays.<sup>21</sup> These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products.
- Individuals recently vaccinated for hepatitis B may give a transient positive result for HBsAg because of its presence in the vaccine.<sup>22</sup>
- HBsAg results should only be used and interpreted in the context of the overall clinical picture. A negative test result does not exclude the possibility of exposure to or infection with hepatitis B virus. Levels of HBsAg may be undetectable both in early infection and late after infection. In rare cases HBsAg tests do not detect certain HBV mutant strains.<sup>23</sup>
- The analytical sensitivity of the *Vitros* HBsAg assay was determined to be 0.085 IU/mL World Health Organization (WHO) I<sup>51</sup> International Reference Standard 80/549), 0.030 PEI Units/mL (commercial ad subtype sensitivity panel), and 0.019 PEI Units/mL (commercial ay subtype sensitivity panel).
- Assay performance characteristics have not been established for any other specimen matrices than serum or heparin, EDTA, and sodium citrate anticoagulated plasma.
- The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture.
- It has been shown that up to 498 µg HBsAg/mL does not create a high dose hook effect that will interfere with this assay.

## Performance Characteristics

### Clinical Performance

A multi-center prospective study was conducted to evaluate the clinical performance of the *Vitros* HBsAg assay with individuals with signs or symptoms of hepatitis. Also included were individuals at high risk of HBV infection due to lifestyle, behavior, occupation, or known exposure events. Specimens were obtained from collection sites located in Miami, FL (32%), Dallas, TX (36%), Chicago, IL (30%), and New York, NY (2%).

The group was Caucasian (27%), African American (44%), and Hispanic (22%), with the remaining 7% represented by other ethnic groups. The group was 54% male and 46% female and ranged in age from five to 96 years. The HBV disease classification for each subject was determined by a single point serological assessment using a hepatitis marker profile consisting of reference assays (previously licensed or approved by the FDA) for the detection of HBsAg, HBeAg, anti-HBc, anti-HBc IgM, anti-HBe, and anti-HBs (quantitative). All reference assays used were from a single manufacturer. The reference assays' procedures were adhered to during the clinical laboratory study. Testing of these specimens occurred at hospital associated diagnostic laboratories located in Miami, FL (32%), Dallas, TX (36%), and Port Jefferson, NY (32%). Agreement of the *Vitros* HBsAg assay was assessed relative to the reference HBsAg confirmed results and the specimen classification using serum samples from 2156 of the 2177 subjects enrolled.\*

\* HBV disease classification could not be determined for 21 of the 2177 subjects due to incomplete reference marker profiles (missing one or more results for the panel of six HBV reference markers). These 21 subjects were excluded from the analysis.



# HBsAg

## Performance Characteristics (continued)

### Results by Specimen Classification

The data were analyzed following the assignment of specimen classification based upon the positive (+) / negative (-) patterns for the six HBV serological reference markers. The table below summarizes how these classifications were derived. There were 24 unique reference marker patterns observed in the *Vitros* HBsAg clinical study.

HBsAg*	HBV Reference Markers					HBV Classification
	HBsAg	IgM Anti-HBc	Total Anti-HBc	Anti-HBe	Anti-HBs (≥10 mIU/mL)	
+	+	+	+	+	-	Acute
+	+	+	+	-	-	Acute
+	-	+	+	+	-	Acute
+	-	-	-	-	-	Acute
+	+	-	+	+	-	Chronic
+	+	-	+	-	-	Chronic
+	-	-	+	+	+	Chronic
+	-	-	+	+	-	Chronic
+	-	-	+	-	-	Chronic
-	-	+	+	+	+	Early Recovery
-	-	+	+	+	-	Early Recovery
-	-	+	+	-	+	Early Recovery
-	-	+	+	-	-	Early Recovery
-	-	-	+	+	-	Early Recovery
-	-	-	+	+	+	Recovery
-	-	-	+	-	+	Recovered
-	-	-	+	-	-	Recovered
-	-	-	-	-	+	HBV Vaccine Response
-	-	-	-	-	-	Not Previously Infected
+	-	-	-	-	+	Uninterpretable
-	+	-	+	-	-	Uninterpretable
-	+	-	-	-	+	Uninterpretable
-	+	-	-	-	-	Uninterpretable
-	-	+	-	-	-	Uninterpretable

\* Positive (+) = Reference HBsAg assay reactive and confirmed by neutralization. Negative (-) = Reference HBsAg assay negative or not confirmed by neutralization.

# HBsAg

## Performance Characteristics (continued)

### Comparison of Results

The table below compares the *Vitros* HBsAg results with the reference HBsAg results by specimen classification for the prospective sample population.

Disease Classification	Final Reference HBsAg Assay Result*				Total
	-		+		
	Final <i>Vitros</i> HBsAg Result †				
	-	+	-	+	
Acute Infection	0	0	5†	20	25
Chronic Infection	0	0	2	51	53
Early Recovery	64	1	0	0	65
Recovery	184	0	0	0	184
Recovered	266	2	0	0	268
Uninterpretable	11	0	1	1	13
HBV Vaccine Response	236	0	0	0	236
Not Previously Infected with HBV	1304	8	0	0	1312
<b>Grand Total</b>	<b>2065</b>	<b>11</b>	<b>8</b>	<b>72</b>	<b>2156</b>

\* Final reference HBsAg assay result is based on the initial test result, and confirmatory testing of repeatedly reactive samples.

† Fourteen specimens were reactive with the initial reference HBsAg assay, but did not confirm (3 - recovery, 2 - HBV vaccine response, 9 - not previously infected with HBV). All other specimens were nonreactive for the reference HBsAg assay.

‡ These five samples were positive only for the reference HBsAg assay. Four of these patients had normal ALT levels and showed no clinical signs or symptoms of HBV infection. Their clinical presentation was not consistent with the results of the reference HBsAg assay. Taking the clinical symptoms and normal ALT levels into consideration the negative *Vitros* HBsAg result appears to be more consistent with their clinical presentation. The remaining sample where HBsAg was the only marker detectable also showed elevated ALT levels and was reference assay anti-HCV positive.

The results are broken out further where initial *Vitros* HBsAg results required repeat testing and confirmation ( $s/c \geq 1.00$  and  $\leq 5.00$ ), and positive samples where no further *Vitros* testing was required ( $s/c > 5.00$ ).

### Samples with Two of Three *Vitros* HBsAg Initial and Repeat Test Results $\geq 1.00$ and $\leq 5.00$

Disease Classification	Final Reference HBsAg Assay Result*				Total
	+		-		
	Vitros HBsAg Interpretation Following Vitros HBsAg Confirmatory Testing				
	+	NT†	+	-	
Acute Infection	0	1	0	0	1
Chronic Infection	1	0	0	0	1
Early Recovery	0	0	0	1	1
Not Previously Infected with HBV	0	0	2	1	3
<b>Grand Total</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>2</b>	<b>6</b>

\* Final reference HBsAg assay result is based on the initial test result, and confirmatory testing of repeatedly reactive samples.

† Vitros HBsAg Confirmatory Testing was not performed.



# HBsAg

## Performance Characteristics (continued)

Samples with Initial or Two of Three *Vitros* HBsAg S/C results > 5.00 where *Vitros* HBsAg Confirmatory Testing was not Required

Disease Classification	Final Reference HBsAg Assay Result*		Total
	+	-†	
	Final <i>Vitros</i> HBsAg Result		
	+	+	
Acute Infection	19	0	19
Chronic Infection	50	0	50
Recovered	0	2	2
Uninterpretable	1	0	1
Not Previously Infected with HBV	0	5	5
<b>Grand Total</b>	<b>70</b>	<b>7</b>	<b>77</b>

\* Final reference HBsAg assay result is based on the initial test result, and confirmatory testing of repeatedly reactive samples.  
 † Initial reference HBsAg assay result was negative.

### Percent Agreement

The table below summarizes the percent agreement between the *Vitros* HBsAg assay and the reference HBsAg assay for each specimen classification for the prospective sample population. The table provides the 95% exact confidence interval.

HBV Classification	Positive Percent Agreement	95% Exact Confidence Interval	Negative Percent Agreement	95% Exact Confidence Interval
Overall	90.00 (72/80)	81.24–95.58	99.47 (2065/2076)	99.05–99.74
Acute	80.00 (20/25)	59.30–93.17	NA	NA
Chronic	96.23 (51/53)	87.02–99.54	NA	NA
Early Recovery	NA	NA	98.46 (64/65)	91.72–99.96
Recovery	NA	NA	100.0 (184/184)	98.02–100.0
Recovered	NA	NA	99.25 (266/268)	97.33–99.91
Uninterpretable	50.00 (1/2)	1.26–98.74	100.0 (11/11)	71.51–100.0
HBV Vaccine Response	NA	NA	100.0 (236/236)	98.45–100.0
Not Previously Infected	NA	NA	99.39 (1304/1312)	98.80–99.74

# HBsAg

## Performance Characteristics (continued)

### Percent Agreement of the *Vitros* HBsAg Assay for Subjects with Clinically Diagnosed Acute or Chronic HBV Infection

The performance of the *Vitros* HBsAg assay was further evaluated among archived serum samples from subjects based on documented clinical status or diagnosis of acute (demonstrated seroconversion or HBV reference marker profile) or chronic (HBsAg present for  $\geq 6$  months) HBV infection. Samples were obtained prospectively and from commercial and site archives.

The table below summarizes the percent agreement of the *Vitros* HBsAg assay in samples from subjects with documented acute or chronic HBV infection.

HBV Infection	Number of Samples	Number (%) of <i>Vitros</i> HBsAg Positive Samples	95% Exact Confidence Interval
Acute	38	36* (94.7)	82.25–99.36
Chronic	32	32 (100.0)	88.43–100.0
Total	68	66 (97.1)	89.78–99.64

\* The two acute samples negative by the *Vitros* HBsAg assay were obtained from patients undergoing seroconversion to HBsAg positive status. Occasional discrepancies may occur between different manufacturer's HBsAg assays when testing samples during early seroconversion.

### Seroconversion Panels

Seventeen HBV seroconversion panels were obtained from two commercial vendors (6280–6293 and PHM920–PHM933). These panels were obtained from donors in the early stages of seroconversion from HBsAg negative to HBsAg positive status and contained individual samples in which HBsAg was the only detectable HBV marker, as determined by historical HBV marker data provided by the manufacturers.

The table below presents a summary of the results of testing of the 17 panels with the *Vitros* and reference HBsAg assays.

Panel ID	Days to HBsAg Reactive Result from Initial Draw Date		Difference in Days to HBsAg Reactive Result (Reference – <i>Vitros</i> )
	Reference HBsAg Assay	<i>Vitros</i> HBsAg Assay	
6280	13	13	0
6282	26	19	7
6283	37	29	8
6284	50	50	0
6285	45	45	0
6286	40	36	4
6287	68	68	0
6288	21	14	7
6289	36	36	0
6290	21	21	0
6291	34	27	7
6292	35	35	0
6293	15	23	-8
PHM920	26	26	0
PHM921	0	0	0
PHM922	16	16	0
PHM933	9	7	2



# HBsAg

## Performance Characteristics (continued)

### Clinical Performance in Pregnant Women

Prospectively collected and archived serum samples from healthy, pregnant women at low risk or high risk for exposure to HBV were tested to assess the clinical performance of the *Vitros* HBsAg assay in screening for hepatitis B infection to identify neonates at high risk of acquiring HBV during the perinatal period.<sup>24,25</sup> A total of 545 samples were prospectively collected during the clinical study in several different locations in the US. An additional 199 frozen archived samples were obtained from a commercial vendor. These frozen archived samples had been prospectively collected from women at low risk for viral hepatitis in several different locations in the US. Of the combined 744 prospectively collected and archived samples, 52% were obtained in Florida, 24% were obtained in Texas, 23% were obtained in California and 1% were obtained in Connecticut. Of the combined population, 35.9% were obtained during the first trimester, 34.1% during the second trimester and 30.0% during the third trimester. The following table furnishes a breakdown of the study population.

### Demographic Profiles of Pregnant Women at Low or High Risk for Viral Hepatitis

Risk	Low N (%)	High N (%)	Total N (%)*
TOTAL†	463 (62.2)	281 (37.8)	744 (100)
TRIMESTER			
First	200 (43.2)	67 (23.8)	267 (35.9)
Second	152 (32.8)	102(36.3)	254 (34.1)
Third	111(24.0)	112 (39.9)	223 (30.0)
ETHNICITY‡			
Caucasian	269 (58.1)	17 (6.0)	286 (38.4)
African-American	53 (11.4)	75 (26.7)	128 (17.2)
Hispanic	125 (27.0)	158 (56.2)	283 (38.0)
Asian	7 (1.5)	1 (0.4)	8 (1.1)
Indian	1 (0.2)	4 (1.4)	5 (0.7)
Haitian	1 (0.2)	16 (5.7)	17 (2.3)
Other	7 (1.5)	6 (2.1)	13 (1.7)
Unknown	0 (0)	4 (1.4)	4 (0.5)
AGE (Years)†			
11-30	268 (57.9)	182 (64.8)	450 (60.5)
31-50	194 (41.9)	99 (35.2)	293 (39.4)
Unknown	1 (0.2)	0 (0)	1 (0.1)

\*The total number (N) of subjects at both low and high risk belonging to the variable category in the left hand column; expressed as a percentage (%) of all analyzed subjects (N=744).

† The number (N) of subjects at low or high risk; expressed as a percentage (%) of analyzed subjects (N= 744).

‡ The number (N) of subjects at low or high risk belonging to the variable category in the left hand column; expressed as a percentage (%) of all subjects at low or high risk.

# HBsAg

## Performance Characteristics (continued)

Agreement of the *Vitros* HBsAg assay was assessed relative to the reference HBsAg results using serum samples obtained from a total of 744 women at low risk or high risk for HBV infection. The tables below compare the *Vitros* and reference HBsAg assays among the overall population of pregnant women by risk and trimester.

### *Vitros* and Reference HBsAg Results Among Low Risk Pregnant Women

<i>Vitros</i> HBsAg Result	First Trimester			Second Trimester			Third Trimester		
	Reference HBsAg Result		Total	Reference HBsAg Result		Total	Reference HBsAg Result		Total
	+	-		+	-		+	-	
+	2	0	2	0	0	0	0	0	
-	0	198	198	0	152	152	0	111	
Total	2	198	200	0	152	152	0	111	

### *Vitros* and Reference HBsAg Results Among High Risk Pregnant Women

<i>Vitros</i> HBsAg Result	First Trimester			Second Trimester			Third Trimester		
	Reference HBsAg Result		Total	Reference HBsAg Result		Total	Reference HBsAg Result		Total
	+	-		+	-		+	-	
+	1	0	1	1	0	1	1	1	
-	0	66	66	0	101	101	0	111	
Total	1	66	67	1	101	102	1	112	

## Overall *Vitros* and Reference HBsAg Results Among Pregnant Women

### Frequency of Reactivity of the *Vitros* HBsAg Assay in Pregnant Women

The table below summarizes the frequency of reactivity of the *Vitros* HBsAg assay from a total of 744 women at low risk and high risk for HBV infection.

<i>Vitros</i> HBsAg Result	Reference HBsAg Result		Total N (%)
	+	-	
+	5 (100)	0 (0.0)	5 (0.7)
-	0 (0.0)	739 (100)	739 (99.3)
Total	5 (0.7)	739 (99.3)	744 (100)

### Positive and Negative Percent Agreement of the *Vitros* and Reference HBsAg Assays in Pregnant Women

The table below summarizes the percent agreement between the *Vitros* HBsAg assay and the reference HBsAg assay for this population. The table provides the 95% exact confidence intervals.

Subjects	Positive Percent Agreement	95% Exact Confidence Interval	Negative Percent Agreement	95% Exact Confidence Interval
Pregnant Women	100 (5/5)	47.82–100.0	100.0 (739/739)	99.50–100.0



# HBsAg

## Performance Characteristics (continued)

### Potentially Cross-Reacting Subgroups

The specificity of the *Vitros* HBsAg assay was evaluated by testing 249 samples from 16 potentially cross-reacting subgroups. All of the samples were previously classified as HBsAg negative in other commercially available assays. Samples found to be  $\geq 1.00$  by the *Vitros* HBsAg assay were retested in duplicate. A summary of the results is given in the table below.

Clinical Category	Number Samples Tested	<i>Vitros</i> HBsAg Assay Result < 1.00	<i>Vitros</i> HBsAg Assay Result $\geq 1.00$
Hepatitis A Infection (HAV)	10	9	1*
Hepatitis C Infection (HCV)	10†	10	0
Hepatitis E Infection (HEV)	10	10	0
Non-viral Liver Disease ‡	50	50	0
Autoimmune Diseases (Rheumatoid Arthritis / Systemic Lupus Erythematosus)	60	60	0
Cytomegalovirus (CMV)	7	7	0
Epstein-Barr Virus (EBV)	10	10	0
Herpes Simplex Virus (HSV) (HSV1 and HSV2 not distinguished)	10	10	0
Parvovirus B19 Infection	10	10	0
Rubella	11	11	0
Syphilis	16	16	0
Toxoplasmosis	10	10	0
Human Immunodeficiency Virus (HIV 1/2)	10	10	0
Human T-cell Lymphotropic Virus (HTLV 1/2)	10	10	0
Heterophilic Antibodies (Human anti-mouse)	5	5	0
Recent Influenza Vaccine Recipients	10	10	0
<b>Total Samples Tested</b>	<b>249</b>	<b>248</b>	<b>1</b>

\* Classified as falsely reactive. The sample did not demonstrate the  $\geq 50\%$  neutralization required to be classified as positive.

† Two of these samples were repeatedly aHCV reactive by EIA and strip immunoblot assay (SIA) positive.

‡ Samples were obtained from individuals with elevated liver enzymes, alcoholic liver disease, and liver cancer.

The specificity of the *Vitros* HBsAg assay was evaluated further by testing samples from the following additional potentially cross-reacting sub-groups: HCV (bDNA positive), HIV-1 (PCR positive), and HIV-2 (antibody positive). Additionally, testing was performed on serum samples spiked with *Toxoplasma gondii* tachyzoites (whole and sonicated), *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Samples were tested with and without an additional spike of HBsAg at a s/c of  $2.00 \pm 1.00$ . The spiked and unspiked samples were assayed in triplicate.

Of the samples tested, none of the unspiked samples were observed to be false reactive and none of the HBsAg spiked samples were observed to be false negative in the *Vitros* HBsAg assay.

# HBsAg

## Performance Characteristics (continued)

### Substances that do not Interfere

As recommended by NCCLS Protocol EP7<sup>26</sup>, the *Vitros* HBsAg assay was evaluated for interference by testing the substances listed in the table below. Testing was performed using matched pairs of negative donor serum and negative donor serum spiked with HBsAg at a target s/c of  $2.00 \pm 1.00$  with two lots of reagent. None of the compounds at the levels tested were found to interfere with the clinical interpretation of the assay.

Compound	Compound Concentration	
Bilirubin	0.35 mmol/L	20 mg/dL
Hemoglobin	0.31 mmol/L	500 mg/dL
Triolein	33.9 mmol/L	3000 mg/dL

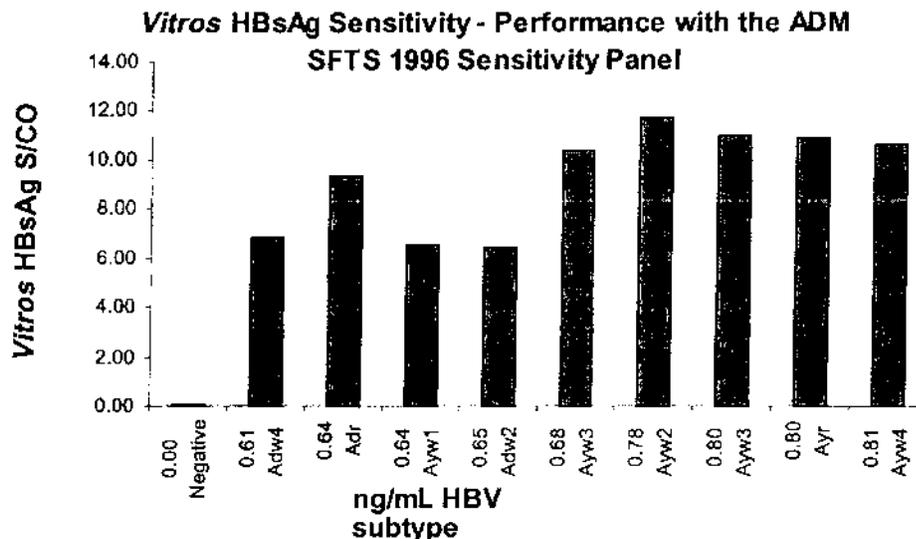
### Analytical Sensitivity

Detection of a known level of HBsAg is a function of the assay's analytical sensitivity (that is, the dependency of the assay result on the HBsAg level) as well as the assay precision. To examine the analytical sensitivity of the *Vitros* HBsAg assay, three standard series with known levels were evaluated. Duplicate determinations of each panel member were obtained using three lots of reagents. The HBsAg level at the assay's cutoff was estimated from a linear regression analysis.

Series	Cutoff (s/c = 1.00)	95% Exact Confidence Interval
WHO 1st International Reference Standard, 80/549	0.085 IU/mL	0.051 - 0.118
Boston Biomedica Inc. HBsAg Sensitivity panel (ad subtype), PHA 805	0.030 PEI* Units/mL	0.007 - 0.054
Boston Biomedica Inc. HBsAg Sensitivity panel (ay subtype), PHA 805	0.019 PEI* Units/mL	0.008 - 0.029

\* Paul Ehrlich Institute

As a demonstration of performance of subtype detection, the *Vitros* HBsAg assay tested with the French ADM SFTS 1996 Sensitivity panel is presented below. The panel contains 20 individual samples representing 10 subtypes with a known, predetermined HBsAg concentration. Single determinations of the panel members with the *Vitros* HBsAg assay were made. The *Vitros* HBsAg assay demonstrated detection of all subtypes in the French ADM SFTS 1996 Sensitivity panel. Ten panel members consisted of the more common ad/ay subtype. Nine panel members represented the less commonly encountered subtypes and are depicted in the graph below.





## HBsAg

**Performance  
Characteristics  
(continued)**
**Precision**

Precision was evaluated on a different *Vitros* ECI System at three external sites using one lot of reagent. With one exception, at least two replicates each of a three member panel were assayed on a single occasion per day on up to 20 different days. The data shown in the table were rounded following all calculations.

	Mean <i>Vitros</i> HBsAg S/C (Ratio)	Within Day*		Between Day†		Total‡		No. Observ.	No. Days
		SD	CV (%)	SD	CV (%)	SD	CV (%)		
Site 1	0.11	0.015	14.5	0.023	21.6	0.027	26.0	42	20
	0.75	0.024	3.2	0.035	4.7	0.043	5.7	39	20
	3.05	0.123	4.0	0.239	7.8	0.269	8.8	42	20
Site 2	0.11	0.014	12.4	0.029	25.7	0.032	28.5	40	20
	0.84	0.019	2.3	0.038	4.6	0.043	5.1	40	20
	3.17	0.047	1.5	0.063	2.0	0.079	2.5	40	20
Site 3	0.13	0.028	21.2	0.015	11.3	0.032	24.0	41	20
	0.84	0.022	2.6	0.051	6.0	0.055	6.6	40	20
	3.10	0.048	1.5	0.084	2.7	0.097	3.1	41	20

\* **Within Day:** Variability of the assay performance from replicate to replicate.

† **Between Day:** Variability of the assay performance from day to day.

‡ **Total:** Variability of the assay performance combining the effects of within day and between day.

Precision was further evaluated incorporating between site and between lot variation. The study was performed at three external sites using three reagent lots. At least five replicates each of a four member panel were assayed on a single occasion per day on six different days. The between site, between lot, and total precision estimates (CV) were derived from a variance component analysis. The data shown in the table were rounded following all calculations.

Mean <i>Vitros</i> HBsAg S/C (Ratio)	Between Site*		Between Lot†		Total‡		No. Observ.
	SD	CV (%)	SD	CV (%)	SD	CV (%)	
0.87	0.058	6.7	0.055	6.4	0.094	10.8	270
0.93	0.057	6.2	0.048	5.1	0.090	9.7	269 **
1.07	0.066	6.2	0.051	4.8	0.106	9.8	270
4.06	0.049	1.2	0.183	4.5	0.249	6.1	270

\* **Between site:** Variability of the assay performance from site to site.

† **Between lot:** Variability of the assay performance from lot to lot, calculated using data across all sites.

‡ **Total:** Variability of the assay performance incorporating factors of site, lot, and day.

\*\*One gross outlier (> 21 SDs from the mean) in 270 observations (0.37%) was excluded from this calculation.

The data presented in both studies are a representation of assay performance and are based on the studies described. Variables such as sample handling and storage, reagent handling and storage, laboratory environment, and system maintenance can affect the reproducibility of assay results.

## HBsAg



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# HBsAg



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Manufactured by  
Ortho-Clinical Diagnostics, Amersham, UK.

Distributed in the US by  
Ortho-Clinical Diagnostics, Inc.  
100 Indigo Creek Drive  
Rochester, NY 14626-5101

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65