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Hepatitis B Virus Core Antigen (Recombinant)
ORTHO® HBc ELISA Test System

Enzyme-Linked Immunosorbent Assay for the Detection of Antibody to Hepatitis B Virus Core Antigen (anti-HBc) in Human Serum or Plasma

REF

933245
933275

Rx ONLY

NAME AND INTENDED USE

ORTHO® HBc ELISA Test System is a qualitative enzyme-linked immunosorbent assay for the detection of total antibody to hepatitis B virus core antigen (anti-HBc) in human serum or plasma indicated for the screening of blood and blood products intended for transfusion and as an aid in the diagnosis of ongoing or previous hepatitis B virus infection. This test is not intended for use on samples of cord blood.

The ORTHO® HBc ELISA Test System is intended for use in a fully manual mode, in semi-automated mode using the ORTHO VERSEIA® Pipetter, or in an automated mode with the ORTHO® Summit System (OSS).

SUMMARY AND EXPLANATION

A variety of serologic markers appear following infection with hepatitis B virus (HBV). The first marker to appear is usually hepatitis B surface antigen (HBsAg). Antibodies to hepatitis B core antigen (anti-HBc) appear next and remain detectable following the clearance of HBsAg and into convalescence. Antibodies to hepatitis B surface antigen (anti-HBs) generally appear a few weeks after the clearance of HBsAg.

The determination of anti-HBc in serum and plasma may be used as an aid to monitor the progress of HBV infection. Anti-HBc appears in virtually all individuals infected with HBV and is an accurate serological marker of recent and past infection.[1,2] During the acute phase of HBV infection, anti-HBc appears shortly after the appearance of HBsAg and persists following HBsAg clearance.[3] In those cases where HBsAg has cleared and the appearance of anti-HBs is delayed, anti-HBc may be the only serological marker of recent HBV infection.[4] Anti-HBc is found in virtually all patients with chronic hepatitis B.[5]

Enzyme-linked immunosorbent assay (ELISA) procedures provide a means for routinely detecting antibodies to specific antigens.[6,7] The detection of total anti-HBc has value considering the association of such antibodies with HBV infections.

PRINCIPLE OF THE PROCEDURE

The assay procedure is a three-stage test carried out in a microwell coated with recombinant-derived hepatitis B core antigen (rHBcAg). The recombinant antigen used in this assay is prepared under U.S. License by Grifols Diagnostic Solutions Inc. under a shared manufacturing arrangement. The recombinant antigen is produced in *Escherichia coli*.

In the first stage, a test specimen is placed directly in the test well containing specimen diluent and incubated for a specified length of time. If anti-HBc is present in the specimen, antigen-antibody complexes will form on the microwell surface. If anti-HBc is not present, complexes will not form and the unbound serum or plasma proteins will be removed in the washing step.

In the second stage, antibody conjugate is added to the test well. The antibody conjugate is a mixture of murine monoclonal antibodies specific for human IgG and IgM. The conjugate will bind specifically to the antibody portion of the antigen-antibody complexes. If antigen-antibody complexes are not present, the unbound conjugate will be removed by washing.

In the third stage, an enzyme detection system composed of *o*-phenylenediamine (OPD) and hydrogen peroxide is added to the test well. If bound conjugate is present, the OPD will be oxidized, resulting in a colored end-product. Sulfuric acid is then added to stop the reaction.

The color intensity depends on the amount of bound conjugate and therefore is a function of the concentration of anti-HBc present in the specimen. The color intensity is measured with a microwell reader.

REAGENTS

Label Abbreviations	480 Test Kit Product Code 933245	2400 Test Kit Product Code 933275	Component Description
HBc	5	25	Hepatitis B Virus Core Antigen (HBcAg) (Recombinant)-Coated Microwell Plates (8 strips of 12 wells each in holder)
CON	1 bottle (125 mL)	5 bottles (125 mL)	Antibody Conjugate: (Murine Monoclonal)—mixture of anti-human IgG and anti-human IgM conjugated to horseradish peroxidase with protein stabilizers Preservative: 1% ProClin® 300
SD	1 bottle (150 mL)	5 bottles (150 mL)	Specimen Diluent—phosphate-buffered saline with bovine protein stabilizers Preservative: 0.1% 2-chloroacetamide
OPD	1 vial (30 tablets)	3 vials (30 tablets)	OPD Tablets—contains *o*-phenylenediamine • 2HCl
SB	1 bottle (190 mL)	5 bottles (190 mL)	Substrate Buffer-G—citrate-phosphate buffer with 0.02% hydrogen peroxide Preservative: 0.1% 2-chloroacetamide
PC	1 vial (1.0 mL)	4 vials (1.0 mL)	Positive Control (Human) Source: Human serum or plasma containing anti-HBc and nonreactive for HBsAg and antibody to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) Preservative: 0.02% thimerosal
NC	2 vials (1.0 mL)	5 vials (1.0 mL)	Negative Control (Human) Source: Human serum nonreactive for anti-HBc, HBsAg, antibody to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) Preservative: 0.02% thimerosal
	21	84	Plate Sealers, disposable

CAUTION: HANDLE AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS.

Store at 2-8°C

FOR IN VITRO DIAGNOSTIC USE

ORTHO® HBc ELISA Test System meets the requirements of the FDA Antibody to Hepatitis B Virus Core Antigen Reference Panel.

PRECAUTIONS

1. **CAUTION:** Some components of this kit contain human blood derivatives. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.[8,9]
2. Wear disposable gloves while handling kit reagents and specimens. Thoroughly wash hands afterward.
3. All specimens should be handled as potentially infectious agents.
4. Handle and dispose of all specimens and materials used to perform the test as if they contain infectious agents. Disposal of all specimens and materials should comply with all local, state and federal waste disposal requirements.[10,11]
5. 4N sulfuric acid (H_2SO_4) (CAS 7664-93-9) is a strong acid. Wipe up spills immediately. Flush the area of the spill with water. If the acid contacts the skin or eyes, flush with copious amounts of water and seek medical attention. Following are the Hazard and Precautionary Requirements.[12] Refer to www.orthoclinical.com for the Safety Data Sheets and for Ortho contact information.

DANGER

Hazard Statements:
Toxic if inhaled.
Causes severe skin burns and eye damage.

Precautionary Statements:
Use only outdoors or in a well-ventilated area.
Do not breathe dust/fume/gas/mist/vapors/spray.
Wash face, hands and any exposed skin thoroughly after handling.
Wear protective gloves/protective clothing/eye protection/face protection.
Immediately call a POISON CENTER or doctor/physician.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Immediately call a POISON CENTER or doctor/physician.
IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
Wash contaminated clothing before reuse.
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
Call a POISON CENTER or doctor/physician.
IF SWALLOWED: Rinse mouth. DO NOT induce vomiting.

6. OPD tablets are light - and moisture-sensitive. Keep vial **tightly** closed when not in use. **Bring vial to room temperature (15 to 30°C) before opening.** The desiccant pouch must be retained in the vial at all times. Do not use tablets which are yellow or broken.
7. Handle OPD tablets with plastic or Teflon®- coated forceps only. Metal forceps may react with tablets and interfere with the test results.

8. Avoid contact of OPD with eyes, skin or clothing, as OPD may cause irritation or an allergic skin reaction. If OPD should come into contact with the skin, wash thoroughly with water. OPD is toxic for inhalation, ingestion, and skin contact. In case of malaise, call a physician.
9. *o*-Phenylenediamine (CAS 95-54-5) dihydrochloride is included in the OPD tablet. Following are the Hazard and Precautionary Requirements.[12] Refer to www.orthoclinical.com for the Safety Data Sheets and for Ortho contact information.

DANGER:

Hazard Statements:
Toxic if swallowed.
Harmful in contact with skin.
Harmful if inhaled.
Causes serious eye irritation.
May cause an allergic skin reaction.
Suspected of causing genetic defects.
Suspected of causing cancer.

Precautionary Statements:
Avoid breathing dust/fume/gas/mist/vapors/spray.
Use only outdoors or in a well-ventilated area.
Wash face, hands and any exposed skin thoroughly after handling.
Do not eat, drink or smoke when using this product.
Obtain special instructions before use.
Do not handle until all safety precautions have been read and understood.
Use personal protective equipment as required.
Contaminated work clothing should not be allowed out of the workplace.
Wear protective gloves/protective clothing/eye protection/face protection.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention.
IF ON SKIN: Wash with plenty of soap and water. Call a POISON CENTER or doctor/physician if you feel unwell. Wash contaminated clothing before reuse. If skin irritation or rash occurs: Get medical advice/attention.
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. Rinse mouth.

10. ProClin® 300 (CAS 55965-84-9) is included as a preservative in the Conjugate. Following are the Hazard and Precautionary Requirements.[12] Refer to www.orthoclinical.com for the Safety Data Sheets and for Ortho contact information.

WARNING:

Hazard Statement:
May cause an allergic skin reaction.

Precautionary Statements:
Avoid breathing dust/fume/gas/mist/vapors/spray.
Contaminated work clothing should not be allowed out of the workplace.
Wear protective gloves.
Wash face, hands and any exposed skin thoroughly after handling.
IF ON SKIN: Wash with plenty of soap and water.
If skin irritation or rash occurs: Get medical advice/attention.
Wash contaminated clothing before reuse.
Take off contaminated clothing and wash before reuse.

11. 2-chloroacetamide (CAS 79-07-2) is included as a preservative in the Specimen Diluent, 20X Wash Buffer Concentrate and Substrate Buffer-G. Following are the Hazard and Precautionary Requirements.[12] Refer to www.orthoclinical.com for the Safety Data Sheets and for Ortho contact information.

WARNING:

Hazard Statements:
May cause an allergic skin reaction.
Suspected of damaging fertility or the unborn child.

Precautionary Statements:
Avoid breathing dust/fume/gas/mist/vapors/spray.
Contaminated work clothing should not be allowed out of the workplace.
Wear protective gloves.
Obtain special instructions before use.
Do not handle until all safety precautions have been read and understood.
Use personal protective equipment as required.
IF ON SKIN: Wash with plenty of soap and water.
If skin irritation or rash occurs: Get medical advice/attention.
Wash contaminated clothing before reuse.

12. Distilled or deionized water must be used for Wash Buffer preparation. Clinical laboratory reagent water Type I or Type II is acceptable.[13] Store the water in nonmetallic containers.
13. Do not mix lot numbers of coated microwell plates, Specimen Diluent, Conjugate Reagent, Negative Control, or Positive Control from kits with different lot numbers. Any lot number of Substrate Buffer-G, OPD tablets, 4N sulfuric acid, and 20X Wash Buffer Concentrate may be used provided they are not used beyond the labeled expiration date.
14. All reagents and components **must** be at room temperature prior to use and kit components returned to 2-8°C after use.
15. The microwell strips are sealed in protective pouches with a humidity indicator desiccant. The desiccant, normally blue/purple in color, will turn pink if moisture is present in the pouch. If the desiccant is pink, the microwell strips should not be used.
16. Desiccant is included in both OPD tablet and Microwell Plate. Synthetic amorphous precipitated silica gel (SiO2) (CAS 112926-00-8) and Cobalt chloride (CAS 7646-79-9) are included in the desiccant. Following are the Hazard and Precautionary Requirements.[12] Refer to www.orthoclinical.com for the Safety Data Sheets and for Ortho contact information.

DANGER:

Hazard Statements:
May cause cancer.
May damage fertility.
Toxic to aquatic life.
Harmful to aquatic life with long lasting effects.

Precautionary Statements:
Obtain special instructions before use.
Do not handle until all safety precautions have been read and understood.
Avoid release to the environment.
Wear protective gloves.
Use personal protective equipment as required.
If exposed or concerned: Get medical advice/attention.
Store locked up.
Dispose of contents/container in accordance with local/regional/national/international regulations.

17. Do not use reagents beyond their labeled expiration date.
18. Cross-contamination between reagents will invalidate the test results. Labeled, dedicated reservoirs for the appropriate reagents are recommended.
19. Ensure that specimen is added to the microwell. Failure to add specimen may produce an erroneous nonreactive result.
20. When using a single-channel micropipette for manual sample addition, use a new pipette tip for each specimen to be assayed. When using a multichannel micropipette, new tips are to be used for each reagent to be added.
21. Strict adherence to the specified wash procedure is crucial to ensure optimum assay performance. (See **Test Procedure**.)
22. Do not allow microwells to become dry once the assay has begun.
23. Do not touch the bottom exterior surface of the microwells. Fingerprints or scratches may interfere with reading the microwells.
24. Ensure that the microwell strips are level in the microwell strip holder during the test procedure. If necessary, wipe the bottom of the microwell strips carefully with a soft, lint-free, absorbent tissue to remove any moisture, dust or debris before reading.
25. Negative or positive control values which are not within the expected range (refer to **Quality Control Procedures** section) may indicate a technique problem or product deterioration.
26. Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell strips during the assay as the color reaction may be inhibited.
27. All pipetting equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.
28. The microwell reader should contain a reference filter with a setting at 620 or 630 nm. If an instrument without a reference filter is used, areas in the bottom of the microwells that are opaque, scratched or irregular may cause elevated readings.
29. Delays in plate processing may affect absorbance values.
30. Room temperature is defined as 15-30°C.
31. Refer to "Precautions" in other ORTHO® instruments User's Manuals:
    a. ORTHO® Summit System User's Guide
    b. ORTHO VERSEIA® Pipetter User's Guide
    c. ORTHO® Summit Processor User's Guide
    d. AutoReader IV User's Guide
    e. ORTHO® Training and Reference Manual
32. Visual inspections of the reagents should be performed prior to use to check for color change, cloudiness, and precipitates.

PREPARATION OF REAGENTS

1. **Preparation of Wash Buffer (1X):** Mix 50 mL of 20X Wash Buffer Concentrate with 950 mL of distilled or deionized water. Wash Buffer (1X) is stable for 30 days when stored at room temperature. For longer storage (up to 60 days), keep at 2-8°C. Record the date the Wash Buffer (1X) is prepared and the expiration date on the container. Discard Wash Buffer (1X) if visibly contaminated.

   NOTE: Any lot number of 20X Wash Buffer Concentrate may be used to prepare this reagent provided it is not used beyond its labeled expiration date.

2. **Preparation of Substrate Solution:** Clean glass or plastic vessels must be used. Prior to the end of the second incubation, transfer a sufficient amount of Substrate Buffer-G to a container and protect the contents from light. Completely dissolve the appropriate number of OPD tablets in Substrate Buffer-G prior to use.

   Each microwell plate requires at least 20 mL of Substrate Solution. More Substrate Solution may be needed depending upon the reagent dispenser used. See the instrument manufacturer's instructions for additional reagent required. Below are guidelines for general use.

Number of Wells	Number of Plates	Number of OPD Tablets	Substrate Buffer-G (mL)
48	0.5	1	12
96	1	2	24
144	1.5	3	36
192	2	4	48
240	2.5	5	60
288	3	6	72

   The Substrate Solution is stable for 8 hours after the addition of OPD tablets when held at room temperature in the dark. Record the time when the OPD tablets are added to the Substrate Buffer-G and when it will expire on the container.

SPECIMEN COLLECTION, STORAGE AND HANDLING

Note: Handle all specimens as if they are capable of transmitting infectious agents.

A. No special preparation of the patient is required prior to blood collection.

B. Blood should be collected by approved medical techniques.

C. Blood specimens collected in glass, plastic or serum separator tubes may be used.

D. Plasma collected with an improper ratio of specimen to anticoagulant should not be used.

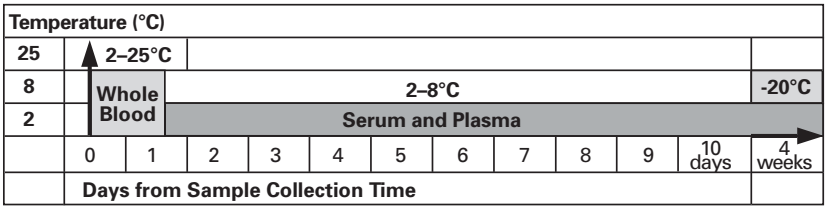
E. Serum or plasma collected in EDTA (glass and plastic tubes, plasma preparation tubes), heparin or citrate-based anticoagulants may be used and should be tested as soon as possible following collection.

F. **Do not use heat-treated specimens.**

G. **Do not use azide to preserve specimens. Do not test patient or donor samples containing azide. Sodium azide inhibits horseradish peroxidase activity.**

H. Whole Blood may be stored up to 25°C for 24 hours from time of draw, and serum and EDTA plasma specimens may be stored up to 10 days from time of draw at 2-8°C prior to centrifugation. Do not freeze whole blood.

I. Serum and plasma specimens may be stored for up to 10 days from time of draw at 2-8°C following centrifugation, or up to 4 weeks at -20°C undergoing 5 freeze/thaw cycles.



J. Storage of specimens in self-defrosting freezers is not recommended.

K. Mix thoroughly after thawing and before testing.

L. Clear, nonhemolyzed specimens are preferred. Precipitates in specimens should be removed by centrifugation.

M. All specimens should be handled as if capable of transmitting infectious agents. If specimens are to be shipped, they must be packed in compliance with federal regulations covering the transportation of etiologic agents.[14]

N. Studies have demonstrated that serum or plasma specimens may be shipped at ambient temperature (up to 37°C) for up to seven days or refrigerated (2-8°C) for up to seven days, and upon arrival should be stored at 2-8°C. For shipments requiring extensive transit times (greater than seven days), specimens should be kept frozen (-20°C or lower).

O. The effect of hemoglobin was evaluated in 30 anti-HBc positively spiked and 30 anti-HBc unspiked matched samples at concentrations up to 800 mg/dL and found to have no effect on the performance of the assay.

P. The effect of triglycerides was evaluated in 30 anti-HBc positively spiked and 30 anti-HBc unspiked matched samples at concentrations up to 3000 mg/dL and found to have no effect on the performance of the assay.

Q. The effect of bilirubin was evaluated in 30 anti-HBc positively spiked and 30 anti-HBc unspiked matched samples at concentrations up to 30 mg/dL and found to have no effect on the performance of the assay.

R. In an archived panel of 20 elevated total protein specimens (≥9.0 g/dL total protein), four specimens were repeatedly reactive with the ORTHO® HBc ELISA Test System. In a second set of 25 elevated protein specimens obtained from a sample vendor, all specimens were nonreactive in the ORTHO® HBc ELISA Test System.

S. No interference from specimens with known human anti-mouse antibodies (HAMA) was observed in a 15 member commercially available HAMA panel. No interference from heterophilic antibodies was observed in a 15 member commercially available panel.

PROCEDURE

Operational Modes

Manual testing is performed with handheld pipette sample handling, AutoReader IV, a microwell incubator capable of maintaining 37°C, and ORTHO® Assay Software (OAS).

Automated testing is performed with the ORTHO® Summit System (OSS), defined as the ORTHO VERSEIA® Pipetter, ORTHO® Summit Processor (OSP), and ORTHO® Assay Software (OAS).

Semi-automated testing is performed with the ORTHO VERSEIA® Pipetter, AutoReader IV, a microwell incubator capable of maintaining 37°C, and ORTHO® Assay Software (OAS).

Under circumstances of limited sample volume or limited number of samples, handheld pipette sample handling may be combined with the ORTHO® Summit Processor (OSP) and ORTHO® Assay Software (OAS).

An ORTHO® Assay Protocol Disk (OAPD) for ORTHO® HBc ELISA Test System is also used in the testing of the samples by all processing methods.

The protocol to run this test on the ORTHO® Summit System (OSS) is contained on the ORTHO® HBc ELISA Test System ORTHO® Assay Protocol Disk (OAPD) for the ORTHO® Assay Software (OAS). The pipetting protocol for the ORTHO VERSEIA® Pipetter is provided by the ORTHO VERSEIA® Pipetter software.

Follow the instructions in the OSS User's Guide.

Materials Provided

ORTHO® HBc ELISA Test System

480 Test Kit (Product Code 933245)
2400 Test Kit (Product Code 933275)

(See **REAGENTS** for complete listing)

Materials Required But Not Provided

1. ORTHO® Assay protocol Disk (OAPD) for Hepatitis B Virus Core Antigen (Recombinant) ORTHO® HBc ELISA Test System (Product Code 938246)
2. Hepatitis B Virus Core Antigen (Recombinant) ORTHO® HBc ELISA Test System Plate and Control Bar Code Labels (Product Code 935138, 1000 plate labels and 300 control tube labels and 935139, 4500 plate labels and 300 control tube labels) required to perform the assay on the ORTHO® Summit System
3. Hepatitis B Virus Core Antigen (Recombinant) ORTHO® HBc ELISA Test System 525 Control Vial Bar Code Labels for ORTHO VERSEIA® Pipetter (Product Code 6904574) required to perform the assay on the ORTHO VERSEIA® Pipetter
4. Hepatitis B Virus Core Antigen (Recombinant) ORTHO® HBc ELISA Test System 420 Specimen Diluent Bar Code Labels for ORTHO VERSEIA® Pipetter (Product Code 6904575) required to perform the assay on the ORTHO VERSEIA® Pipetter
5. ORTHO® Summit System User's Guide (Product Code 936578) and other appropriate OSS user documentation listed in the guide to run the assay on OSS
6. ORTHO® Summit Processor, adjustable multichannel micropipettes, or equivalent reagent dispenser capable of delivering 50 µL and 200 µL with at least ± 5% accuracy
7. ORTHO VERSEIA® Pipetter, a micropipette, or equivalent pipetter-dilutor capable of delivering 10 µL to 15µL with at least ± 10% accuracy and 200 µL to 300 µL with at least ± 5% accuracy
8. 5 µL to 250 µL disposable pipette tips or equivalent
9. Appropriately sized serological pipette or graduated cylinder
10. Multichannel micropipette reservoir or equivalent reagent container
11. ORTHO® Summit Processor or a multichannel microwell aspirator-washer device capable of at least 5 cycles of wash by dispensing and aspirating 300 µL to 800 µL of fluid per well and leaving a full well of fluid to soak at least 20 seconds. (Consult the device operator's manual for additional technical information.)
12. ORTHO® Summit Processor or AutoReader IV or a dual wavelength microwell reader capable of reading at 490 or 492 nm with a reference filter of 620 or 630 nm. If an instrument without a reference filter is used, areas in the bottom of the microwells that are opaque, scratched, or irregular may cause erroneous readings. Linearity of the microwell reader must range from at least 0 to 2.5 absorbance units. Consult the instrument manufacturer's specifications.
13. ORTHO® Summit Processor or equivalent 37°C ± 1°C microwell incubator (dry or humidified)
14. Distilled or deionized water, clinical laboratory reagent water Type I or Type II is acceptable (see **PRECAUTIONS** section)
15. 5.25% sodium hypochlorite (chlorine bleach)
16. 4N sulfuric acid (H_2SO_4) – available in the United States from Ortho-Clinical Diagnostics, Inc. (Product Code 933040) or equivalent. To determine the suitability of another source of acid, prepare Substrate Solution as described under **PREPARATION OF REAGENTS**. Add 200 µL of Substrate Solution to three microwells, then add 50 µL of the 4N H_2SO_4 to be tested to each microwell. Read the microwells at a wavelength of 490 nm or 492 nm with a reference filter of 620 nm or 630 nm at "0 time" and "60 minutes." All absorbance values at each time interval must be less than or equal to 0.050.
17. Black microwell strips or equivalent uncoated microwells
18. 20X Wash Buffer Concentrate (Product Code 933730, 6 x 150 mL: Ortho-Clinical Diagnostics, Inc.) – phosphate buffer with sodium chloride and detergent.

    Preservative: 2% 2-chloroacetamide
19. Plastic or Teflon®-coated forceps

Test Procedure

1. Prior to the beginning of the procedure, bring kit components to room temperature (15-30°C). Invert liquid reagents gently several times, but avoid foaming. Check the incubator temperature; maintain at 37°C ± 1°C.
2. Determine the total number of wells needed for the assay. In addition to specimens, one substrate blank, three negative controls and two positive controls will be included on each plate or partial plate. Unused wells should be stored at 2-8°C in the supplied foil pouch, tightly sealed **with desiccant** and used within 30 days of opening the foil pouch. Record the date the pouch is opened and the expiration date of the unused wells on the pouch.

   Performing the test on less than a full plate is permitted as long as the following conditions are met.

   Microwell strips from different plates can be mixed to assemble full or partial plates as long as they are from the same lot, within the open pouch expiration date and have come from plates that have previously demonstrated proper response to kit controls.

   When assembling a plate which contains strips from a newly opened, previously untested plate, one of these strips should be placed at the beginning of the plate and receive the full complement of kit controls.

   CAUTION: Use caution when assembling partial plates (mixing coated and uncoated) wells in a microplate. The OSP may not be able to differentiate between coated and uncoated (expired) wells and may produce results for any well position with an assigned ID number or control.

   CAUTION: Handle microwell strips with care. Do not touch the bottom exterior surface of the wells.
3. Assemble the microwell strips into the microwell strip holder, if necessary. **Microwell strips must be level in the microwell strip holder.** For incomplete plates, add black or uncoated microwell strips.
4. Prepare a record (plate map) identifying the placement of the controls and specimens in the microwells.

   Arrange the assay control wells so that well 1A is the substrate blank. From well 1A arrange all controls in a horizontal or vertical configuration as follows. Configuration is dependent upon software.

Well 1A	Substrate Blank
	Negative Control
	Negative Control
	Negative Control
	Positive Control
	Positive Control

5. **Verify that any manual dispensing equipment is set to deliver the specified volumes as stated in the procedure, following the equipment manufacturer's instructions.**

   Add controls and specimens to the microwells as follows:

   Sample Addition:

   a. Add 200 µL of Specimen Diluent to all wells, **except 1A** using the ORTHO VERSEIA® Pipetter, a micropipette, or an equivalent pipetter-dilutor capable of delivering 200 µL with at least ± 5% accuracy.

   b. Add 10 µL of the control, or specimens to the appropriate wells using the ORTHO VERSEIA® Pipetter, a micropipette, or an equivalent pipetter-dilutor capable of delivering 10 µL with at least ± 10% accuracy.

   c. If the controls and specimens have been manually delivered, to ensure the complete addition of control, or specimen, mix the sample and Specimen Diluent in the well by flushing the pipette tip several times.

   For Previously Diluted Sample Addition:

   a. Add 300 µL of Specimen Diluent to a tube or container.

   b. Add 15 µL of control or specimen to the tube. Mix thoroughly.

   c. Transfer 210 µL of each previously diluted control or specimen to the appropriate well position.

6. For manual processing of microwell plates, cover the microwell strip holder with a plate sealer. When using an automated microplate processor for incubation, follow the instrument manufacturer's recommendations with regard to microwell plate sealing. Incubate at 37°C ± 1°C for **60 minutes ± 5 minutes**.

7. Level the strips in the microwell holder, if necessary. With an aspirator-washer device, aspirate and wash all wells **five** times with Wash Buffer (1X).

   CAUTION: Strict adherence to the specified wash procedure is crucial to ensure optimum assay performance. Follow the steps specified in order to ensure thorough washing.

   a. Aspirate the sample solutions from microwells and then completely fill wells with Wash Buffer. Do not allow the wells to overflow. Allow approximately 20 seconds between the addition of Wash Buffer and subsequent aspiration.

   b. Complete the aspirate/fill sequence **four** additional times (5 times total).

   c. Completely aspirate wells. Invert the plate and firmly tap on a clean paper towel to remove excess Wash Buffer, if necessary.

8. Add 200 µL of Antibody Conjugate to all wells, **except 1A** using an adjustable multichannel micropipette or equivalent reagent dispenser capable of delivering 200 µL with at least ± 5% accuracy.

9. For manual processing of microwell plates, cover the microwell strip holder with a **new unused plate sealer**. When using an automated microplate processor for incubation, follow the instrument manufacturer's recommendations with regard to microwell plate sealing. Incubate at 37°C ± 1°C for **60 minutes ± 5 minutes**.

10. Prepare **sufficient** Substrate Solution prior to use in Step 12 to allow time for the OPD tablets to **dissolve completely**. Refer to **PREPARATION OF REAGENTS. Do not use more than a single preparation of Substrate Solution on a plate**.

11. After the second incubation, wash the wells as described in Step 7.

12. Add 200 µL of Substrate Solution to all wells, **including 1A** using an adjustable multichannel micropipette or equivalent reagent dispenser capable of delivering 200 µL with at least ± 5% accuracy.

13. Incubate at room temperature in the dark for **30 minutes ± 1 minute**.

14. Add 50 µL of 4N sulfuric acid (H_2SO_4) to all wells, **including 1A** using an adjustable multichannel micropipette or equivalent reagent dispenser capable of delivering 200 µL with at least ± 5% accuracy. To ensure proper mixing, acid should be added forcibly in a steady stream. If necessary, gently tap the plate or use a microwell plate shaker to mix the contents. Care should be taken to avoid splashing of the contents of the microwells. When using an automated microplate processor, follow the instrument manufacturer's instructions with regard to mixing.

15. If necessary, wipe moisture from the bottom of the microwell strips carefully with a soft, lint-free, absorbent tissue before reading. If necessary, level the strips in the microwell holder. Read the microwell strip plate at a wavelength of 490 nm or 492 nm. For dual wavelength readers set the reference wavelength at 620 nm or 630 nm. Blank the reader on well 1A according to the instrument manufacturer's instructions.

    For manual calculation, the user should ensure that the blank value (well 1A) has been subtracted from all control and specimen well values prior to applying the Quality Control criteria.

    NOTE: Microwell strip plates must be read within 60 minutes following the addition of 4N sulfuric acid (H_2SO_4). Plates must be stored in the dark until read.

Quality Control Procedures[15,16]

1. **Substrate Blank Acceptance Criteria**

   A plate is considered valid with respect to the substrate blank if the absorbance value of the substrate blank well (well 1A) is greater than or equal to -0.020 and less than or equal to 0.200.

2. **Negative Control Acceptance Criteria**

   a. Individual negative control values must be less than or equal to 0.350 and greater than or equal to -0.005. Numbers which are between 0.000 and -0.005 inclusive are valid and should be rounded to 0.000 for calculations. If one of the three negative control values is outside either of these limits, recalculate the negative control mean ($NC\bar{x}$) based on the other two acceptable control values. The plate is invalid and the test must be repeated if two or more of the three control values are outside either of the limits.

b. Determine the mean of the negative control values ($NC\bar{x}$).

Example:

Negative Control		Absorbance
1		0.200
2		0.180
3		0.160
Total Absorbance	=	0.540

$$NC\bar{x} = \frac{\text{Total Absorbance}}{3} = 0.180$$

3. **Positive Control Acceptance Criteria**

   The positive control is used to verify that the test kit components are capable of detecting a reactive specimen provided the test procedure has been strictly adhered to.

   A plate is considered valid with respect to the positive control if both positive control values are greater than or equal to **0.800**, within the readable range of the microwell reader and do not differ by more than **0.500**. Any other values for the positive control are considered invalid.

   NOTE: Results beyond the upper limit of the readable range of the microwell reader may appear as "OVER" or "***" or ">".

4. **Calculation of the Cutoff Value**

   Cutoff Value = $NC\bar{x}$ + 0.400

   Example:

Negative Control		Absorbance
1		0.200
2		0.180
3		0.160
Total Absorbance	=	0.540

$$NC\bar{x} = \frac{\text{Total Absorbance}}{3} = 0.180$$

Cutoff Value = 0.180 + 0.400 = 0.580

INTERPRETATION OF RESULTS

1. Specimens with absorbance values less than -0.005 should be retested in a single microwell. Specimens initially tested with the ORTHO VERSEIA® Pipetter may be retested manually or with the ORTHO VERSEIA® Pipetter. The specimen should be considered nonreactive if the retest absorbance value is less than the Cutoff Value, even if the retest absorbance value remains less than -0.005.
2. Specimens with absorbance values less than the Cutoff Value and greater than or equal to -0.005 are considered nonreactive. Further testing is not required.
3. Specimens with absorbance values greater than or equal to the Cutoff Value are considered initially reactive and should be retested in duplicate before final interpretation. Specimens initially tested with the ORTHO VERSEIA® Pipetter should only be retested with the ORTHO VERSEIA® Pipetter. Specimens initially tested manually may be retested manually or with the ORTHO VERSEIA® Pipetter.
4. Upon retesting an initially reactive specimen, the specimen is considered repeatedly reactive for anti-HBc if one or both duplicate determination(s) is (are) reactive (i.e., equal to or greater than the Cutoff Value.)
5. After retesting an initially reactive specimen, the specimen is considered nonreactive for anti-HBc if both duplicate determinations are nonreactive (i.e., less than the Cutoff Value.)

LIMITATIONS OF THE PROCEDURE

ORTHO® HBc ELISA Test System is limited to the detection of anti-HBc in human serum or plasma. The presence of anti-HBc does not constitute a diagnosis of hepatitis B infection but may be indicative of recent and/or past infection by hepatitis B virus. A nonreactive test result does not exclude the possibility of exposure to hepatitis B virus. Levels of anti-HBc may be undetectable in early infection.

The positive control in the test kit is not to be used to quantitate assay sensitivity. The positive control is used to verify that the test kit components are capable of detecting a reactive specimen provided the test procedure has been strictly adhered to.

When positive control values are beyond the linear range of the microwell reader, the positive control cannot be used to assess assay precision.

EXPECTED RESULTS

The frequency of anti-HBc in a population varies widely depending upon the geographic locale and the population under study. In one study of volunteer blood donors nonreactive for HBsAg, 1% was positive for anti-HBc.[2]

SPECIFIC PERFORMANCE CHARACTERISTICS[17]

Reactivity in Presumably Healthy Blood Donors

Three independent clinical study sites tested a total of 3010 specimens from presumably healthy blood donors. The results of reactivity with ORTHO® HBc ELISA Test System are shown in Table 1.

A total of 3010 specimens were tested, 2969 of which were nonreactive. The repeatedly reactive rate of ORTHO® HBc ELISA Test System in this low prevalence population is 1.1%.

Table 1: Detection of Anti-HBc in Serum and Plasma from Presumably Healthy Blood Donors

SITE	NUMBER TESTED	INITIALLY NONREACTIVE		INITIALLY REACTIVE		REPEATEDLY REACTIVE	
		N (%)	Mean S/CO	N (%)	Mean S/CO	N (%)	Mean S/CO
1	1033	1016 (98.4%)	0.233	17 (1.6%)	3.917	16 (1.5%)	4.071
2	1000	989 (98.9%)	0.212	11 (1.1%)	1.953	7 (0.7%)	2.159
3	977	964 (98.7%)	0.166	13 (1.3%)	3.848	10 (1.0%)	4.320
Total	**3010**	**2969 (98.6%)**	**0.204**	**41 (1.4%)**	**3.239**	**33 (1.1%)**	**3.517**

Note: Data presented in Table 1 was collected using manual processing equipment.

Reactivity in Patients with Hepatitis

Specimens from patients with acute hepatitis B infection (A HBV), chronic hepatitis B infection (C HBV), hepatitis A infection (HAV), non-A, non-B hepatitis (HCV) and alcoholic liver disease (LIVER) were tested. Results appear in Table 2.

Table 2: Detection of Anti-HBc in Patients with Hepatitis

GROUP	NUMBER TESTED	INITIALLY NONREACTIVE	INITIALLY REACTIVE	REPEATEDLY REACTIVE
A HBV	25	0	25 (100%)	25 (100%)
C HBV	28	0	28 (100%)	28 (100%)
HAV	10	8 (80%)	2 (20%)	2 (20%)
HCV	10	8 (80%)	2 (20%)	1 (10%)
LIVER	25	12 (48%)	13 (52%)	10 (40%)

Note: Data presented in Table 2 was collected using manual processing equipment.

The two HAV specimens which were anti-HBc reactive were also anti-HBs reactive. The anti-HBc reactivity for HCV and liver disease specimens is probably the result of other risk factors.

Reproducibility

The intra-plate (within plate), inter-plate (between plate), and total reproducibility of the ORTHO® HBc ELISA Test System was evaluated using a six-member reproducibility panel. The reproducibility panel consisted of three HBc IgG reactive members, two HBc IgM reactive members, and one nonreactive member. Testing was conducted at 3 internal sites with one kit lot. The study evaluated the reproducibility of the assay when using manual processing equipment (hand-held precision pipetters, 37°C incubator, AutoWash 96, AutoReader IV, OAS and OAPD). Three replicates of each of the six-member panel were tested on one plate, two times per day, on each of 3 sets of manual processing equipment, for 5 days for a total of 90 replicates per panel member.

Mean signal to cutoff (S/CO), standard deviation (SD), and coefficient of variation (CV%) results are presented in Table 3 for the manual processing equipment.

Table 3: Reproducibility Using Manual Processing Equipment

Manual			Inter-plate*		Intra-plate†		Total‡	
Panel Member	N	Mean S/CO	SD	CV (%)	SD	CV (%)	SD	CV (%)
A (IgM)	90	1.568	0.123	7.8	0.093	5.9	0.201	12.8
B (IgM)	90	2.177	0.131	6.0	0.073	3.4	0.158	7.3
C (IgG)	90	1.207	0.126	10.4	0.008	0.7	0.191	15.8
D (IgG)	90	1.468	0.090	6.1	0.059	4.0	0.207	14.1
E (IgG)	90	1.262	0.076	6.0	0.083	6.6	0.223	17.7
F (Nonreactive)	90	0.144	0.039	27.1	0.000	0.0	0.044	30.6

*Inter-plate/Between Plate: Between run (Day (Site)): Variability of the assay performance from plate to plate, nested within day, with day nested within site.

†Intra-plate/Within Plate: Between Replicate: Variability of the assay performance from replicate to replicate.

‡Total: Sum of the individual components of variance including (1) Inter-plate, (2) Intra-plate, (3) Site to Site, (4) Day to Day

PERFORMANCE CHARACTERISTICS ON THE ORTHO® SUMMIT SYSTEM (OSS)

Specificity in Blood Donors on the OSS

Across three sites (2 US Blood Centers and one internal site), 3179 random donor samples were tested in singleton on both the ORTHO® Summit Sample Handling System and ORTHO VERSEIA® pipetters in an abbreviated specificity study. The study evaluated the specificity of the assay when pipetting with the ORTHO VERSEIA® Pipetter as compared to the ORTHO® Summit Sample Handling System. Assay processing was performed in both cases on the OSP (OSS automated mode). Of the 3179 specimens tested, 3175 were included in the qualitative analysis. Four specimens were excluded due to pipetting errors. Table 4 below summarizes the qualitative and mean S/CO results obtained with the ORTHO® Summit Sample Handling System and ORTHO® Summit Processor, and Table 5 below summarizes the qualitative and mean S/CO results obtained with the ORTHO VERSEIA® Pipetter and ORTHO® Summit Processor. Combined across sites, 99.7% (95% Confidence Interval of 99.5% to 99.9%) of ORTHO® Summit Sample Handling System results were nonreactive and 99.5% (95% Confidence Interval of 99.2% to 99.7%) of ORTHO VERSEIA® Pipetter results were nonreactive.

Of the 3175 random blood donor samples included in the qualitative analysis, 3172 were included in additional quantitative analyses. Three samples were excluded from the quantitative analysis due to optical density (OD) values being above the linear range of the OSP reader. A two-tailed paired t-test at the 5% significance level was conducted to test for equality of the mean S/CO ratio between the random blood donor samples pipetted on the ORTHO VERSEIA® to the random blood donor samples pipetted on the ORTHO® Summit Sample Handling System for each test site and for all sites combined. The results are summarized in Table 6.

Table 4: Detection of Anti-HBc in Serum and Plasma from Random Blood Donors on the ORTHO® Summit Sample Handling System* and ORTHO® Summit Processor

SITE	NUMBER TESTED	INITIALLY NONREACTIVE		INITIALLY REACTIVE		REPEATEDLY REACTIVE		NONREACTIVE	
		N (%)	Mean S/CO	N (%)	Mean S/CO	N (%)	Mean S/CO	N (%)	Mean S/CO
1	1024	1019 (99.5%)	0.182	5 (0.5%)	8.160	5 (0.5%)	8.160	1019 (99.5%)	0.182
2	1100	1097 (99.7%)	0.187	3 (0.3%)	7.734	3 (0.3%)	7.734	1097 (99.7%)	0.187
3	1051	1049 (99.8%)	0.165	2 (0.2%)	2.770	1 (0.1%)	1.394	1050 (99.9%)	0.169
Total	**3175**	**3165 (99.7%)**	**0.178**	**10 (0.3%)**	**6.221**	**9 (0.3%)**	**5.763**	**3166 (99.7%)**	**0.179**

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

Table 5: Detection of Anti-HBc in Serum and Plasma from Random Blood Donors on the ORTHO VERSEIA® Pipetter and ORTHO® Summit Processor

SITE	NUMBER TESTED	INITIALLY NONREACTIVE		INITIALLY REACTIVE		REPEATEDLY REACTIVE		NONREACTIVE	
		N (%)	Mean S/CO	N (%)	Mean S/CO	N (%)	Mean S/CO	N (%)	Mean S/CO
1	1024	1018 (99.4%)	0.203	6 (0.6%)	7.251	5 (0.5%)	8.325	1019 (99.5%)	0.205
2	1100	1092 (99.3%)	0.199	8 (0.7%)	3.758	5 (0.5%)	5.363	1095 (99.5%)	0.201
3	1051	1046 (99.5%)	0.191	5 (0.5%)	1.196	5 (0.5%)	1.196	1046 (99.5%)	0.191
Total	**3175**	**3156 (99.4%)**	**0.197**	**19 (0.6%)**	**4.068**	**15 (0.5%)**	**4.961**	**3160 (99.5%)**	**0.199**

Table 6: Mean S/CO Difference between the ORTHO VERSEIA® Pipetter and the ORTHO® Summit Sample Handling System with Random Blood Donor Sample

SITE	N	ORTHO VERSEIA® Mean S/CO	ORTHO® Summit Mean S/CO	ORTHO VERSEIA® Mean S/CO Difference from ORTHO® Summit	Two-tailed p-value
1	1022	0.209	0.185	0.024*	<0.0001
2	1099	0.206	0.190	0.016*	<0.0001
3	1051	0.195	0.170	0.025*	<0.0001
Total	**3172**	**0.203**	**0.182**	**0.021***	**<0.0001**

*Statistically significant difference (two-tailed p-value is < 0.05), but not clinically significant based on predetermined criteria.

Comparative Studies with Prepared HBc Antibody Positive Specimens

Comparison studies with 114 serum/plasma specimens known to be positive for HBc antibody were performed at 2 US Blood Centers and one internal site using the OSS. The comparison panel consisted of 92 plasma and 22 serum specimens. Of the 114 specimens, 44 were undiluted and the remaining 70 specimens were prepared dilutions ranging from 1:35 to 1:2560 to yield S/CO values across the range of the assay with approximately one third of the panel possessing S/CO values less than 3.000. The study on the OSS evaluated the performance of the assay when pipetting with the ORTHO VERSEIA® Pipetter as compared to the ORTHO® Summit Sample Handling System. The 114 HBc antibody positive specimens were tested in triplicate for a possible total of 342 replicates on the ORTHO VERSEIA® Pipetter and on the ORTHO® Summit Sample Handling System. Assay processing was performed in both cases on the OSP (OSS automated mode). The study demonstrated that assay results are acceptable with the ORTHO® HBc ELISA Test System using either method of pipetting. Table 7 below summarizes the qualitative results obtained with the ORTHO® Summit Sample Handling System and ORTHO® Summit Processor, and Table 8 below summarizes the qualitative results obtained with the ORTHO VERSEIA® Pipetter and ORTHO® Summit Processor.

To evaluate the performance of the assay quantitatively, the 114 HBc antibody positive specimens were tested in triplicate at each of 3 sites for a possible total of 342 replicates on each of the following processing modes per site: ORTHO VERSEIA® Pipetter and ORTHO® Summit Processor, ORTHO® Summit Sample Handling System and ORTHO® Summit Processor, and using the manual processing method (pipetting using hand-held precision pipetters and processing using 37°C incubator, AutoWash 96, AutoReader IV, OAS and OAPD). The automated testing (OSS) was performed at 2 US Blood Centers and one internal site. The manual testing was performed at 3 internal sites by 3 manual operators (one manual operator using one set of manual processing equipment at each of the 3 internal sites). For the data analysis, each manual testing site was matched to an OSS testing site for the quantitative comparison. One ORTHO VERSEIA® replicate was excluded from the quantitative analysis as the OD value was above the linear range of the OSP reader. A second ORTHO VERSEIA® replicate was excluded due to a pipetting error. A two-tailed paired t-test was performed, which compares replicates; therefore the corresponding replicates pipetted on the ORTHO® Summit Sample Handling System and the corresponding replicates pipetted and processed manually were also excluded. The mean S/CO values, mean S/CO differences and mean percent differences for each processing method are summarized in Table 9. A two-tailed paired t-test at the 5% significance level was conducted to test for equality of the S/CO ratios between the HBc antibody positive specimens pipetted on the ORTHO VERSEIA® and the HBc antibody positive specimens pipetted on the ORTHO® Summit Sample Handling System and the HBc antibody positive specimens pipetted and processed using the manual method. This was performed for each test site and for all sites combined. The two-tailed p-values are also shown in Table 9. Any statistically significant differences between processing methods were not clinically significant based on predetermined criteria.

Table 7: Detection of Anti-HBc in a Panel of Known Positive Specimens on the ORTHO® Summit Sample Handling System and ORTHO® Summit Processor

SITE	SAMPLES TESTED	REPLICATES TESTED	REPLICATES NONREACTIVE N (%)	REPLICATES REACTIVE N (%)
1	114	342	1 (0.3%)	341 (99.7%)
2	114	341	0 (0.0%)	341 (100%)
3	114	342	1 (0.3%)	341 (99.7%)
Total	**342**	**1025**	**2 (0.2%)**	**1023 (99.8%)**

Table 8: Detection of Anti-HBc in a Panel of Known Positive Specimens on the ORTHO VERSEIA® Pipetter and ORTHO® Summit Processor

SITE	SAMPLES TESTED	REPLICATES TESTED	REPLICATES NONREACTIVE N (%)	REPLICATES REACTIVE N (%)
1	114	342	0 (0.0%)	342 (100%)
2	114	341	0 (0.0%)	341 (100%)
3	114	342	0 (0.0%)	342 (100%)
Total	**342**	**1025**	**0 (0.0%)**	**1025 (100%)**

Table 9: Mean S/CO Differences with HBc Antibody Positive Specimens Processed Using the ORTHO VERSEIA® Pipetter, ORTHO® Summit Sample Handling System and Manual Pipetting and Processing

SITE	N	Manual	ORTHO® Summit vs. Manual				ORTHO VERSEIA® vs. Manual				ORTHO VERSEIA® vs. ORTHO® Summit		
		Manual Mean S/CO	ORTHO® Summit Mean S/CO	ORTHO® Summit Mean S/CO Difference from Manual	ORTHO® Summit % Difference from Manual	Two-tailed p-value	ORTHO VERSEIA® Mean S/CO	ORTHO VERSEIA® Mean S/CO Difference from Manual	ORTHO VERSEIA® % Difference from Manual	Two-tailed p-value	ORTHO VERSEIA® Mean S/CO Difference from ORTHO® Summit	ORTHO VERSEIA® % Difference from ORTHO® Summit	Two-tailed p-value
1	341	2.787	2.727	-0.060*	-2.15%	0.0062	3.216	0.429*	15.39%	<0.0001	0.489*	17.93%	<0.0001
2	341	2.482	2.892	0.410*	16.52%	<0.0001	3.181	0.699*	28.16%	<0.0001	0.289*	9.99%	<0.0001
3	342	2.618	2.821	0.203*	7.75%	<0.0001	3.236	0.618*	23.61%	<0.0001	0.415*	14.71%	<0.0001
Total	**1024**	**2.629**	**2.814**	**0.185***	**7.04%**	**<0.0001**	**3.211**	**0.582***	**22.14%**	**<0.0001**	**0.397***	**14.11%**	**<0.0001**

Note: Manual Testing Sites were not the same testing sites as the ORTHO® Summit and ORTHO VERSEIA® testing sites, however the same panel was tested across all sites in the study.

*Statistically significant difference (two-tailed p-value is < 0.05), but not clinically significant based on predetermined criteria.

Comparative Analytical Performance with Seroconversion Panels and Dilution Panels

To demonstrate that the analytical performance of the ORTHO® HBc ELISA Test System when using the ORTHO VERSEIA® Pipetter is comparable to the ORTHO® Summit Sample Handling System and to the manual processing method, comparison studies were performed with ten HBV seroconversion panels and ten dilution panels (four dilutions per panel member with target signal to cutoff (S/CO) values ranging from 0.5 to 3.0 of five anti-HBc IgG and five anti-HBc IgM antibody positive samples diluted through the assay cutoff).

The HBV seroconversion panels were pipetted in singleton on each of 3 ORTHO VERSEIA® Pipetters and 3 ORTHO® Summit Sample Handling Systems. Assay processing was performed on the ORTHO® Summit Processor (OSP). To evaluate the correlation of the two pipetting methods, Deming's regression analysis was performed. The regression analysis yielded a slope of 0.96, intercept of 0.01 and Pearson correlation coefficient (r) of 0.99. The analysis indicates a high correlation between the ORTHO VERSEIA® Pipetter and ORTHO® Summit Sample Handling System with the ten HBV seroconversion panels. Additionally, the HBV seroconversion panels were manually pipetted using hand-held precision pipetters and tested in triplicate on one set of manual processing equipment (37°C incubator, AutoWash 96, AutoReader IV, OAS and OAPD). To evaluate correlation of data generated on the OSS (ORTHO VERSEIA® Pipetter/OSP and ORTHO® Summit Sample Handling System/OSP) and the manual processing method, Deming's regression analysis was performed. For the ORTHO® Summit Sample Handling System/OSP vs. manual, the regression analysis yielded a slope of 1.10, intercept of 0.01 and Pearson correlation coefficient (r) of 0.99. For the ORTHO VERSEIA® Pipetter/OSP vs. manual, the regression analysis yielded a slope of 0.94, intercept of 0.05 and Pearson correlation coefficient (r) of 1.00. The regression analysis indicates a high correlation between the OSS and the manual processing method with the ten HBV seroconversion panels.

The dilutional panel samples were pipetted in triplicate on each of 3 ORTHO VERSEIA® Pipetters and 3 ORTHO® Summit Sample Handling Systems. Assay processing was performed on the ORTHO® Summit Processor (OSP). To evaluate the correlation of the two pipetting methods, Deming's regression analysis was performed. The regression analysis yielded a slope of 1.10, intercept of 0.04 and Pearson correlation coefficient (r) of 0.99. The analysis indicates a high correlation between the ORTHO VERSEIA® Pipetter and ORTHO® Summit Sample Handling System with dilutional panel samples with approximate S/CO values ranging from 0.5 to 3.0. Additionally, the dilutional panel samples were manually pipetted using hand-held precision pipetters and tested in triplicate on one set of manual processing equipment (37°C incubator, AutoWash 96, AutoReader IV, OAS and OAPD). The S/CO data generated on the OSS (ORTHO VERSEIA® Pipetter/OSP and ORTHO® Summit Sample Handling System/OSP) was compared to the S/CO data generated with manual pipetting and processing. The Mean S/CO and percent difference data for each processing method is summarized in Table 10 below.

Table 10: Mean S/CO % Difference between the ORTHO VERSEIA® Pipetter/OSP, ORTHO® Summit Sample Handling System/OSP and Manual Processing Equipment with HBc Dilutional Panel Samples

Dilution Level Target S/CO	Manual		ORTHO® Summit				ORTHO VERSEIA®					
	N	Mean S/CO	N	Mean S/CO	ORTHO® Summit Difference from Manual		N	Mean S/CO	ORTHO VERSEIA® Difference from Manual		ORTHO VERSEIA® Difference from ORTHO® Summit	
					%	Two-tailed p-value			%	Two-tailed p-value	%	Two-tailed p-value
0.5	30	0.420	90	0.483	15.00%*	0.002	89	0.563	34.05%*	<0.001	16.56%*	<0.001
1.0	30	1.208	90	1.337	10.68%*	0.037	90	1.582	30.96%*	<0.001	18.32%*	<0.001
2.0	30	2.145	90	2.360	10.02%	0.077	90	2.615	21.91%*	<0.001	10.81%*	<0.001
3.0	30	3.395	90	3.504	3.21%	0.540	90	3.736	10.04%	0.055	6.62%*	<0.001
Total	**120**	**1.790**	**360**	**1.925**	**7.54%**	**0.323**	**359**	**2.128**	**18.88%***	**0.011**	**10.55%***	**<0.001**

*Statistically significant difference (two-tailed p-value is <0.05), but not clinically significant based on predetermined criteria.

Reproducibility on the OSS

The intra-plate (within plate), inter-plate (between plate) and total reproducibility of ORTHO® HBc ELISA Test System was evaluated using a six-member reproducibility panel. The reproducibility panel consisted of three HBc IgG reactive members, two HBc IgM reactive members, and one nonreactive member. Testing was conducted at 2 US Blood Centers and one internal site with one kit lot. The study evaluated the reproducibility of the assay when pipetting with the ORTHO VERSEIA® Pipetter as compared to the ORTHO® Summit Sample Handling System. Assay processing was performed in both cases on the OSP (OSS automated mode). On each pipetter, three replicates each of the six-member panel were tested on one plate, two times per day, for 5 days. Internal testing included 3 ORTHO® Summit Pipetters and 3 ORTHO VERSEIA® Pipetters; external testing included 2 ORTHO® Summit Pipetters and 2 ORTHO VERSEIA® Pipetters (one each per site).

Mean signal to cutoff (S/CO), standard deviation (SD), and coefficient of variation (CV%) results are presented in Table 11 for the two pipetting methods.

Table 11: Reproducibility Panel Testing: ORTHO® Summit System (OSS) [ORTHO® Summit Pipetter and ORTHO VERSEIA® Pipetter, ORTHO® Summit Processor (OSP), and OAS]

				Inter-plate*		Intra-plate†		Total‡	
Platform	Panel Member	N	Mean S/CO	SD	CV (%)	SD	CV (%)	SD	CV (%)
ORTHO® Summit Pipetter	A (IgM)	150	1.719	0.034	2.0	0.155	9.0	0.264	15.4
	B (IgM)	150	2.205	0.198	9.0	0.175	7.9	0.310	14.1
	C (IgG)	150	1.431	0.000	0.0	0.138	9.6	0.279	19.5
	D (IgG)	150	1.648	0.076	4.6	0.333	20.2	0.417	25.3
	E (IgG)	150	1.347	0.068	5.0	0.122	9.1	0.263	19.5
	F (Nonreactive)	150	0.217	0.099	45.6	0.118	54.3	0.162	74.6
ORTHO VERSEIA® Pipetter	A (IgM)	150	1.881	0.052	2.8	0.127	6.8	0.278	14.8
	B (IgM)	150	2.193	0.080	3.6	0.128	5.8	0.259	11.8
	C (IgG)	150	1.692	0.013	0.8	0.148	8.7	0.322	19.0
	D (IgG)	150	1.917	0.076	4.0	0.084	4.4	0.272	14.2
	E (IgG)	150	1.700	0.029	1.7	0.108	6.4	0.285	16.8
	F (Nonreactive)	150	0.199	0.009	4.5	0.032	16.1	0.045	22.6

*Inter-plate/Between Plate: Between run (Day (Site)): Variability of the assay performance from plate to plate, nested within day, with day nested within site.

†Intra-plate/Within Plate: Between Replicate: Variability of the assay performance from replicate to replicate.

‡Total: Sum of the individual components of variance including (1) Inter-plate, (2) Intra-plate, (3) Site to Site, (4) Day to Day, and (5) Pipetter to Pipetter variation.

Table 12 is a compilation of the Reproducibility Panel signal to cutoff (S/CO) data previously presented in Table 3 (Reproducibility Using Manual Processing Equipment) and Table 11 (Reproducibility Panel Testing: ORTHO® Summit System) and provides a summary of the Reproducibility Panel S/CO values and percent differences observed between manual processing equipment (hand-held precision pipetters, 37°C incubator, AutoWash 96, AutoReader IV, OAS and OAPD) and the ORTHO® Summit System with both the ORTHO® Summit Sample Handling System and ORTHO VERSEIA® Pipetter.

Table 12: Mean S/CO % Difference between the ORTHO VERSEIA® Pipetter/OSP, ORTHO® Summit Sample Handling System/OSP and Manual Processing Equipment with Reproducibility Panel Samples

Reproducibility Panel Member	Manual		ORTHO® Summit				ORTHO VERSEIA®					
	N	Mean S/CO	N	Mean S/CO	ORTHO® Summit % Difference from Manual	Two-tailed p-value	N	Mean S/CO	ORTHO VERSEIA® % Difference from Manual	Two-tailed p-value	ORTHO VERSEIA® % Difference from ORTHO® Summit	Two-tailed p-value
A (IgM)	90	1.568	150	1.719	9.6%*	<0.0001	150	1.881	20.0%*	<0.0001	9.4%*	<0.0001
B (IgM)	90	2.177	150	2.205	1.3%	0.4148	150	2.193	0.7%	0.5885	-0.5%	0.7030
C (IgG)	90	1.207	150	1.431	18.6%*	<0.0001	150	1.692	40.2%*	<0.0001	18.2%*	<0.0001
D (IgG)	90	1.468	150	1.648	12.3%*	0.0001	150	1.917	30.6%*	<0.0001	16.3%*	<0.0001
E (IgG)	90	1.262	150	1.347	6.7%*	0.0061	150	1.700	34.7%*	<0.0001	26.2%*	<0.0001
F (Nonreactive)	90	0.144	150	0.217	50.7%*	<0.0001	150	0.199	38.2%*	<0.0001	-8.3%	0.1814

*Statistically significant difference (two-tailed p-value is < 0.05), but not clinically significant based on predetermined criteria.

Technical questions concerning these reagents should be directed to Ortho Care™ Technical Solutions Center at 1-800-421-3311.

SUMMARY OF REVISIONS	
Section	**Revision**
PRINCIPLE OF THE PROCEDURE	Replaced Novartis Vaccines and Diagnostics, Inc. with Grifols Diagnostic Solutions Inc.
Back Page	Replaced CHIRON logo with GRIFOLS logo.

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KEY TO SYMBOLS / LÉGENDE DES SYMBOLES / CLAVE DE LOS SÍMBOLOS

The following symbols may have been used in the labeling of this product. / Les symboles suivants ont pu être utilisés sur l'étiquette de ce produit. / Los siguientes símbolos pueden haber sido empleados en el etiquetado de este producto.

Do Not Reuse / Ne pas réutiliser / No reutilizar

Use by or Expiration Date (Year-Month-Day) / À utiliser avant la date de péremption (année-mois-jour) / Usar antes de o Fecha de caducidad (año-mes-día)

Lot Number / Numéro de lot / Número de lote

Serial Number / Numéro de série / Número de serie

Catalog Number or Product Code / Référence catalogue ou code produit / Referencia de catálogo o Código del producto

Attention: See Instructions for Use / Attention : consulter le feuillet technique / Atención: Consultar las instrucciones de uso

Manufacturer / Fabricant / Fabricante

Authorized Representative in the European Community / Mandataire dans l'Union européenne / Representante autorizado en la Unión Europea

Contains Sufficient for "n" Tests / Suffisant pour << n >> dosages / Contiene suficiente para "n" ensayos

In vitro Diagnostic Medical Device / Pour diagnostic in vitro / Producto sanitario para diagnóstico in vitro

Upper Limit of Temperature / Conserver à une température égale ou inférieure à / Límite superior de temperatura

Lower Limit of Temperature / Conserver à une température égale ou supérieure à / Límite inferior de temperatura

Temperature Limitation / Conserver à une température comprise entre / Limitación de temperatura

Consult Instructions for Use, "n" Version / Consultez le feuillet technique << n >> version / Atención: ver las instrucciones de uso "n" versión

Biological Risks / Risques biologiques / Riesgos biológicos

Do not use if damaged / Ne pas utiliser si endommagé / No usar si está dañado

Health Hazards / Dangereux pour la santé / Riesgos para la salud

Acute Toxicity / Toxique ou mortel / Toxicidad aguda

Serious Health Hazards / Très dangereux pour la santé / Riesgos graves para la salud

Corrosive / Corrosif / Corrosivo

KEY TO SYMBOLS / LÉGENDE DES SYMBOLES / CLAVE DE LOS SÍMBOLOS

Continued / Suite / Continuación

Environmental or Aquatic Toxicity / Dangereux pour l'environnement aquatique / Toxicidad marina o medioambiental

Fragile, Handle with Care / Attention, fragile / Frágil; manipular con cuidado

Keep Dry / Tenir au sec / Mantener seco

This end up / Haut / Este lado hacía arriba

CONTROL +

Positive Control / Contrôle positif / Control positivo

CONTROL −

Negative Control / Contrôle négatif / Control negativo

CALIBRATOR +

Positive Calibrator / Calibrateur positif / Calibrador positivo

CALIBRATOR −

Negative Calibrator / Calibrateur négatif / Calibrador negativo

Confirmatory Control

Confirmatory Control / Contrôle de confirmation / Control de confirmación

Recombinant Antigens Provided by

Recombinant Antigens Provided by / Antigènes recombinants fournis par / Antígenos recombinantes suministrados por

Antibody to Hepatitis B Surface Antigen

Antibody to Hepatitis B Surface Antigen / Anticorps dirigé contre l'antigène de surface du virus de l'hépatite B / Anticuerpo frente al antígeno de superficie de la hepatitis B

Antibody to Hepatitis B Surface Antigen: Peroxidase Conjugate Concentrate

Antibody to Hepatitis B Surface Antigen: Peroxidase Conjugate Concentrate / Anticorps dirigé contre l'antigène de surface du virus de l'hépatite B : conjugué concentré à la peroxydase / Anticuerpo frente al antígeno de superficie de la hepatitis B: concentrado de conjugado a peroxidasa

Der Grüne Punkt (the Green Dot). Manufacturer follows certain packaging material waste disposal management regulations. / Der Grüne Punkt (Point Vert). Le fabricant suit certaines règles de mise au rebut pour les déchets des matériaux d'emballage / Punto Verde (der grüne Punkt). El fabricante sigue la regulación sobre gestión de residuos de los embalajes

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