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

Intended Use
FOR IN VITRO DIAGNOSTIC 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 automated mode with the ORTHO® Summit System (OSS).

Summary and Explanation of the Test
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. During the acute phase of HBV infection, anti-HBc appears shortly after the appearance of HBsAg and persists following HBsAg clearance. 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. Anti-HBc is found in virtually all patients with chronic hepatitis B.

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

Principles 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 Novartis Vaccines and Diagnostics, 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.

Warnings and Precautions

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.
1. Wear disposable gloves while handling kit reagents and specimens. Thoroughly wash hands afterward.
2. All specimens should be handled as potentially infectious agents.
3. 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
4. 4N sulfuric acid (H\textsubscript{2}SO\textsubscript{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.
- 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.

5. 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.
6. Handle OPD tablets with plastic or Teflon\textsuperscript{®}-coated forceps only. Metal forceps may react with tablets and interfere with the test results.
7. 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.
8. \(\alpha\)-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.
9. ProClin® 300 (CAS 55965-84-9) is included as a preservative in the Conjugate. Following are the Hazard and Precautionary Requirements. Refer to www.orthoclinical.com for the Safety Data Sheets and for Ortho contact information.

**WARNING:**

**Hazard Statements:**
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.

10. 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. 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.

11. Distilled or deionized water must be used for Wash Buffer preparation. Clinical laboratory reagent water Type I or Type II is acceptable. Store the water in nonmetallic containers.

12. 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.

13. All reagents and components must be at room temperature prior to use and kit components returned to 2 to 8°C after use.

14. 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.

15. 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. 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.

16. Do not use reagents beyond their labeled expiration date.

17. Cross-contamination between reagents will invalidate the test results. Labeled, dedicated reservoirs for the appropriate reagents are recommended.
18. Ensure that specimen is added to the microwell. Failure to add specimen may produce an erroneous nonreactive result.
19. When using a single-channel micropipette for manual sample addition, use a new pipette tip for each specimen to be assayed. When using a multi-channel micropipette, new tips are to be used for each reagent to be added.
20. Strict adherence to the specified wash procedure is crucial to ensure optimum assay performance (see Test Procedure).
21. Do not allow the microwells to become dry once the assay has begun.
22. Do not touch the bottom exterior surface of the microwells. Fingerprints or scratches may interfere with reading the microwell.
23. 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.
24. 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.
25. Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell strips during the assay because the color reaction may be inhibited.
26. All pipetting equipment should be used with care and calibrated regularly, following the equipment manufacturer’s instructions.
27. 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 erroneous readings.
28. Delays in plate processing may affect absorbance values.
29. Room temperature is defined as 15 to 30°C.
30. 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
31. Visual inspections of the reagents should be performed prior to use to check for color change, cloudiness, and precipitates.

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### Reagents

<table>
<thead>
<tr>
<th>Label Abbreviations</th>
<th>480 Test Kit Product Code 933245</th>
<th>2400 Test Kit Product Code 933275</th>
<th>Component Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBc</td>
<td>5</td>
<td>25</td>
<td>Hepatitis B Virus Core Antigen (HBcAg) (Recombinant)-Coated Microwell Plates (8 strips of 12 wells each in holder)</td>
</tr>
<tr>
<td>CON</td>
<td>1 bottle (125 mL) 5 bottles (125 mL)</td>
<td>Antibody Conjugate: (Murine Monoclonal) – mixture of anti-human IgG and anti-human IgM conjugated to horseradish peroxidase with protein stabilizers. Preservative: 1% ProClin® 300</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>1 bottle (150 mL) 5 bottles (150 mL)</td>
<td>Specimen Diluent – phosphate-buffered saline with bovine protein stabilizers. Preservative: 0.1% 2-chloroacetamide</td>
<td></td>
</tr>
<tr>
<td>OPD</td>
<td>1 vial (30 tablets) 3 vials (30 tablets)</td>
<td>OPD Tablets – contains o-phenylenediamine • 2HCl</td>
<td></td>
</tr>
<tr>
<td>SB</td>
<td>1 bottle (190 mL) 5 bottles (190 mL)</td>
<td>Substrate Buffer-G – citrate-phosphate buffer with 0.02% hydrogen peroxide. Preservative: 0.1% 2-chloroacetamide</td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>1 vial (1.0 mL) 4 vials (1.0 mL)</td>
<td>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: 0.2% thimerosal</td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>2 vials (1.0 mL) 5 vials (1.0 mL)</td>
<td>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: 0.2% thimerosal</td>
<td></td>
</tr>
</tbody>
</table>

21 | N/A | Plate Sealers, disposable |
Caution: HANDLE AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS.

Storage Requirement
Store at 2 to 8 °C.

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

Specimen Collection, Storage and Handling

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

1. No special preparation of the patient is required prior to blood collection.
2. Blood should be collected by approved medical techniques.
3. Blood specimens collected in glass, plastic or serum separator tubes may be used.
4. Plasma collected with an improper ratio of specimen to anticoagulant should not be used.
5. 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.
6. Do not use heat-treated specimens.
7. Do not use azide to preserve specimens. Do not test patient or donor samples containing azide. Sodium azide inhibits horseradish peroxidase activity.
8. 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.
9. 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.

Reagent Preparation

1. Preparation of Wash Buffer (1X): Fractions of bottles are not recommended for dilution. Each bottle of 20X Wash Buffer concentrate will make 3L of working buffer solution.
1. Preparation of Wash Buffer (1X):

   a. In an appropriate sized container, mark the required final diluted volume.
   b. Add approximately 70% of total volume of distilled or deionized water.
   c. Invert 20X bottle(s) to thoroughly mix the contents of the solution of the bottle.
   d. Add the contents of the required bottle(s) of 20X wash buffer concentrate to the container. If crystals are observed in the bottle, proceed to step 1.e. If no crystals are observed, proceed to step 1.g.
   e. Rinse each bottle with distilled or deionized water and add each rinse to the container to ensure any crystals which may be present are transferred from the bottle to the final buffer container.
   f. If crystals remain, continue process until all crystals have been transferred from the bottle(s) to the container.
   g. Add the remaining amount of water to the mark on the container for the required diluted volume.

   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:

   a. 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.
   b. Completely dissolve the appropriate number of OPD tablets in Substrate Buffer-G prior to use.
   c. 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.

<table>
<thead>
<tr>
<th>Number of Wells</th>
<th>Number of Plates</th>
<th>Number of OPD Tablets</th>
<th>Substrate Buffer-G (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>0.5</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>96</td>
<td>1</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>144</td>
<td>1.5</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td>192</td>
<td>2</td>
<td>4</td>
<td>48</td>
</tr>
<tr>
<td>240</td>
<td>2.5</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>288</td>
<td>3</td>
<td>6</td>
<td>72</td>
</tr>
</tbody>
</table>

   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.

Testing 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

- ORTHO® Assay protocol Disk (OAPD) for Hepatitis B Virus Core Antigen (Recombinant) ORTHO® HBc ELISA Test System (Product Code 938246)
- Hepatitis B Virus Core Antigen (Recombinant) ORTHO® HBc ELISA Test System Plate Bar Code Labels (Product Code 935138, 1000 plate labels) required to perform the assay on the ORTHO® Summit System
• Hepatitis B Virus Core Antigen (Recombinant) ORTHO® HBc ELISA Test System Control Vial Bar Code Labels (Product Code 6904574, 255 sets) required to perform the assay on the ORTHO VERSEIA® Pipetter
• Hepatitis B Virus Core Antigen (Recombinant) ORTHO® HBc ELISA Test System Specimen Diluent Bar Code Labels (Product Code 6904575, 420/pkg) required to perform the assay on the ORTHO VERSEIA® Pipetter
• 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
• ORTHO® Summit Processor, adjustable multichannel micropipettes, or equivalent reagent dispenser capable of delivering 50 μL and 200 μL with at least ± 5% accuracy
• 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
• 5 μL to 250 μL disposable pipette tips or equivalent
• Appropriately sized serological pipette or graduated cylinder
• Multichannel micropipette reservoir or equivalent reagent container
• 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.)
• 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.
• ORTHO® Summit Processor or equivalent 37°C ± 1°C microwell incubator (dry or humidified)
• Distilled or deionized water; clinical laboratory reagent water Type I or Type II is acceptable. (See the Warnings & Precautions section.)
• 5.25% sodium hypochlorite (chlorine bleach)
• 4N Sulfuric Acid (H$_2$SO$_4$) - available in the United States from Ortho-Clinical Diagnostics, Inc. (Product Code 933040) or equivalent.

Note:
To determine the suitability of another source of acid, prepare Substrate Solution as described under Reagent Preparation. Add 200 μL of Substrate Solution to three microwells, and then add 50 μL of 4N Sulfuric Acid (H$_2$SO$_4$) to be tested to each microwell. Read the microwells at a wavelength of 490 or 492 nm with a reference filter of 620 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.

• Black microwell strips or equivalent uncoated microwells
• 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.
• 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 must 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.
3. Assemble the microwell strips in 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.

<table>
<thead>
<tr>
<th>Well</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>Substrate Blank</td>
</tr>
<tr>
<td></td>
<td>Negative Control</td>
</tr>
<tr>
<td></td>
<td>Negative Control</td>
</tr>
<tr>
<td></td>
<td>Negative Control</td>
</tr>
<tr>
<td></td>
<td>Positive Control</td>
</tr>
<tr>
<td></td>
<td>Positive Control</td>
</tr>
</tbody>
</table>

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:

a. **Sample Addition:**
   - Add 200 μL of Specimen Diluent to all wells, except 1A using the ORTHO VERSEIA® Pipetter, a micropipette, or an equivalent pipettor-dilutor capable of delivering 200 μL with at least ±5% accuracy.
   - Add 10 μL of the control, or specimens to the appropriate wells using the ORTHO VERSEIA® Pipetter, a micropipette, or an equivalent pipettor-dilutor capable of delivering 10 μL with at least ±10% accuracy.
   - 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.

b. **For Previously Diluted Sample Addition:**
   - Add 300 μL of Specimen Diluent to a tube or container.
   - Add 15 μL of control or specimen to the tube. Mix thoroughly.
   - 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 strip holder, if necessary. With an aspirator-washer device, aspirate and wash all wells **five times** with Wash Buffer (1X).

8. **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 the microwells, then fill completely 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.

9. 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.

10. 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**.

11. Prepare sufficient Substrate Solution prior to use in Step 12 to allow time for OPD tablets to dissolve completely. Refer to Reagent Preparation.

**Note:** Do not use more than a single preparation of Substrate Solution on a plate.

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

13. **Caution:** Do not use more than a single preparation of Substrate Solution on a plate.

14. 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.

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

16. Add 50 μL of 4N sulfuric acid (H₂SO₄) 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 strip 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$_2$SO$_4$). Plates must be stored in the dark until read.

Quality Control

Quality Control Procedures

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 (NCx ) 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 (NCx).

   **Example:**
   
<table>
<thead>
<tr>
<th>Negative Control</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.200</td>
</tr>
<tr>
<td>2</td>
<td>0.180</td>
</tr>
<tr>
<td>3</td>
<td>0.160</td>
</tr>
<tr>
<td>Total Absorbance</td>
<td>0.540</td>
</tr>
<tr>
<td>NCx = Total Absorbance / 3</td>
<td>0.180</td>
</tr>
</tbody>
</table>

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 = NCx + 0.400

   **Example:**
   
<table>
<thead>
<tr>
<th>Negative Control</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.200</td>
</tr>
<tr>
<td>2</td>
<td>0.180</td>
</tr>
<tr>
<td>3</td>
<td>0.160</td>
</tr>
<tr>
<td>Total Absorbance</td>
<td>0.540</td>
</tr>
<tr>
<td>NCx = Total Absorbance / 3</td>
<td>0.180</td>
</tr>
<tr>
<td>Cutoff Value = 0.180 + 0.400</td>
<td>0.580</td>
</tr>
</tbody>
</table>

**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 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

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

<table>
<thead>
<tr>
<th>SITE</th>
<th>NUMBER TESTED</th>
<th>INITIALY NONREACTIVE</th>
<th>INITIALY REACTIVE</th>
<th>REPEATEDLY REACTIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N (%)</td>
<td>Mean S/CO</td>
<td>N (%)</td>
</tr>
<tr>
<td>1</td>
<td>1033</td>
<td>1016 (98.4%)</td>
<td>0.233</td>
<td>17 (1.6%)</td>
</tr>
<tr>
<td>2</td>
<td>1000</td>
<td>989 (98.9%)</td>
<td>0.212</td>
<td>11 (1.1%)</td>
</tr>
<tr>
<td>3</td>
<td>977</td>
<td>964 (98.7%)</td>
<td>0.166</td>
<td>13 (1.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>3010</td>
<td>2969 (98.6%)</td>
<td>0.204</td>
<td>41 (1.4%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NUMBER TESTED</th>
<th>INITIALLY NONREACTIVE</th>
<th>INITIALLY REACTIVE</th>
<th>REPEATEDLY REACTIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A HBV</td>
<td>25</td>
<td></td>
<td>25 (100%)</td>
<td>25 (100%)</td>
</tr>
<tr>
<td>C HBV</td>
<td>28</td>
<td>0</td>
<td>28 (100%)</td>
<td>28 (100%)</td>
</tr>
<tr>
<td>HAV</td>
<td>10</td>
<td>8 (80%)</td>
<td>2 (20%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>HCV</td>
<td>10</td>
<td>8 (80%)</td>
<td>2 (20%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>LIVER</td>
<td>25</td>
<td>12 (48%)</td>
<td>13 (52%)</td>
<td>10 (40%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Panel Member</th>
<th>N</th>
<th>Mean S/CO</th>
<th>SD</th>
<th>CV (%)</th>
<th>SD</th>
<th>CV (%)</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (IgM)</td>
<td>90</td>
<td>1.568</td>
<td>0.123</td>
<td>7.8</td>
<td>0.093</td>
<td>5.9</td>
<td>0.201</td>
<td>12.8</td>
</tr>
<tr>
<td>B (IgM)</td>
<td>90</td>
<td>2.177</td>
<td>0.131</td>
<td>6.0</td>
<td>0.073</td>
<td>3.4</td>
<td>0.158</td>
<td>7.3</td>
</tr>
<tr>
<td>C (IgG)</td>
<td>90</td>
<td>1.207</td>
<td>0.126</td>
<td>10.4</td>
<td>0.008</td>
<td>0.7</td>
<td>0.191</td>
<td>15.8</td>
</tr>
<tr>
<td>D (IgG)</td>
<td>90</td>
<td>1.468</td>
<td>0.090</td>
<td>6.1</td>
<td>0.059</td>
<td>4.0</td>
<td>0.207</td>
<td>14.1</td>
</tr>
<tr>
<td>E (IgG)</td>
<td>90</td>
<td>1.262</td>
<td>0.076</td>
<td>6.0</td>
<td>0.083</td>
<td>6.6</td>
<td>0.223</td>
<td>17.7</td>
</tr>
<tr>
<td>F (Nonreactive)</td>
<td>90</td>
<td>0.144</td>
<td>0.039</td>
<td>27.1</td>
<td>0.000</td>
<td>0.0</td>
<td>0.044</td>
<td>30.6</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>SITE</th>
<th>NUMBER TESTED</th>
<th>INITIALLY NONREACTIVE</th>
<th>INITIALLY REACTIVE</th>
<th>REPEATEDLY REACTIVE</th>
<th>NONREACTIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>Mean S/CO</td>
<td>N (%)</td>
<td>Mean S/CO</td>
<td>N (%)</td>
</tr>
<tr>
<td>1</td>
<td>1024</td>
<td>1019 (99.5%)</td>
<td>8.160</td>
<td>5 (0.5%)</td>
<td>1019 (99.5%)</td>
</tr>
<tr>
<td>2</td>
<td>1100</td>
<td>1097 (99.7%)</td>
<td>7.734</td>
<td>3 (0.3%)</td>
<td>1097 (99.7%)</td>
</tr>
<tr>
<td>3</td>
<td>1051</td>
<td>1049 (99.8%)</td>
<td>2.770</td>
<td>1 (0.1%)</td>
<td>1050 (99.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>3175</td>
<td>3165 (99.7%)</td>
<td>6.221</td>
<td>9 (0.3%)</td>
<td>3166 (99.7%)</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>SITE</th>
<th>NUMBER TESTED</th>
<th>INITIALLY NONREACTIVE</th>
<th>INITIALLY REACTIVE</th>
<th>REPEATEDLY REACTIVE</th>
<th>NONREACTIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>Mean S/CO</td>
<td>N (%)</td>
<td>Mean S/CO</td>
<td>N (%)</td>
</tr>
<tr>
<td>1</td>
<td>1024</td>
<td>1018 (99.4%)</td>
<td>7.251</td>
<td>5 (0.5%)</td>
<td>1019 (99.5%)</td>
</tr>
<tr>
<td>2</td>
<td>1100</td>
<td>1092 (99.3%)</td>
<td>3.758</td>
<td>5 (0.5%)</td>
<td>1095 (99.5%)</td>
</tr>
<tr>
<td>3</td>
<td>1051</td>
<td>1046 (99.5%)</td>
<td>1.196</td>
<td>5 (0.5%)</td>
<td>1046 (99.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>3175</td>
<td>3156 (99.4%)</td>
<td>4.068</td>
<td>15 (0.5%)</td>
<td>3160 (99.5%)</td>
</tr>
</tbody>
</table>

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

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

* 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 pipettors 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

<table>
<thead>
<tr>
<th>SITE</th>
<th>SAMPLES TESTED</th>
<th>REPLICATES TESTED</th>
<th>REPLICATES NONREACTIVE N (%)</th>
<th>REPLICATES REACTIVE N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>114</td>
<td>342</td>
<td>1 (0.3%)</td>
<td>341 (99.7%)</td>
</tr>
<tr>
<td>2</td>
<td>114</td>
<td>341</td>
<td>0 (0.0%)</td>
<td>341 (100%)</td>
</tr>
<tr>
<td>3</td>
<td>114</td>
<td>342</td>
<td>1 (0.3%)</td>
<td>341 (99.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>342</td>
<td>1025</td>
<td>2 (0.2%)</td>
<td>1023 (99.8%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>SITE</th>
<th>SAMPLES TESTED</th>
<th>REPLICATES TESTED</th>
<th>REPLICATES NONREACTIVE N (%)</th>
<th>REPLICATES REACTIVE N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>114</td>
<td>342</td>
<td>0 (0.0%)</td>
<td>342 (100%)</td>
</tr>
<tr>
<td>2</td>
<td>114</td>
<td>341</td>
<td>0 (0.0%)</td>
<td>341 (100%)</td>
</tr>
<tr>
<td>3</td>
<td>114</td>
<td>342</td>
<td>0 (0.0%)</td>
<td>342 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>342</td>
<td>1025</td>
<td>0 (0.0%)</td>
<td>1025 (100%)</td>
</tr>
</tbody>
</table>
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 for 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/OSS vs. manual, the regression analysis yielded a slope of 1.10, intercept of 0.05 and Pearson correlation coefficient (r) of 0.99. The 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 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

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

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

**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.
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 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

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

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

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

<table>
<thead>
<tr>
<th>Platform</th>
<th>Panel Member</th>
<th>N</th>
<th>Mean S/CO</th>
<th>Inter-plate**</th>
<th>Intra-plate**</th>
<th>Total**</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORTHO® Summit Pipetter</td>
<td>A (IgM)</td>
<td>150</td>
<td>1.719</td>
<td>0.034</td>
<td>2.0</td>
<td>0.557</td>
</tr>
<tr>
<td></td>
<td>B (IgM)</td>
<td>150</td>
<td>2.205</td>
<td>0.198</td>
<td>9.0</td>
<td>0.175</td>
</tr>
<tr>
<td></td>
<td>C (IgG)</td>
<td>150</td>
<td>1.431</td>
<td>0.000</td>
<td>0.0</td>
<td>0.138</td>
</tr>
<tr>
<td></td>
<td>D (IgG)</td>
<td>150</td>
<td>1.648</td>
<td>0.076</td>
<td>4.6</td>
<td>0.333</td>
</tr>
<tr>
<td></td>
<td>E (IgG)</td>
<td>150</td>
<td>1.347</td>
<td>0.068</td>
<td>5.0</td>
<td>0.122</td>
</tr>
<tr>
<td></td>
<td>F (Nonreactive)</td>
<td>150</td>
<td>0.217</td>
<td>0.099</td>
<td>45.6</td>
<td>0.118</td>
</tr>
<tr>
<td>ORTHO VERSEIA® Pipetter</td>
<td>A (IgM)</td>
<td>150</td>
<td>1.881</td>
<td>0.052</td>
<td>2.8</td>
<td>0.127</td>
</tr>
<tr>
<td></td>
<td>B (IgM)</td>
<td>150</td>
<td>2.193</td>
<td>0.080</td>
<td>3.6</td>
<td>0.128</td>
</tr>
<tr>
<td></td>
<td>C (IgG)</td>
<td>150</td>
<td>1.692</td>
<td>0.013</td>
<td>0.8</td>
<td>0.148</td>
</tr>
<tr>
<td></td>
<td>D (IgG)</td>
<td>150</td>
<td>1.917</td>
<td>0.076</td>
<td>4.0</td>
<td>0.084</td>
</tr>
<tr>
<td></td>
<td>E (IgG)</td>
<td>150</td>
<td>1.700</td>
<td>0.029</td>
<td>1.7</td>
<td>0.108</td>
</tr>
<tr>
<td></td>
<td>F (Nonreactive)</td>
<td>150</td>
<td>0.199</td>
<td>0.009</td>
<td>4.5</td>
<td>0.032</td>
</tr>
</tbody>
</table>

* 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.
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

<table>
<thead>
<tr>
<th>Reproducibility Panel Member</th>
<th>Manual</th>
<th>ORTHO® Summit</th>
<th>ORTHO VERSEIA®</th>
<th>Difference from Manual</th>
<th>Two-tailed p-value</th>
<th>Difference from ORTHO® Summit</th>
<th>Two-tailed p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (IgM)</td>
<td>1.568</td>
<td>1.719</td>
<td>9.6%</td>
<td>&lt;0.0001</td>
<td>20.0%</td>
<td>&lt;0.0001</td>
<td>4.9%</td>
</tr>
<tr>
<td>B (IgM)</td>
<td>2.177</td>
<td>2.245</td>
<td>1.3%</td>
<td>0.4148</td>
<td>0.7%</td>
<td>0.5885</td>
<td>-0.5%</td>
</tr>
<tr>
<td>C (IgG)</td>
<td>1.207</td>
<td>1.365</td>
<td>18.6%</td>
<td>&lt;0.0001</td>
<td>42.0%</td>
<td>&lt;0.0001</td>
<td>18.2%</td>
</tr>
<tr>
<td>D (IgG)</td>
<td>1.468</td>
<td>1.564</td>
<td>12.3%</td>
<td>0.0001</td>
<td>36.0%</td>
<td>&lt;0.0001</td>
<td>16.3%</td>
</tr>
<tr>
<td>E (IgG)</td>
<td>1.262</td>
<td>1.347</td>
<td>6.7%</td>
<td>0.0001</td>
<td>34.7%</td>
<td>&lt;0.0001</td>
<td>26.2%</td>
</tr>
<tr>
<td>F (Nonreactive)</td>
<td>0.144</td>
<td>0.217</td>
<td>50.7%</td>
<td>&lt;0.0001</td>
<td>38.2%</td>
<td>&lt;0.0001</td>
<td>-8.3%</td>
</tr>
</tbody>
</table>

* 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.

### References

Glossary of Symbols

The following symbols may have been used in the labeling of this product.

- **Do Not Use**
- **Use by or Expiration Date** (Year-Month-Day)
- **Batch Code or Lot Number**
- **Serial Number**
- **Catalog Number or Product Code**
- **Caution**
- **Date of Manufacture**
- **Manufacturer**
- **Authorized Representative in the European Community**
- **Positive Control**
- **Negative Control**
- **Positive Calibrator**
- **Negative Calibrator**
- **Confirmatory Control**

![Symbols Diagram]

- **Contains Sufficient for "n" Tests**
- **In vitro Diagnostic Medical Device**
- **Upper Limit of Temperature**
- **Lower Limit of Temperature**
- **Temperature Limitation**
- **Consult instructions for use**
- **Biological Risks**
- **Health Hazards**
- **Corrosive**
- **Acute Toxicity**
- **Environmental or Aquatic Toxicity**
- **Fragile, Handle with Care.**
- **Keep Dry**
- **This end up**
- **Do Not Use if Damaged**
- **Serious Health Hazard**
- **Antibody to Hepatitis B Surface Antigen**
- **Antibody to Hepatitis B Surface Antigen Recombinant Antigens Provided by**
- **Antibody to Hepatitis B Surface Antigen Peroxidase Conjugate Concentrate**
- **Antibody to Hepatitis B Surface Antigen Peroxidase Conjugate Concentrate**

Revision History

<table>
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<tr>
<th>Date of Revision</th>
<th>Version</th>
<th>Description of Technical Changes*</th>
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<tr>
<td>2020-11-19</td>
<td>e631201329</td>
<td>Reagent Preparation: Updated Preparation of Wash Buffer (1X)</td>
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* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.