

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

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.

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 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.

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 <i>o</i> -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 to 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

- 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}
- Wear disposable gloves while handling kit reagents and specimens. Thoroughly wash hands afterward.
- All specimens should be handled as potentially infectious agents.
- 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}
- 4N sulfuric acid (H₂SO₄) 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.
- Handle OPD tablets with plastic or Teflon®-coated forceps. Metal forceps may react with the tablets and interfere with the test results.
- 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. Following are the Risk and Safety Requirements.¹²

T,N,R: 20/21-25-36-40-43-50/53-68 – Harmful by inhalation and in contact with skin. Toxic if swallowed. Irritating to eyes. Limited evidence of a carcinogenic effect. May cause sensitization by skin contact. Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Possible risks of irreversible effects.

S: 26-36/37-45-60-61 – In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). This material and its container must be disposed of as hazardous waste. Avoid release to the environment. Refer to special instructions/safety data sheets.
- 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 vial. The desiccant pouch must be retained in the vial at all times. Do not use tablets which are yellow or broken.
- 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.
- 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.
- All reagents and components **must** be at room temperature prior to use and kit components returned to 2 to 8°C after use.
- 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.

13. Do not use reagents beyond their labeled expiration date.
14. Cross-contamination between reagents will invalidate the test results. Labeled, dedicated reservoirs for the appropriate reagents are recommended.
15. Ensure that specimen is added to the microwell. Failure to add specimen may produce an erroneous nonreactive result.
16. 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.
17. Strict adherence to the specified wash procedure is crucial to ensure optimum assay performance. (See Step 7 of **Test Procedure**.)
18. Do not allow microwells to become dry once the assay has begun.
19. Do not touch the bottom exterior surface of the microwells. Fingerprints or scratches may interfere with reading the microwells.
20. 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.
21. 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.
22. 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.
23. All pipetting equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.
24. 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.
25. ProClin™ 300 is included as a preservative in the Conjugate Reagent. Following are the Risk and Safety Requirements.¹²
R: 43 – May cause sensitization by skin contact.
S: 24-37 – Avoid contact with skin. Wear suitable gloves.
26. 2-chloroacetamide is included as a preservative in the 20X Wash Buffer Concentrate, Substrate Buffer-G and Specimen Diluent.
 Following are the Risk and Safety Requirements.¹²
R: 43 – May cause sensitization by skin contact.
S: 24-37-60 – Avoid contact with skin. Wear suitable gloves. This material and its container must be disposed of as hazardous waste.

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 to 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 60 minutes after the addition of OPD tablets when held at room temperature in the dark and should be colorless to very pale yellow when used. Record the time when the OPD tablets are added to the Substrate Buffer-G and when it will expire on the container. **If it is noticeably yellow in color, discard and prepare more Substrate Solution as required.**

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques. Plasma collected with an improper ratio of specimen to anticoagulant should not be used. Serum or plasma collected in EDTA, heparin or citrate-based anticoagulants may be used and should be tested as soon as possible following collection. **Do not use heat-treated specimens. Do not use azide to preserve specimens. Do not test patient or donor samples containing azide. Sodium azide inhibits horseradish peroxidase activity.** Serum or plasma may be stored at 2 to 8°C for up to seven days. If longer storage is necessary, the specimens should be frozen (-20°C or lower) to limit possible contamination. Storage of specimens in self-defrosting freezers is not recommended. No effect was observed on reactivity in ORTHO HbC ELISA Test System when 12 weakly reactive and 12 nonreactive specimens were subjected to five freeze-thaw cycles. Clear, nonhemolyzed specimens are preferred. Precipitates in specimens should be removed by centrifugation.

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

PROCEDURE

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. Adjustable multichannel micropipette capable of delivering 50 µL and 200 µL with at least ± 5% accuracy or equivalent reagent dispenser
2. Fixed or adjustable single-channel micropipette capable of delivering 10 µL with at least ± 10% accuracy or equivalent sample dilutor
3. 5 µL to 250 µL disposable pipette tips or equivalent
4. Appropriately sized serological pipette or graduated cylinder
5. Multichannel micropipette reservoir or equivalent reagent container
6. Multichannel aspirator-washer device capable of dispensing and aspirating 300 µL to 800 µL per well. (Consult the device's operator's manual for additional technical information.)
7. Dual wavelength microwell reader capable of reading at 490 nm or 492 nm with a reference filter of 620 nm 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. Linearity of the microwell reader must range from at least 0 to 2.5 absorbance units. Consult the instrument manufacturer's specifications.
8. 37°C ± 1°C incubator (dry or humidified)
9. Distilled or deionized water, clinical laboratory reagent water Type I or Type II is acceptable (see **PRECAUTIONS** section)
10. 5.25% sodium hypochlorite (chlorine bleach)
11. 4N sulfuric acid (H₂SO₄)—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 H₂SO₄ 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.
12. Black microwell strips (Product Code 651-20-003-1: Ortho-Clinical Diagnostics, Inc.) or equivalent uncoated microwells
13. 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
14. Variable speed microwell plate shaker capable of 100 to 400 rpm for use when not using a pipetter-dilutor for sample delivery

Test Procedure

1. Prior to the beginning of the procedure, bring kit components to room temperature (15 to 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. If the entire strip is not needed, an appropriate number of wells can be broken off. Unused wells should be stored at 2 to 8°C in the supplied foil pouch, tightly sealed **with desiccant** and used within 14 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: 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:
 - a. 200 µL of Specimen Diluent to all wells, **except 1A**.
 - b. 10 µL of the controls or specimens to the appropriate wells.
 - c. If the controls and specimens have been manually delivered, after filling the microwells, place the microwell strip holder on a microwell plate shaker to mix for 5 to 10 seconds. The shaker should be used at a slow to moderate speed, taking care to avoid splashing of the contents of the test wells.
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.
 - 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**.
 9. For manual processing of microwell plates, cover the microwell strip holder with a **new 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**.
 13. Incubate at room temperature in the dark for 30 minutes ± 1 minute.
 14. Add 50 µL of 4N sulfuric acid (H₂SO₄) to all wells, **including 1A**. 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.

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. 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 0.050.

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 linear 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 linear range of the microwell reader may appear as "OVER" or "****" or ">".

4. Calculation of the Cutoff Value

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

Example:

Negative Control	Absorbance
1	0.200
2	0.180
3	0.160
Total Absorbance	= 0.540
$\text{NC}\bar{x} = \frac{\text{Total Absorbance}}{3}$	= 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. 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.
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 CHARACTERISTICS¹⁷

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 3,010 specimens were tested, 2,969 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
1	1,033	1,016 (98.4%)	17 (1.6%)	16 (1.5%)
2	1,000	989 (98.9%)	11 (1.1%)	7 (0.7%)
3	977	964 (98.7%)	13 (1.3%)	10 (1.0%)
Total	3,010	2,969 (98.6%)	41 (1.4%)	33 (1.1%)

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%)

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.

SUMMARY OF REVISIONS**Section****Revision****REAGENTS**

Updated component description for SD - Specimen Diluent.

PRECAUTIONS

Removed statement dealing with previous Specimen Diluent.
Number 26: Updated Risk and Safety Statements for 2-chloroacetamide.

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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 / Limite supérieur de temperatura



Lower Limit of Temperature / Conserver à une température égale ou supérieure à / Limite 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



Irritant / Irritant / Irritante



Harmful / Nocif / Nocivo



Toxic / Toxique / Tóxico

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

Continued / Suite / Continuación



*Dangerous for the Environment / Dangereux pour l'environnement /
Peligroso para el medio ambiente*



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



Keep Dry / Tenir au sec / Mantener seco



This end up / Haut / Este lado hacia 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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CHIRON

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