ENGLISH

iStatis

Hepatitis B Surface Antigen Test

Single-use lateral flow immunoassay for the detection of Hepatitis B Surface Antigen

REF **90-1136**

2°C – 30°C **Store at 2°C – 30°C. For *in vitro* diagnostic use only.** IVD

It is recommended that the entire Instructions for Use be read prior to beginning the test procedure. Although the assay is designed to be simple to use, conformance with the test procedure is necessary to ensure accurate results.

INTENDED USE - Not for donor screening

The iStatis Hepatitis B Surface Antigen Test (HBsAg) is a single use, rapid, lateral-flow, in vitro qualitative immunoassay for the detection of Hepatitis B Surface Antigen in human EDTA-whole blood, fingerstick whole blood, serum or plasma. The test is intended for manual operation by trained personnel in medical facilities, clinical laboratories, emergency care situations, and physicians' offices as an aid to diagnosis for HBV infection in adults over 18 years of age by providing results within 15 minutes. Although suitable for near-patient or point-of-care (POC) testing, it is not suitable for home testing.

All required pre- and post-test counseling guidelines must be followed in each setting in which the iStatis Hepatitis B Surface Antigen Test is used. The **iStatis Hepatitis B Surface Antigen Test** will be referred to as iStatis HBsAg Test in the remainder of this Instructions for Use.

SUMMARY

Hepatitis B is a potentially life-threatening liver infection caused by the Hepatitis B Virus (HBV). It can cause chronic infection and puts people at high risk of death from cirrhosis and liver cancer. Hepatitis B can be spread through perinatal transmission, and exposure to infected blood or bodily fluids such as saliva, menstrual, vaginal, and seminal fluids.[1]
Following a reactive result for Hepatitis B Surface Antigens, a venous blood sample must be drawn in an EDTA collection tube (for whole blood or plasma) or red-top tube (for serum) and forwarded to a laboratory for HBV confirmatory testing.

PRINCIPLES OF THE TEST

The **iStatis HBsAg Test** is packaged as a kit containing a single-use cartridge unit along with a chase buffer vial. The test cartridge consists of a sample absorption pad, a conjugate reagent pad with specific anti-HBsAg antibodies, and a test membrane where an anti-HBsAg antibody, as well as a control antibody have been striped on two distinct lines to form the entire test strip. Results are visualized in 15 minutes following the addition of the sample and chase buffer to the test cartridge in the form of blue control (marked "C") and test lines (marked "T"). Results should not be read after 30 minutes.

Hepatitis B Surface Antigen Detection:
Upon addition of a sample to the iStatis HBsAg Test cartridge, the Hepatitis B Surface Antigens bind to the conjugated antibodies. This complex then migrates onto the test strip, aided by chase buffer, and is captured by capture antibodies on the test line immobilized on the nitrocellulose membrane. The test strip is designed to filter, absorb, and retain the test specimen and all the test reagents in such a manner as to limit leakage and exposure of personnel to potentially infectious materials.

Test Complexity: The iStatis HBsAg Test was designed to reduce protocol complexity. The iStatis HBsAg assay does not require sample preparation, a separate reader, or several steps, which include multiple washes and reagents. These requirements increase the complexity of an assay and lead to procedural errors which may adversely affect sensitivity and specificity. Total test time may vary slightly depending on specimen type, but results of valid tests are readable within 15 minutes and up to 30 minutes.

KIT COMPONENTS AND STORAGE

2°C – 30°C iStatis components should be stored at 2-30°C
All kit components are individually packaged for single use only. Each test requires the following materials:

1. **Test cartridge:** individually packaged, prepared in a foil pouch with a desiccant.
2. **Chase Buffer Vial (0.27 mL)**: A vial that contains chase buffer, a phosphate buffer used for aiding the sample across the cartridge.
3. **Buffer Vial Cap:** A dispensing nozzle to dispense the chase buffer into the sample well of the cartridge.
4. **Buffer Vial Holder:** A cardboard holder to hold chase buffer vial during sample collection process.
5. **Alcohol Swab**: For disinfection of the patient's finger prior to pricking.
6. STERILE R **Single-use lancet**: For pricking the patient's finger to allow for fingerstick sample collection.
7. **Single-use capillary pipette (50 µL):** For collection and dispensing of fingerstick blood sample into the sample well of the cartridge.

⚠ Chase buffer solution contains 0.08% (w/v) Sodium Azide as a preservative and is harmful if swallowed. This solution is for single use only and is to be stored at 2°C to 30°C until expiry date specified on the test kit.

MATERIALS REQUIRED BUT NOT PROVIDED

- Personal protective equipment
- Appropriate biohazard waste containers and disinfectants
- Absorbent cotton balls for fingerstick or venipuncture wound closure
- Timer

For venipuncture blood collection and testing:

- Venipuncture apparatus if collecting venous blood samples
- Appropriate blood collection tubes and shipping containers
- Precision pipette capable of delivering 50 µL of sample

MATERIALS AVAILABLE AS AN ACCESSORY TO THE KIT

iStatis HBsAg Positive and Negative Controls are available from bioLytical Laboratories in user-defined amounts, for use in quality control procedures. Please refer to the section on Quality Control, following the Assay Procedure, and the iStatis HBsAg Test Controls Instructions for Use.

WARNINGS

1. Do not use test cartridges or the chase buffer if packaging has been damaged.
2. Do not use if the desiccant in the test cartridge package is exposed or damaged.
3. Do not use the kit beyond the expiry date.
4. This product has been authorized only for the detection of HBsAg, not for any other bacteria, viruses, or pathogens.
5. Do not interchange kit contents from different lots.
6. Avoid microbial contamination and exercise care in handling the kit components.
7. Failure to use the provided buffer solution and vial cap may result in leakage and/or overflow of liquids from the test cartridge.
8. If the kit is refrigerated, ensure it is brought to room temperature (15-30°C) before performing the test. If required, use the iStatis HBsAg Positive/Negative Controls to ensure proper kit performance.
9. ⚠ Sodium azide is present at 0.08% in chase buffer solution. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. If products containing sodium azide are discarded into a drain, flush with large amounts of water to prevent azide build-up. Check with local regulatory agencies to determine at what concentration of sodium azide may cause a product to be regulated as hazardous waste.
10. Individuals with color-impaired vision may not be able to adequately interpret test results.

PRECAUTIONS

1. Wear disposable gloves while handling kit reagents or specimens. Change gloves and wash hands thoroughly after performing each test. Do not pipette by mouth.
2. All specimens should be handled as if capable of transmitting infectious agents.
3. Avoid contact with skin and eyes. If contact occurs, wash affected areas with water.
4. Do not smoke, eat, or drink in areas where specimens or kit reagents are being handled.
5. Avoid forming aerosols.
6. Dispose of used contents as biohazardous wastes in accordance with federal, state, and local requirements.
7. Spills should be cleaned up and decontaminated in accordance with the user facility's established procedures for handling biohazardous spills.
8. Specific training or guidance is recommended if operators are not experienced with specimen collection and handling procedures.
9. Use appropriate precautions in the collection, handling, storage, and disposal of samples and used kit contents.

SPECIMEN STORAGE AND STABILITY

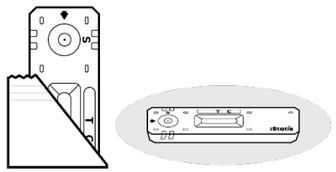
1. For EDTA-whole blood, EDTA-plasma or serum specimens, follow venipuncture blood collection procedures using lavender-top EDTA anticoagulant tubes (for whole blood and plasma) or red-top (no anticoagulant) tubes for serum.
2. If plasma or serum is to be used, separate from the blood cells by centrifugation.
3. Serum or EDTA-plasma may be stored at 2-8°C for up to 14 days.
4. Whole blood specimens collected by venipuncture in EDTA anticoagulant, may be stored at 2-8°C and should be tested within 3 days. **Do not heat or freeze whole blood specimens.**
5. Do not dilute prior to testing.

ASSAY PROCEDURE

NOTE: An iStatis test cartridge must be used immediately once opened from the pouch.

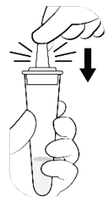
Test Set Up

1. Remove the test cartridge from its packaging and lay flat on the table. For sample identification purposes, the blank space at the top of the cassette may be labelled with the patient's name or number.

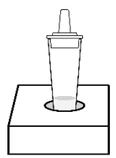


2. Tear off the foil seal of the Buffer Vial.

3. Insert the Buffer Vial Cap into the vial and press to close onto the vial.



4. Place the Buffer Vial into the Vial Holder.



Sampling Venous Whole Blood, Serum, Plasma:

1. Bring specimens to room temperature and mix each specimen thoroughly prior to use.
2. A volume of 50 µL must be used for venous whole blood, serum and plasma (see note).
3. Follow General Procedure after sampling below.

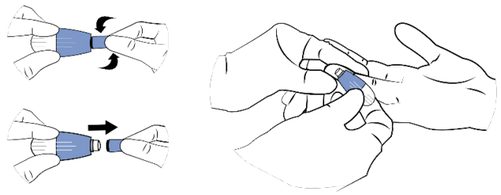
NOTE: In POC settings, it is important to use a 50 µL pipette to add the venous whole blood, plasma, or serum to the cartridge. Do not use the disposable single-use pipette provided for finger stick blood collection.

Sampling Fingerstick Blood:

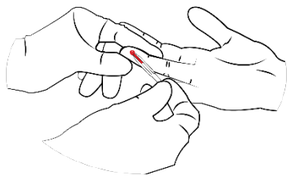
1. Gather support materials (alcohol swab, lancet, pipette), one sealed test pouch containing an iStatis test cartridge, and one vial of the Chase Buffer for each test to be performed.

CAUTION! The amount of sample (fingerstick blood) is critical. To ensure that the proper amount of blood is achieved, follow these instructions carefully:

2. Massage the finger to allow the blood to move to the surface (the fingertip will become pink). Use a heating pad if available to warm the hand. Hand must be positioned at waist level or lower.
3. Wipe the fingertip with the alcohol swab.
4. As soon as the finger is dry, twist and remove the protective insert from the lancet. Press the finger firmly at the point just below where the lancet will be applied. With the other hand, place the lancet on the side of the fingertip and press hard until it clicks. Immediately dispose the used lancet into a proper sharps container.



5. As the blood droplet forms, hold the pipette horizontally and touch the tip of the pipette to the blood sample. Capillary action automatically draws the sample to the fill line and stops. If very little blood trickles out of the puncture, gently apply intermittent pressure below the puncture site to obtain the required blood volume. If blood is inadequate, open a new test kit and perform a second skin puncture using a new lancet.

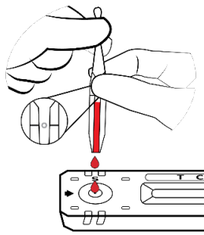


⚠ **CAUTION!** Filling is automatic: Never squeeze the pipette bulb while sampling.

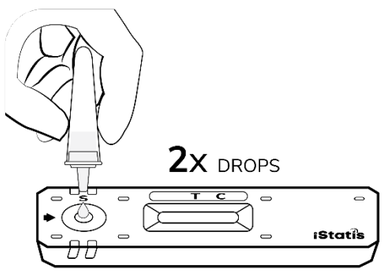
6. Follow General Procedure after Sampling below. Test the sample immediately.

General Procedure after Sampling:

1. Transfer the fingerstick whole blood sample held in the pipette to the sample well (marked "S") of the test cartridge. For serum/plasma samples and venous whole blood samples, transfer 50 µL using a precision pipette. For Fingerstick whole blood collected using the pipette provided in the kit, align the tip of the pipette with the sample well and squeeze the bulb to dispense the sample. **NOTE:** if the sample will not expel, hold the pipette vertically and slide a finger over (without pressing) the vent hold, then squeeze the bulb.



2. Wait for 1 minute, then add 2 drops of Chase Buffer to the sample well of the test cartridge holding the vial vertically.



IMPORTANT: Do not move or lift the cartridge during this step.

3. Start a timer for 15 minutes. The results of the test can be read at 15 minutes. The test result should NOT be read after 30 minutes.

NOTE: iStatis test should be read and interpreted under adequate lighting.

QUALITY CONTROL

The control line is a procedural control and indicates that the chase buffer has been applied successfully and that the active ingredients of the main components on the strip are functional.

Kit Controls:

iStatis HBsAg Test Positive and Negative Controls are available from bioLytical Laboratories for use with the iStatis HBsAg Test. The controls are used to verify iStatis HBsAg test performance and interpretation of results. Kit controls should be run under the following circumstances:

- Once for every new lot of kits and every new operator verification prior to performing testing on patient specimens.
- When temperature during storage of the kit falls outside of 2° to 30°C (35.6°F to 86°F)
- When the temperature of the test area falls outside of 15° to 30°C (59°F to 86°F)
- At regular intervals as determined by the user facility

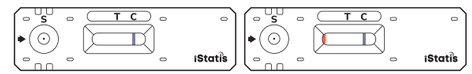
Refer to the iStatis HBsAg Test Controls Package Insert for additional information on the use of these samples. It is the responsibility of laboratory or POC site using the iStatis HBsAg Test to establish an adequate quality assurance program to ensure the performance under their specific locations and conditions of use.

⚠ **CAUTION! It is not recommended to use external controls that have not been validated for the iStatis HBsAg Test as these may not produce the expected results.**

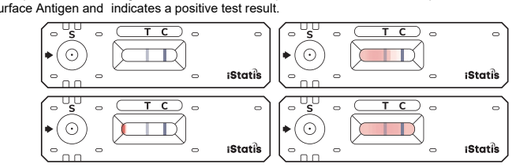
INTERPRETATION OF RESULTS

- Do not read the results if more than 30 minutes have elapsed following the addition of sample and chase buffer to the test cartridge.
- If using the iStatis HBsAg Test control samples provided by bioLytical Laboratories:
  - A HBsAg Positive Control must be positive with the iStatis HBsAg Test.
  - A Negative Control must be negative with the iStatis HBsAg Test.
  - Controls that produce incorrect or invalid results must be re-tested. If results are still incorrect or invalid, inform bioLytical Laboratories immediately.
- Control and test lines appear blue in colour

NEGATIVE ► Control line is clearly visible at "C" marking following development. Additionally, absence of any test line indicates the specimen does not contain any Hepatitis B Surface Antigen and indicates a negative test result.



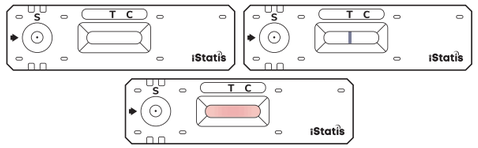
POSITIVE ► Control line is clearly visible at "C" marking following development. Additionally, any test line development at "T" marking, be it faint or distinct is present. This indicates the presence of Hepatitis B Surface Antigen and indicates a positive test result.



NOTE: The Test and Control Lines can be very faint. Any blue line visible here indicates a positive test result.

Following a positive result, a venous blood sample must be drawn in an EDTA collection tube (for whole blood or plasma) or red-top tube (for serum) and forwarded to a laboratory for Hepatitis B Surface Antigen confirmatory testing. Depending on the antigen titer, a reactive specimen may be less intense in color than the procedural control.

INVALID ► Absence of blue control line on the cartridge indicates an invalid result. If the smearing background makes it impossible to read the result, the test is considered invalid. An invalid test result means that the test was run incorrectly, or insufficient specimen was added. Invalid test results cannot be interpreted. Repeat the test with a new iStatis HBsAg Test.



NOTE: Invalid tests with fingerstick blood should be repeated with a fresh sample using a new test kit. Invalid tests with whole blood, plasma or serum samples should be repeated using a new test cartridge and kit components.

LIMITATIONS OF THE TEST

- The performance of the device has not been established in individuals under the age of 18.
- In some instances, samples may exhibit longer than normal flow times (from the time the specimen is added into the sample well, to the time the Chase Buffer has fully flown through the membrane). This is due to various factors such as cellular components, especially with whole blood. In these instances, a venous blood sample should be drawn in an appropriate collection tube and forwarded to a laboratory for HBV confirmatory testing.
- Insufficient data are available to interpret tests performed on other body fluids, pooled blood or pooled serum or products made from such pools; therefore, testing of these specimens is not recommended.
- A negative result at any time does not preclude the possibility of exposure to or infection with hepatitis B virus.

PERFORMANCE CHARACTERISTICS

Analytical Specificity (Cross Reactivity):

Cross reactivity with the potentially interfering medical conditions/diseases was evaluated with the iStatis HBsAg Test. A total of 330 clinical specimens were evaluated individually (n=1). The results are summarized in the following table. All tested samples showed no cross-reactivity with the iStatis HBsAg test.

Medical Conditions/diseases (n=330)	Number of Specimens	iStatis Positive	iStatis Negative
Hepatitis A infection (HAV)	5	0	5
Hepatitis C infection (HCV)	5	0	5
Hepatitis E infection (HEV)	5	0	5
Cytomegalovirus (CMV)	5	0	5
Epstein-Barr virus (EBV)	5	0	5
Herpes Simplex Virus (HSV)	5	0	5
HIV 1/2	5	0	5
HTLV 1/2	5	0	5
Human Papillomavirus	5	0	5
Influenza A and B	5	0	5
Measles	5	0	5
Varicella Zoster Virus (VZV)	5	0	5
Malaria	5	0	5
Visceral Leishmaniasis	5	0	5
Neisseria Gonorrhoeae (NG)	5	0	5
Syphilis	5	0	5
Schistosomiasis	5	0	5
Trichomoniasis	5	0	5
Human African Trypanosomiasis	5	0	5
Influenza Vaccine Recipient	5	0	5
HBV Vaccine Recipient	5	0	5
Yellow Fever Vaccine Recipient	5	0	5
Non-Viral Hepatitis	5	0	5
Non-Alcoholic Fatty Liver Disease (NAFLD)	5	0	5
Alcoholic Fatty Liver Disease (ALD)	5	0	5
Primary Biliary Cirrhosis (PBC)	5	0	5
Pregnancy	200	0	200

Analytical Specificity (Endogenous Interferences):
To evaluate the effect of elevated levels of various endogenous substances on the iStatis HBsAg Test, both plasma and whole blood samples were spiked with the following levels of endogenous interferences and assayed at two HBsAg levels: negative and weak positive. No interference was observed at the following tested concentrations:

Interferent Tested	No Interference up to
Hemoglobin	10 mg/mL
Bilirubin, Conjugated	0.4 mg/mL
Bilirubin, Unconjugated	0.4 mg/mL
Cholesterol	4.0 mg/mL
Albumin	60 mg/mL
Intralipid	20 mg/mL
Hyperglobulinaemia	20 mg/mL

To evaluate the effect of Multiple Transfusions, Human Anti-Mouse Antibodies, Systemic Lupus Erythematous, Anti-Nuclear Antibodies, Rheumatoid Factor, Pregnancy (1st/2nd/3rd Trimester), Multiparous Pregnancy, Sickle Cell Disease, and Autoimmune Hepatitis on iStatis HBsAg Test performance, both plasma or serum and whole blood samples were tested as is (negative) and spiked with HBsAg to a weak positive level. The results are presented in the following table, and no interference was observed for each condition.

Medical Conditions	Negative			Weak Positive		
	N	iStatis Positive	iStatis Negative	N	iStatis Positive	iStatis Negative
Autoimmune Hepatitis	10	0	10	10	10	0
Sickle cell disease	10	0	10	10	10	0
Pregnancy (1st Trimester)	10	0	10	10	10	0
Pregnancy (2nd Trimester)	10	0	10	10	10	0
Pregnancy (3rd Trimester)	10	0	10	10	10	0
Multiparous Pregnancy	10	0	10	10	10	0
Rheumatoid Factor	10	0	10	10	10	0
Anti-Nuclear antibodies	10	0	10	10	10	0
Systemic Lupus Erythematous	10	0	10	10	10	0
Human Anti-Mouse antibodies	10	0	10	10	10	0
Multiple transfusion	10	0	10	10	10	0

Drug Interferences (Exogenous Interference):

A drug interference study was performed with 17 common therapeutic drugs representing common over-the-counter, anti-inflammatory drugs and anti-bacterial and anti-viral drugs. Each drug was evaluated at the highest concentration recommended by CLSI EP07 and as listed in CLSI EP37 and spiked into both plasma and whole blood samples at two HBsAg levels: negative and weak positive. No interference was observed at the following tested concentrations:

Drug Name	Concentration
Acetaminophen	0.156 mg/mL
Acetylsalicylic acid	0.03 mg/mL
Ampicillin	0.075 mg/mL
Cefoxitin	6.6 mg/mL
Chloroquine	789 ng/mL
Darunavir	0.0159 mg/mL
Doxycycline	0.018 mg/mL
Entecavir	0.024 µg/mL
Isoniazid	0.06 mg/mL
Ibuprofen	0.219 mg/mL
Interferon	6,000 IE/mL
Mefloquine	9.84 µg/mL
Metronidazole	0.123 mg/mL
Rifampicin	0.048 mg/mL
Quinine	0.054 mg/mL
Ribavirin	0.011 mg/mL
Ritonavir	0.044 mg/mL

Analytical Sensitivity:

The WHO 3rd International Standard for HBsAg (NIBSC 12/226) was tested with the iStatis HBsAg Test. The LoD was determined to be 1.746 IU/mL for plasma samples, and 0.3959 IU/mL for whole blood samples.

Seroconversion Sensitivity:

Seroconversion sensitivity of the iStatis HBsAg Test was evaluated by testing five commercially available seroconversion panels (SeraCare Life Sciences Hepatitis B Seroconversion Panel PHM926, AccuVert™ HBV Seroconversion Panel PHM936(0605-0038), AccuVert™ HBV Seroconversion Panel PHM937 (0605-0039), ZeptoMetrix HBV Seroconversion Panel Donor No.61042(HBV6273), ZeptoMetrix HBV Seroconversion Panel Donor No.61799(HBV6274)). The test results demonstrated that iStatis HBsAg test can detect samples during seroconversion.

Genotype Performance:

Genotype performance of the iStatis HBsAg test was evaluated by testing the 1st WHO international reference panel for Hepatitis B Virus genotypes for HBsAg assays (PEI 6100/09). The iStatis HBsAg test was able to detect specimens from the following genotypes: A, B, C, D, E, F and H.

Serotype Performance:

Serotype performance of the iStatis HBsAg Test was evaluated by testing three commercially available HBsAg positive specimens from SeraCare Life Sciences. The iStatis HBsAg test was able to detect specimens from the following serotype: *adr, adw* and *ayw*.

Hook effect:

iStatis HBsAg Test was able to detect 10 high titer HBsAg positive samples (defined by Abbott Architect HBsAg Qualitative S/CO of > 5000) as positive.

CLINICAL PERFORMANCE
A prospective clinical performance evaluation in South Africa compared the performance of the iStatis HBsAg test using fingerstick capillary blood, venous whole blood, serum, and plasma specimens with standard laboratory tests. Out of 1400 recruited participants, female contributed 69.4%, male 30.6%, and non-binary 0.1%. Participant age group in decreasing contribution percentage is as follows: 26-35 (34.1%), 36-45 (32.9%), 18-25 (15.2%), 46-55 (12.7%), and >55 (5.1%). iStatis HBsAg Test performance on fingerstick capillary blood, venous whole blood, serum, and plasma from the eligible 1398 participants results are as follows: 100.0% Positive Percent Agreement (PPA) and 100.0% Negative Percent Agreement (NPA) for fingerstick, 99.5% PPA and 100.0% NPA for venous whole blood, serum, and plasma samples.

iStatis HBsAg Test	PPA(%) (95% CI)	NPA (%) (95% CI)
Fingerstick Capillary blood	100.0% [99.1% – 100.0%] N=400 400/(400+0)	100.0% [99.6% - 100.0%] N=998 998/(998+0)
Venous whole blood	99.5% [98.2% – 99.9%] N=400 398/(398+2)	100.0% [99.6% - 100.0%] N=998 998/(998+0)
Plasma	99.5% [98.2% – 99.9%] N=400 398/(398+2)	100.0% [99.6% - 100.0%] N=998 998/(998+0)
Serum	99.5% [98.2% – 99.9%] N=400 398/(398+2)	100.0% [99.6% - 100.0%] N=998 998/(998+0)

BIBLIOGRAPHY

1. World Health Organization (WHO). (2023). Hepatitis B. https://www.who.int/news-room/fact-sheets/detail/hepatitis-b

TECHNICAL INFORMATION
For further information, assistance, or problem reporting, contact Customer Service at +1-604-644-4677.

Reference herein to any specific third party by name, trade name, trade-mark, manufacturer or otherwise does not constitute or imply an endorsement or recommendation of this Kit by such third party, or of the products or services of such third party by bioLytical or that such products or services are necessarily best suited for the intended purpose.

GLOSSARY OF SYMBOLS

2°C 30°C	Store at 2°C to 30°C		Manufacturer
IVD	*In Vitro* Diagnostic Medical Device		Caution Harmful if swallowed
	Consult Package Insert	STERILE R	Sterilization using irradiation
	Do not reuse	Σ N	Contains sufficient for "N" tests
	Do not use if damaged		Keep dry
REF	Catalogue Number		Keep away from direct sunlight
	Use By Date		This side up
LOT	Lot Number		

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