IVD CE

Sclavo-EIA HBsAg

Ref ELI901001

Instructions for Use

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A. INTENDED USE

Sclavo-EIA HBsAg is a one-step 4th generation ELISA kit for the detection of Hepatitis B Virus surface antigen (HBsAg) in human plasma and serum.

This kit can detect viral variants too and can be used for the screening of samples from blood donors in blood banks and for the follow-up of hepatitis B virus (HBV) infected patients in diagnostic laboratories.

B. INTRODUCTION

Hepatitis B Virus, member of the *Hepadnaviridae* family, is a small enveloped double-stranded DNA virus, with a marked tropism for hepatocytes, causing transient and chronic hepatitis. Transient infections can cause serious illness in a small fraction of subjects with fatal, fulminant hepatitis. Chronic infections may also have serious consequences: nearly 25% terminate in untreatable liver cancer.

It is estimated that from 250 to 350 million people are chronically infected with HBV worldwide, with a global prevalence of 3,9%. Approximately 2 billion people have serological evidence of past or present HBV infection.

HBV is transmitted through exposure to infected blood and bodily fluids (particularly semen and vaginal secretions). Most of the new infections worldwide are acquired vertically at birth (perinatal infection), horizontally through transmission between young children below the age of 5, high risk sexual contact, sharing of infected syringes between intravenous drug users, and contaminated blood or blood products and unsafe medical practices.

Hepatitis B surface Antigen or HBsAg is the most important protein of the envelope of Hepatitis B Virus.

The surface antigen contains the determinant “a”, common to all the known viral subtypes, immunologically distinguished by two distinct subgroups (ay and ad).

HBsAg may be detected as early as 1–2 weeks or as late as 11–12 weeks after exposure, and its persistence is a marker of chronicity.

The ability to detect HBsAg with high-sensitive immunoassays in the last years has led to an understanding of its distribution and epidemiology worldwide and to radically decrease the risk of infection in transfusion.

C. PRINCIPLE OF THE TEST

The solid phase (microtiter wells) is sensitized with a mix on murine monoclonal antibodies directed against the determinants “a”, “d” and “y” of HBsAg (capture antibody). Patient’s sample (serum/plasma) and a second mix of mouse monoclonal antibodies, conjugated with Horseradish Peroxidase (HRP) and directed against a different epitope of the determinant “a” and against “preS” are added together in the microtiter well. If HBsAg is present in the sample, it will bridge between the capture antibodies in the solid phase and the HRP-conjugate. At the end of the one-step incubation, microwells are washed to remove unbound serum/plasma proteins and excess HRP conjugate.

After the washing step, the polyclonal anti-human IgG and IgM HRP-conjugate is added to the microtiter well. If antibodies have been captured by the recombinant antigens, the conjugate will bind to the microtiter well.

The presence of the capture antibodies-HBsAg-HRP-conjugate complexes is revealed by adding the colorless substrate TMB to start the colorimetric reaction. The color that is developed is proportional to the quantity of HBsAg bound to the solid phase.

D. COMPONENTS OF THE KIT AND THEIR PREPARATION

The kit is for 96 tests and contains the following materials.

Microtiter plate **MICROPLATE**

12 strips x 8 wells coated with Anti-HBsAg affinity- purified mouse monoclonal antibodies, specific to “a”, “y” and “d” determinants in a resealable pouch with desiccant bag.

Equilibrate the microplate to room temperature (1 hour) before opening the pouch. If the humidity indicator in the desiccant bag has turned dark green, do not use the microplate.

Put the unused strips back into the resealable pouch with the desiccant bag, squeeze to remove the air, firmly close the pouch and store at 2 – 8°C.

After the first opening, strips can be used until the humidity indicator in the desiccant bag has turned yellow to green.

Negative control **CONTROL –**

GHS07

1 vial x 2 ml. Ready to use. Color code: pale yellow. Vortex thoroughly before use.

10 mM Phosphate buffer pH 7,4 containing goat serum. Preservatives: sodium azide (0,09%) and Proclin™ 300 (0,045%).
Warning: ATTENTION – Sensitizing (H317; P261; P280; P362+P364, P333+P313; P321; P501).
Contains reaction mass of 5-chloro-2-methyl-4-iso-thiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1) (CAS 55965-84-9)

Positive control **CONTROL +**

GHS07

1 vial x 2 ml. Ready to use. Color code: green. Vortex thoroughly before use.
10 mM Phosphate buffer pH 7,4 containing goat serum and non-infectious recombinant HBsAg. Preservatives: gentamicin sulphate (0,02%) and Proclin™ 300 (0,045%).
Warning: ATTENTION – Sensitizing (H317; P261; P280; P362+P364, P333+P313; P321; P501).
Contains reaction mass of 5-chloro-2-methyl-4-iso-thiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1) (CAS 55965-84-9)

Calibrator **CAL**

GHS07

1 vial. Freeze-dried. To be reconstituted with the volume of EIA grade water reported in the label. Vortex thoroughly before use.
Non-infectious recombinant HBsAg at 0.5 IU/ml (2nd WHO international standard for HBsAg, NIBSC code 00/588) in 10 mM phosphate buffer pH 7.4. Preservatives: gentamicin sulphate (0,02%) and Proclin™ 300 (0,045%).
Important: *once reconstituted the calibrator is not stable. Make aliquots and store at -20°C for 7 days.*
Warning: ATTENTION – Sensitizing (H317; P261; P280; P362+P364, P333+P313; P321; P501).
Contains reaction mass of 5-chloro-2-methyl-4-iso-thiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1) (CAS 55965-84-9)

Concentrated washing buffer **WASHBUF 20X**

GHS09

GHS07

1 bottle x 60 ml. Concentrated 20 times. Bring to the final volume of 1200 ml using EIA grade water.
Once diluted it contains 10 mM phosphate buffer pH 7.0, 0,5% Tween 20, Na-azide (0.09%) and Proclin™ 300 (0.045%).
Carefully check that there are no undissolved salt crystals, if necessary, mix until completely dissolved.
Important: *avoid the formation of foam during resuspension as it could give rise to false results.*
Important: *once reconstituted the ready to use buffer is stable 1 week at 2-8°C.*

Warning: DANGER – Sensitizing / Dangerous for the environment (H317; H411; P101; P102; P103; P261; P273; P280; P333+P313; P321; P501).
Contains reaction mass of 5-chloro-2-methyl-4-iso-thiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1) (CAS 55965-84-9)

Conjugate-HRP diluent **CONJ DIL**

GHS07

1 Vial x 16 ml. Ready to use. Color code: pink/red. Mix gently before use.
10 mM Phosphate buffer pH 7,4 containing mouse serum and 5% BSA. Preservatives: gentamicin sulphate (0,02%) and Proclin™ 300 (0,045%).
Warning: ATTENTION – Sensitizing (H317; P261; P280; P362+P364, P333+P313; P321; P501).
Contains reaction mass of 5-chloro-2-methyl-4-iso-thiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1) (CAS 55965-84-9)

Conjugate **CONJ 20X**

GHS07

1 Vial x 0,8 ml. 20 times concentrated. To be reconstituted with Conjugate-HRP diluent. After reconstitution, vortex thoroughly before use.
Peroxidase (HRP) labeled mouse monoclonal antibodies to HBsAg, determinant "a" and "preS", 10 mM Tris buffer pH 6.8+/- 0.1, 5% BSA. Preservatives: gentamicin sulphate (0,02%) and Proclin™ 300 (0,045%).
Important: *The working solution is not stable. Prepare only the volume necessary for the work of the day. As an example, dilute 0.1 ml 20X Conjugate with 1.9 ml Conjugate-HRP diluent into a disposable plastic vial and mix carefully before use.*
Warning: ATTENTION – Sensitizing (H317; P261; P280; P362+P364, P333+P313; P321; P501).
Contains reaction mass of 5-chloro-2-methyl-4-iso-thiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1) (CAS 55965-84-9)

Substrate **SUBS TMB**

1 Vial x 25 ml. Ready to use. Vortex thoroughly before use.
0,03% tetramethylbenzidine (TMB), 4% dimethylsulphoxide, and 0.02% hydrogen peroxide (H_2O_2) stabilised in 50 mM citrate buffer (pH 3.8).
Important: *do not expose to strong illumination, oxidizing agents (e. g. hypochlorite fumes), metallic surfaces; store protected from light.*

Sulphuric acid **H_2SO_4 0.3 M**

GHS05

1 Vial x 25 ml. Ready to use. Mix by inversion before use.
0.3 M H_2SO_4 solution.
Warning: DANGER - Corrosive (H314; P303 + P361 + P353; P305 + P351 + P338; P310; P321; P405; P501).
Contains Sulphuric Acid (CAS 7664-93-9)

Legend:

Warning statements H:
H314 - Causes severe skin burns and eye damage.
H317 - May cause an allergic skin reaction.
H411 - Toxic to aquatic life with long lasting effects.

Precautionary statements P:
P101 - In the event of a doctor's consultation, keep the product container or label available.
P102 - Keep out of reach of children.
P103 – Read carefully and follow all instructions.
P261 - Avoid breathing dust / fume / gas / mist / vapors / spray.
P273 - Do not release into the environment.
P280 - Wear protective gloves.
P310 - Immediately call a Poison Center / doctor.
P321 - Specific treatment (see on this label)
P303 + P361 + P353 - IF ON SKIN (or hair): take off immediately all contaminated clothing. Rinse the skin / take a shower.
P305 + P351 + P338 - IF IN EYES: rinse thoroughly for several minutes. Remove any contact lenses if easy to do. Continue rinsing.
P333 + P313 - If skin irritation or rash occurs: Get medical attention.
P362 + P364 - Take off contaminated clothing and wash it before wearing again.

P405 - Store locked up.
P501 - Dispose of contents / container in accordance with local / regional / national / international regulations.

Adhesive films to cover microplate	**2**
Instructions for use booklet	**1**

E. Materials Required But Not Provided

Laboratory glassware: appropriately sized graduated cylinders, pipettes etc.

Adjustable single-channel micropipettes capable of delivering 50 µL, 100 µL and 200 µL and disposable plastic tips.
EIA-grade distilled water.
Dual wavelength microwell reader capable of reading at 450 nm with a reference filter of 620 - 630 nm. If the reference filter is not available, make sure that the bottom of the microtiter wells is clean (do not touch without gloves).
37°C ±1°C incubator (dry or humidified).
Calibrated multichannel ELISA washer device.
Vortex or similar mixing device.
Timer.
Absorbent paper to blot the microtiter plate.

F. PRECAUTIONS AND WARNINGS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at 2 - 8°C into a temperature-controlled refrigerator or cold room.
7. After opening, the stability of the individual reagents is described in Section D
8. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
9. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement
10. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
11 Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
12. Do not use the unopened kit after the expiration date indicated on the outer container and inner labels (vials). After opening, the stability of the individual reagents as described in section D.
13. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.

14. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.

15. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 minutes.

16. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.

17. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water.

18. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: TYPE, PREPARATION AND RECOMMANDATIONS.

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. The use of anticoagulants such as citrate, EDTA and heparin does not interfere with the test.

2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.

3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labelling and electronic reading is strongly recommended.

4. Haemolysed (reddish) and visibly strongly lipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.

5. Sera and plasma can be stored at 2°- 8°C in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C according laboratory validated sample storage procedures. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.

6. If particles are present, centrifuge at 2.000 rpm for 20 minutes or filter using 0.2 - 0.8-micron filters to clean up the sample for testing.

H. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.

2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature Is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.

3. In case of shaking during incubations, the instrument has to ensure 350 rpm +/-150. Amplitude of shaking is very important as a wrong one could give origin to splashes and therefore to some false positive result.

4. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution. The instrument has to be submitted weekly to decontamination according to its manual (NaOH 0.1 M decontamination suggested). Five (5) washing cycles (aspiration + dispensation of 350µl/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing. An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.

5. Incubation times have a tolerance of +/-5%.

6. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth < 10 nm; (b) absorbance range from 0 to > 2.0; (c) linearity to > 2.0; (d) repeatability > 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.

7. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section O "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended for blood screening when the number of samples to be tested exceed 20-30 units per run.

8. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labelled containers to 2° - 8°C, firmly capped.

I. PRE-ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use reagents beyond their stated expiry date.

2.. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.

3. Dilute all the content of the 20x concentrated Wash Solution as described above.

4. Dilute the 20X Conjugate-HRP concentrate with its diluent as described above.

5. Dissolve the Calibrator as described above.

6. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.

7. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturer's instructions. Set the right number of washing cycles as reported in the specific section.

8. Check that the ELISA reader has been turned on at least 20 minutes before reading.

9. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.

10. Check that the micropipettes are set to the required volume.

11. Check that all the other equipment is available and ready to use.

12. In case of problems, do not proceed further with the test and advise the supervisor.

J. TEST PROCEDURE

The assay has to be carried out according to what reported below, it is important to maintain the same incubation time for all the samples in testing.

Important note: Pre washing (1 cycle: dispensation of 350 µl/well of washing solution + aspiration) is fundamental to obtain reliable and specific results both in the manual and in the automatic procedures. Do not omit this step!

Automated assay:

Follow the automated ELISA processor user manual for programming the Sclavo-EIA HBsAg test method.

In case the test is carried out automatically with an ELISA processor, we recommend to make the instrument aspirate first 150 µl of calibrator and positive and negative controls, then 150 µl of each sample and finally 100 µl diluted Enzyme Conjugate.

Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

For the pre-washing step (point 1 of the assay procedure) and all the next operations follow the operative instructions reported below for the Manual Assay.

Manual assay:

1. Place the required number of 8-Microwell strips in the microwell frame depending on the number of samples to be analysed. Holder and wash them once to hydrate wells. Carefully identify the wells for controls, calibrator and samples.
2. Leave the 1st well (A1) empty for the blank substrate.
3. Dispense 150 µl of Negative Control in triplicate,150 µl Calibrator in duplicate, 150 µl Positive Control in single followed by 150 µl in single for each sample in proper wells.

Do not dilute Controls and Calibrator as they are pre-diluted, ready to use!

Important note: *check for the presence of samples in microwells by the naked eye (there is a marked colour difference between empty and full wells) or by reading at 450/620nm. (samples show OD values higher than 0.100).*

4. Pipette 100µl Enzyme Conjugate into each well, except the 1st blanking well (A1), and cover with the adhesive foil.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

5. Following addition of the conjugate, check that the colour of the samples has changed from yellowish to pink/red. Incubate the microplate for 120 min at +37°C.
6. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section H.4).
7. Pipette 200 µl Chromogen/Substrate mixture into each well, blank well A1 included. Then incubate the microplate at room temperature (18-24°C) for 30 minutes.

Important note: *Do not expose to strong direct illumination: high background might be generated.*

8. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 7 to stop the enzymatic reaction. Addition of acid will turn the positive control and positive samples from blue to yellow/brown.

9 Measure the colour intensity of the solution in each well, as described in section H.6, at 450nm filter (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 (mandatory).

Important notes:

1. Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.

2. Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self-oxidation of the chromogen can occur leading to high background.

3. When samples to be tested are not surely clean or have been stored frozen, the assay procedure reported below is recommended in as far as it is far less sensitive to interferences due to haemolysis, hyperlipaemia, bacterial contamination and fibrin microparticles. The assay is carried out in two-steps at +37°C on shaking at 350 rpm +/- 150 as follows:

- dispense 100 µl of controls, calibrator and samples;
- incubate 60 min at +37°C with shaking;
- wash according to instructions;
- dispense 100 µl diluted enzyme tracer;
- incubate 30 min at +37°C with shaking;
- wash according to instructions;
- dispense 100 µl substrate;
- incubate 30 minutes at R.T. with shaking;
- stop and read.

In this procedure the pre-wash can be omitted. This method shows performances similar to the standard one and therefore can be used in alternative.

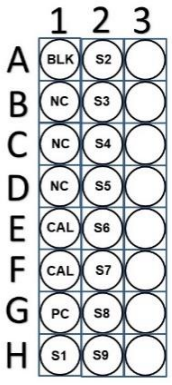
4. The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.

K. ASSAY SCHEME

Operations	Procedure
Pre-washing step	n°1 cycle
Controls&calibrator&samples diluted enzyme conjugate	150 µl 100 µl
1st incubation	**120 min**
Temperature	+37°C
Washing steps	n°5 cycles with 20'' of soaking OR n°6 cycles without soaking
Chromogen/Substrate	200 µl
2nd incubation	**30 min**
Temperature	room
Sulphuric Acid	100 µl
Reading OD	450nm / 620-630nm

An example of dispensation scheme is reported below:

Microplate



Legenda:

BLK = Blank

NC = Negative Control

CAL = Calibrator

PC = Positive Control

S = sample

L. INTERNAL QUALITY CONTROL

A check is carried out on the controls and the calibrator any time the kit is used in order to verify whether their OD450nm values are as expected and reported in the table below.

Parameter	Requirements
Blank well	< 0.100 OD450nm value

Negative Control (NC)	< 0.050 mean OD450nm value after blanking
Calibrator 0.5 IU/ml	S/CO > 2
Positive Control	> 1.000 OD450nm value

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Should these problems happen, after checking, report any residual problem to the supervisor for further actions.

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not got contaminated during the assay.
Negative Control (NC) > 0.050 OD450nm after blanking	1. that the washing procedure and the washer settings are as validated in the pre-qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative one); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to spills of positive samples or of the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate; 6. that the washer needles are not blocked or partially obstructed.
Calibrator S/CO < 2	1. That the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead of calibrator); 3. that the washing procedure and the washer settings are as validated in the pre-qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control < 1.000 OD450nm	1. That the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control. In this case, the negative control will have an OD450nm value > 0.50); 3. that the washing procedure and the washer settings are as validated in the pre-qualification study; 4. that no external contamination of the positive control has occurred.

M. CALCULATION OF THE CUT-OFF

The tests results are calculated by means of a cut-off value determined with the following formula on the mean OD450nm/620 – 630nm value of the Negative Control (NC):

NC + 0.050 = Cut-Off (CO)

The value found for the test is used for the interpretation of results as described in the next paragraph.

Important note: When the calculation of results is done by the operative system of an ELISA automated work station be sure that the proper formulation is used to calculate the cut-off value and generate the right interpretations of results.

N. INTERPRETATION OF RESULTS

Test results are interpreted as ratio of the sample OD450nm and the Cut-Off value (or S/CO) according to the following table:

S/CO	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by HBV and that the blood unit may be transfused.

Any patient showing an equivocal result should be tested again on a second sample taken 1-2 weeks later from the patient and examined. The blood unit should not be transfused.

A positive result is indicative of HBV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:

1. Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.

2. Any positive result must be confirmed first by repeating the test on the sample, after having filtered it on 0,2 – 0,8-micron filter to remove any microparticles interference. Then, if still positive, the sample has to be submitted to a confirmation test before a diagnosis of viral hepatitis is released.

3. When test results are transmitted from the laboratory to another department, attention must be paid to avoid erroneous data transfer.

4. Diagnosis of viral hepatitis infection has to be taken and released to the patient by a suitably qualified medical doctor. An example of calculation is reported below:

The following data must not be used instead or real figures obtained by the user.

NEGATIVE CONTROL	OD 450 nm 0,012 – 0,008 – 0,010
OD 450 nm mean value: 0,010	Lower than 0,050
ACCEPTED	
POSITIVE CONTROL	OD 450 nm 2,489
OD 450 nm higher than 1,000	
ACCEPTED	
CUT-OFF	0,050 + 0,010 = 0,060
CALIBRATOR	OD 450 nm 0,350 – 0,370
OD 450 nm mean value 0,360	S/CO = 6 higher than 2,0
ACCEPTED	
Sample 1 OD 450 nm: 0,028	S/CO < 0,9
NEGATIVE	
Sample 2 OD 450 nm: 1,690	S/CO > 1,1
POSITIVE	

O. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

1. Analytical sensitivity

The limit of detection of the assay has been calculated on the 3rd WHO international standard, NIBSC code 12/226.

In the following table, results are given for three lots (C4T6/5 – C5T7/1 – C5T7/6) of Sclavo-EIA HbsAg

WHO IU/ml	Lot # C4T6/5	Lot # C5T7/1	Lot # C5T7/6

	S/Co	S/Co	S/Co
Negative Control (NC)	-	-	-
Positive Control (PC)	**63.6**	**66.0**	**67.9**
Calibrator (CAL)	**10.2**	**10.5**	**11.2**
Cut-off (=NC+0.050)	**0.052**	**0.052**	**0.053**
*WHO 0.5 IU/ml	**10.0**	**10.4**	**11.2**
*WHO 0.25 IU/ml	**4.7**	**5.3**	**5.5**
*WHO 0.1 IU/ml	**2.0**	**2.3**	**2.6**
*WHO 0.05 IU/ml	**1.3**	**1.2**	**1.2**
*WHO 0.025 IU/ml	**0.6**	**0.7**	**0.7**
Matrice negativa per HbSAg (FCS)	**0.3**	**0.2**	**0.4**

**WHO International Standard – Third international Standard, for HbSAg (HBV genotype B4, HbSAg subtype ayw1/adw2). Code NISBC 12/226 supplied from National Institut for Biological Standards and Controls*

The assay shows an Analytical Sensitivity ≤ 0.05 IU / ml of HBsAg.

In addition, two panels of sensitivity supplied by EFS, France, and by SFTS, France, were tested and gave, in the best conditions, the following results:

Panel EFS Ag HBs HB1-HB6 lot n° 04

Sample ID	Characteristics	ng/ml	S/CO
HB1	diluent	/	0,2
HB2	adw2+ayw3	0.05	0,6
HB3	adw2+ayw3	0.1	**1,0**
HB4	adw2+ayw3	0.2	**1,8**
HB5	adw2+ayw3	0.3	**2,4**
HB6	adw2+ayw3	0.5	**4,2**

Sensitivity panel SFTS, France, Ag HBs 2005

Sample ID	Characteristics	ng/ml	S/CO
231	Adw2 + ayw3	2.21 + 0.15	**15,4**
232	Adw2 + ayw3	1.18 ± 0.10	**8,3**
233	Adw2 + ayw3	1.02 + 0.05	**7.4**
234	Adw2 + ayw3	0.64 + 0.04	**5.2**
235	Adw2 + ayw3	0.49 + 0.03	**3.9**
236	Adw2 + ayw3	0.39 + 0.02	**2.9**
237	Adw2 + ayw3	0.25 + 0.02	**2.1**
238	Adw2 + ayw3	0.11 + 0.02	**1**
239	Adw2 + ayw3	0.06 ± 0.01	***0,6***
240	Adw2 + ayw3	0.03 + 0.01	***0,5***
241	Adw2	0.5 – 1.0	**4,7**
242	Adw4	0.5 – 1.0	**4.1**
243	Adr	0.5 – 1.0	**4.5**
244	Ayw1	0.5 – 1.0	**4.7**
245	Ayw2	0.5 – 1.0	**5.9**
246	Ayw3	0.5 – 1.0	**6.4**
247	Ayw3	0.5 – 1.0	**5.6**
248	Ayw4	0.5 – 1.0	**7.4**
249	Ayr	0.5 – 1.0	**6.2**
250	diluent	/	***0.3***

The panel # 808, supplied by Boston Biomedical Inc., USA, was also tested to define the limit of sensitivity.

Results in the best conditions are as follows:

Sample ID	Characteristics	ng/ml	S/CO
01	ad	2,49	**10,2**
02	ad	1,17	**4,8**
03	ad	1,02	**4,3**
04	ad	0,96	**3,8**
05	ad	0,69	**2,9**
06	ad	0,50	**2,2**
07	ad	0,41	**1,5**
08	ad	0,37	**1,3**
09	ad	0,30	**1,2**
10	ad	0,23	***1,0***
11	ay	2,51	**11,2**
12	ay	1,26	**5,9**
13	ay	0,97	**4,1**

14	ay	0,77	3,7
15	ay	0,63	2,0
16	ay	0,48	2,4
17	ay	0,42	2,0
18	ay	0,33	1,8
19	ay	0,23	1,6
20	ay	0,13	1,1
21	negative	/	0,6

2. Diagnostic sensitivity

The diagnostic sensitivity was tested according to what required by Common Technical Specifications (CTS) of the directive 98/79/EC on IVD for HBsAg testing.

Positive samples, including panel of "s" HBsAg subtypes and a frequent mutants from most collected from mutations, pathologies were and chronic different HBV synthetically, (acute, a-symptomatic positive in the hepatitis B) or produced and were detected assay.

Panel ID	1st sample positive	HBsAg subtype	HBsAg ng/ml	VersionULTRA S/CO	Ref. deviceS/CO
PHM 906	02	ad	0.5	**3.7**	**1.4**
PHM 907 (M)	06	ay	1.0	**4.4**	**2.9**
PHM 909	04	ad	0.3	**1.2**	0.8
PHM 914	04	ad	0.5	**1.1**	**1.1**
PHM 918	02	ad	0.1	**1.8**	0.5
PHM 923	03	ay	< 0.2	**2.2**	**1.2**
PHM 925	03	Ind.	n.d.	**1.4**	*0.9*
PHM 934	01	ad	n.d.	**1.0**	0.8

All the HBsAg known subtypes, "ay" and "ad", and isoforms "w" and "r", supplied by CNTS, France, were tested in the assay and determined positive by the kit as expected.

An overall value of 100% has been found in a study conducted on a total number of more than 400 positive samples tested with a CE marked kit.

A total of 30 sero-conversions were studied, most of them produced by Boston Biomedica Inc., USA.

Results obtained by examining eight panels supplied by Boston Biomedica Inc., USA, are reported below for Sclavo-EIA HBsAg in comparison with the reference device.

3. Diagnostic specificity

It is defined as the probability of the test to obtain a negative result in the absence of a specific analyte. In addition to the first study, where 5043 negative samples from blood donors (two blood centres) were examined, classified negative with the CE marked device in use at the collection laboratory, the diagnostic specificity was recently evaluated by adding 2288 samples for a total of 7331 negative blood donors on seven different batches. A specificity value of 99.8% was found. The discrepant results (n=10) turned out to be False Positives and all came from patients with an altered coagulation pathway: the samples had clots and fibrin particles. After filtration they were negative (section G.4).

Both plasmas, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity.

No false reactivity due to the method of specimen preparation has been observed.

Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.

Samples derived from patients with different viral (HCV, HAV) and non-viral pathologies of the liver that may interfere with the test were examined. No cross reactions were observed.

Frozen specimens have been tested, as well, to check for interferences due to collection and storage. No interference was observed.

4. Precision

It has been calculated for Sclavo-EIA HBsAg on two samples examined in 16 replicates in 3 different runs for three lots.

Results are reported in the following table:

The variability shown in the tables did not result in sample misclassification.

P. LIMITATIONS

Repeatable false positive results were evaluated on freshly collected samples in less than 0.1% of the normal population, mainly due to high-titer heterophilic anti-mouse antibodies (HAMA). Interference was observed in fresh samples even when they were not particle-free or had been poorly collected (see chapter G). Old or frozen samples, resecting fibrin clots, cryoglobulins, micelles or microparticles containing lipids after storage or thawing, may generate false positive results.

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Average values Total n = 144	NegativeSample	Calibrator 0.5 IU/ml
OD450nm	0.026	0.332
Std.Deviation	0.004	0.027
CV %	16%	8%

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Symbols used on labels and packaging

IVD = In vitro medical diagnostic device

REF = Catalog number

LOT = Lot number

 = Manufacturer

 = Expiration date

 = Storage Temperature

 = Instructions for use