IVD CE

Sclavo-EIA HCV Ab

Ref ELI903001

Instructions for Use

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A. INTENDED USE

Sclavo-EIA HCV Ab is a 4th generation ELISA kit for the detection of antibodies (IgG and IgM) against Hepatitis C virus (HCV) in human plasma and serum.
This kit can be used for the screening of samples from blood donors in blood banks and for the follow-up of HCV infected patients in diagnostic laboratories.

B. INTRODUCTION

HCV, identified in 1988 through molecular biology techniques, is a positive-strand RNA virus that belongs to the Hepacivirus genus of the family Flaviviridae of which six major genotypes have been identified. HCV is a major cause of liver disease (hepatitis, cirrhosis, hepatocarcinoma) worldwide; it is estimated that more than 170 million persons in the world have hepatitis C virus infection, of whom more than 70 million have chronic infection.
HCV is transmitted by parenteral routes, of which infected blood and blood derivatives transfusion and the sharing of contaminated syringes for intravenous administration of drugs of abuse are the most frequent.
The principal assays used to determine exposure to HCV infection and evidence of past or current HCV infection rely on detection of antibodies to HCV using serological tests, such as ELISA.
The detection of anti-HCV antibodies in plasma or serum is based on the use of ELISAs, that detect antibodies raised against various HCV immunodominant antigenic epitopes. Specific recombinant antigens are used to capture circulating anti-HCV antibodies onto the wells of microtiter plates. The presence of anti-HCV antibodies is revealed by anti-human IgG and IgM antibodies conjugated with horseradish peroxidase, that catalyses the transformation of a colorless substrate into a colored product, that can be measured with a photometer.

C. PRINCIPLE OF THE TEST

Conserved and immunodominant recombinant Core and Non-Structural proteins (NS3, NS4 and NS5) are used in the solid phase (microtiter wells).
During the first incubation step of the assay, diluted samples are added to the sensitized microwell and antibodies (IgG and IgM) are bound to the recombinant proteins, if present.
After a washing step to remove the unbound proteins, 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 unbound enzymatic conjugate is removed by a washing step and the presence of the antigen-conjugate complexes are revealed by adding the colorless substrate TMB to start the colorimetric reaction. The color that is developed is proportional to the quantity of antibody 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 recombinant Core, NS2, NS3 and NS5 HCV proteins 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. Vortex thoroughly before use.
10 mM Na-citrate buffer pH 6.0 containing 1% goat serum and 0,5% Tween 20. 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. Vortex thoroughly before use.

10 mM Na-citrate buffer pH 6.0 containing human serum positive for HCV antibodies and 0,5% Tween 20. Preservatives: sodium azide (0,09%) and Proclin™ 300 (0,045%).
The anti-HCV serum has been chemically inactivated to reduce the titer of potentially infectious virus. However, no test method can rule out the risk of potential infection; handle as potentially infectious.
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.
10 mM Na-citrate buffer pH 6.0 containing goat fetal proteins and human anti-HCV antibodies, calibrated against the NIBSC Working Standard (ref. 99/588-003-WI). Preservatives: 0,3 mg/mL gentamicin sulphate and Proclin™ 300 (0,045%).
The anti-HCV serum has been chemically inactivated to reduce the titer of potentially infectious virus. However, no test method can rule out the risk of potential infection; handle as potentially infectious.
Important: *once reconstituted the calibrator is not stable. Make aliquots and store at -20°C for up to 6 months.*

Warning: ATTENTION - Sensitizing (H317; H411; 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)

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 **CONJ**

GHS07

1 Vial x 16 ml. Ready to use. Vortex thoroughly before use.
Anti-human IgG and IgM Horse Radish Peroxidase conjugate goat polyclonal antibodies in 10 mM Tris buffer pH 6.8 and 5% BSA. Preservatives: 0,02% gentamicin sulphate 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)

Substrate **SUBS TMB**
1 Vial x 16 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.*

Assay diluent **DILAS**

GHS07

1 Vial x 15 ml. Ready to use. Vortex thoroughly before use.
10 mM tris buffered solution pH 8.0. Preservative: Proclin™ 300 (0.045%). To be used for the pre-treatment of samples and controls in the microplate, blocking interference.

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)

Sulphuric acid **H_2SO_4 0.3 M**

GHS05

1 Vial x 16 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)

Sample diluent **DILSPE**

GHS07

1 vial x 50 ml. Ready to use. Vortex thoroughly before use.
10 mM Na-citrate buffer pH 6.0, containing 1% goat serum proteins and 0,5% Tween 20. Preservatives: sodium azide (0,09%) and Proclin™ 300 (0,045%).
To be used for the dilution of samples.
Important: *the sample diluent turns dark bluish green from olive green when the sample is added.*
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)

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 the 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 10 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

ATTENTION: some components of this kit contain materials of human origin (blood derivatives) therefore, they must be treated as potentially infectious. It is recommended that these reagents and human samples be handled using established good laboratory practices.

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).
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. 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 350ul/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.

4. Incubation times have a tolerance of ±5%.

5. 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.

6. 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 L "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.

7. 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 colourless 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 aluminium pouch, containing the microplate, is not punctured or damaged.

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

4. Dissolve the Calibrator as described above.

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

6. 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.

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

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

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

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

11. 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.

Automated assay:

Follow the automated ELISA processor user manual for programming the Sclavo-EIA HCV Ab test method. In case the test is carried out automatically with an ELISA processor, we recommend to make the instrument aspirate first 200 µl Sample Diluent and then 10 µl sample. All the mixture is then carefully dispensed directly into the appropriate sample well of the microplate. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. Do not dilute controls/calibrator as they are ready to use. Dispense 200 µl controls/calibrator in the appropriate control/calibration wells.

Important note: Visually monitor that samples have been diluted and dispensed into appropriate wells: check that the colour of dispensed samples has turned to dark bluish-green while the colour of the negative control has remained olive green. For the next operations follow the operative instructions reported below for the Manual Assay. 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.

Manual assay:

1. Place the required number of 8-Microwell strips in the microwell frame. Leave the 1st well (A1) empty for the blank substrate.

2. Dispense 200 µl of Negative Control in triplicate, 200 µl Calibrator in duplicate and 200 µl Positive Control in single in proper wells.

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

3. Add 200 µl of Sample Diluent (DILSPE) to all the sample wells; then dispense 10 µl sample in each properly identified well. Mix gently the plate, avoiding overflowing and contaminating adjacent wells, in order to fully disperse the sample into its diluent.

Important note: Check that the colour of the Sample Diluent, upon addition of the sample, changes from light green to dark bluish green, monitoring that the sample has been really added.

4. Dispense 50 µl Assay Diluent (DILAS) into all the controls/calibrator and sample wells. Check that the color of samples has turned to dark blue.

5. Incubate the microplate for 45 min at +37°C.

Important note: Strips have to be sealed with the supplied adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.

6. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section H.3).

7. Pipette 100µl Enzyme Conjugate into each well, except the 1st blanking well, and cover with the adhesive foil. Check that this pink/red coloured component has been dispensed in all the wells, except A1.

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.*

8. Incubate the microplate for 45 min at +37°C.

9. Wash microwells as in step 6.

10. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at room temperature (18-24°C) for 15 minutes.

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

11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10 to stop the enzymatic reaction. Addition of acid will turn the positive control and positive samples from blue to yellow/brown.

12. Measure the colour intensity of the solution in each well, as described in section H.5, at 450nm filter (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 (mandatory).

Important notes:

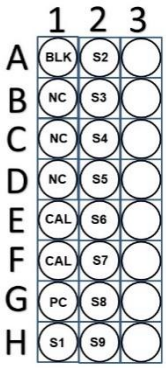
1. Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.
2. Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self-oxidation of the chromogen can occur leading to high background.
3. Shaking at 350 +150 rpm during incubation has been proved to increase the sensitivity of the assay of about 20%.
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

Method	Operations
Controls & Calibrator	200 µl (ready to use)
Samples	200 µl sample diluent +10 µl
Assay Diluent (DILAS)	50 µl on all wells except A1
1st incubation	**45 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Conjugate	100 µl with the exception of A1
2nd incubation	**45 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H_2O_2	100 µl
3rd incubation	**15 min**
Temperature	RT
Sulphuric Acid	100 µl
Reading OD	450nm / 620-630nm within 20 minutes

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.

Check	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	< 0.050 mean OD450nm value after blanking
Calibrator	S/CO > 1.1
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:

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 control); 4. that no contamination of the negative control or of their wells has occurred due to positive samples, to spills or to 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 < 1.1	1. That the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of negative control instead of control serum); 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 control has occurred.
Positive Control < 1.000 OD450nm	1. That the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of positive control. In this case, the negative control will have an OD450nm value > 0.150, too; 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.

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

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 value of the Negative Control (NC):

NC + 0.350 = 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 HCV or 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 HCV 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 should be confirmed by an alternative method capable to detect IgG and IgM antibodies (confirmation test) before a diagnosis of viral hepatitis is formulated.
3. As proved in the Performance Evaluation of the product, the assay is able to detect seroconversion to anti HCV core antibodies earlier than some other commercial kits. Therefore, a positive result, not confirmed with these commercial kits, does not have to be ruled out as a false positive result! The sample has to be anyway submitted to a confirmation test.
4. As long as the assay is able to detect also IgM antibodies some discrepant results with other commercial products for the detection of anti HCV antibodies - lacking anti hIgM conjugate in the formulation of the enzyme tracer and therefore missing IgM reactivity - may be present. The real positivity of the sample for antibodies to HCV should be then confirmed by examining also IgM reactivity, important for the diagnosis of HCV infection.
5. When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.
6. Diagnosis of viral hepatitis infection has to be done and released to the patient only by a 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 0,019 – 0,020 – ,020
OD 450 nm mean value: 0,020	Lower than 0,050
ACCEPTED	
POSITIVE CONTROL	OD 450 nm 2,189
OD 450 nm higher than 1,000	
ACCEPTED	
CUT-OFF	0,020 + 0,350 = 0,370
CALIBRATOR	OD 450 nm 0,550 – 0,530
OD 450 nm mean value 0,540	S/CO = 1,4 higher than 1,1
ACCEPTED	
Sample 1 OD 450 nm: 0,070	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).

O.1. Limit of detection

The limit of detection of the assay has been calculated by means of the British Working Standard for anti-HCV, NIBSC code 99/588-003-WI. The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined**.**

Dilution	Lot # 1	Lot # 2
Factor	S/CO	S/CO
1 X	**2.0**	**2.0**

2 X	**1.1**	**1.2**
4 X	0.7	0.8
8 X	0.5	0.5
Negative plasma	0.3	0.3

In addition, the sample coded Accurun 1 – series 3000 - supplied by Boston Biomedica Inc., USA, has been evaluated "in toto" showing the results below:

Sclavo HCV Lot ID	Accurun 1 Series	S/CO
1201	3000	1.5
0602	3000	1.5
1202	3000	1.9

In addition, n° 7 samples, tested positive for HCV Ab with Ortho HCV 3.0 SAVe, code 930820, lot. # EXE065-1, were diluted in HCV Ab negative plasma in order to generate limiting dilutions and then tested again on Sclavo-EIA HCV Ab, lot. # 1202, and Ortho.

The following table reports the data obtained:

Sample n°	Limit Dilution	Sclavo HCV S/CO	Ortho 3.0 S/CO
1	256 X	**1.9**	**1.3**
2	256 X	**1.9**	**0.7**
3	256 X	**2.4**	**1.0**
4	128 X	**2.5**	**3.2**
5	85 X	**3.3**	**1.4**
6	128 X	2.2	0.8
7	135 X	**3.2**	**2.2**

O.2. Diagnostic specificity:

It is defined as the probability of the assay of scoring negative in the absence of specific analyte. In addition to the first study, where a total of 5043 unselected blood donors (including 1st time donors), 210 hospitalized patients and 162 potentially interfering specimens (other infectious diseases, E. coli antibody positive, patients affected by non-viral hepatic diseases, dialysis patients, pregnant women, haemolyzed, lipemic, etc.) were examined, the diagnostic specificity was recently assessed by testing a total of 2876 negative blood donors on six different lots. A value of specificity of 100% was found.

No false reactivity due to the method of specimen preparation has been observed. Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.

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

O.3. Diagnostic Sensitivity:

It defined as the probability of the assay of scoring positive in the presence of specific analyte.

The diagnostic sensitivity has been assessed externally on a total number of 359 specimens; a diagnostic sensitivity of 100% was found. Internally more than other 50 positive samples were tested, providing a value of diagnostic sensitivity of again 100%. Positive samples from infections carried out by different genotypes of HCV were tested as well.

Furthermore, most of seroconversion panels available from Boston Biomedica Inc., USA, (PHV) and Zeptometrix, USA, (HCV) have been studied.
Results are reported below for some of them:

Panel	N° samples	Sclavo*	Ortho* **
PHV 901	11	9	9
PHV 904	7	2	4
PHV 905	9	3	4
PHV 906	7	7	7
PHV 907	7	3	2
PHV 908	13	10	8
PHV 909	3	2	2
PHV 910	5	3	3
PHV 911	5	3	3
PHV 912	3	1	1
PHV 913	4	2	2
PHV 914	9	5	5

PHV 915	4	3	0
PHV 916	8	4	3
PHV 917	10	6	6
PHV 918	8	2	0
PHV 919	7	3	3
PHV 920	10	6	6
HCV 10039	5	2	0
HCV 6212	9	6	7
HCV 10165	9	5	4

Note: * Positive samples detected ** *HCV v.3.0*

Finally, the Product has been tested on the panel EFS Ac HCV, lot n° 01/08.03.22C/01/A, supplied by the Etablissement Francais Du Sang (EFS), France, with the following results:

EFS Panel Ab HCV

Sample	Lot # 1 S/CO	Lot # 2 S/CO	Lot # 2 S/CO	Expected results
HCV 1	**2.2**	**2.4**	**2.6**	**positive**
HCV 2	**1.6**	**2.0**	**2.1**	**positive**
HCV 3	**1.5**	**1.7**	**1.6**	**positive**
HCV 4	**5.2**	**6.5**	**5.5**	**positive**
HCV 5	**1.6**	**1.8**	**1.6**	**positive**
HCV 6	**0.4**	**0.4**	**0.4**	**Negative**

O.4. Precision:

It has been calculated on two samples, one negative and one low positive, examined in 16 replicates in three separate runs. Results are reported as follows:

Lot # 1202

Negative Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.094	0.099	0.096	0.096
Std.Deviation	0.008	0.007	0.008	0.007
CV %	8.7	6.6	7.9	7.7

Cal # 2 – 7K (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.396	0.403	0.418	0.406
Std.Deviation	0.023	0.029	0.027	0.026
CV %	5.9	7.1	6.4	6.5
S/Co	1.1	1.1	1.2	1.1

Lot # 0602

Negative Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average
OD 450nm	0.097	0.096	0.094	0.096
Std.Deviation	0.009	0.010	0.008	0.009
CV %	8.9	10.1	8.4	9.1

Cal # 2 – 7K (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.400	0.395	0.393	0.396
Std.Deviation	0.021	0.025	0.026	0.024
CV %	5.4	6.2	6.6	6.1
S/CO	1.2	1.2	1.1	1.2

Lot # 0602/2

Negative Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average

OD 450nm	0.087	0.091	0.088	0.089
Std.Deviation	0.009	0.007	0.008	0.008
CV %	10.0	8.2	8.6	8.9

Cal # 2 – 7K (N = 16)

Mean values	1st run	2nd run	3rd run	Average
OD 450nm	0.386	0.390	0.391	0.389
Std.Deviation	0.023	0.021	0.023	0.022
CV %	6.0	5.3	5.8	5.7
S/CO	1.1	1.2	1.2	1.2

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

P. LIMITATIONS

Repeatable false positive results, not confirmed by RIBA or similar confirmation techniques, were assessed as less than 0.1% of the normal population. Frozen samples containing fibrin particles or aggregates after thawing have been observed to generate some false results.

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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