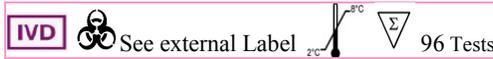


AccuDiag™
HCV IgM ELISA

REF 1708-6



Test	HCV IgM
Method	Enzyme-linked Immunosorbent Assay
Principle	Indirect ELISA
Type	Quantitative OR Qualitative
Sample	10 µL human plasma/sera
Total Time	~150 min
Relative Specificity	> 98%
Relative Sensitivity	> 98%

INTENDED USE

The Diagnostic Automation, Inc. AccuDiag™ HCV IgM ELISA is designed for the quantitative or qualitative determination of IgM antibodies to Hepatitis C Virus in human plasma and sera.

This kit is intended for the follow-up of HCV chronic patients submitted to anti-viral pharmaceutical treatment.

SUMMARY AND EXPLANATION

Antiviral drugs, such as interferon, taken by itself or in combination with Ribavirin, can be used for the treatment of individuals with chronic viral hepatitis C.

Treatment with interferon alone is effective in about 10% to 20% of patients. Combined with Ribavirin, the effectiveness is improved to about 30% to 50% of patients. Ribavirin does not appear to be effective when used alone.

Active production of HCV antigens in the liver of chronic patients generates spikes of IgM antibodies production and release of liver specific enzymes, similar to that in HBV chronic patients. The presence of anti-viral IgM is often correlated to a phase of suffering and cellular damage of the liver.

During pharmaceutical treatment, HCV IgM may represent a marker for the follow-up of the efficiency of the drug itself, monitoring the balance between its effectiveness and the side effects that may often be heavy for the patient.

TEST PRINCIPLE

Microplates are coated with HCV immunodominant synthetic antigens (core peptide, recombinant NS3, NS4 and NS5 peptides).

In the first incubation, the solid phase is treated with diluted samples and anti HCV IgM are captured, if present, by the antigens. After washing out all the other components of the sample, in the second incubation, bound anti-HCV IgM are detected by the addition of anti-hIgM antibody, which are labeled with peroxidase (HRP). The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti-HCV IgM antibodies present in the sample.

The presence of IgM in the sample may therefore be quantitated by means of a calibration curve able to determine the content of the antibody in arbU/mL.

Neutralization of IgG anti-HCV, carried out directly in the well, is performed in the assay in order to block interferences due to this class of antibodies in the determination of IgM.

SPECIMEN PREPARATION

1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of sample preparation for clinical laboratory analysis. No influence has been observed in the preparation of samples with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Hemolyzed ("red") and visibly hyperlipemic ("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.
4. Sera and plasma can be stored at 2 °C - 8 °C for up to five days after collection. For longer storage periods, samples can be stored frozen at -20 °C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2000 rpm for 20 min or filter using 0.2 - 0.8µm filters to clean up the sample for testing.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. **Microplate:** 12 strips X 8 microwells coated with HCV-specific synthetic antigens (core, NS4 and NS5 peptides and recombinant NS3). Plates are sealed into a bag with desiccant.
2. **Standards:** 6 X 2.0 mL/vial. Ready to use and color coded. Standard curve calibrated on an Internal Gold Standard (in absence of a defined international one) or IGS, ranging:
 - a) CAL 1 = 0 arbU/mL
 - b) CAL 2 = 10 arbU/mL
 - c) CAL 3 = 25 arbU/mL
 - d) CAL 4 = 50 arbU/mL
 - e) CAL 5 = 100 arbU/mL
 - f) CAL 6 = 250 arbU/mL

It contains chemically inactivated HCV IgM positive human plasma, 100mM Tris buffer pH 7.4 ± 0.1, 0.2% Tween 20, 0.09% sodium azide, and 0.1% Kathon GC as preservatives. The Calibration Curve is coded with blue alimentary dye.

Important Note: Even if plasma has been chemically inactivated, handle this component as potentially infectious.

3. **Wash Buffer Concentrate:** 1 X 60 mL/bottle, 20X concentrated solution. Once diluted, the wash solution contains 10mM phosphate buffer pH 7.0 ± 0.2, 0.05% Tween 20 and 0.05% Kathon GC.
4. **Enzyme Conjugate:** 1 X 16 mL/vial. Ready to use and red color coded. It contains horseradish peroxidase conjugated polyclonal antibodies to human IgM, 5% BSA, 10mM Tris buffer pH 6.8 ± 0.1, 0.1% Kathon GC and 0.02% gentamicin sulfate as preservatives.
5. **TMB Substrate Solution:** 1 X 15 mL/vial. It contains 50 mM citrate-phosphate buffer pH 3.5 - 3.8, 4% dimethyl sulfoxide, 0.03% tetramethylbenzidine (TMB) and 0.02% hydrogen peroxide (H₂O₂).

Note: To be stored protected from light as sensitive to strong illumination.

6. **Sulfuric Acid:** 1 X 15 mL/vial. Contains 0.3 M H₂SO₄ solution. Also known as sulphuric acid. **Attention:** Irritant (H315, H319; P280, P303+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).
7. **Specimen Diluent:** 2 X 60 mL/vial. It contains 2% casein, 10 mM sodium citrate buffer pH 6.0 ± 0.1, 0.1% Tween 20, 0.09% sodium azide, and 0.1% Kathon GC as preservatives. To be used to dilute the sample.



8. **Neutralizing Reagent:** 1 X 8 mL/vial. It contains goat anti-hlgG, 2% casein, 10 mM sodium citrate buffer pH 6.0 ± 0.1, 0.1% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives.
9. **Plate sealing foils** X 2

Materials required but not provided

1. Calibrated micropipettes (10, 100, and 1000 µL) and disposable plastic tips.
2. ELISA grade water (bidistilled or deionized, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper towels.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (±0.5 °C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and possibly with 620 – 630 nm (blinking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

WARNINGS & PRECAUTIONS

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. 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.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. 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 (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2 - 8°C into a temperature controlled refrigerator or cold room.
6. 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.
7. 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.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 6 months.
11. 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.
12. 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.
13. 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.

14. 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.
15. The sulfuric acid is an irritant. In case of spills, wash the surface with plenty of water.
16. 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.

PREPARATION OF COMPONENTS

Microplate:

Allow the microplate to reach room temperature (about 1 hour) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of storing.

In this case call Diagnostic Automation, Inc.

Unused strips have to be placed back into the aluminum pouch, in presence of desiccant supplied, firmly zipped and stored at 2 °C - 8 °C. When opened the first time, residual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

Standards:

Ready to use components. Mix carefully on vortex before use.

Wash Buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

Note: Once diluted, the wash solution is stable for 1 week at 2 °C - 8 °C.

Enzyme Conjugate:

Ready to use. Mix well on vortex before use.

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.

If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:

Ready to use. Mix well on vortex before use.

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.

Do not expose to strong illumination, oxidizing agents and metallic surfaces.

If this component has to be transferred use only plastic, possible sterile disposable container.

Sample Diluent:

Ready to use component. Mix carefully on vortex before use.

Neutralizing Reagent:

Ready to use component. Mix carefully on vortex before use.

Sulfuric Acid:

Also known as sulphuric acid. Ready to use. Mix well on vortex before use.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363)

Warning H statements:

H315 – Causes skin irritation.

H319 – Causes serious eye irritation.

Precautionary P statements:

P280 – Wear protective gloves/protective clothing/eye protection/face protection.

P302+P352 – IF ON SKIN: Wash with plenty of soap and water.

P332+P313 – If skin irritation occurs: get medical advice/attention.

P305+P351+P338 – IF IN EYES: Rinse cautiously with water for several minutes.

Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 – If eye irritation persists: Get medical advice/attention.

P362+P363 – Take off contaminated clothing and wash it before reuse.

INSTRUMENTS AND TOOLS

- 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.
- 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.
- The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350 µL/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section “Internal Quality Control”. Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
- Incubation times have a tolerance of ± 5%.
- The **ELISA microplate reader** has to be equipped with a reading filter of 450 nm and ideally with a second filter (620-630 nm) 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; repeatability 1%. Blanking is carried out on the well identified in the section “Internal Quality Control”. 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.
- 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 “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 when the number of samples to be tested exceed 20-30 units per run.
- DAI’s customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

PRE-ASSAY CONTROLS & OPERATIONS

- Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
- Check that the liquid components are not contaminated by visible particles or aggregates.
- Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
- Check that no leakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
- Dilute all the content of the 20x concentrated Wash Solution as described above.
- Allow all the other components to reach room temperature (about 1 hour) and then mix gently on vortex all liquid reagents.

- 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 found in the validation of the instrument for its use with the kit.
- Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
- If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
- Check that the micropipettes are set to the required volume.
- Check that all the other equipment is available and ready to use.
- In case of problems, do not proceed further with the test and seek advice from the supervisor.

INTERNAL QUALITY CONTROL

A validation check is carried out on the controls and the calibrator any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

Check	Requirements
Blank well	< 0.100 OD _{450nm} value
Calibrator 0 arbU/mL	< 0.200 OD _{450nm} value after blanking
Calibrator 10 arbU/mL	OD _{450nm} > OD _{450nm} CAL 0 arbU/mL + 0.100
Calibrator 250 arbU/mL	3.500 > OD _{450nm} > 2.000

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 perform the following checks:

Problem	Check
Blank well > 0.100 OD _{450nm}	1. That the Chromogen/Substrate solution has not become contaminated during assay
Calibrator 0 arbU/ml > 0.200 OD _{450nm} 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 calibrators instead of the CAL 0 arbU/ml); 4. That no contamination of the CAL 0 arbU/ml, or of the wells where this was dispensed, has occurred due to positive samples, to spills or to the enzyme conjugate; 5. That micropipettes have not become contaminated with positive samples or with the enzyme conjugate; 6. That the washer needles are not blocked or partially obstructed.
Calibrator 10 arbU/ml < CAL 0 + 0.100	1. That the procedure has been correctly executed; 2. That no mistake has occurred during its distribution 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.
Calibrator 250 arbU/ml < 2.000 OD _{450nm}	1. That the procedure has been correctly executed; 2. That no mistake has occurred during its distribution 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.
Calibrator 250 arbU/ml > 3.500 OD _{450nm} 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; 4. That no contamination of the CAL 250 arbU/ml, or of the wells where this was dispensed, has occurred due to positive samples, to spills or to the enzyme conjugate; 5. That micropipettes have not become contaminated with positive samples or with the enzyme conjugate;

6. That the washer needles are not blocked or partially obstructed.

If any of the above problem has occurred, report the problem to the supervisor for further actions.

ASSAY PROCEDURE

The assay has to be carried out according to instructions below, and care must be taken to maintain the same incubation time for all samples in testing.

Two methods of analysis are possible, as described below:

Quantitative Assay

- Place the required number of strips in the plastic holder and carefully identify the wells for calibrators and samples.
- Dilute samples 1:101 dispensing 1 mL Sample Diluent into a disposable tube and then 10 µL sample; mix on vortex before use. Do not dilute the Standards as they are ready to use.
- Leave the A1 + B1 wells empty for blanking purposes.
- Dispense 50 µL Neutralizing Reagent in all the wells, except A1+ B1 wells used for blanking operations and the wells used for the Standards.
- In the identified positions pipette 100 µL of the Calibrators in duplicate followed by 100 µL of diluted samples. Check that Calibrators and samples have been correctly added.
- Incubate the microplate for **60 min at +37 °C**.

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

- When the first incubation is finished, wash the microwells as previously described (section 9.3)
- In all the wells, except A1+B1, pipette 100 µL Enzyme Conjugate. Incubate the microplate for **60 min at +37 °C**.

Important note: Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.

- When the second incubation is finished, wash the microwells as previously described (section 9.3)
- Pipette 100 µL Chromogen/Substrate into all the wells, A1 + B1 included.

Important note: Do not expose to strong direct light. High background might be generated.

- Incubate the microplate protected from light **at room temperature (18 °C - 24 °C) for 20 minutes**. Wells dispensed with positive samples and with positive calibrators will turn from clear to blue.
- Pipette 100 µL Sulfuric Acid into all the wells using the same pipetting sequence as in step 10 to block the enzymatic reaction. Addition of the stop solution will turn the positive calibrators and the positive samples from yellow to blue.
- Measure the color intensity of the solution in each well, as described in section 9.5 using a 450 nm filter (reading) and if possible a 620-630 nm filter (background subtraction), blanking the instrument on A1 or B1 or both.

Qualitative Assay

- Place the required number of strips in the plastic holder and carefully identify the wells for calibrators and samples.
- Dilute samples 1:101 dispensing 1 mL Sample Diluent into a disposable tube and then 10 µL sample; mix on vortex before use. Do not dilute the Standards as they are ready to use.
- Leave the A1 well empty for blanking purposes.
- Dispense 50 µL Neutralizing Reagent in all the wells, except A1 well used for blanking operations and the wells used for the Standards.
- Then pipette 100 µL of Calibrator 0 arbU/mL in duplicate, 100 µL of Calibrator 10 arbU/mL in duplicate and finally 100 µL of diluted samples. Check that Calibrators and samples have been correctly added.
- Incubate the microplate for 60 min at +37 °C.

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

- When the first incubation is finished, wash the microwells as previously described (section 9.3)
- In all the wells, except A1, pipette 100 µL Enzyme Conjugate. Incubate the microplate for 60 min at +37 °C.
- Important note:** Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.
- When the second incubation is finished, wash the microwells as previously described (section 9.3)
- Pipette 100 µL Chromogen/Substrate into all the wells, A1 included.

Important note: Do not expose to strong direct light. High background might be generated.

- Incubate the microplate protected from light **at room temperature (18 °C - 24 °C) for 20 minutes**. Wells dispensed with positive samples and with positive calibrators will turn from clear to blue.
- Pipette 100 µL Sulfuric Acid into all the wells using the same pipetting sequence as in step 10 to block the enzymatic reaction. Addition of the stop solution will turn the positive calibrators and the positive samples from yellow to blue.
- Measure the color intensity of the solution in each well, as described in section 9.5 using a 450 nm filter (reading) and if possible a 620-630 nm filter (background subtraction), blanking the instrument on A1 or B1 or both.

General Important notes:

- If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450 nm. Fingerprints could generate false positive results on reading.
- 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.

ASSAY SUMMARY & SCHEME

Methods	Operations
Neutralizing Reagent	50 µL
Calibrators	100 µL
Samples diluted 1:101	100 µL
1st incubation	60 min
Temperature	+37 °C
Wash step	4-5 cycles
Enzyme conjugate	100 µL
2nd incubation	60 min
Temperature	+37 °C
Wash step	4-5 cycles
TMB/H ₂ O ₂	100 µL
3rd incubation	20 min
Temperature	Room temp.
Sulfuric acid	100 µL
Reading OD	450 nm

An example of dispensation scheme in **quantitative** assays is reported below:

	Microplate												
	1	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S3										
B	BLK	CAL4	S4										
C	CAL1	CAL5	S5										
D	CAL1	CAL5	S6										
E	CAL2	CAL6	S7										
F	CAL2	CAL6	S8										
G	CAL3	S1	S9										
H	CAL3	S2	S10										

Legenda: BLK = Blank CAL = Calibrator S = Sample

An example of dispensation scheme in **qualitative** assays is reported below:

		Microplate												
		1	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S4												
B	CAL1	S5												
C	CAL1	S6												
D	CAL2	S7												
E	CAL2	S8												
F	S1	S9												
G	S2	S10												
H	S3	S11												

Sample 2: 1.800 OD_{450nm}
 Sample 1: S/Co < 1.0 = negative
 Sample 2: S/Co > 1.0 = positive

PERFORMANCE CHARACTERISTICS

Evaluation of Performances has been conducted on selected panels carried out in a clinical external center and internally.

RESULTS

If the test turns out to be valid, interpretation of results is carried out in the **quantitative assay** from the mean OD_{450nm} value of the Calibration Curve elaborated with an appropriate curve fitting system (suggested: 4 parameters).

In the **qualitative assay** interpretation of results is done on the mean OD_{450nm} value of the Calibrator 10 arbU/ml (or CAL 2) by means of the following formulation:

Mean OD_{450nm} CAL 2 = cut-off (Co)

Important note: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to generate the correct interpretation of results.

INTERPRETATION OF RESULTS

A. Quantitative Assay

Concentrations in arbU/mL are obtained elaborating OD 450 nm of samples on the fitted calibration curve.

The concentration of IgM is from Literature correlated proportionally with the liver damage produced by antibodies to HCV upon virus replication in hepatocytes.

A decrease in IgM concentration upon pharmacological treatment is usually clinically acknowledged as a sign of recovery and therapeutic efficacy.

B. Qualitative Assay

Test results are interpreted as a ratio of the sample OD_{450nm} value (S) and the cut-off value (Co), or S/Co, according to the following table:

S/Co	Interpretation
< 1.0	Negative
> 1.0	Positive

A negative result indicates that the patient has not developed IgM antibodies to HCV.

A positive result is indicative of an ongoing HCV active infection.

Important notes:

- Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.
- When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.
- Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.
- The results of this ELISA assay should be anyway implemented with other diagnostic and clinical tests.

An example of calculation is reported below.

The following data must **NOT** be used instead of real figures obtained by user.

CAL 1: 0.060 – 0.080 OD_{450nm}
 Mean value: 0.070 OD_{450nm}
 Lower than 0.200 – Accepted

CAL 2: 0.200 – 0.220 OD_{450nm}
 Mean value: 0.210 OD_{450nm}
 Higher than CAL1 + 0.100 = accepted
 Cut-Off or Co = 0.210

Sample 1: 0.080 OD_{450nm}

A. Limit of detection

No international standard for HCV IgM Antibody detection has been defined so far by the European Community.

In its absence, an Internal Gold Standard (or IGS), derived from a patient with a history of chronic HCV infection, has been defined in order to provide the device with a constant and excellent sensitivity.

B. Clinical Sensitivity and Specificity

The diagnostic performances were evaluated in a study conducted in an external clinical center, with excellent experience in the diagnosis of infectious diseases and HCV.

The Diagnostic Sensitivity was studied on about 200 samples, pre-tested positive with an analytical system developed in house by the clinical laboratory where the study was conducted. Positive samples were collected from patients with a clinical history of HCV infection (acute and chronic). In addition some Seroconversion Panels, purchased from Boston Biomedica Inc., USA, were examined.

The diagnostic specificity was determined on panels of more than 300 negative samples from normal individuals and blood donors, classified negative for anti HCV antibodies with the reference kit in use in the laboratory, including potentially interfering specimens.

A panel of potentially interfering samples (RF+, hemolyzed, lipemic, etc.) was also examined. No interference was observed on the samples examined.

Both plasma, 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.

The Performance Evaluation provided the following values:

Sensitivity	> 98%
Specificity	> 98%

C. Reproducibility

It has been calculated on two samples examined in replicates in different runs. Results are reported below summarized in a table:

Average values N=48	Calibrator 2 10 arbU/ml	Calibrator 5 100 arbU/ml
OD 450nm	0.241	1.632
Std. Deviation	0.027	0.113
CV%	11.3	6.9

LIMITATIONS OF PROCEDURE

- False positivity has been assessed on less than 2% of the normal population, mostly due to high titers of RF.
- Frozen samples containing fibrin particles or aggregates may generate false positive results.

STORAGE

1. Store the unopened kit between 2 and 8 °C.
2. Coated microwell strips: Store between 2 and 8 °C. Extra strips should be immediately resealed in the aluminum pouch with desiccant and returned to proper storage at 2 – 8 °C. Strips are stable until the humidity indicator in the desiccant bag turns to green from yellow.
3. Conjugate: Store between 2 and 8°C. **DO NOT FREEZE.**
4. Calibrator, Positive Control and Negative Control: Store between 2 and 8°C.
5. TMB: Store between 2 and 8°C. Protect from light.
6. Wash Buffer concentrate: Store between 2 and 8 °C. Diluted wash solution is stable for 1 week at 2 – 8 °C.
7. Sample Diluent: Store between 2 and 8°C.
8. Stop Solution: Store between 2 and 25°C.

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