HCV-Ab Confirmatory (EIA-3970)

Not for Sale in the USA

INTRODUCTION

Hepatitis C Virus or HCV is an enveloped RNA virus recently classified in the family of Flaviviridae. The genome encodes for structural components, a nucleocapsid protein and two envelope glycoproteins, and functional constituents involved in the virus replication and protein processing. The nucleocapsid-encoding region seems to be the most conservative among the isolates obtained all over the world. HCV accounts for about 95% of hepatitis infections in recipients of blood transfusion and 50% of cases of sporadic NANB hepatitis. HCV commonly gives origin to asymptomatic hepatitis and chronicity develops in a high number of cases, sometime evolving in severe forms of illness, as hepato-carcinoma. The determination of antibody to HCV has become mandatory in the screening of blood units to prevent post-transfusion hepatitis. It is also currently used to follow-up risk individuals and patients under treatment with interferon. Conformation of any positive result is strongly recommended in the clinical laboratory practice before considering the patient truly positive for anti HCV antibodies.

PRINCIPLE OF THE ASSAY

Strips coat Microplates with HCV-specific synthetic antigens derived from "core", "ns" and "env" regions encoding for conservative immunodominant antigenic determinants (Core, NS3, NS4, NS5 & Env). Antigens are adsorbed to the wells composing the strips as follows:

Position	Antigen	Composition
A	None	Well for blanking operations
B	Casein	Negative internal control
C	Core	Specific synthetic antigen
D	NS3	Specific synthetic antigen
E	NS4	Specific synthetic antigen
F	NS5	Specific synthetic antigen
G	Env	Specific synthetic antigen
H	hIgG	Positive Internal Control

The strip is first treated with the sample turned out to be positive in the screening assay. Anti HCV antibodies are captured, if present, by the specific antigens. After washing out all the other components of the sample, in the 2nd incubation bound HCV Ab are detected by the addition of anti hIgG&M antibody, 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 antibodies present in the sample. Controls are included to provide an internal check of the analytical system. The sample is confirmed positive if at least two specific reactivities are present.

TEST CONDITIONS AND NOTICES

1. All the reagents contained in the kit are for "in vitro" diagnostic use only.
2. Do not use the kit or reagents after the expiry date stated on labels. Do not mix reagents of different lots.
3. Procedures should be performed carefully in order to obtain reliable results and clinical interpretations.
4. Bring all the reagents to room temperature for at least 60 min, before the test is started.
5. Avoid any contamination of reagents when taking them out of vials. We recommend using automatic pipettes and disposable tips. When dispensing reagents, do not touch the wall of microplate wells with tips, in order to avoid any cross-contamination.
6. In the washing procedure, use only the Washing Solution provided with the kit and follow carefully the indications reported in the "WASHING INSTRUCTIONS" section of this insert.
7. Ensure that the Substrate/Chromogen mixture does not come in contact with oxidizing agents or metallic surfaces; avoid any intense light exposure during the incubation step or the reagent preparation.
   When preparing the Substrate/Chromogen mixture for the analysis use only plastic, disposable, clean or sterile containers.

8. Samples and materials potentially infective have to be handled with care as they could transmit infection. All objects come in direct contact with samples and all residuals of the assay should be treated or wasted as potentially infective. Best procedures for inactivation are treatments with autoclave at 121°C for 30 min or with sodium hypochlorite at a final concentration of 2.5% for 24 hr. This last method can be used for the treatment of the liquid waste after that it has been neutralized with NaOH.
9. Avoid any contact of liquids with skin and mucosas.
10. Use always-protective talk-free gloves, glasses and laboratory coats, according to the safety regulations.

CONTENT OF THE KIT

a. Strips Microplate n° 1

12x8 wells strips coated by strip with synthetic HCV antigens. Strips are contained in a sealed bag with a dessicant and a frame. Bring the strips necessary to the test to room temperature before use, and close firmly the bag to prevent any moisture formation inside.

b. Enzymatic Enzym conjugate n° 1 vial of 16 ml

Proteic buffer solution containing a specific anti-hIgG&M antibody, labeled with HRP, ready to use.
It contains proteic stabilizers, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC.

c. Washing Solution n° 1 vial of 60 ml

20x concentrated solution to be diluted up to 1200 ml with EIA grade water. It contains a phosphate buffer, Tween 20 and Kathon GC as preservative. The diluted solution, when stored at room temperature, is stable for at least 1 week.

d. Chromogen/Substrate n° 1 vial of 16 ml

The solution contains tetramethylbenzidine (TMB) and hydrogen peroxide (H2O2) with activators and stabilizers, diluted in a phosphate/citrate buffer. The solution is ready to use. **Warning**: Store protected from light.

e. Stop Solution n° 1 vial of 16 ml

It contains a solution of 0.3 M sulphuric acid. **Warning**: Irritant! (Xi. R36/38 S2, 26,30)

f. Negative Control n° 1 vial of 3 ml

Human serum base not reactive for anti-HCV antibodies. It contains 0.2 mg/ml gentamicine sulphate and 0.3% Kathon GC as preservatives.

g. Positive Control n° 1 vial of 3 ml

Human serum base highly reactive for HCV Ab. It contains 0.2 mg/ml gentamicine sulphate and 0.3% Kathon GC as preservatives.

h. Sample Diluent n° 1 vial of 20 ml

Proteic solution for the preparation of samples. It contains a detergent, proteic stabilizers, 0.1% sodium azide and 0.3% Kathon GC as preservatives. **Note:** All human serum derived materials have been tested as negative for HBsAg, and HIV antibodies with FDA approved kits. The Positive Control has been inactivated for HCV. Anyway, handle these components as potentially infective.

STORAGE AND STABILITY

1. The kit has to stored at 2-8°C and used before the expiration date declared on the external label.
2. Unused strips have to be returned into their bag containing the dessicant and firmly sealed with tape before store them back at 2-8°C.
3. After the bag has been opened the first time, strips are stable till the dessicant turns pink.
4. The diluted washing solution is stable at room temperature for 1 week or for 3 weeks at 2-8°C.

MATERIALS NOT PROVIDED

1. Micropipettes of 10, 100 and 1000 ul.
2. Vortex mixer and adsorbent papers and ELISA grade water for dilutions.
3. Timer.
4. Photometric ELISA supplied with filters of 450nm and 620-630 nm.
5. Incubator set at +37°C
6. Automatic microplate washer or manual apparatus capable to deliver 300 ul and aspirate.

SAMPLES

Either fresh sera or plasma can be used for the assay. If not used immediately, they can be stored at 2-8°C for 1 week. In case of longer storage freeze them at –30°C. Samples should be clear and not contaminated by microrganisms. Do not freeze and thaw samples more than one time, as antibodies may get damaged. If necessary, remove contaminating particles by centrifugation at 2000 g x 20 min at r.t. or by filtration on 0.22u filters. Highly lipemic, icteric or hemolized samples should not be used as they can give false results in the assay.

PREPARATION OF REAGENTS

WASHING INSTRUCTIONS

Washing Solution: The concentrated solution has to be diluted 20x in ELISA grade water before use. A good washing procedure is essential to obtain correct and precise analytical data. We therefore recommend to use a good quality ELISA microplate washer, maintained at the best level of washing performances. Generally, 4-5 automatic washing cycles of 300 ul/well are sufficient to avoid false positive reactions and high background. Anyhow, we recommend calibrating the washing system on the kit itself so to match the declared analytical performances. In case of manual washing, we suggest to carry out 5 cycles, dispensing 300 ul/well and aspirating the liquid for 5 times. In any case, the liquid aspirated out the strips must be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hrs, before liquids are wasted in an appropriate way.

ASSAY PROCEDURE

Important notes: At least 1 hr before use, bring all the components of the kit to r.t. and mix the liquid reagents carefully on vortex. Do not mix reagents of different lots. We recommend distributing the calibrators in duplicate. Distribution and incubation times should be the same for all the wells. Avoid long interruptions among the different steps of the assay. It is suggested to eliminate the excess of washing solution from wells by blotting them gently on a paper adsorbent pad. The color developed in the last incubation is stable for maximum 1 hr in the dark. We recommend to read the microplate at 450 nm (reading filter) and subtract the blank at 620-630 nm (blanking filter). Use well A1 to carry out blanking operations. Leave the A1 well empty for blanking operations. Dilute 10-ul samples to confirm with 1 ml diluent (1:101 dilution). Do not dilute controls (if tested) as they are prediluted and ready-to-use.

1. Dispense the diluted sample to confirm into one strip module according to the following table:

POSITION	SAMPLE
A1	blanking well
B1	100 ul diluted sample to confirm
C1	100 ul diluted sample to confirm
D1	100 ul diluted sample to confirm
E1	100 ul diluted sample to confirm
F1	100 ul diluted sample to confirm
G1	100 ul diluted sample to confirm
H1	100 ul diluted sample to confirm

2. Cover the strip with the sealer and incubate the strip module for 60 min at +37°C.
3. Peel out the plate sealer and wash the strip module according to instructions. In the meantime prepare the Enzyme conjugate by diluting it 1:20 as described.
4. Add 100 ul diluted Enzyme conjugate to all the wells, except A1. Incubate the module sealed for 60 min at +37°C.
5. Peel out the plate sealer and wash the strip according to the instructions. In the meantime prepare the Chromogen/Substrate mixture as described.
6. Add 100 ul of the Chromogen/Substrate mixture to all the wells, A1 included. Incubate the strip module for 20 min at r. temp, protected from light.
7. Stop the enzymatic reaction by adding 100 ul Stop Solution to all the wells, A1 included.
8. Read the strip module at 450nm and 620-630nm blanking the instrument on A1 well.

VALIDITY OF THE ASSAY

The assay is considered valid if:

1. The OD450nm in A1 blank well is < 0.100.
2. After blanking, the OD450nm in H1 well is > 0.800
3. After blanking, the OD450nm mean value of the negative control (if tested) is < 0.200.
4. After blanking, the OD450nm value of the positive control (if tested) is > 0.800 in at least two positions from B1 to F1

In case data above do not match the correct values, before repeating the test check carefully the expiration date of the kit, the performances of the instruments used for the assay and the procedure of distribution of controls and samples.

INTERPRETATION OF RESULTS

If the validity of the assay is confirmed, examine the following table for the interpretation of results.

Classification	Results
Negative	Wells from C1 to G1 with OD450nm < B1 + 0.250
False Positive	B1 well with OD450nm > 0.250
Indeterminate	One well from C1 to G1 with OD450nm > B1 + 0.250. B1 well must have OD450nm < 0.250
Positive	At least 2 wells from C1 to G1 with OD450nm > B1 + 0.250. B1 well must have OD450nm < 0.250

REFERENCES

1. Ellner PD,Neu HC. Viral agents of gastroenteritis. In “Understanding infectious disease. St.Louis: Mosby-Year Book, 1992, pp183-186.
2. Hollinger FB, Dreesman GR. Hepatitis virus. In “Rose NR, de Macario EC, Fahey JL, et al. (eds), Manual of clinical laboratory immunology, 4th ed. Washington, DC: ASM, 1992, pp634-650.
3. Fody EP, Johnson DF. The serologic diagnosis of viral hepatitis. J Med Technol 1987, 4:54-59.
4. Evans AS. Viral infections of humans. Epidemiology and control. New York; Plenum, 1976.