Monolisa™ HCV Ag-Ab ULTRA

1 plate - 96 tests 72556
5 plates - 480 tests 72558

SCREENING KIT FOR THE DETECTION OF HCV (HEPATITIS C VIRUS) INFECTION IN HUMAN SERUM/PLASMA BY ENZYME IMMUNOASSAY

Manufacturer Quality Control

All manufactured and commercialised reagents are under complete quality system starting from reception of raw material to the final commercialisation of the product. Each lot is submitted to a quality control and only is released on the market when conforming to the acceptance criteria. The records relating to production and control of each single lot are kept within our company.
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1 - INTENDED USE
Monolisa™ HCV Ag-Ab ULTRA assay is an enzyme immunoassay for the detection of HCV infection, based on the detection of capsid antigen and antibodies associated with an infection by Hepatitis C virus in patient serum or plasma.

2 - PRINCIPLE OF THE TEST
Monolisa™ HCV Ag-Ab ULTRA Microplate solid phase is coated with:
• Monoclonal Antibodies against capsid protein of Hepatitis C virus.
• 2 recombinant proteins produced by *E.coli* from NS3 region : genotype 1 and 3a.
• One recombinant antigen from the nonstructural region NS4.
• A mutated peptide from the capsid of structural area of the hepatitis C virus genome.
The conjugates used are:
• Conjugate 1 (R6) : Mouse biotinilated monoclonal antibodies against the hepatitis C capsid. This monoclonal does not react against the hepatitis C capsid mutated peptide coated on the microplate.
• Conjugate 2 (R7) : Mouse peroxidase-labelled antibodies to human IgG and peroxidase-labelled streptavidin.
The performance of the test includes the following reaction steps:
1) The conjugate 1 and samples to be tested and the control sera are added to the wells. If antibodies to HCV are present, they will bind to the antigens fixed on the solid phase and if hepatitis C capsid antigen is present, this antigen is bind by the monoclonal antibodies coated on microplate and by the Biotinilated monoclonal antibodies against the capsid hepatitis C antigen (conjugate 1).
2) After incubation at 37°C during 90 minutes and a washing step, the peroxidase-labeled antibodies to human IgG and streptavidine –peroxidase (conjugate 2) are added. Streptavidin/peroxidase conjugate reacts with biotinilated monoclonal antibodies against Hepatitis C capsid antigen if presents. Anti human IgG conjugate to peroxidase reacts with anti HCV antibodies if present.
3) After 30 minutes incubation at 37°C, the unbound enzymatic conjugate is removed by washing step and the antigen-antibody complex is revealed by addition of substrate.
4) After 30 minutes the reaction has been stopped, the absorbance values are read using a spectrophotometer at 450/620-700 nm. The absorbance measured for a sample allows to detect the presence or absence of antibody or/and capsid antigen to HCV. The colour intensity is proportional to the quantity of antibody or antigen to HCV bound on the solid phase.
3 - COMPOSITION OF THE KIT

<table>
<thead>
<tr>
<th>LABEL</th>
<th>TYPE OF THE REAGENTS</th>
<th>PRESENTATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 plate</td>
</tr>
<tr>
<td>R1</td>
<td>MICROPLATE</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>12 strips of 8 wells coated with monoclonal antibodies against hepatitis C capsid and purified recombinant antigens and capsid mutated peptide specific for Hepatitis C virus</td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>CONCENTRATED WASHING SOLUTION (20X)</td>
<td>1 vial 70 ml</td>
</tr>
<tr>
<td></td>
<td>Tris NaCl buffer, pH 7.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preservative : Proclin™ 300 (0.04%)</td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>NEGATIVE CONTROL</td>
<td>1 vial 1 ml</td>
</tr>
<tr>
<td></td>
<td>Tris HCl buffer containing BSA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preservative : Proclin™ 300 (0.1%)</td>
<td></td>
</tr>
<tr>
<td>R4</td>
<td>POSITIVE CONTROL</td>
<td>1 vial 1.5 ml</td>
</tr>
<tr>
<td></td>
<td>Human serum containing antibodies to HCV, negative for HBs Antigen and for anti-HIV1 and anti-HIV2 antibodies diluted in Tris HCl buffer containing BSA, photochemically inactivated.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preservative : Proclin™ 300 (0.1%)</td>
<td></td>
</tr>
<tr>
<td>R5a</td>
<td>ANTIGEN POSITIVE CONTROL</td>
<td>1 vial sqf</td>
</tr>
<tr>
<td></td>
<td>Antigen positive control (Capsid synthetic peptide). Lyophilised</td>
<td></td>
</tr>
<tr>
<td>R5b</td>
<td>ANTIGEN DILUENT</td>
<td>1 vial 1 ml</td>
</tr>
<tr>
<td></td>
<td>Antigen diluent for R5 a. Water with ProClin™ 300 (0.5%)</td>
<td></td>
</tr>
<tr>
<td>R6</td>
<td>CONJUGATE 1</td>
<td>1 vial 15 ml</td>
</tr>
<tr>
<td></td>
<td>Mouse biotinilated monoclonal antibodies against capsid HCV antigen. Purple colored.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preservative : Sodium Azide (&lt; 0.1%), Cosmocil 0.025%</td>
<td></td>
</tr>
<tr>
<td>R7</td>
<td>CONJUGATE 2</td>
<td>1 vial 15 ml</td>
</tr>
<tr>
<td></td>
<td>Mouse antibody directed against human IgG/peroxidase and streptavidine/peroxidase Green colored.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preservative : ProClin™ 300 (0.5%)</td>
<td></td>
</tr>
<tr>
<td>R8</td>
<td>PEROXIDASE SUBSTRATE BUFFER</td>
<td>1 vial 60 ml</td>
</tr>
<tr>
<td></td>
<td>Citric acid and Sodium acetate solution pH 4.0 containing H₂O₂ (0.015%) and DMSO (4%)</td>
<td></td>
</tr>
<tr>
<td>R9</td>
<td>CHROMOGEN</td>
<td>1 vial 5 ml</td>
</tr>
<tr>
<td></td>
<td>Solution containing tetramethyl benzidine (TMB)</td>
<td></td>
</tr>
<tr>
<td>R10</td>
<td>STOP SOLUTION</td>
<td>1 vial 28 ml</td>
</tr>
<tr>
<td></td>
<td>1 N sulfuric acid solution</td>
<td></td>
</tr>
</tbody>
</table>

4 - MATERIAL REQUIRED BUT NOT PROVIDED
- Distilled or deionized water.
- Sodium hypochlorite (bleach) and sodium bicarbonate.
- Absorbent paper.
- Disposable gloves.
- Protective glasses.
- Disposable tubes.
- Automatic or semi-automatic adjustable or fixed pipettes capable of delivering 50 µl, 80 µl, 100 µl, 200 µl and 1 ml.
- Graduated cylinders of 10 ml, 200 ml and 1000 ml capacity.
- Vortex mixer.
Automatic*, semi-automatic* or manual microplate washing system.
Water bath or Dry incubator*, thermostatically set at 37°C ± 1°C.
Container for contaminated residues.
Microplate reading device* (equipped with 490,620,450/620 to 700 nm filters).

(*) Contact your representative for further Information on the equipment validated by our technical services.

5 - HEALTH AND SAFETY INSTRUCTIONS
All the reagents included in the kit are intended for “in vitro” diagnostic use.

- Wear disposable gloves when handling reagents and samples and thoroughly wash your hands after having handled them.
- Do not pipette by mouth.
- The positive control R4 is photochemically inactivated.
- Human source material used in the preparation of the positive control (R4) has been tested and found non-reactive for hepatitis B surface antigen (HBs Ag), and antibodies to Human Immunodeficiency Viruses (HIV-1 and HIV-2 Ab). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious diseases.
- Consider any material directly in contact with samples and reagents of human origin, as well as washing solutions, as infectious materials.
- Avoid spilling samples or solutions containing samples.
- Spills must be rinsed with bleach diluted to 10%. If the contaminating fluid is an acid, spills must be initially neutralized with sodium bicarbonate, then cleaned with bleach and dried with absorbent paper. The material used for cleaning must be discarded in a contaminated residue container.
- Samples, reagents of human origin, as well as contaminated material and products should be discarded after decontamination
  - either by immersion in bleach at the final concentration of 5% of sodium hypochlorite (1 volume of bleach for 10 volumes of contaminated fluid or water) for 30 minutes,
  - or by autoclaving at 121°C for 2 hours minimum.
CAUTION : do not introduce solutions containing sodium hypochlorite into the autoclave.
- Avoid any contact of the substrate buffer, the chromogen and the stop solution with the skin or mucosa. The safety data sheet are available upon request.
- Do not forget to neutralize and/or autoclave the solutions or washing wastes or any fluid containing biological samples before discarding them into the sink.
- Some reagents contain sodium azide. This compound may react with lead or copper pipes to form highly explosive metal azides. In order to avoid the accumulation of such azides in pipes, when discarding these reagents in a sink, neutralize them and wash the sink abundantly with water.
- Some reagents contain ProClin™ 300 (0.04%, 0.1% and/or 0.5%)

IRRITANT PRODUCT
R43 : may cause sensitisation by skin contact
S28-37 : After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

6 - PRECAUTIONS
The quality of results is dependent upon the following good laboratory practices :

- The name of the test, as well as a specific identification number for the test, are written on the frame of each microtiterplate. This specific identification number is stated on each strip too.

Monolisa™ HCV Ag-Ab ULTRA : Specific ID number = 55

Verify the specific identification number before use. If the identification number is missing, or different from the stated number above, the strip should not be used.
- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
REMARK : For washing solution (R2, label identification : 20X coloured green), peroxidase substrate buffer (R8, label identification : TMB buf, coloured blue), chromogen (R9, label identification : TMB 11X, coloured purple) and stopping solution (R10, label identification : 1N coloured red), it is possible to use other lots than those contained in the kit, provided the same lot is used within a given test run. These reagents can be used with some other products of our company. In addition, the wash solution (R2, label identification : 20X coloured green) can be mixed with the 2 other wash solutions included in various Bio-Rad Reagent kits (R2, label identifications : 10X coloured blue or 10X coloured orange) when properly reconstituted, provided only one mixture is used within a given test run. Contact our technical service for detailed information.

• Before use, wait for 30 minutes for the reagents to stabilize at room temperature.
• Carefully reconstitute the reagents avoiding any contamination.
• Do not carry out the test in the presence of reactive vapours (acid, alkaline, aldehyde vapours) or dust that could alter the enzymatic activity of the conjugate.
• Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
• Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
• The enzymatic reaction is very sensitive to any metals or metal ions. Consequently, no metal element must be allowed to come into contact with the various solutions that contain conjugate or substrate solution.
• The development solution (substrate buffer + chromogen) must be coloured pink. The modification of this pink colour within a few minutes after reconstitution indicates that the reagent cannot be used and must be replaced.

Preparation of the development solution can be made in a clean disposable single use plastic tray or glass container that has first been pre-washed with 1N HCl and rinsed thoroughly with distilled water and dried. This reagent must be stored in the dark.

• Use a new distribution tip for each serum.
• Well washing is a critical step in this procedure : respect the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
• Never use the same container to distribute the conjugate and the development solution.

7 - SAMPLES
Collect a blood sample according to the usual practice. The test should be performed on undiluted serum or plasma (collected in EDTA, citrate, ACD, based anticoagulents). extract it as soon as possible to avoid haemolysis. Samples containing aggregates must be clarified by centrifugation prior to testing. Suspended fibrine aggregates or particles may produce falsely positive results. The samples should be stored at +2-8°C if the screening is carried out within 7 days or deep-frozen at -20°C. Avoid repeated freezing/thawing. If the samples are to be shipped, pack them in accordance with the regulations regarding the transport of aetiologic agents transport them preferably frozen.
DO NOT USE CONTAMINATED, HYPERLIPAEMIC OR HYPERHAEMOLYSED SERA.

REMARK : Samples containing up to 90 g/l albumin, 50 µg/l biotin and 100 mg/l bilirubin, lipemic samples containing up to the equivalent of 36 g/l triolein, and hemolyzed samples containing up to 87 g/l hemoglobin do not affect the results.

Negative samples, HCV Ab positive and HCV Antigen positive samples were tested before and after heat treatment 56°C for 30 minutes and after 3 freezing/thawing cycles. None of the treatments impacted the HCV Ab detection. However, the heat-treatment was shown to significantly reduce the recorded Monolisa™ HCV Ag-Ab ULTRA Absorbency for Antigen detection.

8 - RECONSTITUTION OF THE REAGENTS - VALIDITY - STORAGE
Before using the reagents of the Monolisa™ HCV Ag-Ab ULTRA assay kit, allow them to stabilize at room temperature for 30 minutes.
1) Ready-for-use reagents

**Microplate (R1)**
Each frame support containing 12 strips is wrapped in a sealed foil bag. Cut the bag using scissors or a scalpel 0.5 to 1 cm above the sealing. Open the bag and take out the frame. Put the unused strips back into the bag. Close the bag carefully and put it back into storage at +2-8°C.

**Conjugate 1 :** ready-for-use (R6)
Invert to homogenize before use.

**Conjugate 2 :** Ready-for-use (R7)
Invert to homogenize before use.

2) Reagents to be reconstituted

**Concentrated washing solution R2 (20X)**
Dilute the concentrated washing solution R2 1:20 in distilled water. Prepare 800 ml for one plate of 12 strips.

**Working diluted substrate solution (R8 + R9)**
Dilute reagent (R9) 1:11 using reagent R8 (example : 1 ml of R9 reagent in 10 ml of R8 reagent). 10 ml are necessary and sufficient for 1 to 12 strips. Homogenize.

**Working Antigen positive control (R5a + R5b)**
Pour the content of R5b diluent in the lyophilized Ag R5 a vial. Recap the vial and let stand for 10 minutes with gently shaking and inverting form time to time to ease dissolution.

3) Validity

The kit should be stored at +2-8°C. When stored at this temperature, each reagent contained in the kit can be used until the expiry date mentioned on the package (except for specific instructions).

**R1 :** After the vacuum-sealed bag has been opened, the microwell strips stored at +2-8°C in the carefully resealed bag can be used for 1 month.

**R2 :** The diluted washing solution can be stored at +2-30°C during 2 weeks. The concentrated washing solution (R2) can be stored at +2-30°C.

**R5a + R5b :** The Working Antigen positive control can be stored at +2-8°C during 1 month and at -20°C during 2 month (thawed for up to 5 times after frozen at –20°C).

**R8 + R9 :** After the reconstitution, the reagent stored in the dark can be used for 6 hours at room temperature (18-30°C)

9 - ASSAY PROCEDURE

- Strictly follow the protocol.
- Use negative and positive control sera for each test, in order to validate the test quality.
- Apply good laboratory practice.

**Methods**

1) Carefully define the sample distribution and identification plan.
2) Prepare the diluted washing solution (diluted R2) and Working Antigen positive control (R5a + R5b)
3) Remove the microplate frame and strips (R1) from their protective bag.
4) Add directly and in succession, without prior washing of the microplate :
   - 100 µl of conjugate 1 (R6) into each well,
   - 50 µl of negative control serum (R3) in well A1,
   - 50 µl of Antibodies positive control serum (R4) in wells B1, C1, D1,
   - 50 µl of the working Antigen positive control solution (R5a + R5b) in wells E1,
   - 50 µl of the first sample in well F1,
   - 50 µl of the second sample in well G1, etc.

Homogenize the reaction mixture (by a minimum of 3 aspirations or with microplate shaker during 5 second)

If the sample distribution takes over 10 min, it is recommended to distribute the negative and positive controls after the samples.

Depending on the used system, it is possible to modify the position of controls or the order of distribution.

*NB : After the samples distribution, the well containing sample (or controls) turns purple to blue. It is possible to verify the presence of the (samples + conjugate 1) in the wells by spectrophotometric
5) Cover if it is possible the wells with adhesive film by pressing over the whole surface to ensure tightness.

6) Incubate the microplate in a thermostat-controlled water bath or in a dry microplate incubator for: 90 ± 5 minutes at 37°C ± 1°C.

7) Remove the adhesive film. Aspirate the contents of all wells into a liquid waste container and add a minimum of 0.370 ml of washing solution to each well.

Aspirate again. Repeat the washing step 4 times (minimum of 5 washes).

The residual volume must be lower than 10 µl (if necessary blot the microplate by turning it upside down on absorbent paper).

8) Distribute quickly 100 µl of the conjugate 2 solution (R7) into all wells. The conjugate must be shaken gently before use. Cover, if it is possible, with a new adhesive film and incubate for: 30 ± 5 minutes at 37°C ± 1°C.

NB: The conjugate is coloured green. It is possible to verify the presence of conjugate in the wells by spectrophotometric reading at 620 nm (refer to section 12 SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENT PIPETING).

9) Remove the adhesive film, empty all wells by aspiration and wash 5 times as previously described.

10) Prepare the enzymatic development solution (see chapter 8, reagent (R8 + R9).

11) Quickly dispense into each well 80µl of prepared development solution (R8 + R9), freshly prepared before use. Allow the reaction to develop in the dark for 30 ± 5 minutes at room temperature (18 - 30°C). Do not use adhesive film during this incubation.

N.B.: The distribution of the development solution, which is coloured pink, can be visually controlled at this step of the manipulation: There is a clear difference of colouration between empty well and well containing the pink substrate solution. (refer to section 12 for automatic verification: SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENT PIPETING).

12) Add 100µl stopping solution (R10) by using the same sequence and rate of distribution as for the substrate solution. Homogenize the reaction mixture.

N.B.: The distribution of the stopping solution, which is not coloured, can be visually controlled at this step of the manipulation. After the addition of the stopping solution the pink colouration of the substrate disappears (for the negative samples) or turns from blue to yellow (for the positive samples).

13) Carefully wipe the plate bottom. Read at the optical density at 450/620-700 nm using a plate reader at least 4 minutes after stopping solution addition and within 30 minutes of stopping the reaction.

14) Before recording the results, check the correspondence between the reading and the microplate and sample distribution and identification plan.

10 - SYSTEM ADAPTATION

WASHING: Carefully follow the washing procedures described to obtain maximum test performance. For some instrument it can be necessary to increase the number of washing step to achieve an acceptable negative background.

11 - CALCULATION AND INTERPRETATION OF THE RESULTS

The presence or absence of antibodies to HCV or/and HCV capsid antigen is determined by comparing for each sample the recorded absorbance with that of the calculated cut-off value.

1. Calculate the mean of the measured absorbance values for the positive control serum (OD R4)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Optical density</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>1.636</td>
</tr>
<tr>
<td>C1</td>
<td>1.704</td>
</tr>
<tr>
<td>D1</td>
<td>1.650</td>
</tr>
<tr>
<td>Total</td>
<td>4.990</td>
</tr>
</tbody>
</table>
2. Calculation of the cut off value (CO)

\[
\text{Mean OD R4} = \frac{\text{Total optical density}}{3} = \frac{4.990}{3} = 1.663
\]

Example: \(\text{Mean OD R4} = 1.663\)

\[
\text{CO} = \frac{1.663}{4} = 0.415
\]

3. The validation criteria are as follows

a) For the negative control R3: measured absorbance value must be less than: O.D. cut off x 0.6.

b) For the antibodies positive control R4
   The mean of the measured absorbance value must be greater than, or equal to, 0.800 and less than, or equal to 2.400.
   If one of the antibodies positive control R4 individual values differs by more than 30% from the mean value, disregard the value and carry out the calculation again with the two remaining positive control values.

c) For the working Antigen positive control solution (R5a + R5b):
   The measured absorbance value must be greater than 0.500.
   The test is invalidated if the negative control R3, the working Antigen positive control solution (R5a + R5b), and/or if more than one positive control R4 fall outside the limits shown above.

4. Interpretation of the results

Samples with an optical density less than the cut off value are considered to be negative with the Monolisa™ anti-HCV Ag-Ab ULTRA assay test.

Results just below the cut-off value (C.O -10% < OD < C.O) should however, be interpreted with caution (it is advisable to retest in duplicate the corresponding samples when the systems and laboratory procedures permit).

Samples with an optical density higher than, or equal to, the cutoff value are considered to be initially positive and should be retested in duplicate before the final interpretation.

After retesting, the sample is considered to be positive with the Monolisa™ HCV Ag-Ab ULTRA assay test if the second or third measurement is positive, i.e. higher than, or equal to, the cut off value. The sample is considered to be negative if both values are less than the cut off value.

12 - SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENT PIPETING (OPTIONAL)

VERIFICATION OF THE FIRST STEP : CONJUGATE 1 (R6) AND SAMPLE PRESENCE

The presence of conjugate 1 (R6) + sample into the well can be verify by automatic reading at 620 nm. Each well containing sample and conjugate 1 (R6) must have an OD greater than 0.800.

REMARK : After sample addition, conjugate 1 (R6) turn purple to blue.

VERIFICATION OF THE SECOND STEP : CONJUGATE 2 (R7) PRESENCE

The conjugate 2 (R7) is coloured green
The presence of conjugate 2 (R7) into the wells can be controlled by automatic reading at 620 nm:

The OD value of each well must be greater than 0.300 (a value lower this norm indicates a poor dispensing of the conjugate).

DEVELOPMENT SOLUTION PIPETTING VERIFICATION

It is possible to verify the presence of pink development solution into the well by automatic reading at 490 nm: a well with development solution must have an optical density greater than 0.100 (a lower OD indicates a poor dispensing of the development solution).

There is a significative colour change for the empty wells from uncoloured to pink after addition of prepared substrate chromogen solution.

13 - PERFORMANCES OF THE TEST

A - SPECIFICITY STUDIES

Specimens from blood donors and patients were tested with the Monolisa™ HCV Ag-Ab ULTRA assay, as well as with the current assay used, in routine testing at different sites to ascertain the specificity of the assay.
Specificity on blood donors
Investigations were conducted at 3 different sites. Serum specimens from established or new donors were tested.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Rungis B.B France</th>
<th>Bordeaux B.B France</th>
<th>Donors from Montpellier France</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nb of tested samples</td>
<td>2410</td>
<td>2503</td>
<td>2248</td>
<td>7161</td>
</tr>
<tr>
<td>Nb repeat Reactive samples</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>12</td>
</tr>
</tbody>
</table>

Of these 7161 blood donors specimens, 12 samples were reproduced Monolisa™ HCV Ag-Ab ULTRA reactive according to the package insert recommendation, but were not confirmed HCV positive by HCV PCR or HCV PLUS Deciscan™ immunoblot.

The specificity on blood donors is 99.83% (99.71% to 99.91% with 95% confidence interval).

Specificity on Hospitalised patients
469 of the prospective sera collected from hospitalized patients were assessed at 2 sites. 440 samples were found negative with routine HCV Ab EC registered assay and with Monolisa™ HCV Ag-Ab ULTRA assay. 22 were found positive with Monolisa™ HCV Ag-Ab ULTRA and the routine assays from which 21 confirmed positive and 1 positive probable with Deciscan™.

For 7 samples, results were found discordant between the Monolisa™ HCV Ag-Ab ULTRA assay, HCV PLUS Deciscan™ Immunoblot assay and PCR assay.

For these 7 samples, results were found as follow:
- 2 samples positive with the assay use in routine (negative with HCV PCR assay and HCV PLUS Deciscan™ immunoblot assay) and negative with Monolisa™ HCV Ag-Ab ULTRA were considered as false positive with the routine assay.
- For two samples no conclusion can be drawn as the PCR was not interpretable. These samples were excluded from the following calculation.
- One sample was found positive with the test use in routine, negative with Monolisa™ HCV Ag-Ab ULTRA assay, indeterminate with HCV PLUS Deciscan™ immunoblot assay and negative with PCR assay.
- Only 2 samples positives with Monolisa™ HCV Ag-Ab ULTRA assay (with low ratio below 1.7) and with routine HCV Ab assay were negative with PCR assay and indeterminate with HCV Deciscan™ immunoblot (NS4 low positive band).

The specificity calculated on the basis of this study is 99.5% (443/445) if the last two samples are considered as false positives and 100% if they are considered as true positives due to the presence of residual antibodies following a former infection (indeterminate data with immunoblot).

Specificity on potentially interfering samples
Additional 429 samples likely to induce cross reactivity in immunoassay testing were studied. They were collected from:
- patients suffering from different infectious diseases such as hepatitis B, A, Rubella, Toxoplasmosis, Mumps, Measles, CMV, HSV, EBV, VZV, HTLV I, HIV, Chagas, Flavivirus, Flu vaccinated patients
- rheumatoid factor positive patients,
- auto-immune diseases suffering patients (SLE), Myelome, HAMA & ANA positive samples
- Pregnant women, cirrhotic, chronic renal failure (CRF) and dialysis patients

Among these 429 samples, 1 was reproduced Monolisa™ HCV Ag-Ab ULTRA reactive and HCV positive with an HCV Ab EC registered assay.

The subsequent assay specificity when these tricky samples are tested achieved 100% (428/428).
B - SENSITIVITY STUDY

The assay sensitivity was assessed by testing a large series of HCV positive samples, from commercial seroconversion panels, Bio-Rad or the experts site. The cumulated findings are summarised thereafter:

**Sensitivity for HCV confirmed positive samples**

A total of 646 HCV positive samples mostly from chronically infected people (presence of anti HCV antibodies) was tested including 405 HCV genotyped samples. All of these 646 samples were highly reactive with Monolisa™ HCV Ag-Ab ULTRA giving a sensitivity of 100%.

**Sensitivity for genotyped HCV positive samples**

A total of 405 genotyped samples were tested : 133 genotyped from various origin and 272 genotyped from hospital laboratories

The repartition of the tested genotypes is as follows:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>1a</th>
<th>1b</th>
<th>1c</th>
<th>1d</th>
<th>1a/b</th>
<th>2</th>
<th>2a</th>
<th>2b</th>
<th>2c</th>
<th>2i</th>
<th>2a/c</th>
<th>3</th>
<th>3a</th>
<th>3b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>53</td>
<td>83</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>31</td>
<td>7</td>
<td>18</td>
<td>4</td>
<td>1</td>
<td>17</td>
<td>25</td>
<td>77</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>3a/b</th>
<th>4a</th>
<th>4c</th>
<th>4d</th>
<th>4h</th>
<th>4k</th>
<th>4f</th>
<th>4c/d</th>
<th>5a</th>
<th>6a</th>
<th>6d</th>
<th>1b, 2a/2</th>
<th>1a/b, 2a/c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>2</td>
<td>10</td>
<td>21</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>24</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

All these samples were found positive : 100% sensitivity.

**Specimens from acute infected patients**

The sensitivity was evaluated on samples from the acute phase of HCV infection (initial phase of infection). A total of 53 seroconversion panels corresponding to 421 samples were tested with the Monolisa™ HCV Ag-Ab ULTRA kit and compared to an E.C. registered anti-HCV antibody detection kit.

The Monolisa™ HCV Ag-Ab ULTRA kit detects infection earlier on practically all the panels : 119 additional samples detected compared to an anti-HCV antibody detection kit (anti-HCV kit).

<table>
<thead>
<tr>
<th>53 seroconversion panels (421 samples)</th>
<th>anti-HCV kit</th>
<th>Monolisa™ HCV Ag-Ab ULTRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of positive samples</td>
<td>152</td>
<td>271</td>
</tr>
</tbody>
</table>

Of these 53 seroconversion panels, 11 start with a non-viremic sample, enabling a more accurate estimation of the serological window phase without anti-HCV antibodies.
In these 11 panels, the Monolisa™ HCV Ag-Ab ULTRA kit detects HCV infection on average 24 days earlier compared to an anti-HCV kit. This benefit is essentially due to the HCV capsid antigen detection used in the Monolisa™ HCV Ag-Ab ULTRA kit.

**Antigen sensitivity**

HCV Ag detection sensitivity was also appraised mostly by testing some seroconversion panel, BBI 917 for example. The following graph compares the sensitivity of Monolisa™ HCV Ag-Ab ULTRA to a competitor HCV Ab EIA assay.

<table>
<thead>
<tr>
<th>Panel</th>
<th>Genotype</th>
<th>Time required to obtain positive result from first bleeding (in days)</th>
<th>Difference between anti-HCV kit and Monolisa™ HCV Ag-Ab ULTRA kit (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HCV RNA**</td>
<td>anti-HCV kit</td>
</tr>
<tr>
<td>BCP-6211</td>
<td>1a</td>
<td>140</td>
<td>186</td>
</tr>
<tr>
<td>BCP-6213</td>
<td>1a</td>
<td>11</td>
<td>43</td>
</tr>
<tr>
<td>BCP-6222</td>
<td>1a</td>
<td>17</td>
<td>40</td>
</tr>
<tr>
<td>BCP-6225</td>
<td>1a</td>
<td>45</td>
<td>78</td>
</tr>
<tr>
<td>BCP-6227</td>
<td>1a</td>
<td>42</td>
<td>74</td>
</tr>
<tr>
<td>BCP-9041</td>
<td>1a</td>
<td>24</td>
<td>62</td>
</tr>
<tr>
<td>BBI-PHV919</td>
<td>1a</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td>BCP-9054</td>
<td>3a</td>
<td>52</td>
<td>60</td>
</tr>
<tr>
<td>BCP-9055</td>
<td>NG*</td>
<td>31</td>
<td>68</td>
</tr>
<tr>
<td>BCP-6216</td>
<td>NG*</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>NABI-SC90</td>
<td>NG*</td>
<td>10</td>
<td>52</td>
</tr>
<tr>
<td>Mean in days</td>
<td></td>
<td>38.2</td>
<td>64.9</td>
</tr>
</tbody>
</table>

* Not genotyped

** The RNA test results are those indicated by the panel suppliers.

Reproducibility study

Intra and inter assays reproducibility studies were performed using 7 samples: one non-reactive, 3 HCV Ab reactive and 3 Antigen reactive.
For intra assay reproducibility, the samples were tested 30 times in the same assay.
For inter-assay reproducibility, the samples were tested by two technicians, during 20 days. (Following NCCLS EP5 procedure)
The ratio means, the standard deviations and the coefficients of variation were calculated and are listed below:

**Table 1 : Intra assay reproducibility**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean Ratio</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0.14</td>
<td>0.01</td>
<td>6.1</td>
</tr>
<tr>
<td>HCV Ab Low positive</td>
<td>1.12</td>
<td>0.04</td>
<td>3.65</td>
</tr>
<tr>
<td>HCV Ab Medium positive</td>
<td>2.39</td>
<td>0.08</td>
<td>3.39</td>
</tr>
<tr>
<td>HCV Ab High positive</td>
<td>4.88</td>
<td>0.18</td>
<td>3.7</td>
</tr>
<tr>
<td>Ag Low positive</td>
<td>1.14</td>
<td>0.03</td>
<td>3.04</td>
</tr>
<tr>
<td>Ag Medium positive</td>
<td>1.97</td>
<td>0.06</td>
<td>3.02</td>
</tr>
<tr>
<td>Ag High positive</td>
<td>5.86</td>
<td>0.14</td>
<td>2.38</td>
</tr>
</tbody>
</table>

The CVs obtained with the 6 positive samples are below 10%.

**Table 2 : Inter assay reproducibility**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean Ratio</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0.16</td>
<td>0.02</td>
<td>12.4</td>
</tr>
<tr>
<td>HCV Ab Low positive</td>
<td>1.2</td>
<td>0.08</td>
<td>6.65</td>
</tr>
<tr>
<td>HCV Ab Medium positive</td>
<td>2.39</td>
<td>0.13</td>
<td>5.38</td>
</tr>
<tr>
<td>HCV Ab High positive</td>
<td>4.77</td>
<td>0.17</td>
<td>3.58</td>
</tr>
<tr>
<td>Ag Low positive</td>
<td>1.13</td>
<td>0.09</td>
<td>7.97</td>
</tr>
<tr>
<td>Ag Medium positive</td>
<td>2.0</td>
<td>0.15</td>
<td>7.73</td>
</tr>
<tr>
<td>Ag High positive</td>
<td>5.99</td>
<td>0.37</td>
<td>6.2</td>
</tr>
</tbody>
</table>

The CVs obtained with the 6 positive samples are below 15%

**14 - LIMITS OF THE TEST**

Given the diversity of the immunological reactions of infected patients by the Hepatitis C Virus (especially during seroconversions), some differences of detection between tests can be observed depending on the kind of antigenic proteins used. A negative result with a screening test does not exclude the possibility of exposition or infection by Hepatitis C Virus.

All ELISA techniques are liable to produce false positive reactions. It is recommended to verify the specificity of the reaction for any sample found to be a repeatable positive, according to the interpretation criteria of the Monolisa™ HCV Ag-Ab ULTRA kit, using a suitable method: using an ELISA anti-HCV antibody screening test or with an immunoblot anti-HCV antibody detection test to prove the presence of anti-HCV antibodies. If need be, use an HCV viral genome detection test or a specific HCV antigen test followed by a neutralization test.

The colorimetric method for the samples, conjugate and development solution deposition verification does not allow to verify the accuracy of the dispensed volumes. This method only shows the presence of sample, conjugate and development solution into wells. The rate of wrong answers with this method is closely linked to the accuracy of the utilized system (cumulated coefficient of variation of dispensing and reading over 10% significantly decrease the quality of the verification).

**15 - LITERATURE**


Centers for Disease Control. Guidelines for Laboratory testing and result reporting of antibody to hepatitis C virus. MMWR 2003, 52: RR-3.


For in vitro diagnostic use

Catalogue number

Authorised Representative

Expiry date YYYY/MM/DD

Batch code

Date of peremption AAAA/MM/JJ

Code du lot

Date ESTABLE hasta AAAA/MM/DD

Código de lote

Da utilizare prima del AAAA/MM/GG

Codice del lotto

Data de expiración AAAA/MM/DD

Chargen-Bezeichnung

Ungültig bis AAAA/MM/DD

Código do lote

Utlope datot AAAA/MM/DD

Batchnr

Applicato fino AAAA/MM/DD

Batchkoden

Datum expirare RRRR/MM/DD

Numer serii

Data valoarei YYYY/MM/DD

Serijos numeris

Garantie termin AAAA/MM/DD

Codów partródo

Cifre de garantie YYYY/MM/DD

Partikode

Tilgjenteliggjárn enns AAAAA/MM/DD

Numár de lot

Data utjuprandi AAAA/MM/DD

Partidenum

Skrappningsdatum AAAA/MM/DD
- Storage temperature limitation
- Limites de températures de stockage
- Temperatura limite
- Limiti di temperatura di conservazione
- Lagertemperatur
- Limites de temperatura de armazenamento
- Temperaturbegränsning
- Temperaturbegränsning
- Perioratismos thermokratias apothekes
- Temperatura przechowywania
- Saugojimo temperatūriniai apribojimai
- Tárolási hőmérsékleti határok
- Piirangud sālištustemperatuurile
- Skladovacia teplota od do
- Teplotní rozmezí od do
- Oppbevaringstemperatur
- Limitele de temperatură la stocare
- Температурни граници на съхранение
- Consult Instruction for use
- Consulter le mode d’emploi
- Consulte las instrucciones de uso
- Consultare le istruzioni per uso
- Siehe Gebrauchsanweisung
- Consulte o folheto informativo
- Se bruksanvisningen
- Se instruktion før brug
- Σύμβολευτείς τις οδηγίες χρήσης
- Sprawdź instrukcję
- Ieškokite informacijos vartojimo instrukcijoje
- Olvassa el a használati utasítást
- Kasutamisel vaata instruktsiooni
- Katalógové číslo
- Viz návod k použití
- Se bruksanvisninger
- Consultati prospectul de utilizare
- Виж инструкцията за употреба