Monolisa™ Anti-HBc PLUS
1 plate - 96 tests 72315
5 plates - 480 tests 72316

DETECTION KIT FOR ANTIBODIES TO NUCLEOCAPSID ANTIGEN (CORE) OF THE HEPATITIS B VIRUS IN HUMAN SERUM OR PLASMA BY ENZYME IMMUNOASSAY

IVD For In Vitro Diagnostic Use

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 - CLINICAL INTEREST
During primary infection by hepatitis B virus, the first antibodies to appear after HBs and HBe antigens are antibodies to HBC. IgM antibodies are the primary response to infection and remain detectable for several weeks, disappearing during convalescence. IgG antibodies which are produced later, continue for many years after recovery.
The detection of antibodies to hepatitis B nucleocapsid or core antigen is a major marker for the presence of past (anti-HBc Total) or recent (anti-HBc IgM) infection by the hepatitis B virus.
Patients with chronic hepatitis B virus infection usually show high levels of anti-HBC. The anti-HBc antibodies can persist a long time and may be observed in at least three clinical situations: associated with HBs Ag, associated with anti-HBs antibodies and, in some cases, alone.
The testing of blood donations and derivative products for anti-HBc antibodies is a useful tool for preventing transmission of the hepatitis B virus.

2 - PRINCIPLE OF THE TEST
Monolisa™ Anti-HBc PLUS is an enzyme immunoassay (indirect ELISA type) for the simultaneous detection of total antibodies to hepatitis B virus core in human serum or plasma.
Monolisa™ Anti-HBc PLUS is based upon the use of a solid phase prepared with recombinant HBC antigen.
Steps of the manipulation:
1. The sera to be tested and the control sera are added to the wells. If antibodies to HBC are present, they will bind to the antigens fixed on the solid phase.
2. The peroxidase-labelled antibodies to human IgG and IgM are added after a washing step. They in turn bind to the specific antibodies captured on the solid phase.
3. After removal of the unbound enzymatic conjugate, the antigen-antibody complex is revealed by addition of substrate.
4. After 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 the presence or absence of antibodies to HBC to be determined. The colour intensity is proportional to the quantity of anti-HBc antibodies bound on the solid phase.
### 3 - COMPOSITION OF THE KIT

<table>
<thead>
<tr>
<th>LABEL</th>
<th>REAGENT COMPOSITION</th>
<th>PRESENTATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td><strong>MICROPLATE</strong> : 12 strips of 8 wells coated with purified recombinant antigen (expressed in <em>E.Coli</em>)</td>
<td>1 microplate</td>
</tr>
<tr>
<td>R2</td>
<td><strong>CONCENTRATED WASHING SOLUTION (20X)</strong> Tris NaCl buffer, pH 7.4 Preservative : ProClin™ 300 (0.04%)</td>
<td>1 vial</td>
</tr>
<tr>
<td>R3</td>
<td><strong>NEGATIVE CONTROL SERUM</strong> Human serum negative for anti-HBc antibodies Preservative : Sodium Azide (0.1%)</td>
<td>1 vial</td>
</tr>
<tr>
<td>R4</td>
<td><strong>POSITIVE CONTROL SERUM</strong> Human serum containing anti-HBc antibodies. Photochemically inactivated. Preservative : Sodium Azide (0.1%)</td>
<td>1 vial</td>
</tr>
<tr>
<td>R6</td>
<td><strong>SAMPLE DILUENT</strong> : PBS buffer with a coloured control for sample deposition (purple) Preservative : ProClin™ (0.1%) and Ciprofloxacin 10 µg/ml.</td>
<td>1 vial</td>
</tr>
<tr>
<td>R7</td>
<td><strong>CONJUGATE</strong> Peroxidase-labelled goat antibody directed against human IgG and IgM (green) Preservative : ProClin™ (0.1%) and Ciprofloxacin 10 µg/ml</td>
<td>1 vial</td>
</tr>
<tr>
<td>R8</td>
<td><strong>PEROXIDASE SUBSTRATE BUFFER</strong> Citric acid and Sodium acetate solution pH 4.0 containing H₂O₂ (0.015 %) and DMSO (4%)</td>
<td>1 vial</td>
</tr>
<tr>
<td>R9</td>
<td><strong>CHROMOGEN</strong> Solution containing tetramethylbenzidine (TMB).</td>
<td>1 vial</td>
</tr>
<tr>
<td>R10</td>
<td><strong>STopping SOLUTION</strong> 1 N sulfuric acid solution</td>
<td>1 vial</td>
</tr>
</tbody>
</table>

### 4 - MATERIAL NECESSARY 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 20 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 or 40°C ± 1°C.
- Container for contaminated waste.
- Microplate reading device* (equipped with 450/620-700 nm filters).

(*) For further information on the equipment validated by our technical service department, contact our technical services.
5 - HEALTH AND SAFETY INSTRUCTIONS

All the reagents included in the kit are intended for “in vitro” diagnostic use.

- 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™ Anti-HBc PLUS : Specific ID number = 14.**

Verify the specific identification number before any use. If the identification number is missing, or different from the stated number above, the strip should not be used.

- Wear disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.

- Do not pipette by mouth.

- Human origin material used in the preparation of the negative control (R3) has been tested and found non reactive for hepatitis B surface antigen (HBs Ag), anti-HBc antibodies and antibodies to hepatitis C virus (HCV) and human immunodefiency virus (HIV 1 and HIV 2).

- Human origin material used in the preparation of the positive control (R4) has been tested and found reactive for hepatitis B surface antigen (HBs Ag) and anti-HBc antibodies and non reactive for antibodies to hepatitis C virus (HCV) and human immunodefiency virus (HIV 1 and HIV 2). It has been photochemically inactivated.

- No method can absolutely guarantee the absence of the HIV, HBV, HCV viruses or other pathogens. Consider these reagents as well as patient samples, as potentially infectious and handle them with the customary precautions.

- 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 at 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 waste 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 or the stopping solution with the skin or mucosa.

- 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 with copious amounts of water.

- Some reagents contain ProClin™ 300 (0.04%, 0.1% and/or 0.5%)

  **Xi Irritant**

  R43 : may cause sensitisation by skin contact

  S28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- Manufacturer security data sheet of each reagent are available on request.

6 - PRECAUTIONS

The quality of results is dependent upon following good laboratory practice :

- 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.

- All reagents must be brought to room temperature before proceeding (30 minutes).
- 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 pipette tip for each serum.
- Well washing is a critical step in this procedure: follow the recommended number of wash 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.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay procedure.

7 - SAMPLES
Collect a blood sample according to the usual practice. The test shall be performed on serum or plasma (collected in EDTA, heparin, citrate, ACD, CPD and CPDA-based anticoagulants). Extract it as soon as possible to avoid haemolysis. A severe haemolysis may alter the performance of the test.
Samples containing aggregates must be clarified by centrifugation prior to testing. Suspended fibrin aggregates or particles may produce false 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 etiologic agents, transport them preferably frozen.
DO NOT USE CONTAMINATED, HYPERLIPEMIC OR HYPERHAEMOLYSED SERA.
REMARK: Samples containing up to 30 g/l albumin, 200 mg/l bilirubin, lipemic samples containing up to the equivalent of 36 g/l triolein, and hemolyzed samples containing up to 5 g/l hemoglobin do not affect the results.

8 - RECONSTITUTION OF THE REAGENTS - VALIDITY - STORAGE
Before using the reagents of the Monolisa™ Anti-HBc PLUS kit, allow them to stabilize at room temperature (18-30°C) for 30 minutes.

1) Ready-for-use reagents
- HBc Ag 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.
• Negative control serum (R3)
• Positive control serum (R4)
• Sample diluent (R6)
  Invert gently to homogenize before use.
• Conjugate (R7)
  Invert gently to homogenize before use.

2) Reagents to be reconstituted
• Washing solution (20X concentrate) : R2
  Dilute 1:20 in distilled water to obtain the ready-for-use washing solution. 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.

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

9 - METHOD
• Strictly follow the protocol.
• Use negative and positive control sera for each test, in order to validate the test quality.
• Apply Good Laboratory Practice.

Two methods are available with Monolisa™ Anti-HBc PLUS :

<table>
<thead>
<tr>
<th></th>
<th>METHOD 1</th>
<th>METHOD 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample incubation</td>
<td>37 ± 1°C</td>
<td>40 ± 1°C</td>
</tr>
<tr>
<td></td>
<td>30 ± 5 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>water-bath dry incubator</td>
<td></td>
</tr>
<tr>
<td>Conjugate incubation</td>
<td>37 ± 1°C</td>
<td>40 ± 1°C</td>
</tr>
<tr>
<td></td>
<td>60 ± 5 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>water-bath dry incubator</td>
<td></td>
</tr>
<tr>
<td>Enzymatic revelation</td>
<td></td>
<td>30 ± 5 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>room temperature 18 - 30°C (in the dark)</td>
</tr>
</tbody>
</table>

1. Carefully define the sample distribution and identification plan.
2. Prepare the wash solution to working strength.
3. Remove the microplate frame and ready to use strips (R1) from their protective bag.
4. Add quickly, directly and in succession :
   4.1  200 µl of diluent (R6) into each well
   4.2  20 µl of negative control serum (R3) in A1, B1
   20 µl of positive control serum (R4) in C1, D1, E1
   20 µl of the first sample in F1 if this well is not used as a reagent control for the sample addition monitoring
   20 µl of the second sample in G1, etc ...

Depending on the utilized system, it is possible to modify the position of the controls.
Homogenize the reaction mixture by a minimum of 3 aspirations with the 20 µl pipette or by shaking the microplate after the pipetting step.
It is also possible to dispense 220 µl of a sample previously diluted to 1:11. If the sample distribution is over 10 min, it is recommended to distribute the negative and positive controls after the samples to be tested.

NB : After the samples distribution, the purple diluent turns blue.

It is possible to verify the presence of the samples in the wells by spectrophotometric reading at 620 nm (refer to section 11 SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENT PIPETING).

5. Cover 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 :
   - Method 1 : 30 min ± 5 min at 37°C ± 1°C
   - Method 2 : 30 min ± 5 min at 40°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 three times (4 washes). The residual volume must be lower than 10 µl (if necessary, blot the microplate by turning it upside down on absorbent paper).

   If an automatic washing device is used, follow the same operating cycle.

8. Distribute quickly 200 µl of the conjugate solution into all wells. The conjugate must be shaken gently before use.

   NB : The conjugate is coloured green.

   It is possible to verify the presence of conjugate in the wells by spectrophotometric reading at 450 nm. (refer to section 11 SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENT PIPETING)

9. Cover with new adhesive film and incubate for :
   - Method 1 : 60 min ± 5 min at 37°C ± 1°C
   - Method 2 : 60 min ± 5 min at 40°C ± 1°C

10. Remove the adhesive film, empty all wells by aspiration and wash 4 times as previously described. The residual volume must be lower than 10 µl (if necessary, blot the microplate by turning it upside down on absorbent paper).

11. Prepare the substrate solution (see section 8, reagent R8 + R9).

12. Quickly dispense into each well 100µl 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 11 for automatic verification : SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENT PIPETING)

13. Add 100 µl 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).

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

15. Before recording the results, check the correlation between the reading and the microplate and sample distribution and identification plan.

10 - CALCULATION AND INTERPRETATION OF THE RESULTS

The presence or absence of anti-HBc antibodies is determined by comparing for each sample the recorded absorbance with that of the calculated cut-off value.

1. Calculate the mean of the absorbance values for the positive control serum (OD R4)
   Example : Positive control R4
Well with positive control serum  |  Optical density
--- | ---
C1  | 1.796
D1  | 1.802
E1  | 1.852
Total  | 5.450

Mean of OD R4 = \frac{\text{Total optical density}}{3} = \frac{5.450}{3} = 1.817

2. Calculation of the cut-off value (Vs)

\[ \text{Vs} = \frac{\text{mean of OD R4}}{5} \]

Example: mean of OD R4 = 1.817

\[ \text{Vs} = \frac{1.817}{5} = 0.363 \]

The validation criteria are as follows

a) For the negative control: each individual measured absorbance value must be less than 0.100.

b) For the positive control

- Each absorbance value must be greater than, or equal to, 1.000 and less than, or equal to 2.900.
- If one of the positive control value is out of these norms or differs by more than 30% from the mean value, carry out the calculation again with the two remaining positive control values.

The test should be repeated if more than one positive control value is outside the limits set above.

Interpretation of the results

Samples with an optical density less than the cut-off value are considered to be negative with the Monolisa™ Anti-HBc PLUS test.

Samples with an optical density higher than, or equal to, the cut-off value are considered to be initially positive with the Monolisa™ Anti-HBc PLUS test and must be retested in duplicate before the final interpretation.

However, results just below the cut-off value Vs -10% < OD should be interpreted with care (it is advised to retest the corresponding samples in duplicate when the utilized systems and laboratory procedures allow it).

For initial reactive or doubtful (0.9<ratio<1) samples, after retesting, the sample is considered to be positive with the Monolisa™ Anti-HBc PLUS test if at least one of the both measurements is positive, i.e. higher than, or equal to, the cut-off value. The sample is considered to be negative with the Monolisa™ Anti-HBc PLUS test if both values are less than the cut-off value.

11 - SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENT PIPETTING

(OPTIONAL)

VERIFICATION OF THE SAMPLE PIPETING

After the sample distribution, the purple diluent turns blue.

Method 1: without the use of a control reagent

The presence of sample into the well can be verify by automatic reading at 620 nm: compare the measured optical densities for each well, after the distribution of sample diluent (R6) and after the sample pipeting:

- the OD values of the wells containing sample diluent (R6) only must be over 0.500.
- each well containing sample must have an OD variation greater than 0.200 (an OD variation strictly lower than 0.200 indicates a poor dispensing of the sample).

Method 2: using a control reagent

The presence of sample into the wells can be verify by automatic reading at 620 nm with the use of a control well containing the sample diluent (R6) only: read the plate and compare the OD obtained for each sample with the OD obtained for the control well:

- the OD value of the control well containing sample diluent (R6) only must be over 0.500.
- the OD variation between well with sample and the control well must be greater than 0.200 (an OD variation strictly lower than 0.200 indicates a poor dispensing of the sample).
VERIFICATION OF CONJUGATE PRESENCE
The conjugate (R7) is coloured green.
The presence of conjugate (R7) into the wells can be controlled by automatic reading at 450 nm:
- the OD value of each well must be greater than 0.300 (a value lower than 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 \(490\, \text{nm}\): 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).

12 - PERFORMANCES OF THE TEST
PRELIMINARY REMARK: given the lack of confirmatory test for anti-HBc antibodies, it is difficult to clearly determine the true status of results given by screening kits. Consequently, the following percentages are determined by comparison with reference kits.

Sensitivity studies with Monolisa™ Anti-HBc PLUS test have been performed on 430 positive samples from follow-up of clinical patients with hepatitis B and on 2 sensitivity panels with documented samples from patients recently infected by hepatitis B virus. The diagnostic sensitivity, performed on 430 positive samples with EIA reference test was 99.53\% (428/430). Three samples were found negative. Among these 3 samples, one sample has not been confirmed with the second reference test. The 2 other samples, close to the cut-off value (1.08 and 1.04 ratio) with the second reference test correspond to only one patient. When performing the evaluations, the sensitivity was evaluated using the Paul Ehrlich Institute IgG and IgM standards, and the limit of detection was estimated at 0.5 U PEI/ml and 8 U PEI/ml for the IgG and IgM respectively.

The specificity of the test on non selected blood bank donors was 99.9\% on 5071 tested samples. The specificity of the test evaluated on 439 patients from hospital was 99.5\%.

Out of 220 patients with pathologies or conditions unrelated to the hepatitis B virus (pregnant women, rhumatoid factor, anti-nuclear Ig or other viral infections) 16 samples were found positive with Monolisa™ Anti-HBc PLUS test. Among these 16 samples, 15 were also found positive with one or two another tests and one sample (HAV IgM positive) was found negative with one reference test, volume being insufficient to be confirmed with one second technique.

The accuracy of Monolisa™ Anti-HBc PLUS test has been determined by the analysis of 4 samples: 1 negative sample (sample 1), 2 low anti-HBc positive samples (samples 2 and 3) and 1 high anti-HBc positive sample (sample 4). The intra assay reproducibility has been evaluated by testing these 4 samples 30 times in the same run, the inter assay reproducibility has been evaluated by testing these 4 samples 3 times on 2 microplates performed on 2 independent runs each day during 5 days. Results are shown in the following tables:

Table 1: Intra assay reproducibility

<table>
<thead>
<tr>
<th>n = 30</th>
<th>sample 1</th>
<th>sample 2</th>
<th>sample 3</th>
<th>sample 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean of ratios</td>
<td>0.20</td>
<td>2.09</td>
<td>2.71</td>
<td>6.44</td>
</tr>
<tr>
<td>standard deviation (SD)</td>
<td>0.02</td>
<td>0.08</td>
<td>0.18</td>
<td>0.27</td>
</tr>
<tr>
<td>CV (%) ratios</td>
<td>7.77 %</td>
<td>3.72 %</td>
<td>6.82 %</td>
<td>4.15 %</td>
</tr>
</tbody>
</table>

Table 2: Inter assay reproducibility

<table>
<thead>
<tr>
<th>n = 30</th>
<th>sample 1</th>
<th>sample 2</th>
<th>sample 3</th>
<th>sample 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean of ratios</td>
<td>0.22</td>
<td>2.18</td>
<td>2.69</td>
<td>6.41</td>
</tr>
<tr>
<td>standard deviation (SD)</td>
<td>0.05</td>
<td>0.28</td>
<td>0.10</td>
<td>0.20</td>
</tr>
<tr>
<td>CV (%) ratios</td>
<td>23.42 %</td>
<td>12.78 %</td>
<td>3.53 %</td>
<td>3.17 %</td>
</tr>
</tbody>
</table>
13 - LIMITS OF THE TEST

A negative result indicates that the tested sample does not contain detectable antibodies anti-HBc with Monolisa™ Anti-HBc PLUS. However, such a result does not preclude the possibility of exposure to an Hepatitis B virus infection. 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).

In case of very poor washing efficiency after the conjugate incubation, the automatic verification of the development solution pipetting (by reading OD of wells at 490 nm) may provide wrong results with OD above 0.100 in the absence of development solution. However this phenomena has not been observed during evaluation on 939 tested samples.

14 - LITERATURE

1. HOOFNAGLE J.H., GERETY R.J., BARKER L.F. 
   Antibody To Hepatitis-B-Virus Core in Man. Lancet (1973) 2 : 869-873
2. SZMUNESS W., HOFFNAGLE J.H., STEVENS C.E., PRINCE A.M. Antibody Against The Hepatitis Type B Core Antigen Am. J. of Epidemiology (1976) 104 (3) : 256-262
4. SLADE B.A., VROON D.H. 
   Anti-HBc To Screen For Susceptibility To Hepatitis B. Lancet (1984) 1 : 1246-1247
5. TIOLLAIS P., POURCEL C., DEJEAN A. 
   Follow-up of anti-HBc titers in healthy HBs Ag carriers and patients with chronic inflammatory liver diseases . Digestion, (1981), 22 , 289-293
7. GERLICH W.L. : LOER W and THOMSSEN R. 
   Diagnosis of acute and inapparent Hepatitis B virus infections by measurement of IgM antibody to Hepatitis B core antigen - J. Infect. Dis. (1980) 142 (1) : 95-101
8. LEMON S.M. ; GATES N.L. ; SIMMS T.E. and BANCROFT W.H. 
   (1981) - IgM antibody to Hepatitis B core antigen as a diagnostic parameter of acute infection with Hepatitis B virus - J. Infect. Dis. 143 (6) : 803-809
10. NAKAJIMA E. ; TSUJI T. ; KACHI K. ; KAGAWA K. ; OKANOUE T. and TAKINO T. 
   Immunoglobulin M antibody to hepatitis B core antigen (IgM anti-HBc) as a marker of interferon therapy in patients with persistent Hepatitis B virus infection - Biken Journal (1987) 30 ; 17-23
11. LEMON S.M. 
12. NEURATH A.R., SZUMNESS W., STEVENS C.E., STRICK N., HARLEY E.J. 
   Radioimmunoassay and Some Properties of Human Antibodies to Hepatitis B Core Antigen J. gen Virology (1978) 38 : 549-559
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