Monolisa™ Anti-HBs PLUS

192 tests 72566

ENZYME IMMUNOASSAY (EIA) FOR THE DETECTION AND LEVEL DETERMINATION OF ANTIBODY TO HEPATITIS B SURFACE ANTIGEN (ANTI-HBs) IN HUMAN SERUM OR PLASMA

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.

BIO-RAD

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1 - INTENDED USE

Monolisa™ Anti-HBs PLUS is an enzyme immunoassay intended for use in the qualitative and quantitative detection of total antibodies to Hepatitis B surface antigen (anti-HBs) in human serum or plasma.

2 - PRESENTATION OF THE TEST

The presence of antibodies to Hepatitis B surface antigen is an important factor in the diagnosis and prognosis of Hepatitis B virus (HBV) infection. In patients with acute Hepatitis B infection, anti-HBs is found in almost 80% of subjects 1 to 3 months after the appearance of Hepatitis B surface antigen (HBsAg). Anti-HBs is used in epidemiological surveillance, to assess past exposure to Hepatitis B in potential Hepatitis B vaccine recipients, for the monitoring of vaccinations and for the selection of plasma with high antibody concentrations for the preparation of specific immunoglobulins.

Monolisa™ Anti-HBs PLUS is a direct, antibody sandwich enzyme immunoassay which utilizes polystyrene microwells coated with native HBsAg (subtypes ad and ay) as the solid phase and a conjugate, containing horseradish peroxidase-labeled HBsAg (human, subtypes ad and ay). The determination of anti-HBs levels has been standardized by use of the WHO Anti-HBs Reference Preparation expressed in milli-International Units per milliliter (mIU/ml). A level greater than or equal to 10 mIU/mL is generally considered as the standard for demonstrating post-vaccination protection against HBV. The verification of at least a minimum anti-HBs titer of 10 mIU/mL, i.e., an immunity threshold titer, is crucial for the appropriate management of vaccinated individuals who may subsequently be exposed to HBs Ag-positive fluids and specimens.

3 - PRINCIPLE OF THE PROCEDURE

In the assay procedure, patient specimens and controls are incubated with the antigen-coated microwells. If antibodies to HBs are present in a specimen or control, they bind to the antigen. Excess sample is removed by a wash step. The conjugate is then added to the microwells. The conjugate binds to any antigen-antibody complexes present in the microwells. Excess conjugate is removed by a wash step, and a chromogen/substrate solution is added to the microwells and allowed to incubate.

If a sample contains anti-HBs, the bound enzyme (HRP) causes the coloration of tetramethyl-benzidine (TMB) in the chromogen solution wich turns blue. The blue color turns yellow after the addition of a stopping solution.

If a sample does not contain anti-HBs, the chromogen/substrate solution in the well remains colorless during the substrate incubation, and after addition of the stopping solution.

The color intensity, measured spectrophotometrically, is proportional to the amount of anti-HBs present in the specimen.

Absorbance value readings for patient specimens are compared to a cutoff value determined by the 10 mIU/mL calibrator.

4 - COMPOSITION OF THE KIT

All reagents are exclusively for *in vitro* diagnostic use.

LABEL	NATURE OF THE REAGENTS	PRESENTATION
R1	**MICROPLATE :** 12 strips of 8 wells sensitized by a mixture of Hepatitis B surface antigen, subtype ad and ay (human origin)	2 microplates
R2	**CONCENTRATED WASHING SOLUTION (20X)** Tris NaCl buffer pH 7.4 Preservative agent : ProClin™ 300 (0,04%)	1 vial 235 ml
C0	**ANTI-HBS NEGATIVE CONTROL** Buffer with fetal calf serum and protein stabilizers Preservative agent : ProClin™ 300 (0.5%)	1 vial 2.2 ml
C1	**10 mIU/ml CALIBRATOR *** Buffer with Anti-HBs antibodies of human origin, fetal calf serum, protein stabilizers and sample indicator dye Preservative agent : ProClin™ 300 (0.5%)	1 vial 3 ml
C2	**100 mIU/ml CALIBRATOR – POSITIVE CONTROL** Buffer with Anti-HBs antibodies of human origin, fetal calf serum, protein stabilizers and sample indicator dye Preservative agent : ProClin™ 300 (0.5%)	1 vial 2.2 ml
C3	**400 mIU/ml CALIBRATOR** Buffer with Anti-HBs antibodies of human origin, fetal calf serum, protein stabilizers and sample indicator dye Preservative agent : ProClin™ 300 (0.5%)	1 vial 2.2 ml
C4	**1000 mIU/ml CALIBRATOR** Buffer with Anti-HBs antibodies of human origin, fetal calf serum, protein stabilizers and sample indicator dye Preservative agent : ProClin™ 300 (0.5%)	1 vial 2.2 ml
R6	**SPECIMEN DILUENT** Buffer with fetal calf serum, protein stabilizers and sample indicator dye Preservative agent : ProClin™ 300 (0.1%)	1 vial 27 ml
R7a	**CONCENTRATED CONJUGATE (11X)** Buffer with HBsAg (human ad and ay subtypes) coated with peroxydase and protein stabilizers Preservative agent : ProClin™ 300 (0.5%)	1 vial 2.5 ml
R7b	**CONJUGATE DILUENT** Buffer with calf serum and protein stabilizers Preservative agent : ProClin™ 300 (0.1%)	1 vial 25 ml
R8	**SUBSTRATE BUFFER** Citric acid and Sodium acetate solution pH 4.0 Containing H_2O_2 (0.015 %) and DMSO (4%)	1 vial 60 ml
R9	**CHROMOGEN** Solution containing Tetramethyl Benzidine (TMB)	1 vial 5 ml
R10	**STOPPING SOLUTION** 1 N sulfuric acid solution	1 vial 28 ml

** The Controls are calibrated according to an internal reference, which is calibrated according to 1st IRP WHO 1977 reference.*

Store the kit at 2-8°C. Bring all reagents except Conjugate Concentrate to room temperature (18-30°C) before use. Return reagents to 2-8°C immediately after use. Store all unused strips/plates in pouch and reseal. Do not remove desiccant. Store strip plates at 2-8°C.

5 - 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™ Anti-HBs PLUS : Specific ID number = 63.

  Verify the specific identification number before any use. If the identification number is missing, or different from the stated number corresponding to the assay to be tested, 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.

6 - HEALTH AND SAFETY INSTRUCTIONS

Xi Irritant

Some reagents contain ProClin™ 300 (0.04%, 0.1% and/or 0.5%)
R43 : May cause sensitization by skin contact.
S28-37 : After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- Monolisa™ Anti-HBs PLUS contains human blood components that have been tested and found non-reactive for Hepatitis B surface antigen (HBsAg), antibodies to Hepatitis C virus (HCV), and antibodies to Human immunodeficiency viruses (HIV-1 and HIV-2). 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.
- Wear disposable gloves when handling reagents and samples and thoroughly wash your hands after having handled them.
- Do not pipette by mouth.
- 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.

  Autoclaving at 121°C, for 1 hour minimum, is the best method for HIV and HBV virus inactivation.

  CAUTION : DO NOT INTRODUCE SOLUTIONS CONTAINING SODIUM HYPOCHLORITE INTO THE AUTOCLAVE.

  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.
- 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.
- The Security Data Sheet is available on request.

7 - MATERIAL REQUIRED BUT NOT PROVIDED

- Distilled or deionised water.
- Sodium hypochlorite (bleach) and sodium bicarbonate.
- Automatic or semi-automatic adjustable or fixed pipettes capable of delivering 10 µl to 200 µl, 1 ml, 5 ml and 10 ml.
- Graduated cylinders of 25 ml, 100 ml and 1000 ml capacity.
- Container for contaminated residues.
- Water bath or Dry incubator, thermostatically set at 37°C ± 1°C.
- Automatic, semi-automatic or manual microplate washing system.
- Microplate reading device (equipped with 490, 450 and 620 nm filters).
- Absorbent paper.
- Disposable gloves.
- Clean polypropylene containers for TMB preparation.

8 - PREPARATION OF REAGENTS

Before using the reagents of the Monolisa™ Anti-HBs PLUS 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 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.

Specimen Diluent (R6)

Anti-HBs Negative Control (C0)

10 mIU/ml Calibrator (C1)

100 mIU/ml Calibrator – Positive Control (C2)

400 mIU/ml Calibrator (C3)

1000 mIU/ml Calibrator (C4)

Homogenize reagents before use by vortex or invert gently.

2) Reagents to be reconstituted

Concentrated Washing Solution (20X) : R2

Dilute 1:20 in distilled water to obtain the ready-for-use washing solution. Prepare 800 ml for one plate of 12 strips.

Conjugate working solution (R7a + R7b)

Bring Conjugate Diluent (R7b) to room temperature.

Invert Conjugate Diluent (R7b, colorless to pale straw) and Conjugate Concentrate (R7a, green) to mix before using.

Prepare a 1:11 dilution for each strip to be tested (example : add 100 µl of Conjugate Concentrate (R7a) to each 1 ml of Conjugate Diluent (R7b) in a clean, polypropylene tube). Use the following table as a guide. Mix well but gently to avoid foaming.

Working Conjugate Solution should be protected from light, both at room temperature and at +2-8°C.

Working Conjugate Solution should be green. It remains stable 8 hours at room temperature and 24 hours when stored at +2-8°C.

Conjugate Solution can be prepared by pipetting the entire contents of the Conjugate Concentrate vial (R7a) into the Conjugate Diluent (R7b). Always mix working solution by inverting just prior to use.

Return unused Conjugate Concentrate (R7a) to the refrigerator immediately after use.

To avoid contamination of Conjugate, wear clean gloves and do not touch tips of pipettes.

Conjugate working solution preparation by strip

Number of Strips to be used	1	2	3	4	5	6	7	8	9	10	11	12*	24**
Amount of Concentrated Conjugate R7a (µl)	100	200	300	400	500	600	700	800	900	1000	1100	1200	2400
Amount of Conjugate Diluent R7b (ml)	1	2	3	4	5	6	7	8	9	10	11	12	24

** 1 Complete Plate ** 2 Complete Plates*

Working diluted substrate solution (R8 + R9)

Bring Chromogen (R9) and Substrate Buffer (R8) to room temperature.

Invert the Chromogen and Substrate Buffer to mix before using.

Dilute Chromogen (R9) 1:11 using Substrate Buffer (R8) for each strip to be tested (example : add 1 ml of R9 reagent in 10 ml of R8 reagent). 10 ml are necessary and sufficient for 1 to 12 strips. Homogenize.

Mix Working Diluted Substrate Solution gently prior to use. Wait 5 minutes before use. Working Diluted Substrate Solution should be used within 8 hours of preparation and kept in the dark at room temperature.

Chromogen (R9) should be pink. Another color indicates a reagent contamination : in this case, Chromogen has not to be used. Prepare only the amount of the reagent to be used within 6 hours, ensuring that the volume of diluted reagent will be adequate for the entire run. Use the following table as a guide :

Preparation of Working diluted substrate solution by strip

Number of Strips to be used	1	2	3	4	5	6	7	8	9	10	11	12*	24**
Amount of Chromogen R9 (µl)	100	200	300	400	500	600	700	800	900	1000	1100	1200	2400
Amount of Substrate Buffer R8 (ml)	1	2	3	4	5	6	7	8	9	10	11	12	24

** 1 Complete Plate ** 2 Complete Plates*

9 - STORAGE AND 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.
- **R7a + R7b : Working Conjugate Solution should be protected from light, both at room temperature and at +2-8°C.** After the reconstitution, working conjugate solution can be used for 8 hours at room temperature (+18-30°C) and for 24 hours if stored at +2-8°C.
- **R8 + R9 :** After the reconstitution, the reagent stored in the dark can be used for 6 hours at room temperature (18-30°C)

After opening all the other reagents are stable until the expiration date indicated on the box when stored at +2-8°C.

10 - SAMPLES

Collect a blood sample according to the usual practice.

The test should be performed on serum or plasma. Only the following samples have been tested : serum collected in standard tube or tube containing separative gel, plasma collected with EDTA or heparin. In case of use of plasma collected with citrate or ACD, results are lower than those obtained with serum for 20%.

Samples containing aggregates must be clarified by centrifugation prior to testing. Suspended fibrin 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. Samples that have been frozen and defrozen more than 3 times cannot be used. If the samples are to be shipped, pack them in accordance with the regulations regarding the transport of etiologic agents transport them preferably frozen.

REMARK : Samples containing up to 90 g/l albumin and 100 mg/l bilirubin, lipemic samples containing up to the equivalent of 36 g/l triglycerides, and hemolyzed samples containing up to 2.55 g/l hemoglobin do not affect the results.

Do not use samples after treatment at 56°C for 30 minutes.

11 - 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. Bring all of the reagents to room temperature before beginning the assay procedure.
3. Prepare Conjugate Working Solution (R7a + R7b), Working Diluted Substrate Solution (R8 + R9) and Diluted Washing Solution (diluted R2).
4. Remove the microplate frame and strips (R1) from their protective bag. Remove strips not needed for the assay and replace them with labeled Null Strips, as necessary.
5. Dilute specimens, calibrators and controls 3:4 in the Specimen Diluent R6, following one of the two procedures here below :
   a. Specimens, Calibrators and Controls may be diluted in-well (Add 25 µl of Specimen Diluent R6 to each well first, followed by 75 µl of specimen or control within 15 minutes, then mix gently by a minimum of 2 aspirations to avoid foaming).
   b. Specimens, calibrators and controls may be prediluted 3:4 in the Specimen Diluent R6 prior to addition to the well (for example, dilute 150 µl of specimen in 50 µl of Specimen Diluent R6, mix gently to avoid foaming, and then transfer 100 µl to the well).

   NOTE : After adding the sample, the diluent will change from purple to a blue color. It is possible to verify the presence of samples in the wells by spectrophotometric reading at 620 nm (Refer to section 14 : SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENT PIPETTING).
6. Add directly, without prior washing of the plate, and in succession depending on the method selected :

Qualitative determination

- Anti-HBs Negative Control (C0) in well A1,
- 10 mIU/ml Calibrator (C1) in wells B1, C1 and D1,
- 100 mIU/ml Calibrator-Positive Control (C2) in well E1,
- Samples in wells F1, G1, etc.

Quantitative determination

- Anti-HBs Negative Control (C0) in well A1,
- 10 mIU/ml Calibrator (C1) in wells B1 and C1,
- 100 mIU/ml Calibrator-Positive Control (C2) in well D1,
- 400 mIU/ml Calibrator (C3) in well E1,
- 1000 mIU/ml Calibrator (C4) in well F1,
- Samples in wells G1, H1, etc.

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

7. Cover, if it is possible, the wells with adhesive film by pressing over the whole surface to ensure tightness.
8. Incubate the plate for 60 ± 5 minutes at 37 ± 2°C.
9. Remove the adhesive film. Aspirate the contents of all wells into a liquid waste container and add immediately a minimum of 0.375 ml of Washing Solution to each well. Soak each well for 30 to 60 seconds between each wash cycle. 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 plate by turning it upside down on absorbent paper).
10. If an automatic washer is used, follow the same procedure.
11. Add quickly 100 µl of the Conjugate Working Solution (R7a + R7b) to each well. Cover, if it is possible, the wells with a new adhesive film and incubate for 60 ± 5 minutes at 37°C ± 1°C.
    NOTE : The conjugate is colored green. It is possible to verify the presence of conjugate in the wells by spectrophotometric reading at 620 nm (Refer to section 14 : SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENT PIPETTING).
12. Remove the adhesive film. Aspirate the contents of all wells into a liquid waste container and add immediately a minimum of 0.375 ml of Washing Solution to each well. Soak each well for 30 to 60 seconds between each wash cycle. 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 plate by turning it upside down on absorbent paper).
13. If an automatic washer is used, follow the same procedure.
14. Add quickly 100 µl of the Working Diluted Substrate Solution (R8 + R9) to each well. 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.
    NOTE : The Working Diluted Substrate Solution is colored pink. It is possible to verify the presence of conjugate in the wells by spectrophotometric reading at 490 nm (Refer to section 14 : SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENT PIPETTING).
15. Add 100µl Stopping Solution (R10) by using the same sequence and rate of distribution as for the Working Diluted Substrate Solution. Homogenize the reaction mixture.
16. Carefully wipe the plate bottom. At least 4 minutes after stopping solution addition and within 30 minutes of stopping the reaction, read at the optical density at 450/620-700 nm and 405/620-700 nm using a plate reader.

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

12 - VALIDATION OF THE RESULTS FOR QUALITATIVE AND QUANTITATIVE METHOD

The mean absorbance of the 10 mIU/ml Calibrator (C1) is the Cutoff Value for the assay.

For Anti-HBs Negative Control (C0)

The measured absorbance value must be greater than 0.000 and less than or equal to 0.070 ($0.000 < OD_{C0} \leq 0.070$).

For Positive **Control (C2)**

The measured absorbance value must be greater than or equal to 0.400 ($OD_{C2} \geq 0.400$).

For Negative Control (C0) and Positive Control (C2), if any one of the above criteria is not met for qualitative and quantitative method, the assay is invalid and must be repeated.

For 10 mIU/ml Calibrator (C1)

The measured absorbance value must be greater than or equal to 0.050 and less than or equal to 0.200 ($0.050 \leq OD_{C1} \leq 0.200$).

Each measured absorbance values must be greater than or equal to 1.5 the OD of the absorbance value of the Negative Control (C0) : $OD_{C1} \geq (1.5 \times OD_{C0})$.

In case of Qualitative method

If one of the 10 mIU/ml Cutoff Calibrator value is outside the acceptable range (the measured absorbance value must be greater than or equal to 0.050 and less than or equal to 0.200), the mean absorbance should then be calculated from the two remaining absorbance values. The assay is valid.

If several OD_{C1} measured values are outside the acceptable range, the assay is invalid and must be repeated.

In case of Quantitative method

If one of the two OD_{C1} measured values is outside the acceptable range (the measured absorbance value must be greater than or equal to 0.050 and less than or equal to 0.200), the assay is invalid and must be repeated.

13 - CALCULATION AND INTERPRETATION OF THE RESULTS

For each sample, the comparison of measured absorbance values to the calculated cut-off value allows the determination of the presence or absence of anti-HBs antibodies.

1. Qualitative method

Calculate the mean of the measured absorbance values for the 10 mIU/ml Calibrator (C1)

Example : 10 mIU/ml Calibrator (C1)

	B1	0.078
	C1	0.079
	D1	0.089
Total	**=**	**0.246**

Mean $OD_{C1} = 0.246 / 3 = 0.082$

If one of the measured absorbance value is outside the acceptable range (the measured absorbance value must be greater than or equal to 0.050 and less than or equal to 0.200), the mean absorbance should then be calculated from the two remaining absorbance values.

Calculation of the cut off value (C0)

The Cutoff Value for the assay is the mean absorbance of the 10 mIU/ml Calibrator (C1) :

$CO = \text{Mean } OD_{C1}$

Interpretation of the results

Specimens with absorbance values greater than or equal to the cutoff value are considered reactive.
Specimens with absorbance values less than the cutoff value are considered non-reactive.
Those with values greater than the upper linearity limits of the reader should be reported as reactive.

REMARK : Due to the diversity of antibodies and antigen used in each assay, results could be different depending on the assay.

Vaccination strategies : different recommendations are proposed depending on regions and countries involved.

In case of change of analysis method during vaccination follow-up, anti-HBs antibodies concentration have to be determined with both methods during a transitional period.

2. Quantitative method

To determine the concentration of anti-HBs antibodies in serum and plasma specimens, the following Anti-HBs Calibrator must be used : C0 (0 mIU/ml), C1 (10 mIU/ml), C2 (100 mIU/ml), C3 (400 mIU/ml) and C4 (1000 mIU/ml). Calibrators are added directly in each well, without prior washing of the plate, and in succession as described in the assay procedure (Refer to section 11 : ASSAY PROCEDURE).

Read at the optical density at 450/620-700 nm using a plate reader (A450).
For more samples with absorbance values (A450) greater than or equal to C3 measured absorbance value : A450 ≥ ODC3), read at the optical density at 405/620-700 nm.
The A450 of four Calibrators C0, C1, C2 an C3 are graphed versus their assigned concentrations, using a polynomial (quadratic) regression. Please note that the A450 of the 1000 mIU/ml Calibrator (C4) can not be used in this graph, as that absorbance value should be outside the range of the spectrophotometer, hence the necessity of a second graph. Samples with measured absorbance values less than ODC3 are interpreted with the graph obtained with the A450 of the four calibrators.
The A405 of C3 (400 mIU/ml) and C4 (1000 mIU/ml) calibrators are graphed versus their assigned concentrations, using point to point. A straight line is drawn through the points. Then the anti-HBs concentration (mIU/ml) for each sample is read at the intersection of the respective absorbance values. The A405 curve is used to determine the concentrations of serum or plasma samples whose concentrations are greater than 400 mIU/ml and less than or equal to 1000 mIU/ml.
Samples with anti-HBs concentrations greater than 1000 mIU/ml can be diluted using Diluted Washing Solution (diluted R2) and re-assayed.

14 - SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENT PIPETTING (OPTIONAL)

VERIFICATION OF SPECIMEN DILUENT (R6) AND SAMPLE PIPETTING

After sample addition, Specimen Diluent R6 change from purple to a blue colour.
The presence of sample and Specimen Diluent (R6) in the well can be verified by spectrophotometric reading at 620 nm : the OD value of each well containing sample or control diluted in Specimen Diluent must be greater than or equal to 0,150 (a value lower than this indicates poor dispensing of the sample or control).

VERIFICATION OF THE CONJUGATE WORKING SOLUTION (R7a + R7b) PIPETTING

The Conjugate Working Solution (R7a +R7b) is coloured green.
The presence of Conjugate Working Solution in the well can be verified by spectrophotometric reading at 620 nm : the OD value of each well must be greater than or equal to 0.070 (a value lower than this indicates poor dispensing of the Conjugate Working Solution).

VERIFICATION OF THE WORKING DILUTED SUBSTRATE SOLUTION (R8 + R9) PIPETTING

The Working Diluted Substrate Solution (R8 + R9) is coloured pink.
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 Working Diluted Substrate Solution.

15 - LIMITS OF THE TEST

- The procedure and the interpretation of the results must be followed when testing serum or plasma specimens for the presence of antibodies to HBs. The user of this kit is advised to read the package insert carefully prior to conducting the test. In particular, the test procedure must be carefully followed for sample and reagent pipetting, plate washing, and timing of the incubation steps.
- Failure to add specimen or reagent as instructed in the procedure may produce false negative results. It is advice to retest samples where a suspicion of procedural error occurs.
- Factors that can affect the validity of results include failure to add the specimen to the well, inadequate microplate washing, failure to follow stated incubation times and temperatures, addition of wrong reagents to wells, the presence of metals, or splashing of bleach into wells.
- Due to the variability of immunological reaction from a patient to another one, as well after HBV infection as further vaccination or therapeutic immunoglobulin injection, it is advised to carefully interpret results with low value.

16. PERFORMANCES

Analytical performances here below have been obtained during Monolisa™ Anti-HBs PLUS assay evaluations. Results obtained in laboratory can be different than these.

1. Intra-assay reproducibility : five positive samples and one negative sample have been tested 10 times in triplicate inside the same series.

Sample	Mean of OD	SD	CV%
Anti-HBs Negative	0.023	0.003	13.6
Anti-HBs Positive ~ 20 mIU/ml	0.143	0.005	3.4
Anti-HBs Positive ~ 50 mIU/ml	0.358	0.012	3.3
Anti-HBs Positive ~ 100 mIU/ml	0.715	0.019	2.6
Anti-HBs Positive ~ 150 mIU/ml	1.231	0.037	3.0
Anti-HBs Positive ~ 300 mIU/ml	1.982	0.048	2.4

2. Inter-assay reproducibility

3 positive samples and one negative sample have been tested 2 times per day in duplicate during 20 days following the EP5 NCCLS procedure(National Commitee for Clinical Laboratory Standards)

Sample	Mean of OD	SD	CV%
Anti-HBs Negative	0.023	0.004	15.5
Anti-HBs Positive ~ 20 mIU/ml	0.146	0.008	5.5
Anti-HBs Positive ~ 150 mIU/ml	1.284	0.060	4.9
Anti-HBs Positive ~ 300 mIU/ml	2.015	0.067	3.6

3. Accuracy

Quantitative results are given in mIU/ml and calibrated according to an internal reference, which is calibrated according to the 1st IRP WHO Reference Standard 1977.

A correlation study has been performed by testing the 0, 10, 100, 400 and 1000 mIU/ml concentrations obtained with WHO international standard versus kits calibrators.

Correlation coefficient and the slope of the obtained correlation line are R2 = 0.99 and a = 0.96.

4. Analytical Sensitivity was lower than 2 mIU/ml according to the NCCLS procedure.

REMARK : Analytical Sensitivity corresponds to the lower detection limit which corresponds to the lower measurable analyte concentration distinct from zero, calculated from means and standard deviations obtained with points 0 and 10 mIU/ml.

5. Method linearity

5 high Ant-HBs Ab positive samples (924 mIU/ml, 330 mIU/ml, 326 mIU/ml, 544 mIU/ml and 857 mIU/ml) have been two fold diluted up to 1/32 or 1/64. The covering rates lie between 92% and 116%.

6. Measurement range

The measurement range is between 2 and 1000 mIU/ml; it is bounded by the lower detection limit and by the maximum value of the reference curve. Titres below the detection limit are expressed as follows: < 2.00 mIU/ml, and titres higher than the measurement range are expressed as follows: > 1000 mIU/ml.

7. Hook effect

4 undiluted samples with high anti-HBs antibodies concentrations (200000 mIU/ml, 5500 mIU/ml, 28000 mIU/ml and 9000 mIU/ml) have been tested.

Concentration	200000 mIU/ml	28000 mIU/ml	9000 mIU/ml	5500 mIU/ml
DO 450	Read at 405 nm	Read at 405 nm	Read at 405 nm	Read at 405 nm
DO 405	1.342	1.596	1.629	1.310
Result	>1000 mIU/ml	> 1000 mIU/ml	> 1000 mIU/ml	> 1000 mIU/ml

Any hook effect has been displayed during performances evaluation.

8. Specificity

a) 179 patients showing different pathologies (hepatitis A, hepatitis C, VIH1, VIH2, HTLV, HSV, EBV, Rubella, Toxoplasmosis, Myelome (IgG, IgM), RF, ANA, HAMA, Cirrhosis and multitransfusion) have been tested with Monolisa™ Anti-HBs PLUS.

21 samples have been found positive with Monolisa™ Anti-HBs PLUS and two others CE marked anti-HBs detection assays. 1 ANA positive sample has been found positive for anti-HBs Ab with Monolisa™ Anti-HBs PLUS only. 2 other samples (1 HIV1 and 1 HIV2) have been found positive for anti-HBs Ab with Monolisa™ Anti-HBs PLUS and another competitor assay.

b) The specificity of Monolisa™ Anti-HBs PLUS test has been determined by the analysis of blood donors samples and non-selected clinical patients.

	Site 1 Donnors & hospitalised	Site 2 Donnors	Site 3 Hospitalised	TOTAL
Nb Samples	511	300	240	1051
Nb Positive	3	0	3	6
% Specificity	99.4%	100%	98.7%	99.4%

The specificity is 99.4% (98.8% to 99.8% with 95% confidence interval).

9. Sensitivity

654 samples from different populations of vaccinated or naturally infected patients have been tested with Monolisa™ Anti-HBs PLUS and another anti-HBs assay. Discordant samples have been retested with a third CE marked technique.

		Monolisa™ Concordance	
Patients Profil	**N**	**Test 1 CE marked**	**Test 2 CE marked***
Vaccinated	347	98.8%	99.1%
Resolved Hepatitis B	71	95.8%	100%
Chronic Hepatitis B	37	100%	Not tested
Hospitalised Patients	199	98.6%	Not tested
Total	654	98.5%	99.5%

Among these 654 tested patients, 617 have been found anti-HBs positive and 37 negative (chronic hepatitis affected patients) with test1. For these 617 patients, concordance is 98.4% (97% to 99.2% with 95% confidence interval).

**The sensitivity of Monolisa™ Anti-HBs PLUS, after analysis of discordant samples with test 2, is 99.2% (98.1% to 99.7% with 95% confidence interval).*

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(GB) - CE marking (European directive 98/79/CE on *in vitro* diagnostic medical devices)
(FR) - Marquage CE (Directive européenne 98/79/CE relative aux dispositifs médicaux de diagnostic *in vitro*)
(ES) - Marcado CE (Directiva europea 98/79/CE sobre productos sanitarios para diagnóstico *in vitro*)
(IT) - Marchiatura CE (Direttiva europea 98/79/CE relativa ai dispositivi medico-diagnostici *in vitro*)
(DE) - CE Konformitätskennzeichnung (Europäische Richtlinie 98/79/EG über *In-vitro*-Diagnostika)
(PT) - Marcação CE (Directiva europeia 98/79/CE relativa aos dispositivos médicos de diagnóstico *in vitro*)
(SE) - CE-märkning (Europeiskt direktiv 98/79/EG om medicintekniska produkter för *in vitro*-diagnostik)
(DK) - CE-mærkningen (Europa direktiv 98/79/EF om medicinsk udstyr til *in vitro*-diagnostik)
(GR) - Χαρακτηρισμος CE (ευρωπαικη οδηγια 98/79/CE περι *in vitro* διαγνωστικες ιατρικες συσκευες)
(PL) - CE oznaczenie (Dyrektywa unijna 98/79/CE dotycząca produktów medycznych do badań *in vitro*)
(LT) - CE ženklas (Europos sąjungos direktyva 98/79/CE dėl *in vitro* diagnostikos medicinos prietaisų)
(HU) - CE jelzés (98/79/CE Európai Irányelv az *in vitro* orvosi diagnosztikai eszközökről)
(EE) - CE märgistus (Euroopa direktiiv 98/79/CE *in vitro* diagnostikameditsiiniseadmete kohta)
(SK) - CE označenie o zhode (Európska direktíva 98/79/CE pre *in vitro* diagnostické zdravotnícke postupy)
(CZ) - CE značka (Evropská direktiva 98/79/CE o diagnostických zdravotnických prostředcích *in vitro*)
(NO) - CE-merking (EU-direktiv 98/79/CE om medisinsk utstyr til *in vitro*-diagnostikk)
(RO) - Marca CE (Directiva europeana 98/79/CE pentru dispozitive medicale de diagnostic *in vitro*)
(BG) - CE маркировка (Европейска директива 98/79/CE за *ин витро* диагностичните медицински изделия)

IVD

(GB) - For *in vitro* diagnostic use
(FR) - Pour diagnostic *in vitro*
(ES) - Para diagnóstico *in vitro*
(IT) - Per uso diagnostico *in vitro*
(DE) - *In-vitro*-Diagnostikum
(PT) - Para uso em diagnóstico *in vitro*
(SE) - *In vitro*-diagnostik
(DK) - *In vitro* diagnose
(GR) - Για *in vitro* διαγνωστικη χρηση
(PL) - Do stosowania *in vitro*
(LT) - *in vitro* diagnostikai
(HU) - Csak *in vitro* diagnosztikai alkalmazásra
(EE) - *In vitro* diagnostiliseks kasutamiseks
(SK) - Na diagnostiku *in vitro*
(CZ) - Pro diagnostiku *in vitro*
(NO) - Til *in vitro*-diagnostikk
(RO) - Pentru diagnostic *in vitro*
(BG) - За *ин витро* диагностика

REF

(GB) - Catalogue number
(FR) - Référence catalogue
(ES) - Número de catálogo
(IT) - Numero di catalogo
(DE) - Bestellnummer
(PT) - Número de catálogo
(SE) - Katalognummer
(DK) - Katalognummer
(GR) - Αριθμος καταλογου
(PL) - Numer katalogu
(LT) - Katalogo numeris
(HU) - Cikkszám
(EE) - Katalooginumber
(SK) - Katalógové číslo
(CZ) - Katalogové číslo
(NO) - Katalognummer
(RO) - Număr de catalog
(BG) - Каталожен номер

(GB) - Manufacturer
(FR) - Fabricant
(ES) - Fabricante
(IT) - Produttore
(DE) - Hersteller
(PT) - Fabricante
(SE) - Tillverkad av
(DK) - Fremstillet af
(GR) - Κατασκευαστης
(PL) - Producent
(LT) - Gamintojas
(HU) - Gyártó
(EE) - Tootja
(SK) - Výrobca
(CZ) - Výrobce
(NO) - Produsent
(RO) - Producător
(BG) - Производител

EC REP

(GB) - Authorised Representative
(FR) - Représentant agréé
(ES) - Representante autorizado
(IT) - Distributore autorizzato
(DE) - Bevollmächtigter
(PT) - Representante Autorizado
(SE) - Auktoriserad representant
(DK) - Autoriseret repræsentant
(GR) - Εξουσιοδοτημενος αντιπροσωπος
(PL) - Upoważniony Przedstawiciel
(LT) - Įgaliotasis atstovas
(HU) - Meghatalmazott Képviselő
(EE) - Volitatud esindaja
(SK) - Autorizovaný zástupca
(CZ) - Zplnomocněný zástupce
(NO) - Autorisert representant
(RO) - Reprezentant autorizat
(BG) - Упълномощен представител

LOT

(GB) - Batch code
(FR) - Code du lot
(ES) - Código de lote
(IT) - Codice del lotto
(DE) - Chargen-Bezeichnung
(PT) - Código do lote
(SE) - Batchnr
(DK) - Batchkoden
(GR) - Κωδικας παρτιδας
(PL) - Numer serii
(LT) - Serijos numeris
(HU) - Gyártási szám
(EE) - Partii kood
(SK) - Číslo šarže
(CZ) - Číslo šarže
(NO) - Partikode
(RO) - Număr de lot
(BG) - Партиден номер

(GB) - Expiry date YYYY/MM/DD
(FR) - Date de peremption AAAA/MM/JJ
(ES) - Estable hasta AAAA/MM/DD
(IT) - Da utilizzare prima del AAAA/MM/GG
(DE) - Verwendbar bis JJJJ/MM/TT
(PT) - Data de expiração AAAA/MM/DD
(SE) - Utgångsdatum ÅÅÅÅ/MM/DD
(DK) - Anvendes før ÅÅÅÅ/MM/DD
(GR) - Ημερομηνια ληξης YYYY/MM/DD
(PL) - Data ważności YYYY/MM/DD
(LT) - Galioja iki YYYY/MM/DD
(HU) - Szavatossági idő ÉÉÉÉ/HH/NN
(EE) - Aegumistähtaeg AAAA/KK/PP
(SK) - Použiteľné do RRRR/MM/DD
(CZ) - Datum exspirace RRRR/MM/DD
(NO) - Utløpsdato ÅÅÅÅ/MM/DD
(RO) - Data expirarii AAAA/LL/ZZ
(BG) - Срок на годност година/месец/ден

(GB) - Storage temperature limitation
(FR) - Limites de températures de stockage
(ES) - Temperatura límite
(IT) - Limiti di temperatura di conservazione
(DE) - Lagertemperatur
(PT) - Limites de temperatura de armazenamento
(SE) - Temperaturbegränsning
(DK) - Temperaturbegrænsning
(GR) - Περιορισμος θερμοκρασιας αποθηκευσης
(PL) - Temperatura przechowywania
(LT) - Saugojimo temperatūriniai apribojimai
(HU) - Tárolási hőmérsékleti határok
(EE) - Piirangud säilitustemperatuurile
(SK) - Skladovacia teplota od do
(CZ) - Teplotní rozmezí od do
(NO) - Oppbevaringstemperatur
(RO) - Limitele de temperatură la stocare
(BG) - Температурни граници на съхранение

(GB) - Consult Instruction for use
(FR) - Consulter le mode d'emploi
(ES) - Consulte las instrucciones de uso
(IT) - Consultare le istruzioni per uso
(DE) - Siehe Gebrauchsanweisung
(PT) - Consulte o folheto informativo
(SE) - Se bruksanvisningen
(DK) - Se instruktion før brug
(GR) - Συμβουλευθειτε τις οδηγιες χρησης
(PL) - Sprawdź instrukcję
(LT) - Ieškokite informacijos vartojimo instrukcijoje
(HU) - Olvassa el a használati utasítást
(EE) - Kasutamisel vaata instruktsiooni
(SK) - Katalógové číslo
(CZ) - Viz návod k použití
(NO) - Se bruksanvisninger
(RO) - Consultati prospectul de utilizare
(BG) - Виж инструкцията за употреба

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