Monolisa™ HBs Ag ULTRA Confirmatory

72408
25 tests

TEST FOR THE CONFIRMATION OF HBs Ag IN HUMAN SERUM OR PLASMA

IVD For *In Vitro* Diagnostic Use

Manufacturer Quality Control

All products manufactured and commercialised by Bio-Rad are subject to a complete quality system starting from reception of raw materials 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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CONTENTS

1 - INTENDED USE

The Monolisa™ HBs Ag ULTRA Confirmatory test is used to confirm the presence of hepatitis B surface Ag (HBs Ag) in samples of human serum or plasma found reactive by the Monolisa™ HBs Ag ULTRA screening test (codes 72346/72348).

2 - PRINCIPLE OF THE Monolisa™ HBs Ag ULTRA Confirmatory TEST

The Monolisa™ HBs Ag ULTRA Confirmatory test uses the principle of neutralization by excess of antibodies to anti-HBs (anti-HBs diluent: neutralization reagent) of the Ag HBs found in serum or plasma samples.

It is recommended to test samples repeatedly found positive by the Monolisa™ HBs Ag ULTRA screening test with the reagents from the Monolisa™ HBs Ag ULTRA Confirmatory kit.

If a sample is positive in HBs antigen, the anti-HBs antibodies in the neutralization reagent saturate the HBs antigen determinants of the sample which can no longer bind to the antibody fixed on the solid phase. A decrease in optical density will be observed when comparing the same sample in which the neutralization reagent is replaced by a negative control diluent not containing anti-HBs antibodies. Several dilutions of the sample will be analyzed, as required, to take into account variations in HBs Ag concentration.

The test includes the following reactional phases :

1) Incubation of controls and samples positive in HBs Ag with the neutralization reagent and the conjugate in the presence of anti-HBs antibodies fixed on the solid phase.
   For each sample, control wells are provided by replacing the neutralization reagent with a negative control diluent.

The remainder of the procedure is identical to the protocol for the Monolisa™ HBs Ag ULTRA kit :

2) Washing, then development of the enzyme activity bound to the solid phase by addition of the substrate.
3) Stop of the development step, then reading of the optical densities at 450/620-700 nm and interpreting the results.

3 - COMPOSITION OF THE KIT

LABEL	NATURE OF THE REAGENTS	PRESENTATION
RA	**Neutralization reagent** Tris NaCl buffer (pH 8.0) containing sheep serum and human serum negative in HBs antigen and in anti-HCV and anti-HIV1/2 antibodies and supplemented with anti-HBs antibodies. Ready-to-use reagent Preservative : ProClin™ 300 (0.25%)	1 vial 0.7 ml
RB	**Negative control diluent** Tris NaCl buffer (pH 8.0) containing sheep serum and human serum negative in HBs antigen and in anti-HCV and anti-HIV1/2 antibodies. Ready-to-use reagent Preservative : ProClin™ 300 (0.25%)	1 vial 0.7 ml
RC	**Sample diluent** 0.85% physiological saline solution. Ready-to-use reagent Preservative : ProClin™ 300 (0.1%)	1 vial 27 ml

4 - PRECAUTIONS

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

- Do not use expired reagents.
- Do not mix or combine reagents from Monolisa™ HBs Ag ULTRA Confirmatory kits with different batch numbers during the same procedure.
- Before use, allow the reagents to stabilize at ambient temperature (18-30°C) for 30 minutes.
- Use glassware that is thoroughly washed and rinsed in distilled water or, preferably, use disposable material.
- Refer to Monolisa™ HBs Ag ULTRA kit package insert.

5 - HEALTH AND SAFETY INSTRUCTIONS

- All of the reagents included in the kit are intended for in vitro diagnostic use.
- The material of human origin used for the preparation of reagents RA and RB has been tested and found negative in HBs antigen and in anti-HIV1, anti-HIV2 and anti-HCV antibodies. Since no method can absolutely guarantee the absence of HIV, HBV or HCV viruses or other infectious agents, consider these reagents, as well as patient samples, as potentially infections and handle them with precaution.
- For common health and safety instructions, refer to the Monolisa™ HBs Ag ULTRA kit package insert.

  Some reagents contain ProClin™ 300 (0.25% and 0.1%)

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 protective gloves.

6 - MATERIAL REQUIRED BUT NOT PROVIDED

Refer to the Monolisa™ HBs Ag ULTRA kit package insert.

7 - SHELF LIFE - STABILITY

- Store the kit between +2°C and +8°C before use.
- Once opened, all of the reagents can be used up to the expiry date indicated on the box if they are stored between +2°C and +8°C.

8 - SAMPLES

- Refer to the Monolisa™ HBs Ag ULTRA kit package insert.
- Samples with a very high concentration of HBs Ag can not be neutralized by the neutralization regent if they are tested in undiluted form.

  Refer to section 10 “CALCULATION AND INTERPRETATION OF RESULTS”, sub-section “Test validation criteria”.

NOTE : samples with a high HBs Ag concentration, with an optical density greater than 3.000 with the Monolisa™ HBs Ag ULTRA test, can be tested directly when diluted to 1/100 in the sample diluent.

9 - ASSAY PROCEDURE

Proceed as follows using the reagents from the Monolisa™ HBs Ag ULTRA kit.

1. Prepare the washing solution (R2) and the reconstituted conjugate solution (R6+R7) : refer to section 8 “PREPARATION OF REAGENTS”, sub-section “Reagents to be reconstituted” in the Monolisa™ HBs Ag ULTRA kit package insert.
2. Remove the microplate frame and the number of required strips (reagent R1) from the protective packing.
3. Distribute in the wells in the following order :
   - Wells A1, A2 : 100 µl of the negative control (R3) from the Monolisa™ HBs Ag ULTRA kit.
   - Wells B1, B2 : 100 µl of the positive control (R4) from the Monolisa™ HBs Ag ULTRA kit.
   - Wells C1, C2, D1, D2 : 100 µl of the first sample to be confirmed.
   - Wells E1, E2, F1, F2 : 100 µl of the second sample to be confirmed.

- Wells G1, G2, H1, H2 : 100 µl of the third sample to be confirmed, and so forth.

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

4. Add 20 µl of the negative control diluent (RB) in wells A1, B1, C1, D1, E1, F1, G1, H1 and 20 µl of the neutralization reagent (RA) in wells A2, B2, C2, D2, E2, F2, G2, H2, etc.
5. Distribute 50 µl of the reconstituted conjugate solution (R6+R7) in all the wells. Homogenize each mixture using a minimum of 3 aspirations.
6. Follow with step 7 and all subsequent steps contained in section 11 “PROCEDURE” in the Monolisa™ HBs Ag ULTRA kit package insert.

10 - CALCULATION AND INTERPRETATION OF RESULTS

1) Reactivity ratio of the controls and of the samples

1. For the positive control (R4) and the negative control (R3) from the Monolisa™ HBs Ag ULTRA kit, calculate :

$$\text{Reactivity ratio} = \frac{\text{OD controls with RB}}{[(\text{OD R3 with RB} + \text{OD R3 with RA})/2] + 0.050}$$

2. For each sample, calculate :

$$\text{Reactivity ratio} = \frac{\text{Mean OD samples with RB}}{[(\text{OD R3 with RB} + \text{OD R3 with RA})/2] + 0.050}$$

2) Inhibition percentage

1. Calculate the inhibition percentages of the optical densities recorded for the positive control (R4) and the negative control (R3) treated with the neutralization reagent (RA) and the negative control diluent (RB).

$$\text{Inhibition percentage} = \frac{(\text{OD controls with RB}) - (\text{OD controls with RA})}{(\text{OD controls with RB})} \times 100$$

2. Calculate the inhibition percentages of the optical densities recorded for the samples treated with the neutralization reagent (RA) and the negative control diluent (RB).

$$\text{Inhibition percentage} = \frac{(\text{mean OD samples with RB}) - (\text{mean OD samples with RA})}{(\text{mean OD samples with RB})} \times 100$$

3) Test validation criteria

1. The values of the negative control (R3) with addition of negative control diluent (RB) or neutralization reagent (RA) must be less than or equal to 0.080 OD units.

   For this same negative control (R3), the reactivity ratio must be less than 0.8.

   For the positive control (R4) with addition of negative control diluent (RB), the recorded optical density must be greater than 0.800.

   For this same positive control (R4), the inhibition percentage must be greater than or equal to 50%.
2. For samples with an inhibition percentage < 50% and with a reactivity ratio ≥ 0.8, it is necessary to re-test these samples diluted in the sample diluent (1/100; 1/10,000; 1/1,000,000; etc., 100 X dilution) in order to obtain a reactivity ratio < 0.8 (sample not confirmed in this case) or confirmation of the neutralization of the sample tested (inhibition percentage ≥ 50%).

4) Interpretation of results

A sample will be confirmed positive for HBs Ag if the reactivity ratio is greater than or equal to 0.8 and the inhibition percentage is greater than or equal to 50%.

Sample reactivity ratio	Inhibition percentage	Interpretation
≥ 0.8	≥ 50%	Confirmed
≥ 0.8	< 50%	Not confirmed; to be diluted
< 0.8	Whatever result	Not reactive*

** Not confirmed for diluted samples*

Examples :

Test	OD values with RA	OD values with RB	Sample reactivity ratio	Inhibition percentage	Interpretation
Positive control (R4)	0.095	2.025	32.66	95%	Confirmed
Negative control (R3)	0.011	0.013	0.21	15%	Not reactive
Sample 1	0.009 0.007	0.027 0.023	0.40	68%	Not reactive
Sample 2	2.585 2.570	3.906 3.880	63.00	34%	Not confirmed; to be diluted
Sample 2 Dil. 1/100	0.049 0.045	2.079 2.058	33.36	98%	Confirmed
Sample 3	0.132 0.140	0.151 0.161	2.52	13%	Not confirmed; to be diluted
Sample 3 Dil. 1/100	0.032 0.028	0.036 0.032	0.55	12%	Not confirmed

11 - PERFORMANCES

The performances of Monolisa™ HBs Ag ULTRA Confirmatory assay associated with Monolisa™ HBs Ag ULTRA has been determined by testing samples from clinical patients with acute and chronic hepatitis B infection or with diseases unrelated to hepatitis B infection, and from commercial samples and seroconversion panels.

More over the sensitivity limit has been tested using the French EFS HB panel, and the WHO standards (NIBSC code 00/588).

Analytical Sensitivity

All diluted sample concentrations from 0.06 ng/ml to 2.2 ng/ml from the EFS HB sensitivity panel and all positive results from WHO NIBSC panel were all confirmed with Monolisa™ HBs Ag ULTRA Confirmatory assay.

An HBs Ag concentration higher than 10 mg/ml was neutralised after dilution of this sample.

Sensitivity

Sensitivity studies have been performed on 337 positive samples from follow-up of chronic or acute HBV infected. All the positive samples were confirmed with Monolisa™ HBs Ag ULTRA Confirmatory assay.

A total of 15 well documented commercial HBV seroconversion panels (45 samples) were also studied. All the positive samples were neutralised.

Specificity and Cross Reactivity

An internal panel of 16 false positive samples with Monolisa™ HBs Ag ULTRA was tested with Monolisa™ HBs Ag ULTRA Confirmatory assay and were not confirmed.

The analysis of 102 patients showing different pathologies or status not linked to the hepatitis B (pregnant women, rheumatoid factor, anti-nuclear antibodies, anti-mouse Ig or other viral or bacterial infections) were tested with Monolisa™ Ag HBs ULTRA Confirmatory and confirmed negative .

More over, 37 samples positive with HBs Ag and also positive with different markers (Rheumatoid factors samples, antinuclear antibody samples, anti mouse Ig or viral infection samples) were tested with Monolisa™ HBs Ag ULTRA Confirmatory assay; all these samples were confirmed positive for HBs Ag.

23 HBs Ag positive samples from the co infection Panel PCA201 BBI (with HBV, HIV, HCV and /or HTLV) were all confirmed with Monolisa™ HBs Ag ULTRA Confirmatory assay pure or after dilution.

Assay Reproducibility

The reproducibility of Monolisa™ HBs Ag ULTRA Confirmatory assay has been determined, by the analysis of 4 samples : 1 negative sample (sample 1), 2 HBs Ag positive samples (samples 2 and 3) and 1 high HBs Ag 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 in duplicate during 20 days on 2 independent runs each days.

Results are shown in the following tables :

Table 1 : Intra assay reproducibility

n = 30	sample 1 (ratio 0.37)	sample 2 (ratio =1.32)	sample 3 (ratio =3.39)	sample 4 (ratio =18.39)
Mean of percent of inhibition	4	85.37	94.89	98.38
Standard deviation (SD)	12.6	1.59	0.66	0.21
CV (%)	NA	1.86%	0.69%	0.22%

Table 2 : Inter assay reproducibility

n = 40	sample 1 (ratio 0.37)	sample 2 (ratio =1.32)	sample 3 (ratio =3.39)	sample 4 (ratio =18.39)
Mean of percent of inhibition	3.74	81.46	92.76	98.08
Standard deviation	15.84	7.07	3.43	0.75
CV (%)	NA	8.68%	3.70%	0.76%

The results obtained during these reproducibility studies were conformed to those expected.

12 - LIMITS OF THE TEST

The Monolisa™ HBs Ag ULTRA Confirmatory test is strictly limited to the confirmation of the presence of the hepatitis B surface antigen (HBs Ag) in human serum and plasma.

Samples which repeatedly test weakly positive (ratio less than 2) with the Monolisa™ HBs Ag ULTRA test and which are determined not reactive for an undiluted sample with the Monolisa™ HBs Ag ULTRA Confirmatory test (reactivity ratio less than 0.8) must be interpreted with precaution. It is recommended to re-test these patients with another method or with a second sample taken later.

Heterophilic and HAMA antibodies in serum or plasma samples may cause interferences in the assay. These antibodies may be present in samples from individuals regularly exposed to animals or who have been treated with animal serum products. Inconsistent results with clinical observations require additional testing.

13 - LITERATURE

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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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