VIDAS® HBs Ag Ultra Confirmation

IVD

Supplementary VIDAS HBs Ag Ultra kit for confirmation of the presence of hepatitis B surface antigen in human serum or plasma found to be repeatedly positive with the VIDAS HBs Ag Ultra kit (ref. 30 315).

SUMMARY AND EXPLANATION

The VIDAS HBs Ag Ultra test is used in conjunction with the VIDAS HBs Ag Ultra screening test ref. 30 315. It enables confirmation of a repeatedly positive result obtained using VIDAS HBs Ag Ultra.

PRINCIPLE

The VIDAS HBs Ag Ultra test contains a “neutralizing” antibody (R1: see content of the kit) with anti-HBs antibodies. The HBs antigen, if present in the sample, is immunocomplexed using rabbit serum containing anti-HBs antibodies. The neutralizing antibody is preincubated in the instrument with the sample before testing with VIDAS HBs Ag Ultra. The same sample is incubated in parallel with a negative serum (R2) containing no anti-HBs antibodies.

If the sample is truly positive, then the anti-HBs antibodies contained in R1 will "neutralize" the HBs antigen causing a decrease in the signal between the neutralizing antibody R1 and the negative serum R2. When this decrease is greater than or equal to 50%, the sample is considered to be truly positive. If necessary, the samples can be diluted with the dilution buffer (R3) provided in the kit.

CONTENT OF THE KIT (30 tests) – RECONSTITUTION OF REAGENTS:

Neutralizing antibody (lyophilized) 1 x 0.6 mL	R1	Positive anti-HBs rabbit polyclonal serum + preservatives.	Reconstitute using **0.6 mL distilled water.** Leave for 15 minutes at room temperature and shake using a vortex-type mixer before use. Reagents are stable for 1 month at 2-8°C after reconstitution. If longer storage is required, freeze at -25 ± 6°C. Reagents are stable for 12 months at -25 ± 6°C. Five freeze/thaw cycles are possible.
Negative serum (lyophilized) 1 x 0.6 mL	R2	Negative rabbit serum + preservatives.	
Dilution buffer (liquid) 2 x 6 mL	R3	Human serum base* + 1 g/l sodium azide.	
1 Package insert provided in the kit or downloadable from www.biomerieux.com/techlib			

* This product has been tested and shown to be negative for HBs antigen, antibodies to HIV1, HIV2 and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.

REAGENT, MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- VIDAS HBs Ag Ultra reagent Ref. 30 315.
- Pipette with disposable tip to dispense 600 µl, 150 µl and 20 µl.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User’s Manual.
- VIDAS family instrument.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- **This kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (see Laboratory biosafety manual - WHO - Geneva - Latest edition).**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- **Sample to sample contamination through contact with gloves: since high concentrations of HBs Ag may be encountered, it is strongly recommended to use pre-racked pipette tips to avoid any contact with the tip. It is also recommended to keep a separate tube of sample for testing HBs antigen.**
- Do not use reagents after the expiration date indicated on the label.
- Do not mix reagents (or disposables) from different lots.
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.

- Spills should be wiped up thoroughly after treatment with liquid detergent or a solution of household bleach containing at least 0.5% sodium hypochlorite. See the User's Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (see the User's Manual).

STORAGE CONDITIONS

- Store the VIDAS HBs Ag Ultra Confirmation kit at 2-8°C.
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label. Refer to the kit composition table for special storage conditions.

SPECIMENS

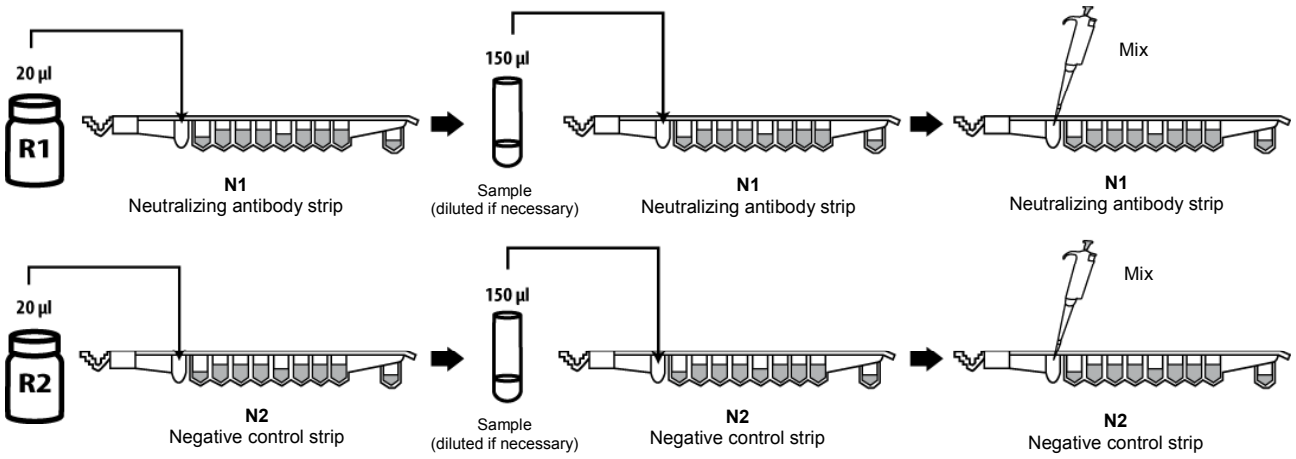
Refer to the VIDAS HBs Ag Ultra package insert (Ref. 30 315).

INSTRUCTIONS FOR USE AND INTERPRETATION

Samples found to be repeatedly positive with VIDAS HBs Ag Ultra must be confirmed using the same protocol (short or long) as that used for determination with VIDAS HBs Ag Ultra.

Pretreatment of specimens:

1. Prepare 2 "HBS" SPRs® and 2 "HBS" strips for each sample to be tested (refer to the VIDAS HBs Ag Ultra package insert).
2. Label the first strip **N1** (neutralizing antibodies) and the second strip **N2** (negative control) for each sample.
   a) in strip **N1**: dispense 20 µl of neutralizing antibody R1 into the sample well.
   b) in strip **N2**: dispense 20 µl of negative control R2 into the sample well.
3. Dispense 150 µl of sample (diluted, if necessary, according to the following table) into the sample well of each strip and, using a pipette, mix by aspirating / dispensing the sample several times.



4. Incubate the strips for at least 10 minutes in the instrument before starting the assay.
5. The test is identified by the "HBS" or "HBSN" code for the short protocol and the "HBL" or "HBLN" code for the long protocol according to the VIDAS family of instruments used. Follow the procedure given in the Instrument User's Manual.
6. When the assay has been completed, the percentage of neutralization (P) is calculated:

$$P = \left(1 - \frac{N1}{N2}\right) \times 100$$

P = percentage of neutralization.

N1 = RFV obtained for the sample supplemented with **R1**.

N2 = RFV obtained for the sample supplemented with **R2**.

Depending on the RFV obtained using VIDAS HBs Ag Ultra Ref. 30 315, it is recommended to perform the following steps:

RFV VIDAS HBs Ag Ultra	**Sample dilution**	**Result of VIDAS HBs Ag Ultra Confirmation test**
SHORT PROTOCOL		
• RFV ≤ 5000	Do not dilute the sample before neutralization.	**Situation A** % of neutralization ≥ 50%: **true positive** **Situation B** % of neutralization < 50% ↓ - If neutralizing antibody RFV < 2000: non neutralized HBs Ag: **negative** - If neutralizing antibody RFV ≥ 2000: see note below.
• RFV > 5000	Dilute the sample **1/50** in R3 diluent (in the kit) before neutralization.	
LONG PROTOCOL		
• RFV ≤ 8000	Do not dilute the sample before neutralization	**Situation A** % of neutralization ≥ 50%: **true positive** **Situation B** % of neutralization < 50% ↓ - If neutralizing antibody RFV < 2000: non neutralized HBs Ag: **negative** - If neutralizing antibody RFV ≥ 2000: see note below.
• RFV > 8000	Dilute the sample **1/100** in R3 diluent (in the kit) before neutralization	

Note: In situation B, if the neutralizing antibody RFV is ≥ 2000, calculation of the % of neutralization is not applicable, in which case the confirmation test should be repeated after dilution of the sample 1/1000 in R3 diluent (a hook effect may be observed if there is a high concentration of HBs Ag).

QUALITY CONTROL

A positive C1 control is included in each VIDAS HBs Ag Ultra kit (ref. 30 315).
This control must be performed each time a new VIDAS HBs Ag Ultra Confirmation kit (ref. 30 317) is opened to ensure that reagent performance has not been altered.
The user should check that the neutralization percentage for C1 is ≥ 50%.
If the positive control is not neutralized, then the reagents have deteriorated and the results of the confirmation test cannot be validated.

Note

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

LIMITATIONS OF THE METHOD

Refer to the VIDAS HBs Ag Ultra package insert (Ref. 30 315).

It should be noted that in very rare cases, the confirmation test may give an erroneous result (false positive samples that have been mistakenly neutralized). It is therefore recommended to take into account the patient's history and the results of any other biological markers for hepatitis B, to confirm the diagnosis.

PERFORMANCE

The results of studies performed using VIDAS HBs Ag Ultra are the following:

1. Diagnostic sensitivity

A study was performed using 381 samples known to be positive, including 13 subtyped or genotyped samples and 12 samples from patients with acute hepatitis. All the samples that were positive with the VIDAS HBs Ag Ultra reagent were confirmed to be positive, using either the short or the long protocol with the VIDAS HBs Ag Ultra confirmation reagent.

The "standard" sample pre-treatment procedure (predilution of the sample by 1/50 with the short protocol and 1/100 with the long protocol) was followed for 311 of the 312 non-selected positive samples tested (99.7% of cases). One of the 312 samples was not diluted before neutralization.

2. Analytical sensitivity

A study using the SNTS panel (panel 99) showed that samples (SNTS 88) with a concentration of 0.25 ng/mL were neutralized using the short protocol (HBS) and samples (SNTS 89) with a concentration of 0.12 ng/mL were neutralized using the long protocol (HBL).

A study using the international standard NIBSC 00/588 showed that samples with a concentration of 0.075 UI/mL, were neutralized using the short protocol (HBS), and samples with a concentration of 0.05 UI/mL were neutralized using the long protocol (HBL).

3. Sensitivity on seroconversion panels

A total of 14 seroconversion panels and one low HBs Ag titer panel were tested using the two VIDAS HBs Ag Ultra confirmation protocols. All the samples, which gave a positive result with the VIDAS HBsAg Ultra reagent were confirmed to be positive using the VIDAS HBs Ag Ultra confirmation reagent.

4. Diagnostic specificity

Only one sample was found to be false positive with VIDAS HBsAg Ultra Confirmation on the overall number of samples tested. This sample was not neutralized with VIDAS HBsAg Ultra Confirmation.

5. Precision

During the repeatability and reproducibility studies, the standard deviation of the neutralization percentage was consistently below 5%.

6. Cross-reactivity

Fifty samples from patients whose physiological status is likely to affect the confirmation of HBs antigen, were, when necessary, spiked with HBs antigen in order to obtain a positive HBs antigen result. They were then tested with the VIDAS HBs Ag Ultra confirmation reagent using the short and the long protocols. All the samples were confirmed to be positive with both protocols.

	VIDAS HBs Ag Ultra positive confirmation
HAV IgG +	5/5
EBV +	1/1
HSV +	3/3
CMV IgG +	4/4
Rubella IgG +	5/5
HIV +	4/4
HTLV +	5/5
HCV +	4/4
Toxoplasmosis IgG +	5/5
Rheumatoid factor	4/4
Anti-nuclear antibodies	5/5
Children less than 15 years old	1/1
Pregnant women (multipara)	4/4

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced according to their type and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

Refer to the VIDAS HBs Ag Ultra package insert (Ref. 30 315).

INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalogue number
IVD	In Vitro Diagnostic Medical Device
	Manufacturer
	Temperature limitation
	Use by
LOT	Batch code
	Consullt Instructions for Use
	Contains sufficient for <n> tests

WARRANTY

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