WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: DS-EIA-HBsAg-0,01, ENZYME IMMUNOASSAY FOR DETECTION AND CONFIRMATION OF HEPATITIS B SURFACE ANTIGEN
WHO reference number: PQDx 0120-038-00

DS-EIA-HBsAg-0,01 ENZYME IMMUNOASSAY FOR DETECTION AND CONFIRMATION OF HEPATITIS B SURFACE ANTIGEN with product codes **B-1254/1.2, B-1252/1.2, B-1255/1.2, B-1256/1.2, B-231/1.2**, manufactured by **RPC Diagnostics Systems Ltd**, **Rest-of-World regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 4 April 2016.

Summary of WHO prequalification assessment for DS-EIA-HBsAg-0,01

	Date	Outcome
PQ listing	4 April 2016	listed
Dossier review	3 March 2015	MR
Site inspection(s) of the quality management system	29 September 2021	MR
Laboratory evaluation of performance and operational characteristics	16 December 2015	MR

MR: Meets requirements

Report amendments and product changes

This report has since been amended. Amendments may have risen because of changes to the prequalified product for which WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and each amendment's details are provided below.

Version	Summary of amendments	Date of amendment
2.0	Minor grammatical corrections.	4 April 2016
3.0	The report was amended to reflect that the manufacturer removed the CE mark from its product. The change only affected the CE mark, product codes and identification code (code 1.1.GB is excluded) and has no impact on the quality, safety and performance, as supported in the submitted prequalification documentation.	1 March 2019
4.0	Substituted 1-methyl-2-pyrrolidone for dimethyl sulfoxide (DMSO) in the preparation procedure of TMB and updated the contact information (telephone numbers of EU Representatives) in IFU and on the label.	1 December 2022

Intended use:

According to RPC Diagnostics Systems Ltd, "*DS-EIA-HBsAg-0.01 is a solid-phase enzyme immunoassay for the qualitative detection and confirmation by neutralization reaction of wild types and mutant variants Hepatitis B surface antigen in human blood serum, plasma in vitro. It is intended for screening of individual human blood donors and the follow-up of HBV-infected patients. The results of this or any other diagnostic assay should be used and interpreted only in the context of the overall clinical picture".*

Assay description:

According to RPC Diagnostics Systems Ltd., *" "DS-EIA-HBsAg-0.01" is one-step "sandwich" assay based on a direct, non-competitive solid-phase enzyme immunoassay with biotin as the marker of antibodies. Significant increase of sensitivity is due to signal amplification that is achieved by streptavidin-biotin technology.*
If present in the patient's specimen, HBsAg will bind with the anti-HBsAg immobilized on the wells and simultaneously will be bound with the biotin-conjugated anti-HBsAg. The horseradish peroxidase labeled streptavidin will be attached to this complex. Unbound conjugates are removed by washing. After that colourless enzyme substrate (H2O2) and chromogen (TMB) are added. The enzyme reaction of chromogen/substrate produces a coloured solution. Enzyme-chromogen/substrate reaction is terminated with acid (H2SO4). The colour intensity is directly proportional to the concentration of HBsAg in a patient sample. The confirmatory assay is an in vitro neutralization assay for confirmation of the presence of HBsAg in blood serum and plasma samples found repeatedly reactive for HBsAg by the screening assay. For each test sample two assay wells are assigned. The "DS-EIA-HBsAg-0.01" confirmatory assay is run according to the usual procedure except that the Control Reagent

added in the control well and HBsAg Confirmatory reagent added in the specific well. During the first incubation the anti-HBsAg in the HBsAg Confirmatory reagent will compete with the antibodies immobilized on the well for any HBsAg present in the sample and will reduce the amount of HBsAg binding to the well; in the control well there is no competition and the HBsAg will bind normally. Conjugates are then added and the assay completed in the normal way. In samples containing HBsAg there will be a significant difference between the OD (optical density) generated in control and specific wells, if the inhibition in the specific well exceeds 50%, the sample is considered to be confirmed reactive".

Product Test kit contents:

Component	Product code B-1254/1.2	Product code B-1252/1.2	Product code B-1255/1.2	Product code B-1256/1.2	Product code B-231/1.2
Anti-HBsAg Coated Strips Polystyrene stripped 96-well plate (breakable wells) coated with monoclonal mouse antibodies to HBsAg (anti-HBsAg).	96 tests/ 1 plate	192 tests/ 2 plates	480 tests/ 5 plates	1 plate 96 (for detection) or 48 (for confirmation)	200 tests
Conjugate-1 (concentrated 11-fold) Monoclonal mouse antibodies to HBsAg (anti-HBsAg), conjugated with biotin. Transparent or slightly opalescent liquid, violet colored. Preserving agent: 0.02 % phenol, 0.016 % gentamycin sulfate.	1 vial 1.4 ml	1 vial 1.4 ml	1 vial 3.5 ml	1 vial 1.4 ml	-
Conjugate-2 (concentrated 11-fold) Peroxidase labeled streptavidin. Transparent or slightly opalescent liquid, blue colored. Preserving agent: 0.02 % phenol.	1 vial 1.4 ml	1 vial 1.4 ml	1 vial 3.5 ml	1 vial 1.4 ml	-
Conjugate-1 Diluent Opaque green color liquid, sediment may form which completely dissolves at shaking. Preserving agent: 0.01 % thimerosal.	1 vial 14.0 ml	1 vial 14.0 ml	2 vials 18.0 ml	1 vial 14.0 ml	-
Conjugate-2 Diluent Transparent yellow liquid, sediment may form which	1 vial 14.0 ml	1 vial 14.0 ml	2 vials 18.0 ml	1 vial 14.0 ml	-

completely dissolves at shaking. Preserving agent: 0.01 % thimerosal.					
Positive Control, Inactivated Recombinant HBsAg in human blood serum negative for anti-HBsAg, anti-HIV-1,2 and anti-HCV. Transparent or slightly opalescent liquid, red colored. Preserving agent: 0.1 % thimerosal, 0.01 % gentamycin sulfate.	1 vial 2.5 ml	1 vial 2.5 ml	1 vial 2.5 ml	1 vial 2.5 ml	-
Low Positive Control, Inactivated Native or recombinant HBsAg with concentration of 0.02 ± 0.01 IU/ml in human blood serum negative for anti-HBsAg, anti-HIV-1,2 and anti-HCV. Preserving agent: 0.1 % thimerosal. **Liquid, ready for use** Transparent or opalescent liquid, orange colored.	1 vial 2.5 ml	1 vial 2.5 ml	1 vial 2.5 ml	1 vial 2.5 ml	-
Negative Control, Inactivated Heat-inactivated human serum negative for HBsAg, anti-HIV-1,2 and anti-HCV. Transparent or opalescent liquid, green colored. Preserving agent: 0.005% cinnamaldehyde.	1 vial 2.5 ml	1 vial 2.5 ml	1 vial 4.5 ml	1 vial 2.5 ml	-
Washing Solution (concentrated 25-fold) Transparent or slightly opalescent liquid, colorless or pale yellow, sediment may form that dissolves at 35-39 °C and shaking.	1 vial 50.0 ml	1 vial 120.0 ml	2 vials 120.0 ml	1 vial 50.0 ml	-
Substrate buffer Citric acid and sodium acetate solution, pH 4.1-4.3, containing H_2O_2. Transparent colorless liquid. Preserving agent: 0.04 % ProClin 300.	1 vial 25.0 ml	1 vial 25.0 ml	2 vials 50.0 ml	1 vial 25.0 ml	-
TMB (concentrated 11-fold) Solution containing Tetramethylbenzidine (TMB). Transparent colorless liquid.	1 vial 2.5 ml	1 vial 2.5 ml	2 vials 3.5 ml	1 vial 2.5 ml	-
Stopping Reagent 0.2 M/L sulphuric acid solution.	1 vial 25.0 ml	1 vial 50.0 ml	2 vials 50.0 ml	1 vial 25.0 ml	-

Transparent colorless liquid.					
HBsAg Confirmatory reagent, Inactivated Human blood serum negative for HBsAg, anti-HBsAg, anti-HIV-1,2 and anti-HCV with addition of goat anti-HBsAg immunoglobulin. Transparent, pink colored liquid. Preserving agent: 0.01 % thimerosal.	-	-	-	1 vial 2.0 ml	1 vial 5.0 ml
Control reagent, Inactivated Human blood serum negative for HBsAg, anti-HBsAg, anti-HIV-1,2 and anti-HCV with addition of native goat immunoglobulin. Transparent blue colored liquid. Preserving agent: 0.01 % thimerosal.	-	-	-	1 vial 2.0 ml	1 vial 5.0 ml
Plate lid or	1	2	3	1	-
Protective film for EIA plates	3	6	15	3	-
Plastic clip or polyethylene bag with a zip lock	1	2	3	1	-
Plastic dish for liquid reagents	2	4	10	2	-
Disposable tips	16	32	80	16	-
Instructions for use	1	1	1	1	1

Storage:
The test kit must be stored at 2 - 8 °C.

Shelf-life upon manufacture:
24 months.

Warnings/limitations:
Please refer to the instructions for use attached at the end of the public report.

Prioritization for prequalification:
RPC Diagnostic Systems submitted an application for prequalification of DS-EIA-HBsAg-0,01. Based on the established criteria, DS-EIA-HBsAg-0,01 was accepted for WHO prequalification assessment.

Product dossier assessment

The Manufacturer submitted a product dossier for DS-EIA-HBsAg-0,01 as per the "Instructions for compilation of a product dossier" (PQDx_018 v1). The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

The Manufacturer's responses to the nonconformities found during dossier screening and assessment findings were accepted on 3 March 2015.

Based on the product dossier screening and assessment findings, the product dossier for DS-EIA-HBsAg-0,01 meets WHO prequalification requirements.

Manufacturing site inspection

A comprehensive inspection was performed at the site of manufacture (22 Yablonevaya, Nizhniy Novgorod, 603093, Russia; and AB Diagnostic Systems GmbH, Sportfliegerstrasse 4, Berlin, Germany) of DS-EIA-HBsAg-0,01 in September 2021 as per the "Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics" (PQDx_014 v1). The inspection found that the Manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality.

The Manufacturer's responses to the nonconformities found at the time of the inspection were accepted on 11 March 2022 and 13 May 2022 for the Russian and German sites, respectively.

Based on the site inspection and corrective action plan review, the quality management system for DS-EIA-HBsAg-0,01 meets WHO prequalification requirements.

Product performance evaluation

DS-EIA-HBsAg-0.01 was evaluated by WHO in the 3rd quarter of 2015 using plasma specimens. From this evaluation, we drew the following conclusions:

DS-EIA-HBsAg-0.01 is an enzyme immunoassay for the detection of HBsAg in human serum or plasma specimens. A volume of 100µl of specimen is needed to perform the assay. This

type of assay requires laboratory equipment and cannot be performed in laboratories with limited facilities. Reading of the results must be performed with a spectrophotometer.

In this limited performance evaluation on a panel of 514 specimens, we found an initial sensitivity (95% CI) of 100.0% (98.2 - 100.0%) and an initial specificity (95% CI) of 97.1% (94.6 – 98.7%) compared to the reference results. The final sensitivity (95% CI) was 100.0% (98.2 - 100.0%) and the final specificity (95% CI) was 99.0% (98.8 –100%) compared to the reference results. In this study, 0% of the results were recorded as indeterminate. Lot to lot variation was in the acceptance range.

For six seroconversion panels, DS-EIA-HBsAg-0.01 assay detected HBsAg seroconversion on average 1.3 specimens earlier than the benchmark assay (Monolisa Ag HBs Plus [Bio-Rad Laboratories]). For the low titer panel, DS-EIA-HBsAg-0.01 correctly classified all specimens.

For the WHO International Biological Reference Preparation for Hepatitis B surface antigen [NIBS code 5086/08], DS-EIA-HBsAg-0.01 detected the 0.13IU/mL. Hepanostika HBsAg ULTRA also detected to 0.13 IU/mL.

Performance characteristics in comparison with an agreed reference standard		
	Initial (95% CI)	**Final (95% CI)**
Sensitivity %	100 (98.2 - 100)	100. (98.2 - 100)
Specificity %	97.1 (94.6 – 98.7)	99.0 (98.8 –100)
Invalid rate %	0	
Inter-reader variability %	Not applicable	

Additional performance characteristics	
Sensitivity during seroconversion on 6 seroconversion panels in comparison with a benchmark assay; Monolisa Ag HBs Plus (Bio-Rad Laboratories)	Seroconversion sensitivity index of -1.3, therefore detection is 1.3 specimens earlier than the benchmark assay
Analytical sensitivity on WHO International Biological Reference Preparation for Hepatitis B surface antigen (NIBS code 5086/08)	0.13 IU/mL
Lot to lot variation on a dilution panel in comparison with an agreed reference standard	Acceptable

Key operational characteristics	
Validated specimen types	Serum, plasma (heparin, EDTA, citrate)
Number of steps	Depends on which test procedure, 9 steps (Procedure 1 and 2), 8 steps (Procedure 3 and 4)
Time to result	Approximately 3h30 for one run
Endpoint stability	Test results remain stable for reading within at least 10 minutes
Internal QC	Two positive and one negative control are supplied ready to use. It is recommended to add controls as following: 1-2 strips/up to 16 wells: 2 wells – Negative Control, 1 well – Positive Control, 2 wells – Low Positive Control. 3 strips and more/17 wells and more: 4 wells – Negative Control, 1 well – Positive Control, 3 wells – Low Positive Control If the kit is used with an automated EIA analyzer the manufacturer recommends that specimen addition is checked by measurement at a wavelength of 450nm.
In-use stability of reagents	After opening the vials with unused reagents of the kit: Positive Control, Low Positive Control (liquid, ready for use), Negative Control, Conjugate-1 (concentrated 11-fold), Conjugate-2 (concentrated 11-fold), Conjugate-1 diluent, Conjugate-2 diluent, HBsAg Confirmatory reagent, Control reagent, Washing Solution (concentrated 25-fold), Substrate Buffer, TMB (concentrated 11-fold), Stopping Reagent can be stored in tightly sealed vials until the kit expiration date at 2-8°C. Anti-HBsAg Coated Strips are stable until the kit expiration date after opening when stored at 2 - 8°C in a closed foil bag.

Labelling

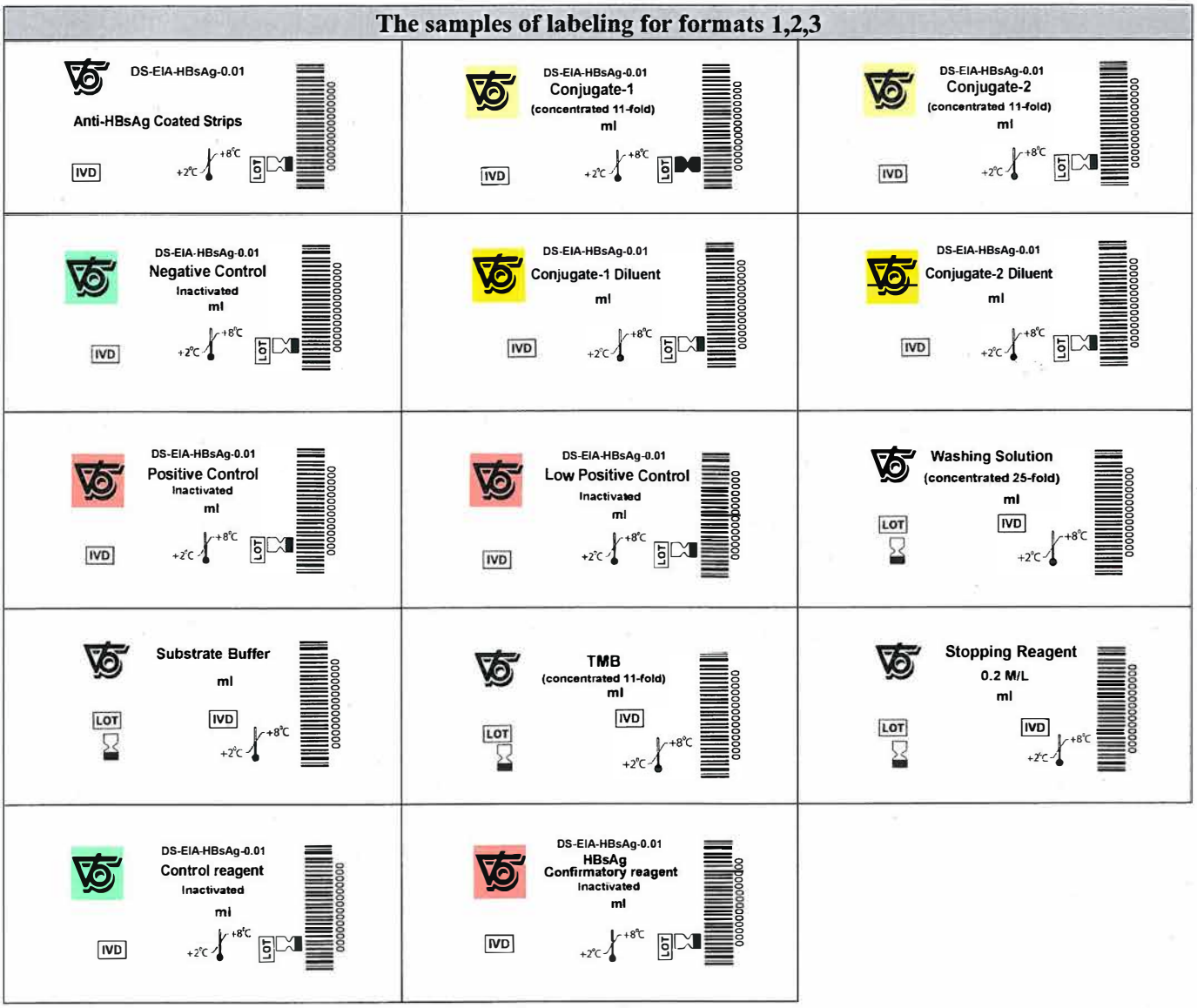
1. **Labels**
2. **Instructions for use**

1. Labels

Models of labeling

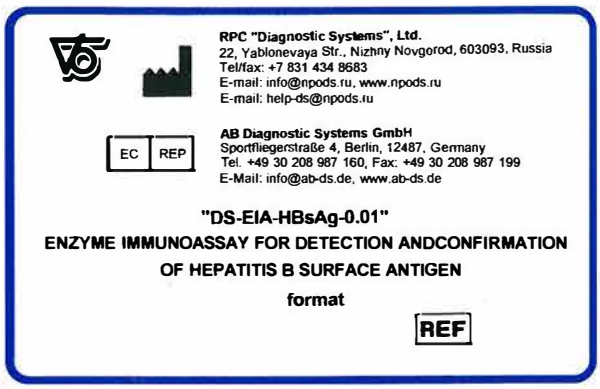
1. Inside labeling

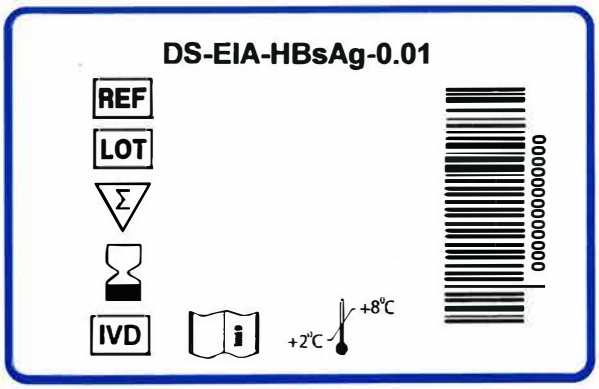
The samples of labeling for formats 1,2,3



2. Outside labeling

The samples of labeling for formats 1,2,3,4,5





The sample of labeling for formats 1,2,3

Kit contents	format Σ	
1. Anti-HBsAg Coated Strips	plate(s)	
2. Conjugate-1	vial	ml
3. Conjugate-2	vial	ml
4. Conjugate-1 Diluent	vial(s)	ml
5. Conjugate-2 Diluent	vial(s)	ml
6. Positive Control, Inactivated	vial	ml
7. Low Positive Control, Inactivated	vial	ml
8. Negative Control, Inactivated	vial	ml
9. Washing Solution	vial(s)	ml
10. Substrate Buffer	vial(s)	ml
11. TMB	vial(s)	ml
12. Stopping Reagent	vial(s)	ml

The sample of labeling for formats 4

Kit contents	format Σ	
1. Anti-HBsAg Coated Strips	plate	
2. Conjugate-1	vial	ml
3. Conjugate-2	vial	ml
4. Conjugate-1 Diluent	vial	ml
5. Conjugate-2 Diluent	vial	ml
6. Positive Control, Inactivated	vial	ml
7. Low Positive Control, Inactivated	vial	ml
8. Negative Control, Inactivated	vial	ml
9. HBsAg Confirmatory reagent, Inactivated	vial	ml
10. Control reagent, Inactivated	vial	ml
11. Washing Solution	vial	ml
12. Substrate Buffer	vial	ml
13. TMB	vial	ml
14. Stopping Reagent	vial	ml

The sample of labeling for format 5

Kit contents	format Σ	
1. HBsAg Confirmatory reagent, Inactivated	vial	ml
2. Control reagent, Inactivated	vial	ml

2. Instructions for use[1]

[1] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

RESEARCH AND PRODUCTION COMPANY
DIAGNOSTIC SYSTEMS Ltd.

REF	**B-1254/1.2**	Σ	**96**
REF	**B-1252/1.2**	Σ	**192**
REF	**B-1255/1.2**	Σ	**480**
REF	**B-1256/1.2**	Σ	**96 (for detection) or 48 (for confirmation)**
REF	**B-231/1.2**	Σ	**200**

IVD **For *In vitro* Diagnostic Use**

INSTRUCTIONS FOR USE
"DS-EIA-HBsAg-0.01"

ENZYME IMMUNOASSAY FOR DETECTION AND CONFIRMATION OF HEPATITIS B SURFACE ANTIGEN

Table of contents

I. INTENDED USE

"DS-EIA-HBsAg-0.01" is a solid-phase enzyme immunoassay for the qualitative detection and confirmation by neutralization reaction of wild types and mutant variants Hepatitis B surface antigen in human blood serum, plasma ***in vitro***. It is intended for screening of individual human blood donors and the follow-up of HBV-infected patients.

The results of this or any other diagnostic assay should be used and interpreted only in the context of the overall clinical picture[1-5].

II. CLINICAL VALUE

Detection of Hepatitis B surface antigen (HBsAg) in blood serum, which is the main markers of the disease and which is detectable long before the clinical signs of the disease, is of decisive importance for Hepatitis B diagnostics by the use of specific tests. Mutations in the virus genome, coinfection, and the beginning and termination of the infectious process may be followed by the reduction of HBsAg concentration in patient blood up to the level which is lower than the sensitivity level of the most test-systems allowed for use.

Therefore the increase of "DS-EIA-HBsAg-0.01" sensitivity allows reducing the period of diagnostic (serologic) window of viral hepatitis type B. It will enhance the quality of hepatitis B diagnostics, allow to reduce the quantity of latent infections both with the presence of complementary markers of anti-HBs and with the absence of any markers and it may also correct the therapy of double infection. High sensitivity of the test-system allows a more effective detection of mutant variants of HBsAg. The use of a highly-sensitive test improves the quality of donor blood screening, allows reducing the risk of post transfusion hepatitis B infection.

III. PRINCIPLE OF THE TEST

"DS-EIA-HBsAg-0.01" is one-step "sandwich" assay based on a direct, non-competitive solid-phase enzyme immunoassay with biotin as the marker of antibodies. Significant increase of sensitivity is due to signal amplification that is achieved by streptavidin-biotin technology.

If present in the patient's specimen, HBsAg will bind with the anti-HBsAg immobilized on the wells and simultaneously will be bound with the biotin conjugated anti-HBsAg. The horseradish peroxidase labeled streptavidin will be attached to this complex. Unbound conjugates are removed by washing. After that colourless enzyme substrate (H_2O_2) and chromogen (TMB) are added. The enzyme reaction of chromogen/substrate produces a coloured solution. Enzyme-chromogen/substrate reaction is terminated with acid (H_2SO_4). The colour intensity is directly proportional to the concentration of HBsAg in a patient sample.

The confirmatory assay is an *in vitro* neutralization assay for confirmation of the presence of HBsAg in blood serum and plasma samples found repeatedly reactive for HBsAg by the screening assay. For each test sample two assay wells are assigned. The "DS-EIA-HBsAg-0.01" confirmatory assay is run according to the usual procedure except that the Control Reagent added in the control well and HBsAg Confirmatory reagent added in the specific well. During the first incubation the anti-HBsAg in the HBsAg Confirmatory reagent will compete with the antibodies immobilized on the well for any HBsAg present in the sample and will reduce the amount of HBsAg binding to the well; in the control well there is no competition and the HBsAg will bind normally. Conjugates are then added and the assay completed in the normal way. In samples containing HBsAg there will be a significant difference between the OD (optical density) generated in control and specific wells, if the inhibition in the specific well exceeds 50%, the sample is considered to be confirmed reactive.

IV. CONTENTS OF THE "DS-EIA-HBsAg-0.01"

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION				
		Format 1	Format 2	Format 3	Format 4	Format 5
Anti-HBsAg Coated Strips	Polystyrene stripped 96-well plate (breakable wells) coated with monoclonal mouse antibodies to HBsAg (anti-HBsAg).	1 plate	2 plates	5 plates	1 plate	-
Conjugate-1 (concentrated 11-fold)	Monoclonal mouse antibodies to HBsAg (anti-HBsAg), conjugated with biotin. Transparent or slightly opalescent liquid, violet colored. Preserving agent: 0.02% phenol, 0.016% gentamycin sulfate.	1 vial 1.4 ml	1 vial 1.4 ml	1 vial 3.5 ml	1 vial 1.4 ml	-
Conjugate-2 (concentrated 11-fold)	Peroxidase labeled streptavidin. Transparent or slightly opalescent liquid, blue colored. Preserving agent: 0.02% phenol.	1 vial 1.4 ml	1 vial 1.4 ml	1 vial 3.5 ml	1 vial 1.4 ml	-
Conjugate-1 Diluent	Opaque green color liquid, sediment may form which completely dissolves at shaking. Preserving agent: 0.01% thimerosal.	1 vial 14.0 ml	1 vial 14.0 ml	2 vials 18.0 ml	1 vial 14.0 ml	-
Conjugate-2 Diluent	Transparent yellow liquid, sediment may form which completely dissolves at shaking. Preserving agent: 0.01% thimerosal.	1 vial 14.0 ml	1 vial 14.0 ml	2 vials 18.0 ml	1 vial 14.0 ml	-
Positive Control, Inactivated	Recombinant HBsAg in human blood serum negative for anti-HBsAg, anti-HIV-1,2 and anti-HCV. Preserving agent: 0.1% thimerosal, 0.01% gentamycin sulfate. Transparent or slightly opalescent liquid, red colored.	1 vial 2.5 ml	1 vial 2.5 ml	1 vial 2.5 ml	1 vial 2.5 ml	-
Low Positive Control, Inactivated	Native or recombinant HBsAg with concentration of 0.02 ± 0.01 IU/ml in human blood serum negative for anti-HBsAg, anti-HIV-1,2 and anti-HCV. Preserving agent: 0.1% thimerosal. Transparent or opalescent liquid, orange colored. Low Positive Control is used for kit sensitivity assessment. The kit sensitivity is determined once for each plate.	1 vial 2.5 ml	1 vial 2.5 ml	1 vial 2.5 ml	1 vial 2.5 ml	-
Negative Control, Inactivated	Heat inactivated human serum negative for HBsAg, anti-HIV-1,2 and anti-HCV. Transparent or opalescent liquid, green colored. Preserving agent: 0.005% cinnamaldehyde.	1 vial 2.5 ml	1 vial 2.5 ml	1 vial 4.5 ml	1 vial 2.5 ml	-
Washing Solution (concentrated 25-fold)	Transparent or slightly opalescent liquid, colorless, or pale yellow, sediment may form that dissolves at 35-39 °C and shaking.	1 vial 50.0 ml	1 vial 120.0 ml	2 vials 120.0 ml	1 vial 50.0 ml	-

Substrate Buffer	Citric acid and sodium acetate solution, pH 4.1-4.3, containing H_2O_2. Transparent colorless liquid. Preserving agent: 0.04% ProClin 300.	1 vial 25.0 ml	1 vial 25.0 ml	2 vials 50.0 ml	1 vial 25.0 ml	-
TMB (concentrated 11-fold)	Solution containing Tetramethylbenzidine (TMB). Transparent colorless liquid.	1 vial 2.5 ml	1 vial 2.5 ml	2 vials 3.5 ml	1 vial 2.5 ml	-
Stopping Reagent	0.2 M/L sulphuric acid solution. Transparent colorless liquid.	1 vial 25.0 ml	1 vial 50.0 ml	2 vials 50.0 ml	1 vial 25.0 ml	-
HBsAg Confirmatory reagent, Inactivated	Human blood serum negative for HBsAg, anti-HBsAg, anti-HIV-1,2 and anti-HCV with addition of goat anti-HBsAg immunoglobulin. Transparent, pink colored liquid. Preserving agent: 0.01% thimerosal.	-	-	-	1 vial 2.0 ml	1 vial 5.0 ml
Control reagent, Inactivated	Human blood serum negative for HBsAg, anti-HBsAg, anti-HIV-1,2 and anti-HCV with addition of native goat immunoglobulin. Transparent blue colored liquid. Preserving agent: 0.01% thimerosal.	-	-	-	1 vial 2.0 ml	1 vial 5.0 ml
Plate lid		1	2	3	1	-
or Protective film for EIA plates		3	6	15	3	-
Plastic clip or polyethylene bag with a zip lock		1	2	3	1	-
Plastic dish for liquid reagents		2	4	10	2	-
Disposable tips		16	32	80	16	-

V. PRECAUTIONS

The reliability of the results depends on correct implementation of the following:

- The operating temperature in the laboratory should be 18-24 °C.
- Inspect the contents of the box: check the vials and labels integrity. If labels lost or labels/vials are damaged, vials should be disposed and kit cannot be used.
- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- Before use, it is necessary to wait 30 minutes for the reagents to stabilize to room temperature (18-24 °C).
- 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 enzyme activity of the conjugates.
- 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 enzyme reaction is very sensitive to metallic ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solutions.
- Use a new distribution tip for each sample.
- 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 conjugate and other solutions.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay procedure.
- Use high quality water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.
- Do not reuse Anti-HBsAg Coated Strips.
- Once the assay has been started, all subsequent steps should be performed without interruption. Do not let the wells dry once the assay has been started.

VI. HEALTH AND SAFETY INSTRUCTIONS

- All reagents included in the kit are intended for "*in vitro* diagnostic use".
- Human origin material used in the preparation of the Negative Control, the Positive Control, the Low Positive Control, HBsAg Confirmatory reagent and the Control reagent has been tested and found non reactive for Hepatitis B surface antigen (HBsAg), antibodies to hepatitis C and antibodies to human immunodeficiency virus (HIV-1 and HIV-2), antigen p24 HIV-1.
- Because no known test method can offer complete assurance that infections agents are absent, handle reagents and patients samples as if capable of transmitting infections disease.
- Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled.
- Do not pipette by mouth.
- Any equipment directly in contact with specimens and reagents as well as washing solutions should be considered as contaminated products and treated as such.
- Wear lab coats and disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
- Avoid spilling samples or solutions containing samples.
- Avoid any contact of the substrate buffer, the chromogen and the stopping solution with the skin and mucosa.
- Provide adequate ventilation.
- Do not forget to neutralize and/or autoclave the washing wastes or any fluids containing biological samples before discarding them into the sink. Solid wastes (used plates, tips, bottles, glassware, etc.) should be disinfected autoclaving for 1 hour at temperature 124-128 ºC and pressure 1.5 kgf/cm^2 (0.15 MPa). Solid wastes can be disinfected by steeping into 3% of chloramine B solution (disinfection time is not less than 1 hour) or other disinfecting agent authorized for production and use. Liquid wastes (washing water) should be disinfected by dry chloramine B added in concentration 30 g/l (disinfection time is not less than 2 hours). Also liquid wastes can be disinfected by boiling treatment for 30 min or by autoclaving for 1 hour at temperature 124-128 ºC and pressure 1.5 kgf/cm^2 (0.15 MPa). Tools and equipment should be wiped 2 times by 70% ethanol before and after work.

Danger!

Stopping Reagent contains 0.2 M/L sulphuric acid.
H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/ eye protection/face protection.
P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/ physician.

VII. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE TEST:

- Microplate reader equipped with 450 nm or with and 620-680 nm filters.
- Pipettes, single-channel and multi-channel.
- Disposable pipette tips.
- Microplate incubator or thermoshaker at (42.0 ± 1.0) ºC or (37.0 ± 1.0) ºC.
- Automatic microplate washer.
- Deionised or distilled water.
- Laboratory filter paper.
- Medical disposable gloves.

VIII. COLLECTION AND HANDLING OF SPECIMENS

Collect blood samples according to the current practices. Serum or heparin/EDTA/citrate plasma may be used. Separate serum or plasma from blood cells as soon as possible to avoid any haemolysis. **Extensive haemolysis may affect test performance.** Specimens with observable particulate matter should be clarified by centrifugation prior to testing. **Suspended fibrin particles or aggregates may yield falsely positive results.**

Samples can be stored at 2-8 ºC not more than for 48 hours; they may be deep-frozen at -20 ºC or below and stored within several months. Plasma must be quickly thawed by warming for a few minutes at 40 ºC (to avoid fibrin precipitation). Heating of samples at over 40 ºC for inactivation is prohibited. Avoid repeated freeze/thaw cycles. Samples that have been frozen and thawed more than 1 time cannot be used. Do not use contaminated, hyperlipaemic or hyperhaemolysed sera or plasma which has been preserved by sodium azide.

No influence of the anticoagulants (i.e. citrate, heparin, EDTA) on the results of sample testing was found.

NOTE: Samples containing up to 25 mg/l bilirubin, lipemic samples containing up to the equivalent of 20 g/l triglyceride, and haemolysed samples containing up to 10 g/l hemoglobin do not affect the results.

IX. PREPARATION OF THE REAGENTS

1. Ready for use reagents:

- Negative Control
- Positive Control
- Low Positive Control
- Conjugate-1 diluent

- Conjugate-2 diluent
- HBsAg Confirmatory reagent (neutralization reagent)
- Control reagent
- Stopping reagent

2. Reagents to prepare

Anti-HBsAg Coated Strips. Each plate containing 12 strips (breakable wells) is wrapped in a sealed foil-lined bag. Open the bag and remove the plate. Select the number of coated strips/wells required for the assay. Unused strips/wells should be placed back in the bag. After the bag has been opened, Anti-HBsAg Coated Strips are stable until the kit expiration date at 2-8 ºC, provided that the foil-lined bag is resealed with the clip or the foil-line bag is resealed in zip locked plastic bag. The silica gel bag should not be removed from the foil packaging.

Working Washing Solution. Thoroughly mix the content of the bottle with concentrated Washing Solution (concentrated 25-fold). Dilute the required volume of concentrated Washing Solution **with** corresponding volume of purified water prior to use (See Tables 2, 3). Mix the solution thoroughly. The prepared Washing Solution is stable at least for 14 days at 18-24 ºC or for 28 days at 2-8 ºC.

Working Solution of Conjugate-1. Dilute the necessary volume of thoroughly mixed concentrate of Conjugate-1 with the corresponding volume of Conjugate-1 diluent (See Tables 2, 3). Mix thoroughly until diluted avoiding foaming. Do not apply intensive mixing. Prepare before use. Working Solution of the Conjugate-1 is stable at least for 12 hours at 18-24 ºC in a dark place.

Working Solution of Conjugate-2. Dilute the necessary volume of thoroughly mixed concentrate of Conjugate-2 with the corresponding volume of Conjugate-2 diluent (See Tables 2, 3). Mix thoroughly until diluted avoiding foaming. Do not apply intensive mixing. Prepare **before** use. Working Solution of the Conjugate-2 is stable at least for 12 hours at 18-24 ºC in a dark place.

Substrate Mixture. Dilute the required volume of TMB (concentrated 11-fold) with the corresponding volume of Substrate Buffer (1:10 ratio) (See Tables 2, 3). Mix thoroughly until diluted. The **Substrate** Mixture should be prepared before use. Prepared mix is stable at least for 10 hours in a dark place at 18-24 ºC in chemically neutral vial or in reagent container used in open automatic EIA analyzer. **Substrate Mixture should be colorless!**

3. Storage of unused reagents

After opening the Anti-HBsAg Coated Strips and the vials with unused reagents of the kit: Positive Control, Low Positive Control, Negative Control, Conjugate-1 (concentrated 11-fold), Conjugate-2 (concentrated 11-fold), Conjugate-1 diluent, Conjugate-2 diluent, HBsAg Confirmatory reagent, Control reagent, Washing Solution (concentrated 25-fold), Substrate Buffer, TMB (concentrated 11-fold), Stopping Reagent can be stored in closed foil bag and tightly sealed vials respectively, until the kit expiration date at 2-8 °C.

X. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature (18 – 24 ºC) for 30 min.

Calculation of volumes of reagents, when using the number of wells not specified in Table 2, should be performed using the data specified for one well.

Table 2

Consumption of reagents for the manual test-procedure (Formats 1, 2, 3, 4)

Number of used strips/ wells	Working Washing Solution		Working Solution of Conjugate		Substrate Mixture	
	Washing Solution (concentrated 25-fold) (ml)	Purified water (ml)	Conjugate-1,2 (concentrated 11-fold) (ml)	Conjugate-1,2 diluents (ml)	TMB (concentrated 11-fold) (ml)	Substrate Buffer (ml)
1 well	0.2	4.8	0.005	0.05	0.01	0.1
Use of the whole strips						
1 strip	3.0	72.0	0.05	0.5	0.1	1.0
2 strips	6.0	144.0	0.1	1.0	0.2	2.0
3 strips	9.0	216.0	0.15	1.5	0.3	3.0
4 strips	12.0	288.0	0.2	2.0	0.4	4.0
5 strips	15.0	360.0	0.25	2.5	0.5	5.0
6 strips	18.0	432.0	0.3	3.0	0.6	6.0
7 strips	21.0	504.0	0.35	3.5	0.7	7.0
8 strips	24.0	576.0	0.4	4.0	0.8	8.0
9 strips	27.0	648.0	0.45	4.5	0.9	9.0
10 strips	30.0	720.0	0.5	5.0	1.0	10.0
11 strips	33.0	792.0	0.55	5.5	1.1	11.0
12 (one plate)	40.0	960.0	0.70	7.0	1.2	12.0

The volumes mentioned in Table 3 are recommended and may vary depending on the other models of used EIA automated analyzers.

Table 3

Consumption of reagents for the test-procedure using an open type automated analyzers

Number of used strips	Working Washing Solution		Working Solution of Conjugate		Substrate Mixture	
	Washing solution (concentrated 25-fold) (ml)	Purified water (ml)	Conjugate-1,2 (concentrated 11-fold) (ml)	Conjugate-1,2 diluents (ml)	TMB (concentrated 11-fold) (ml)	Substrate Buffer (ml)
4 (for format 1 only)	16.0	384.0	0.45	4.5	0.7	7.0
8 (for format 1 only)	32.0	768.0	0.70	7.0	1.2	12.0
12 (a plate) (for all formats)	40.0	960.0	0.70	7.0	1.2	12.0

Attention! Incubation is possible as four alternative procedures. It is very important that the following assay step should be carried out in the same incubation mode. The combination of incubation modes is not supposed. See Table 4.

Table 4

EIA procedures

<table>
<tr><th>№</th><th>Procedure 1
microplate incubator
at (42.0 ± 1.0) ºC</th><th>Procedure 2
microplate incubator
at (37.0 ± 1.0) ºC</th><th>Procedure 3
thermoshaker
at (42.0 ± 1.0) ºC</th><th>Procedure 4
thermoshaker
at (37.0 ± 1.0) ºC</th></tr>
<tr><td>1</td><td colspan="4">Depending on the quantity of strips/wells used in the assay it is recommended to add 100 µl of controls per well as following:
<u>1-2 strips/up to 16 wells (including control wells)</u>: 2 wells – Negative Control, 1 well – Positive Control, 2 wells – Low Positive Control*.
<u>3 strips and more/17 wells and more (including control wells)</u>: 4 wells – Negative Control, 1 well – Positive Control, 3 wells – Low Positive Control*.
Add 100 µl of undiluted specimens into the rest of the wells.</td></tr>
<tr><td>1.1.</td><td colspan="4"><u>For confirmatory assay procedure</u>
Depending on the quantity of strips/wells used in the assay it is recommended to add 100 µl of controls per well as following:
<u>2 strips/up to 16 wells (including control wells):</u> 4 wells – Negative Control, 3 wells - Positive Control, 1 well – Low Positive Control*.
<u>3 strips and more / 17 wells and more (including control wells)</u>: 5 wells – Negative Control, 3 wells – Positive Control, 2 wells – Low Positive Control*.
Add 100 µl of repeatedly reactive samples into 2 wells of the plate (1 well is used for Control reagent adding, 1 well is for HBsAg Confirmatory reagent adding). Add 25 µl of Control reagent and HBsAg Confirmatory reagent in the wells of Anti-HBsAg Coated Strips according to Tables 5-6.</td></tr>
<tr><td rowspan="2">2</td><td colspan="2">Cover the plate by a plate lid or protective film and incubate the plate in microplate incubator:</td><td colspan="2" rowspan="2">See p.3.</td></tr>
<tr><td>30 min
(42.0 ± 1.0) °C</td><td>60 min
(37.0 ± 1.0) °C</td></tr>
<tr><td>3</td><td colspan="4">Without removing the contents of the wells and washing the wells add 50 µl of Working Solution of Conjugate-1 into each well. The color of the content should change from green to grey. Sera and plasma with an acid pH will produce a bright yellow colour. Avoid accidental introduction of samples from one well into another through the tips when adding the Working Solution of Conjugate-1. Mix the contents of the wells by careful tapping on the edge of the plate.</td></tr>
<tr><td rowspan="2">4</td><td colspan="2">Cover the plate by a plate lid or protective film and incubate the plate in microplate incubator:</td><td colspan="2">Incubate the plate without a plate lid or protective film in a thermoshaker at 500 rpm:</td></tr>
<tr><td>45 min
(42.0 ± 1.0) °C.</td><td>60 min
(37.0 ± 1.0) °C.</td><td>40 min
(42.0 ± 1.0) °C.</td><td>60 min
(37.0 ± 1.0) °C.</td></tr>
<tr><td>5</td><td colspan="4">Without removing the contents of the wells and washing the wells add 50 µl of Working Solution of Conjugate-2 into each well.
Avoid accidental introduction of samples from one well into another through the tips when adding the Working Solution of Conjugate-2. Mix the contents of the wells by careful tapping on the edge of the plate.</td></tr>
</table>

6	Cover the plate by a plate lid or protective film and incubate the plate in microplate incubator:		Incubate the plate **without a plate lid or protective film** in a thermoshaker at 500 rpm:	
	45 min (42.0 ± 1.0) °C.	30 min (37.0 ± 1.0) °C	20 min (42.0 ± 1.0) °C.	30 min (37.0 ± 1.0) °C.
7	Remove the content of the wells into the container with disinfectant solution. Add into each well **not less than 400 µl** of Working Washing Solution. **Allow a soak time at least 40 seconds and aspirate. Perform this procedure four times.** *Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision.* Dry the plate by turning it upside down on absorbent paper.			
8	Add 100 µl of Substrate Mixture into each well. Keep the plates in the dark place during 20 min at 18-24 ºC.			
9	Add 150 µl of Stopping Reagent into each well and read the optical density in 2-3 min at 450/620-680 nm using a plate reader. Reading the absorbance at 450 nm only is possible. **Test results remain stable for reading within at least 10 minutes.**			

*Low Positive Control is used for the kit sensitivity assessment as an additional validity point. When using the plate factionary, it is sufficient to test Low Positive Control once.

Table 5

Scheme of reagent addition for 2 strips

	1	2
A	**Negative Control**	**Sample 1**, Control reagent
B	**Negative Control**	**Sample 1**, HBsAg Confirmatory reagent
C	**Negative Control**, Control reagent	**Sample 2**, Control reagent
D	**Negative Control**, HBsAg Confirmatory reagent	**Sample 2**, HBsAg Confirmatory reagent
E	**Positive Control**	**Sample 3,** Control reagent
F	**Positive Control**, Control reagent	**Sample 3,** HBsAg Confirmatory reagent
G	**Positive Control**, HBsAg Confirmatory reagent	**Sample 4,** Control reagent
H	**Low Positive Control**	**Sample 4**, HBsAg Confirmatory reagent

Table 6

Scheme of reagent addition for 3 strips and more

	1	2	3
A	**Negative Control**	**Low Positive Control**	**Sample 4**, Control reagent
B	**Negative Control**	**Low Positive Control**	**Sample 4**, HBsAg Confirmatory reagent
C	**Negative Control**	**Sample 1,** Control reagent	**Sample 5**, Control reagent
D	**Negative Control**, Control reagent	**Sample 1**, HBsAg Confirmatory reagent	**Sample 5**, HBsAg Confirmatory reagent
E	**Negative Control**, HBsAg Confirmatory reagent	**Sample 2**, Control reagent	**Sample 6**, Control reagent
F	**Positive Control**	**Sample 2**, HBsAg Confirmatory reagent	**Sample 6**, HBsAg Confirmatory reagent
G	**Positive Control**, Control reagent	**Sample 3**, Control reagent	**Sample 7**, Control reagent
H	**Positive Control**, HBsAg Confirmatory reagent	**Sample 3**, HBsAg Confirmatory reagent	**Sample 7**, HBsAg Confirmatory reagent

Algorithm of EIA procedure is represented in Annex.

Spectrophotometric verification of samples and reagents dispensing when using the kit "DS-EIA-HBsAg-0.01" on automated EIA-analyzers.

1. We recommend to **check up entering of samples into the wells by measurement** at wavelength of 450 nm, criterion: OD > 0.125.

2. We recommend to verify the Conjugate-1 dispensing at wavelength of 620 nm, criterion: OD > 0.265.

3. We recommend to verify the Conjugate-2 dispensing at wavelength of 405 nm, criterion: OD > 1.200.

XI. RESULTS

The presence or absence of Hepatitis B surface antigen (HBsAg) is determined by the ratio of the OD of each sample to the calculated Cut-Off value.

For the assay to be valid, OD value of Positive Control should be not less than 0.6, average OD value of Negative Control should be not greater than 0.12. If OD of one of Negative Controls is > 0.120, it should be excluded from the Cut-Off value calculation. If more than one OD value of Negative control is to be excluded the analysis should be repeated.

Low Positive Control is used for the kit sensitivity assessment as an additional validity point.

OD of Low Positive Control should be ≥ Cut-Off.

OD value of Negative Controls after the adding of Control reagent and HBsAg Confirmatory reagent should be < Cut-Off.

Calculate Cut-Off value as:

Cut-Off = average OD value of Negative Control + 0.060,

where 0.060 is a coefficient defined by manufacturer during statistical processing for each lot. Value of coefficient is specified for each lot and indicated in the instruction of the kit application and in the CQA.

Negative: if the OD value is < Cut-off

Initially reactive: if the OD value is ≥ Cut-Off

Initially reactive specimens should be retested in duplicates to validate the initial results. If, after repeat testing, the OD value of both duplicate specimens is less than the cut-off value, the specimen may be considered negative for HBsAg. If, after repeat testing, the OD value of either of the duplicates is greater than or equal to the cut-off, the initial result is reproduced. All repeatedly reactive samples should be studied using confirmation tests which are based on neutralizing reaction.

The presence of Hepatitis B surface antigen (HBsAg) in the confirmatory test is determined by the neutralization value that is the ratio of sample OD values after control reagent addition to sample OD values after confirmatory reagent addition.

The value of neutralization (%) is calculated according to the formula:

$$\frac{\text{(OD c – OD HBsAg Cr)}}{\text{(OD c – Negative Control average OD)}} \text{ X 100 \%}$$

where OD c - the optical density of the sample after the Control reagent addition;

OD HBsAg Cr - the optical density of the sample after HBsAg Confirmatory reagent addition;

Negative Control average OD - average optical density of the Negative Controls.

Neutralization value of Positive Control OD should be ≥ 50%.

The sample is positive if:

1. The OD value of the sample after the Control reagent addition is ≥ Cut-off.
2. Neutralization value of the sample OD is not less than 50%.

The sample with OD ≥ 1.0 which has not undergone neutralization should be diluted 25 times (1:25) with working washing solution and the test should be repeated. If after 25 times dilution (1:25) the OD value is still high and the sample is not neutralizing then it is recommended to dilute the initial sample 50 (1:50) and more times and analyze it. If after 50 (1:50) and more times dilution sample is subjected to neutralization more than 50% the sample should be considered positive. If the neutralization value is less than 50% the sample should be considered negative.

The neutralizing reagent contained in the assay proved suitable for the confirmation of low and high reactive samples such as contained in the seroconversion series.

XII. PERFORMANCE CHARACTERISTICS

Diagnostic sensitivity

Results of the diagnostic sensitivity of the kit "DS-EIA-HBsAg-0.01" are represented in Tables 7, 8.

Table 7

Diagnostic sensitivity of the kit "DS-EIA-HBsAg-0.01"

№	The category of samples	The number of tested samples	Results	Source of results
1.	**Samples from different stages of HBV infection:**			
	Patients with acute hepatitis B	34	Sensitivity was 100%.	Results of RPC "Diagnostic Systems", February, 2005
	Patients with chronic hepatitis B	419	Sensitivity was 100%.	Results of RPC "Diagnostic Systems", February, 2005
2.	Serum samples contained different HBsAg subtype			
	HBsAg subtype ad (including adw2, adw4, adr)	48	Sensitivity was 100%.	Results of RPC "Diagnostic Systems", February, 2005 Results of Paul-Ehrlich-Institute, October 2008
	HBsAg subtype ay (including ayw1, ayw2, ayw3 var B, ayw3 var A, ayw4)	28	Sensitivity was 100%.	Results of RPC "Diagnostic Systems", February, 2005 Results of Paul-Ehrlich-Institute, October 2008
3.	HBV seroconversion panels (BBI Inc., ZeptoMetrix, USA)	398 (37 Panels)	The results are represented in Table 5	Results of RPC "Diagnostic Systems", February, 2005 Results of Paul-Ehrlich-Institute, October 2008

4.	HBsAg Mixed Titer Performance Panels			
	HBsAg Mixed Titer Performance Panel PHA 205 (BBI,USA)	25	Sensitivity was 100%.	Results of RPC "Diagnostic Systems", February, 2005
	HBsAg Low Titer Performance Panel PHA 106 (BBI,USA)	15	Sensitivity was 100%.	Results of RPC "Diagnostic Systems", February, 2005
	Hepatitis B surface antigen sensitivity panel BBI-PHA 807	20	Sensitivity was 100%.	Results of RPC "Diagnostic Systems", February, 2005
	Hepatitis B surface antigen sensitivity panel BBI-PHA 808	20	Sensitivity was 100%.	Results of RPC "Diagnostic Systems", February, 2005
	HBsAg qualification panel BBI-QHA711	6	Sensitivity was 100%.	Results of RPC "Diagnostic Systems", February, 2005
	Control panel of recombinant HBsAg mutant variants subtypes ayw1 and adw2 (RPC "Diagnostic systems")	26	Sensitivity was 100%. The results are represented in Table 6	Results of RPC "Diagnostic Systems", February, 2005

Table 8

Diagnostic sensitivity in seroconversion panels

Panel	**PCR HBV DNA*** **	**"DS-EIA-HBsAg-0.01"**	**Any test with earliest detection*** **
	The number of positive results in comparison with the number of tested samples		
BBI PHM 932	8/16	12/16*	9/16
BBI PHM 933	5/6	6/6*	5/6
BBI PHM 934	6/6	6/6*	6/6
BBI PHM 935A(M)	17/20	15/20*	14/20
BBI PHM 935B	6/12	11/12*	10/12
BBI PHM 928	6/7	6/7*	5/7
BBI PHM 911	12/25	8/25*	5/25
ZMC HBV 6277	8/11	9/11*	6/11
ZMC HBV 6278	11/11	10/11*	8/11
ZMC HBV 6279	4/7	5/7*	2/7
ZMC HBV 6281	10/12	11/12*	6/12
BBI PHM 914	-	5/6**	5/6
BBI PHM 916	-	5/11**	3/11
BBI PHM 919	-	8/9**	5/9
BBI PHM 925	3/5	5/5**	4/5
BBI PHM 926	6/8	7/8**	6/8
BBI PHM 927	6/6	6/6**	5/6
BBI PHM 929	7/9	7/9**	5/9
BBI PHM 930	5/5	5/5**	4/5
BBI PHM 931	6/8	7/8**	5/8
ZMC HBV 6271	5/5	5/5**	3/5
ZMC HBV 6272	-	7/26**	7/26
ZMC HBV 6273	3/6	3/6**	3/6
ZMC HBV 6274	-	7/7**	7/7

ZMC HBV 6275	3/7	7/7**	2/7
ZMC HBV 11000	-	7/9**	6/9
ZMC HBV 11001	4/8	6/8**	4/8
ZMC HBV 11002	4/6	6/6**	4/6
ZMC HBV 11003	5/8	5/8**	3/8
ZMC HBV 11006	8/17	8/17**	6/17
ZMC HBV 11007	-	7/14**	5/14
ZMC HBV 11008	7/18	7/18**	5/18
ZMC HBV 11009	-	7/23**	6/23
ZMC HBV 11011	6/14	6/14**	7/14
ZMC HBV 11012	3/6	4/6**	3/6
ZMC HBV 11016	-	6/10**	5/10
ZMC HBV 11017	7/14	8/14**	6/14
Total 37	181/283 (64%)	260/398 (65.3%)	200/398 (50.3%)

* - Results of RPC "Diagnostic systems", Ltd
** - Results of Paul-Ehrlich-Institute
*** - Panel certificate data.

Table 9

Control panel of recombinant HBsAg mutant variants of ayw1 and adw2 subtypes

Sample №	Mutant variant of HBsAg	Sample dilution	"DS-EIA-HBsAg-0.01" S/Co	Test of comparison S/Co
1	**ayw1 G145R**	(1)	39.0	3.9
		(2)	19.2	0.6
2	**adw2 G145R**	(1)	32.2	3.0
		(2)	12.4	0.9
3	**adw2 Q129R**	(1)	14.5	1.9
		(2)	10.9	1.5
4	**adw2 Q129H**	(1)	33.7	3.8
		(2)	14.9	0.98
5	**adw2 Q129L**	(1)	36.2	3.0
		(2)	22.9	1.1
6	**adw2 T143K**	(1)	16.9	0.9
		(2)	3.9	0.5
7	**adw2 T126N**	(1)	25.0	2.6
		(2)	9.4	1.0
8	**adw2 T126S**	(1)	16.0	3.3
		(2)	18.2	2.0
9	**adw2 D144A**	(1)	13.1	0.8
		(2)	7.2	0.5
10	**adw2M133H**	(1)	31.2	1.9
		(2)	8.5	0.5
11	**adw2 M133L**	(1)	31.3	4.1
		(2)	22.5	0.8
12	**adw2 K141E**	(1)	31.2	2.2
		(2)	12.8	0.6
13	**adw2 P142S**	(1)	35.4	5.6
		(2)	32.9	1.5

Conclusion: Sensitivity of the "DS-EIA-HBsAg-0.01" is excellent in acute HBV infection as demonstrated by the seroconversion study results. On the 37 panels tested the "DS-EIA-HBsAg-0.01" detected HBV infection on general earlier than any other CE-marked HBsAg assay. As a result the diagnostic window is on average shortened by 5 days which is a great improvement in early HBV diagnostics. Moreover, the assay is able to recognize all different HBV subtypes and mutants included in the study.

Analytical sensitivity

For test-system analytical sensitivity assessment different dilutions of "Second International Standard for HBsAg Subtype adw2, genotype A" (NIBSC, UK, Code: 00/588) have been used. The test-system sensitivity level is 0.01 IU/ml when detecting hepatitis B surface antigen (HBsAg) (results of RPC "Diagnostic Systems", Ltd).

Table 10

Evaluation of "DS-EIA-HBsAg-0.01" sensitivity

"Second International Standard for HBsAg Subtype adw2, genotype A" (NIBSC, UK).	
IU/ml	**S/Co**
0.1	16.47
0.05	8.95
0.04	7.78
0.02	3.96
0.01	1.95
0.005	0.93

Specificity

Specificity of the kit "DS-EIA-HBsAg-0.01" is shown in Table 11 (results of RPC "Diagnostic Systems", Ltd).

Table 11

Specificity of the kit "DS-EIA-HBsAg-0.01"

№	The category of samples	The quantity of tested samples	The quantity of false-positive samples	Specificity,%
1	Primary donors	5348	11	99.8
2	Patients with non-infectious diseases	285	3	98.9
3	Samples containing rheumatoid factor	182	1	99.5
4	Pregnant women	477	3	99.4
5	Patients with infectious diseases HAV, HCV, HIV-infections, ARD, pneumonia, angina, herpes, cytomegalovirus, Chlamydia, syphilis)	1929	13	99.3

Reproducibility

The intra-assay reproducibility has been evaluated by testing 3 positive samples 70 times each in the same batch.

Between batches reproducibility has been evaluated by testing 3 positive samples during 10 days with 2 different operators. During the assays every sample has been tested 16 times.

The coefficient of variation for reactivity value (OD/Cut off value) of positive samples was less than 10 %.

The performance evaluation of blood serum and plasma equivalency was carried out. Results showed the absence of significant differences between testing blood sera and plasma.

XIII. LIMITS OF THE TEST

- The "DS-EIA-HBsAg-0.01" assay and the interpretation of results must be followed when testing serum, plasma for the presence of HBsAg. The user of this kit is advised to read the instruction for use 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.
- A sample should not be considered to be positive for HBsAg based on a single reactive test results. Additional testing, such as confirmatory testing, is required to establish the specificity of any specimens reactive by the screening procedure.
- All highly sensitive immunoassays have a potential for non-specific reactions which can lead to false positive results. The proportion of false positive results will depend on the sensitivity and specificity of the kit. Refer to the "PERFORMANCE CHARACTERISTICS" section of this package insert for assay performance characteristics.
- Negative results can occur if the quantity of marker present in the sample is too low for the detection limit of the assay, or if the marker which is detected is not present during the stage of disease in which a sample is collected.
- Testing additional HBV markers is recommended for the final diagnosis of the infection.
- A reactive HBsAg result does not exclude co-infection by another hepatitis virus[5].
- This assay was designed and validated for use with human plasma or serum from individual patient and donor specimens. **Pooled specimens must not be used since the accuracy of their test results has not been validated.**
- Specimens containing red blood cells may give inconsistent results, and therefore must be centrifuged prior to testing.
- Some specimens that have undergone multiple freeze-thaw cycles may result in erroneous or inconsistent test results.
- Do not use heat-inactivated specimens.
- All the reagents are for professional *in vitro* diagnostic use only.

XIV. CONDITIONS OF STORAGE AND TRANSPORTATION

Expiry date is indicated on the packaging. Keep in a dark dry place at 2-8 °C.

Transportation may be done by all kinds of covered transport at temperature 9-20 °C not more than during ten (10) days. Freezing is prohibited.

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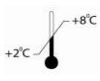
XVI. EXPLANATION OF SYMBOLS

	Manufacturer		
EC REP	Authorized representative in the European Community		Storage temperature limitations
IVD	For *in vitro* diagnostic use		Expiry date CCYY-MM-DD
REF	Catalogue number		Consult Instructions for use
	Sufficient for		Symbol "corrosion"
LOT	Batch code	**Danger!**	Signal word

Annex

Scheme of the assay

1	**Add**	100 µl of Negative control, Positive Control, Low Positive Control
2	**Add**	100 µl of tested samples
3	**Add**	25 µl of Control reagent, HBsAg Confirmatory reagent (use p. 3 only with formats 4, 5)
4	**Incubate**	**Procedure 1:** 30 min at (42.0 ± 1.0) °C, microplate incubator **Procedure 2:** 60 min at (37.0 ± 1.0) °C, microplate incubator (do not apply p. 4 when using a Procedure 3, Procedure 4)
5	**Add**	**50 µl of Working Solution of Conjugate-1**
6	**Incubate**	**Procedure 1:** 45 min at (42.0 ± 1.0) °C, microplate incubator **Procedure 2:** 60 min at (37.0 ± 1.0) °C, microplate incubator **Procedure 3:** 40 min at (42.0 ± 1.0) °C, 500 rpm, thermoshaker **Procedure 4:** 60 min at (37.0 ± 1.0) °C, 500 rpm, thermoshaker
7	**Add**	**50 µl of Working Solution of Conjugate-2**
8	**Incubate**	**Procedure 1:** 45 min at (42.0 ± 1.0) °C, microplate incubator **Procedure 2:** 30 min at (37.0 ± 1.0) °C, microplate incubator **Procedure 3:** 20 min at (42.0 ± 1.0) °C, 500 rpm, thermoshaker **Procedure 4:** 30 min at (37.0 ± 1.0) °C, 500 rpm, thermoshaker
9	**Wash the plate**	**Working Washing Solution, not less than 400 µl, at least 40 seconds, 4 times**
10	**Add**	100 µl of Substrate Mixture
11	**Incubate**	20 min, 18-24 °C in a dark place
12	**Add**	150 µl of Stopping Reagent
13	**Read the optical density**	450 nm/620-680 nm or 450 nm