WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: DS-EIA-HIV-AGAB-SCREEN
WHO reference number: PQDx 0106-038-00

DS-EIA-HIV-AGAB-SCREEN with product codes **I-1654/1.2, I-1652/1.2** and **I-1656/1.2**, manufactured RPC Diagnostic Systems Ltd., **Rest-of-World** regulatory version, was accepted for the WHO list of prequalified *in vitro* diagnostics and was listed on 13 July 2015.

Summary of prequalification status for DS-EIA-HIV-AGAB-SCREEN

	Date	Outcome
Status on PQ list	13 July 2015	listed
Dossier assessment	2 March 2015	MR
Site inspection(s) of the quality management system	27-29 September 2021	MR
Product performance evaluation	9 March 2015	MR

MR: Meets Requirements

Report amendments and product changes

This public report has since been amended. Amendments may have arisen 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 details of each amendment are provided below.

Version	Summary of amendments	Date of report amendment
2.0	Minor grammatical corrections.	13 July 2015
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. Correction on the product codes for the	24 November 2022

	RoW version from I-1654, I-1652 and I-1656 to I-1654/1.2, I-1652/1.2 and I-1656/1.2	

Intended use:

According to the claim of RPC Diagnostic Systems', "*the "DS-EIA-HIV-AGAB-SCREEN" kit is a 'fourth-generation' immunoassay utilizing a mixture of antigens and antibodies for the qualitative simultaneous detection of IgG, IgM and IgA antibodies to major subtypes of HIV-1, including HIV-1 group O, HIV-2, and the HIV-1 p24 antigen at the early stage of infection in human serum or plasma. This kit is intended as an aid in the diagnosis of HIV-1 and/or HIV-2 infection, including acute or primary HIV-1 infection. Results from the "DS-EIA-HIV-AGAB-SCREEN" cannot be used to distinguish between the presence of HIV-1 p24 antigen, HIV-1 antibodies, or HIV-2 antibodies in a specimen. This kit is for diagnostic use by a trained laboratory professional and will not be sold to the general public. The sensitivity of the kit is 0.5 IU/ml ("HIV-1 p24 ANTIGEN 1st international reference reagent", NIBSC Code: 90/636) for HIV-1 p24 antigen detection. Recombinant antigens gp160 (HIV-1), gp41 (HIV-1 group O), p31 (HIV-1), gp36 (HIV-2) and antibodies to HIV-1 p24 antigen with the highest diagnostic potential were selected for test development."*

Assay description:

According to RPC Diagnostic Systems claim, "*DS-EIA-HIV-AGAB-SCREEN is a one-step" sandwich" assay based on micro wells coated by recombinant antigens representing immunodominant regions of HIV-1, HIV-1 (O), HIV-2 proteins and antibodies raised against p24 of HIV-1. The Conjugate is a mixture of the other viral antigen epitopes (gp41, p31 HIV-1, gp41 HIV-1 group O, gp36 HIV-2) all labelled with biotin and horseradish peroxidase, and monoclonal antibodies, also raised against p24, labelled with biotin. Serum or plasma specimens are added to these wells and if p24 antigens and/or antibodies specific for HIV-1, HIV-1 (O) and HIV-2 are present in the specimen, they form stable complexes with the HIV antigens or antibodies attached to the well. Antigen/antibody complexes are then identified by the addition of: (1) biotinylated antigens and antibodies and (2) horseradish peroxidase (HRP) streptavidin conjugate and HRP labelled antigens. Unbound conjugates are removed by washing. After adding of a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide wells with bound Conjugate develop a blue colour which is converted into yellow colour which may be read at 450nm or 450/620nm after the reaction has been stopped with sulphuric acid*".

Product test kit contents:

	Product code I-1654/1.2	**Product code I-1652/1.2**	**Product code I-1656/1.2**
HIV AGAB Coated Strips Polystyrene stripped 96-well (breakable wells) plate coated with a mixture of purified recombinant antigens – gp160, p31 HIV-1, gp41 HIV-1 group O, gp36 HIV-2, and antibodies to antigen p24 HIV-1.	96 tests/ 1 plate	192 tests/ 2 plates	480 tests/ 5 plates
Conjugate-1 (concentrated 11-fold) Mixture of biotinylated recombinant antigens gp41, p31 HIV-1, gp41 HIV-1 group O, gp36 HIV-2 and antibodies to antigen p24 HIV-1. Preserving agent 0.01% gentamycin sulfate, 0.02% thimerosal.	1 vial of 1.2 ml	1 vial of 1.2 ml	1 vial of 3.6 ml or 3 vials of 1.2 ml
Conjugate-2 (concentrated 11-fold) Mixture of horseradish peroxidase labelled recombinant antigens gp41, gp36 HIV-2, and horseradish peroxidase labelled streptavidin. Preserving agent 0.02% phenol (Transparent or slightly opalescence liquid of colourless or pale yellow colour.)	1 vial of 2.0 ml	1 vial of 2.0 ml	2 vials of 2.0 ml or 1 vial of 4.0 ml
Conjugate-1 diluent Transparent or slightly opalescent liquid, orange-coloured pellet dissolved at shaking may form. Preserving agent 0.01% thimerosal, 0.01% gentamycin sulfate, 0.02% phenol.	1 vial of 12.0ml	1 vial of 12.0ml	3 vials of 12.0ml
Conjugate-2 diluent Transparent or slightly opalescent liquid, blue-coloured. Preserving agent 0.01% thimerosal and 0.001 Gentamycin Sulfate.	1 vial of 20.0 ml	1 vial of 20.0 ml	2 vials of 20.0 ml
AB Positive Control, Inactivated Heat-inactivated human plasma positive for anti-HIV, negative for antigen p24 HIV-1, HBsAg and anti-HCV. Transparent or slightly opalescent liquid, orange-coloured. Preserving agent 0.01% gentamycin sulfate, 0.02% thimerosal.	1 vial of 2.0ml	1 vial of 2.0ml	1 vial of 4.0ml or 2 vials of 2.0 ml
AG Positive Control, Inactivated Purified recombinant antigen p24 HIV-1 in heat-inactivated human plasma negative for anti-HIV, anti-HCV, HBsAg and p24 HIV-1 antigen.	1 vial of 2.0ml	1 vial of 2.0ml	1 vial of 4.0ml or 2 vials of 2.0 ml

Transparent or slightly opalescent liquid, crimson-red coloured. Preserving agent 0.01% gentamycin sulfate, 0.05% thimerosal.			
Negative Control, Inactivated Heat-inactivated human plasma negative for anti-HIV, negative for antigen p24 HIV-1, HBsAg and anti-HCV. Transparent or slightly opalescent liquid, green-coloured. Preserving agent 0.06% thimerosal.	1 vial of 2.5ml	1 vial of 2.5ml	2 vials of 2.5ml or 1 vial of 5.0 ml
Washing Solution (concentrated 25-fold) Transparent or slightly opalescent liquid, colourless or pale yellow, sediment may form that dissolves at 35-39 °C and shaking.	1 vial of 50.0ml	1 vial of 120.0ml	2 vials of 120.0ml
Substrate Buffer Citric acid and sodium acetate solution containing H_2O_2. Transparent colourless liquid. Preserving agent 0.04% ProClin 300.	1 vial of 25.0ml	1 vial of 25.0ml	3 vials of 25.0ml or 2 vials of 50.0ml
TMB (concentrated 11-fold) Solution containing Tetramethyl-benzidine (TMB). Transparent colourless liquid.	1 vial of 2.5ml	1 vial of 2.5ml	3 vials of 2.5 ml or 2 vials of 3.5ml
Stopping Reagent 0.2M sulphuric acid solution. Transparent colourless liquid.	1 vial of 25.0ml	2 vials of 25.0ml or 1 vial of 50.0ml	4 vials of 25.0 ml or 2 vials of 50.0ml
Protective film for EIA plates	2 pcs	4 pcs	10 pcs
Plastic clip or polyethylene bag with a zip lock	1 pc	2 pcs	3 pcs
Plastic dish for liquid reagents	2 pcs	4 pcs	10 pcs
Disposable tips	16 pcs	32 pcs	80 pcs
Instructions for use	1 pc	1 pc	1 pc

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

Shelf-life:
24 months.

Prioritization for prequalification

RPC Diagnostic Systems submitted an application for prequalification of DS-EIA-HIV-AGAB-SCREEN. Based on the established eligibility criteria, DS-EIA-HIV-AGAB-SCREEN was accepted for WHO prequalification assessment.

Product dossier assessment

RPC Diagnostic Systems submitted a product dossier for DS-EIA-HIV-AGAB-SCREEN as per the "Instructions for compilation of a product dossier" (PQDx_018 v1). The information submitted in the product dossier was reviewed by WHO staff and external experts (assessors) appointed by WHO in accordance with the internal report on the screening and assessment of a product dossier (PQDx_009 v2). Based on the product dossier screening and assessment findings, a recommendation was made to accept the product dossier for DS-EIA-HIV-AGAB-SCREEN for prequalification.

Manufacturing site inspection

A comprehensive inspection was performed at the site of manufacture (22 Yablonevaya, Nizhniy Novgorod, 603093, Russia) of DS-EIA-HIV-AGAB-SCREEN 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.

Product performance evaluation

DS-EIA-HIV-AGAB-SCREEN (RPC Diagnostic Systems) is a fourth-generation immunoassay for the detection of HIV-1/2 antibodies and HIV-1 p24 antigen in human serum and plasma. A volume of 70 µl 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 1120 specimens, we found an initial sensitivity (95% CI) of 100% (99.2 - 100%) and an initial specificity (95% CI) of 98.3% (97.0 - 99.2%) compared to the reference results. The final sensitivity (95% CI) was 100% (99.2 - 100%) and the final specificity (95% CI) was 98.8% (97.6 - 99.5%) compared to the reference results. Acceptance criteria for sensitivity and specificity were set at 100% sensitivity and ≥98% specificity. Therefore, the performance characteristics were acceptable. The lot-to-lot variation observed was within the acceptance range of ≤5%

For eight seroconversion panels, DS-EIA-HIV-AGAB-SCREEN detected on average 1.25 specimens earlier than the benchmark assay (Enzygnost Anti-HIV 1/2 Plus [Siemens Healthcare Diagnostics]) and on average 0.750 specimens earlier than Vironostika HIV Ag/Ab (bioMérieux) EIA.

For the mixed titer panel, DS-EIA-HIV-AGAB-SCREEN classified one specimen as false positive; all other specimens were correctly identified. For the HIV-1 p24 antigen panel, DS-EIA-HIV-AGAB-SCREEN correctly classified all specimens. For the HIV culture supernatant panel, DS-EIA-HIV-AGAB-SCREEN detected all HIV-1 subtypes, and the HIV-2 culture isolate was not detected.

For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], DS-EIA-HIV-AGAB-SCREEN detected all subtypes tested (HIV-1 A, HIV-1 B, HIV-C, HIV-1 CRF01_AE, HIV-1 O and HIV-2). For the HIV-1 p24 antigen standard [NIBSC code 90/636], DS-EIA-HIV-AGAB-SCREEN detected 0.39 international units). In contrast, Vironostika HIV Ag/Ab (bioMérieux) detected to 12.5 international units.

In this study, 0% of the results were recorded as indeterminate. The invalid rate was 0%.

Labelling

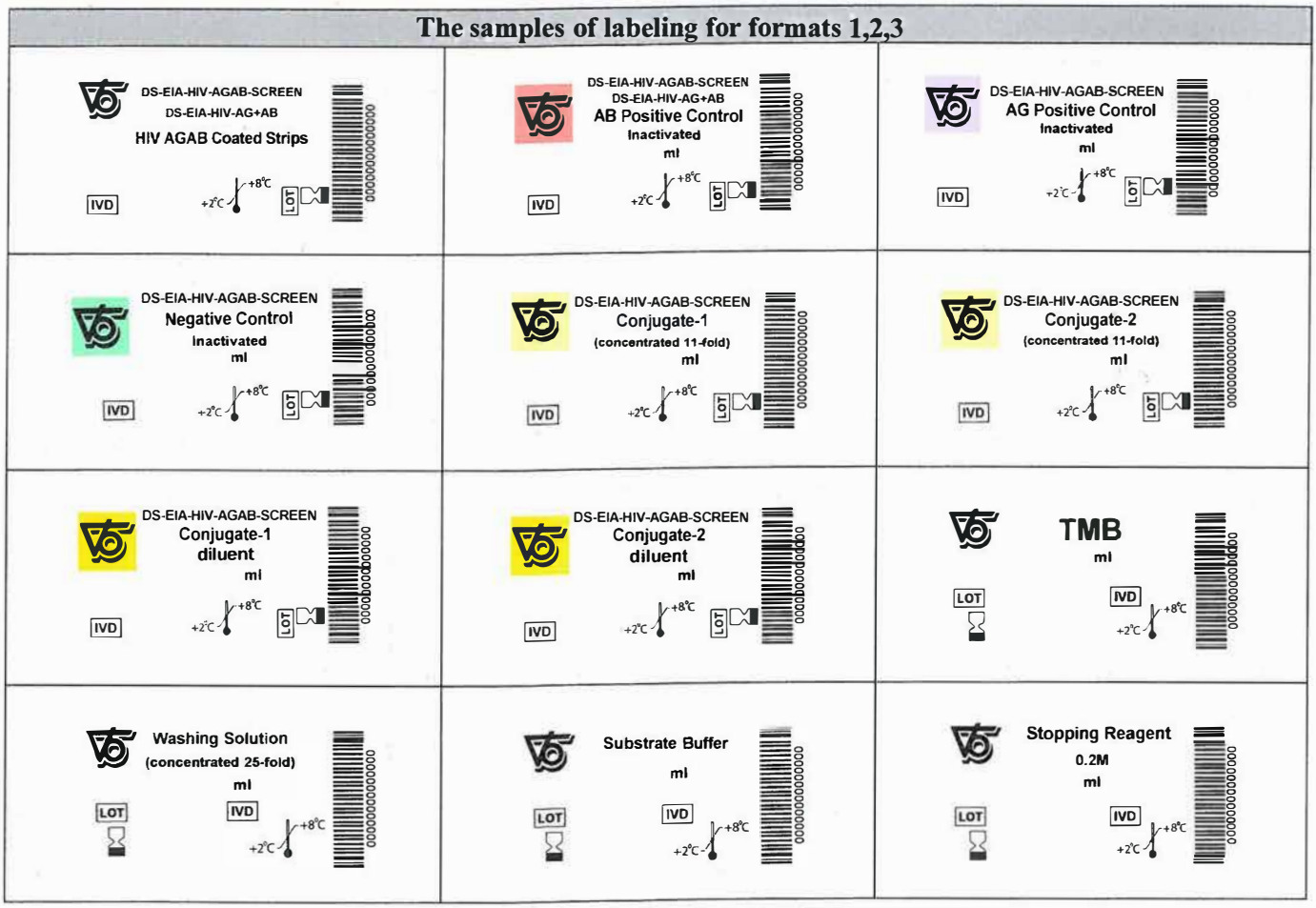
1. **Labels**
2. **Instructions for use**

1.1 Labels

Models of labeling

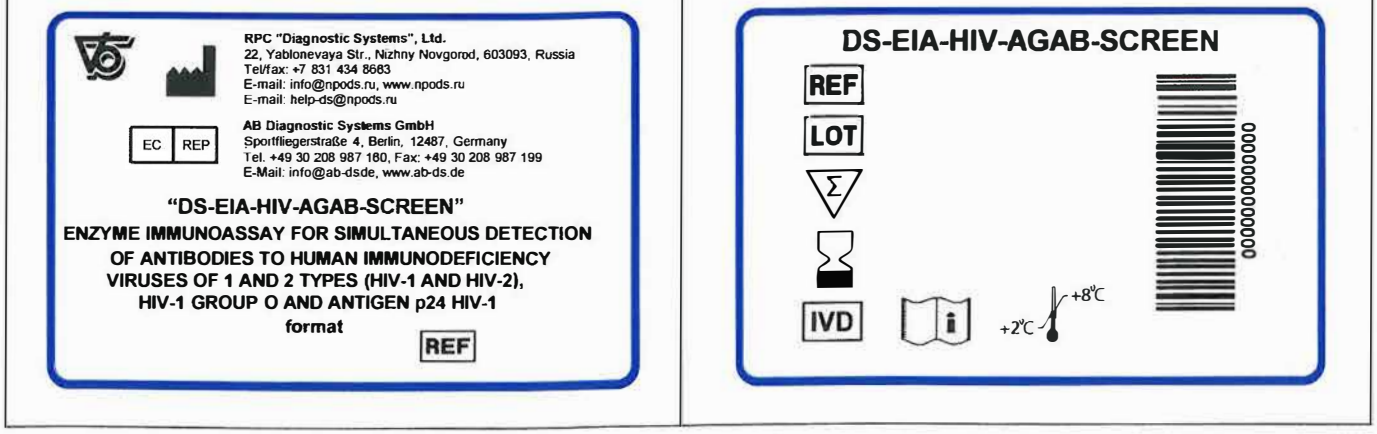
1. Inside labeling

The samples of labeling for formats 1,2,3



2. Outside labeling

The samples of labeling for formats 1,2,3



The sample of labeling for format 1

	Kit contents	format Σ	
1	HIV AGAB Coated Strips	plate	
2	Conjugate-1 (concentrated 11-fold)	ml	vial
3	Conjugate-2 (concentrated 11-fold)	ml	vial
4	Conjugate-1 diluent	ml	vial
5	Conjugate-2 diluent	ml	vial
6	AB Positive Control, Inactivated	ml	vial
7	AG Positive Control, Inactivated	ml	vial
8	Negative Control, Inactivated	ml	vial
9	Washing Solution	ml	vial
10	Substrate Buffer	ml	vial
11	TMB	ml	vial
12	Stopping Reagent	ml	vial

The sample of labeling for format 2

	Kit contents	format Σ	
1	HIV AGAB Coated Strips	plates	
2	Conjugate-1 (concentrated 11-fold)	ml	vial
3	Conjugate-2 (concentrated 11-fold)	ml	vial
4	Conjugate-1 diluent	ml	vial
5	Conjugate-2 diluent	ml	vial
6	AB Positive Control, Inactivated	ml	vial
7	AG Positive Control, Inactivated	ml	vial
8	Negative Control, Inactivated	ml	vial
9	Washing Solution	ml	vial
10	Substrate Buffer	ml	vial
11	TMB	ml	vial
12	Stopping Reagent	ml	vials
		or	
		ml	vial

The sample of labeling for format 3

	Kit contents	format Σ
1	HIV AGAB Coated Strips	plates
2	Conjugate-1 (concentrated 11-fold)	ml vials or ml vial
3	Conjugate-2 (concentrated 11-fold)	ml vials or ml vial
4	Conjugate-1 diluent	ml vials
5	Conjugate-2 diluent	ml vials
6	AB Positive Control, Inactivated	ml vials or ml vial
7	AG Positive Control, Inactivated	ml vials or ml vial
8	Negative Control, Inactivated	ml vials or ml vial
9	Washing Solution	ml vials
10	Substrate Buffer	ml vials or ml vials
11	TMB	ml vials or ml vials
12	Stopping Reagent	ml vials or ml vials

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 **I-1654/1.2** Σ **96**

REF **I-1652/1.2** Σ **192**

REF **I-1656/1.2** Σ **480**

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

INSTRUCTIONS FOR USE
"DS-EIA-HIV-AGAB-SCREEN"

ENZYME IMMUNOASSAY FOR SIMULTANEOUS DETECTION OF ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUSES OF 1 AND 2 TYPES (HIV-1 AND HIV-2), HIV-1 GROUP O AND ANTIGEN p24 HIV-1

Table of contents

I. INTENDED USE

"DS-EIA-HIV-AGAB-SCREEN" kit is a 'fourth-generation' immunoassay utilizing a mixture of antigens and antibodies for the qualitative simultaneous detection of IgG, IgM and IgA antibodies to major subtypes of HIV-1, including HIV-1 group O, HIV-2, and the HIV-1 p24 antigen at the early stage of infection in human serum or plasma. This kit is intended as an aid in the diagnosis of HIV-1 and/or HIV-2 infection, including acute or primary HIV-1 infection. Results from the "DS-EIA-HIV-AGAB-SCREEN" cannot be used to distinguish between the presence of HIV-1 p24 antigen, HIV-1 antibodies, or HIV-2 antibodies in a specimen. This kit is for diagnostic use by a trained laboratory professional and will not be sold to the general public.

The sensitivity of the kit is 0.5 IU/ml ("HIV-1 p24 ANTIGEN 1st international reference reagent", NIBSC Code: 90/636) for HIV-1 p24 antigen detection.

Recombinant antigens gp160 (HIV-1), gp41 (HIV-1 group O), p31 (HIV-1), gp36 (HIV-2) and antibodies to HIV-1 p24 antigen with the highest diagnostic potential were selected for test development.

II. CLINICAL VALUE

Detection of antibodies to HIV proteins or simultaneous detection of antibodies and p24 core protein is the most prevalent method of laboratory diagnostics of HIV infection. HIV antigens and antibodies appear and are detectable at different stages of the seroconversion and of the infection. Using a test-system for simultaneous detection of antibodies and p24 antigen allows reduction in the phase of serological window on the average to 4–6 days due to detection of p24 antigen, the earliest marker of HIV infection. The diagnostic significance of p24 antigen is important also at last stage of infection, when the functional ability of immune system is low.

The diagnostic efficiency of EIA tests depends on the sensitivity for p24 antigen detection.

III. PRINCIPLE OF THE TEST

"DS-EIA-HIV-AGAB-SCREEN" is a one-step "sandwich" assay based on microwells coated with recombinant antigens representing immunodominant regions of HIV-1, HIV-1 (O), HIV-2 proteins and antibodies raised against p24 of HIV-1. The Conjugate is a mixture of the viral antigen epitopes (gp41, p31 HIV-1, gp41 HIV-1 group O, gp36 HIV-2) all labelled with biotin and horseradish peroxidase, and monoclonal antibodies, also raised against p24 antigen labelled with biotin. Serum or plasma samples are added to the wells and if p24 antigens and/or antibodies specific for HIV-1, HIV-1 (O) and HIV-2 are present in the sample, they form stable complexes with the HIV antigens or antibodies immobilized in the well. Antigen-antibody complexes are then identified by the addition of: (1) biotinylated antigens and antibodies and; (2) horseradish peroxidase (HRP) streptavidin conjugate and HRP labelled antigens. The assay "sandwich" design minimises the potential for the "hook effect". Unbound conjugates are removed by washing. After adding of a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide wells with bound Conjugate develop a blue colour which is converted to an yellow colour which may be read at 450 nm or 450/620 nm after the reaction has been stopped with sulphuric acid.

IV. CONTENTS OF THE "DS-EIA-HIV-AGAB-SCREEN"

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION		
		Format 1	Format 2	Format 3
HIV AGAB Coated Strips	Polystyrene stripped 96-well plate (breakable wells) coated with mixture of purified recombinant antigens - gp160, p31 HIV-1, gp41 HIV-1 group O, gp36 HIV-2 and antibodies to antigen p24 HIV-1. Store at 2-8 °C until expiration date.	1 plate	2 plates	5 plates
Conjugate-1 (concentrated 11-fold)	Mixture of biotinylated recombinant antigens gp41, p31 HIV-1, gp41 HIV-1 group O, gp36 HIV-2 and antibodies to antigen p24 HIV-1. Transparent or slightly opalescence liquid of colourless or pale yellow colour. Preserving agent: 0.01% gentamicin sulfate, 0.02% thimerosal. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 1.2 ml	1 vial 1.2 ml	3 vials 1.2 ml or 1 vial 3.6 ml
Conjugate-2 (concentrated 11-fold)	Mixture of horseradish peroxidase labelled recombinant antigens gp41, gp36 HIV-2 and horseradish peroxidase labelled streptavidin. Transparent or slightly opalescence liquid of colourless or pale yellow colour. Preserving agent: 0.02% phenol. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.0 ml	1 vial 2.0 ml	2 vials 2.0 ml or 1 vial 4.0 ml
Conjugate-1 diluent	Transparent or slightly opalescent liquid, orange coloured, pellet dissolved at shaking may form. Preserving agent: 0.01% thimerosal, 0.01% gentamicin sulfate, 0.02% phenol. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 12.0 ml	1 vial 12.0 ml	3 vials 12.0 ml
Conjugate-2 diluent	Transparent or slightly opalescent liquid, blue coloured. Preserving agent: 0.01% thimerosal, 0.001% gentamicin sulfate. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 20.0 ml	1 vial 20.0 ml	2 vials 20.0 ml
AB Positive Control, Inactivated	Heat inactivated human plasma positive for anti-HIV, negative for antigen p24 HIV-1, HBsAg and anti-HCV. Transparent or slightly opalescent liquid, orange coloured. Preserving agent: 0.02% thimerosal, 0.01% gentamicin sulfate. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.0 ml	1 vial 2.0 ml	2 vials 2.0 ml or 1 vial 4.0 ml
AG Positive Control, Inactivated	Purified recombinant antigen p24 HIV-1 in heat inactivated human plasma negative for anti-HIV and anti-HCV, HBsAg, p24 HIV-1 antigen. Transparent or slightly opalescent liquid, crimson-red coloured. Preserving agent: 0.05% thimerosal, 0.01% gentamicin sulfate. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.0 ml	1 vial 2.0 ml	2 vials 2.0 ml or 1 vial 4.0 ml

Negative Control, Inactivated	Heat inactivated human plasma negative for antigen p24 HIV-1, HBsAg, anti-HIV and anti-HCV. Transparent or slightly opalescent liquid, green coloured. Preserving agent: 0.06% thimerosal. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.5 ml	1 vial 2.5 ml	2 vials 2.5 ml or 1 vial 5.0 ml
Washing Solution (concentrated 25-fold)	Transparent or slightly opalescent liquid, colourless or pale yellow, sediment may form that dissolves at 35-39 °C and shaking. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 50.0 ml	1 vial 120.0 ml	2 vials 120.0 ml
Substrate Buffer	Citric acid and sodium acetate solution, containing H_2O_2. Transparent colourless liquid. Preserving agent: 0.04% ProClin 300. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 25.0 ml	1 vial 25.0 ml	3 vials 25.0 ml or 2 vials 50.0 ml
TMB (concentrated 11-fold)	Solution containing Tetramethyl-benzidine (TMB). Transparent colourless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.5 ml	1 vial 2.5 ml	3 vials 2.5 ml or 2 vials 3.5 ml
Stopping Reagent	0.20 M/L sulphuric acid solution. Transparent colourless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 25.0 ml	2 vials 25.0 ml or 1 vial 50.0 ml	4 vials 25.0 ml or 2 vials 50.0 ml
Protective film for EIA plates		2 pcs	4 pcs	10 pcs
Plastic clip or polyethylene bag with a zip lock		1 pc	2 pcs	3 pcs
Plastic dish for liquid reagents		2 pcs	4 pcs	10 pcs
Disposable tips		16 pcs	32 pcs	80 pcs

V. PRECAUTIONS

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

- 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 are lost or labels/vials are damaged, vials should be disposed, and the 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.

- Never use the same container to distribute conjugate and other solutions.
- The enzyme reaction is very sensitive to metallic ions. Consequently, do not allow any metal element to come into contact with various conjugate or substrate solutions.
- Do not reuse HIV AGAB 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.
- 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 development solution.
- 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.

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 has been tested and found non reactive for hepatitis B surface antigen (HBsAg), antigen p24 HIV-1, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2).
- Human origin material used in the preparation of the AB Positive Control has been tested and found non reactive for hepatitis B surface antigen (HBsAg), antigen p24 HIV-1 and antibodies to hepatitis C virus.
- Human origin material used in the preparation of the AG Positive Control has been tested and found non reactive for hepatitis B surface antigen (HBsAg), antigen p24 HIV-1 and antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2).
- As there is no known test method ensuring the absence of infectious agents, handle the reagents and patients samples as if they are infection carriers.
- 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 samples 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. Wipe spills immediately and decontaminate affected surfaces.
- Avoid any contact of the Substrate Buffer, the TMB and the Stopping Reagent 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 °C 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 °C 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.

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

- Deionised or distilled water.
- Automatic or semiautomatic, adjustable or preset pipettes or multipipettes.
- Disposable pipette tips.
- Medical disposable gloves.
- Microplate incubator or shaker thermostatically set at (37.0 ± 1.0) °C.
- Automatic microplate washer.
- Microplate reader equipped with 450 nm and with 620-680 nm filters.

VIII. COLLECTION AND HANDLING OF SAMPLES

Collect blood samples according to the current practices. The test should be performed on undiluted serum or heparin/EDTA/citrate plasma. Separate the serum or plasma from the clot or red cells as soon as possible to avoid any haemolysis. Extensive haemolysis may affect test performance. Samples with observable particulate matter should be clarified by centrifugation prior testing. Suspended fibrin particles or aggregates may yield false positive results. Do not heat the samples. The samples can be stored at 2-8 °C if testing is performed within 3 days or they may be deep-frozen at -20 °C or below and stored within several months. The plasma must be quickly thawed by warming for a few minutes in a water bath at 40 °C (to avoid fibrin precipitation). Due to HIV Ag instability, the temperatures over 40 °C are not allowed. Avoid repeated freeze/thaw cycles. Samples that have been frozen and thawed more than 3 times cannot be used. Do not use contaminated, hyperlipaemic or hyperhaemolysed sera or plasma which has been preserved by sodium azide.

IX. PREPARATION OF THE REAGENTS

1. Ready for use reagents:

- Negative Control
- AB Positive Control
- AG Positive Control
- Conjugate-1 diluent
- Conjugate-2 diluent
- Stopping Reagent

2. Reagents to prepare:

- **HIV AGAB Coated Strips.** Each plate containing 12 strips is wrapped in a sealed foil-lined bag. Open the bag and remove the plate. Select the number of coated strips required for the assay. Return the unused strips in the bag. After the airtight foil pack has been opened, the strips are stable until the kit expiration date at 2-8 °C, provided that the foil pack is resealed with the clip or the foil-lined bag is resealed in zip locked polyethylene bag. The silica gel bag should not be removed from the foil packaging.
- **Working Washing Solution.** Thoroughly mix the contents of the vial with the Washing Solution (concentrated 25-fold). Dilute the required volume of the concentrated Washing Solution with the corresponding volume of purified water prior to use (See Tables 2 and 3). Mix thoroughly by inversion. The prepared Washing Solution is stable at least 14 days at 18-24 °C or for 28 days at 2-8 °C.
- **Working Solution of Conjugate-1.** Prepare before use. Dilute the necessary volume of thoroughly mixed Conjugate-1 (concentrated 11-fold) with the corresponding volume of Conjugate-1 diluent (See Tables 2 and 3). Mix thoroughly until diluted, avoiding foaming. Do not apply intensive mixing. Keep the Working Solution of Conjugate-1 within not less than 10 min at 18-24 °C before use. Working Solution of the Conjugate-1 is stable at least 12 hours at 18-24 °C in dark place.
- **Working Solution of Conjugate-2.** Prepare before use. Dilute the necessary volume of thoroughly mixed Conjugate-2 (concentrated 11-fold) with the corresponding volume of Conjugate-2 diluent (See Tables 2 and 3). Mix thoroughly until diluted, avoiding foaming. Do not apply intensive mixing. Keep the Working Solution of Conjugate-2 within not less than 10 min at 18-24 °C before use. Working Solution of the Conjugate-2 is stable at least 12 hours at 18-24 °C in dark place.
- **Substrate Mixture.** Prepare before use. Dilute the required volume of TMB (concentrated 11-fold) with the corresponding volume of Substrate Buffer (1 : 10 ratio) (See Tables 2 and 3). Mix thoroughly until dilution. Substrate Mixture is stable at least 10 hours when stored in a dark place at 18-24 °C in clean vials or a special container intended for EIA procedure using an automated analyzer.

  Substrate Mixture should be colourless!

3. Storage of unused reagents:

After opening the vials the unused reagents of the kit: HIV AGAB Coated Strips, Conjugate-1 (concentrated 11-fold), Conjugate-2 (concentrated 11-fold), Negative Control, AB and AG Positive Controls, Conjugate-1 diluent, Conjugate-2 diluent, Washing Solution (concentrated 25-fold), Substrate Buffer, Stopping Reagent, TMB (concentrated 11-fold) can be stored in tightly sealed vials until the kit expiration date at 2-8 °C.

X. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature (18 – 24 °C) for 30 min.
The required volumes of reagents depending on the number of used strips/wells are represented in the tables 2 and 3.
The volumes mentioned in Table 3 are recommended and may vary depending on the instrument used.

Table 2

Consumption of reagents for the manual test-procedure

Number of strips to be used	Working Washing Solution		Working Solution of Conjugate-1		Working Solution of Conjugate-2		Substrate Mixture	
	Washing Solution (concentrated 25-fold) (ml)	Purified Water (ml)	Conjugate-1 (concentrated 11-fold) (ml)	Conjugate-1 diluent (ml)	Conjugate-2 (concentrated 11-fold) (ml)	Conjugate-2 diluent (ml)	Substrate Buffer (ml)	TMB (concentrated 11-fold) (ml)
1	3.0	72.0	0.03	0.3	0.05	0.5	1.0	0.1
2	6.0	144.0	0.06	0.6	0.10	1.0	2.0	0.2
3	9.0	216.0	0.09	0.9	0.15	1.5	3.0	0.3
4	12.0	288.0	0.12	1.2	0.20	2.0	4.0	0.4
5	15.0	360.0	0.15	1.5	0.25	2.5	5.0	0.5
6	18.0	432.0	0.18	1.8	0.30	3.0	6.0	0.6
7	21.0	504.0	0.21	2.1	0.35	3.5	7.0	0.7
8	24.0	576.0	0.24	2.4	0.40	4.0	8.0	0.8
9	27.0	648.0	0.27	2.7	0.45	4.5	9.0	0.9
10	30.0	720.0	0.30	3.0	0.50	5.0	10.0	1.0
11	33.0	792.0	0.33	3.3	0.55	5.5	11.0	1.1
12 (a plate)	40.0	960.0	0.60	6.0	0.70	7.0	12.0	1.2

Table 3

Consumption of reagents for the test procedure using automatic analyzer for EIA

Number of strips to be used	Working Washing Solution		Working Solution of Conjugate-1 (ml)		Working Solution of Conjugate-2 (ml)		Substrate Mixture	
	Washing Solution (concentrated 25-fold) (ml)	Purified Water (ml)	Conjugate-1 (concentrated 11-fold) (ml)	Conjugate-1 Diluent (ml)	Conjugate-2 (concentrated 11-fold) (ml)	Conjugate-2 Diluent (ml)	Substrate Buffer (ml)	TMB (concentrated 11-fold) (ml)
4 (for format 1 only)	16.0	384.0	0.40	4.0	0.45	4.5	7.0	0.7
8 (for format 1 only)	32.0	768.0	0.55	5.5	0.7	7.0	12.0	1.2
12 (a plate) (for all formats)	40.0	960.0	0.6	6.0	0.7	7.0	12.0	1.2

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

EIA procedure (Procedure 1 - microplate shaker thermostatically)

1. Add 30 µl of Working Solution of Conjugate-1 in each well. The Working Solution of Conjugate-1 should be added to the wells directly before addition of the control samples and tested samples. Depending on the quantity of strips used in the assay it is recommended to add 70 µl of controls per well as following:

1-2 strips/up to 16 wells (including control wells) – AB Positive Control in 1 well, AG Positive Control in 1 well, Negative Control in 2 wells;

3 strips and more/17 wells and more (including control wells) – AB Positive Control in 1 well, AG Positive Control in 1 well, Negative Control in 3 wells.

Add 70 µl of undiluted samples into the rest of the wells. Orange colour of working solution of conjugate-1 should change to pink after samples have been added. At testing samples of sera or plasma with acid pH orange colour of working solution of conjugate-1 may be change to yellow. At testing samples of sera or plasma with neutral pH change of conjugate-1 colour may be absent.

2. Incubate samples with Working Solution of Conjugate-1 for 45 minutes in shaker at 500 rpm at (37.0 ± 1.0) °C.

3. Without removing the contents of the wells and washing the wells add 50 µl of Working Solution of Conjugate-2 into each well. Avoid contact with the liquid surface during the pipetting, or use a new tip to prevent the contamination. The pink colour should change to green. Incubate plate for 20 minutes in shaker at 500 rpm at (37.0 ± 1.0) °C.

4. 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 aspire. Perform this procedure four times.** Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision.

5. Add 100 µl of Substrate Mixture into each well. Keep uncovered plates in the dark place for 20 minutes at 18-24 °C or for 15 minutes at (37.0 ± 1.0) °C.

6. Add 150 µl of Stopping Reagent into each well and read the optical density after at least 3-4 minutes at 450/620-680 nm using a plate reader. Reading the absorbance at 450 nm only is possible.

Scheme of the assay is represented in Annex.

EIA procedure (Procedure 2 - microplate incubator)

1. Add 30 µl of Working Solution of Conjugate-1 in each well. The Working Solution of Conjugate-1 should be added to the wells directly before addition of the control samples and tested samples. Depending on the quantity of strips used in the assay it is recommended to add 70 µl of controls per well as following:

1-2 strips/up to 16 wells (including control wells) – AB Positive Control in 1 well, AG Positive Control in 1 well, Negative Control in 2 wells;

3 strips and more/17 wells and more (including control wells) – AB Positive Control in 1 well, AG Positive Control in 1 well, Negative Control in 3 wells.

Add 70 µl of undiluted samples into the rest of the wells. Orange colour of working solution of conjugate-1 should change to pink after samples have been added. At testing samples of sera or plasma with acid pH orange colour of working solution of conjugate-1 may be change to yellow. At testing samples of sera or plasma with neutral pH change of conjugate-1 colour may be absent.

2. Mix the contents of the wells by careful tapping on the edge of the plate, then cover the plate with the protective film and incubate in a microplate incubator for 60 minutes at (37.0 ± 1.0) °C.

3. Without removing the contents of the wells and washing the wells add 50 µl of Working Solution of Conjugate-2 into each well. Avoid contact with the liquid surface during the pipetting, or use a new tip to prevent the contamination. The pink colour should change to green. Mix the contents of the wells by careful tapping on the edge of the plate, then cover the plate with the protective film and incubate in microplate incubator for 30 minutes at (37.0 ± 1.0) °C.

4; 5; 6 As in the Procedure 1 **4; 5; 6.**

Scheme of the assay is represented in Annex.

XI. AUTOMATED ANALYZERS

Validated protocols for automated analyzers can be obtained from your representative. For the instrumentation without established validated protocol follow the section "TEST PROCEDURE" and ensure all requirements described in the section "PRECAUTIONS" are fulfilled. All protocols for automated analyzers must be fully validated before use.

XII. SPECTROPHOTOMETRIC VERIFICATION

1. We recommend to verify the Conjugate-1 and tested samples dispensing at wavelength of 450 nm, criterion: OD > 0.450 (a lower OD indicates poor dispensing of the Conjugate 1 or the tested sample).

2. We recommend to verify the Conjugate-2 dispensing at wavelength of 620 nm, criterion: OD > 0.110 (a lower OD indicates poor dispensing of the Conjugate 2).

3. We recommend to verify the Substrate Mixture dispensing at wavelength of 405 nm, criterion: OD > 0.050 (a lower OD indicates poor dispensing of the Substrate Mixture).

XIII. RESULTS

Read plate within 10 minutes of addition of the Stopping Reagent.

The presence or absence of detectable HIV-1 p24 antigen or antibodies to HIV-1 and/or HIV-2 is determined by comparing the absorbance measured for each sample to the calculated cut-off value.

For the assay to be valid, OD (optical density) values of each Negative Control must be not greater than 0.200 and in wells with AB Positive Control and AG Positive Control – not less than 0.800.

Reactive sample: sample OD value ≥ cut-off

Samples with absorbance values more or equal to the cut-off value are considered to be positive in "DS-EIA-HIV-AGAB-SCREEN".

Non-reactive sample: sample OD value < cut-off

Samples with absorbance values less than the cut-off value are considered to be negative in "DS-EIA-HIV-AGAB-SCREEN".

Calculate Cut-Off value as:

Cut-Off = average OD value of Negative Control + 0.150

0.150 – is a coefficient defined by manufacturer. The coefficient may change provided that the performance characteristics are maintained. The coefficient value is specified for each lot and indicated in the Instructions for use and in the Certificate of Analysis issued by the Quality Control Department.

XIV. PERFORMANCE CHARACTERISTICS

Analytical sensitivity

Different dilutions of "HIV-1 ANTIGEN STANDARD" (BIO-RAD, France, cat.№ 72217) and "HIV-1 p24 ANTIGEN 1st International Reference Reagent", NIBSC Code: 90/636 have been used for evaluation of analytical sensitivity of the assay. As shown in table 4 the sensitivity limit has been estimated at 10 pg/ml HIV-1 antigen ("HIV-1 ANTIGEN STANDARD", BIO-RAD France) and 0.5 IU/ml ("HIV-1 p24 ANTIGEN 1st International Reference Reagent", NIBSC).

Table 4

Evaluation of the "DS-EIA-HIV-AGAB-SCREEN" kit sensitivity

HIV- 1 ANTIGEN STANDARD (BIO – RAD)		**HIV-1 p24 ANTIGEN 1st international reference reagent**	
pg/ml	OD/CO	IU/ml	OD/CO
80	**10.8**	4	**11.0**
40	**5.2**	2	**5.4**
20	**2.7**	1	**2.9**
10	**1.2**	0.5	**1.4**
5	0.6	0.25	0.7

Analytical sensitivity of the "DS-EIA-HIV-AGAB-SCREEN" was investigated with BBI - HIV Antigen Sensitivity Panel № 801. Sensitivity limit has been estimated at 0.5 IU/ml antigen p24 HIV-1.

Diagnostic sensitivity of the test "DS-EIA-HIV-AGAB-SCREEN"

1. 3276 patients infected with HIV-1 and 107 patients infected by HIV-2 have been tested and found positives with the kit "DS-EIA-HIV-AGAB-SCREEN". Kit demonstrated 100% sensitivity with these samples.
2. 38 commercial seroconversion panels (BBI, ZeptoMetrix, USA) were studied. The results presented in the table below were excellent (See Table 5).
3. Performance panel WWRP 302 (M), containing samples of the HIV-1 M group (subtype A to G), one sample of subtype O, one recombinant form, three samples of HIV-2. The sensitivity is 100%.
4. 1st International Reference Panel for anti-HIV, NIBSC Code: 02/210. The sensitivity is 100%.
5. Anti-HIV 1/2 Combo Performance panel BBI PRZ206. The sensitivity is 100%.
6. HIV 1 Incidence/Prevalence Performance panel BBI PRB601. The sensitivity is 100%.
7. Anti-HIV 1 Mixed Titer Performance Panel PRB204 (M). The sensitivity is 100%.
8. Anti-HIV 1/2 Qualification Panel BBI QRZ 761. The sensitivity is 100%.

Table 5

Diagnostic sensitivity of the test "DS-EIA-HIV-AGAB-SCREEN" when testing seroconversion panels

Panel	DS-EIA-HIV-AGAB-SCREEN	4th generation HIV AG/AB kit, (CE-marked)
	The number of positive results compare with number of tested samples	
BBI PRB926	4/6	NT
BBI PRB929	5/7	5/7
BBI PRB930	4/4	4/4
BBI PRB931	5/9	4/9
BBI PRB934	3/3	3/3
BBI PRB939	4/9	3/9
BBI PRB940	8/8	7/8
BBI PRB941	4/6	4/6
BBI PRB942	1/4	1/4
BBI PRB944	5/6	4/6
BBI PRB947	3/4	3/4
BBI PRB948	2/4	1/4
BBI PRB949	2/5	2/5
BBI PRB951	4/6	4/6
BBI PRB952	4/6	4/6
BBI PRB960	2/9	2/9
BBI PRB961	2/9	2/9
BBI PRB962	2/6	2/6
BBI PRB963	2/7	2/7
BBI PRB964	1/6	1/6
BBI PRB965	5/6	5/6
BBI PRB966	3/10	3/10
ZMC HIV6240	7/13	6/13
ZMC HIV6244	3/15	3/15
ZMC HIV6245	6/11	6/11
ZMC HIV6247	4/10	4/10
ZMC HIV6246	9/21	8/21
ZMC HIV9079	17/25	17/25
ZMC HIV12008	6/13	5/13
ZMC HIV9077	17/28	17/28
ZMC HIV6243	4/9	4/9

ZMC HIV9022	3/9	2/9
ZMC HIV9032	8/14	5/14
ZMC HIV9030	3/16	NT
ZMC HIV9021	4/17	NT
ZMC HIV9018	2/11	NT
ZMC HIV9017	4/6	3/6
ZMC HIV9014	6/7	5/7
Total	**181/372**	**152/312**

Specificity of the test "DS-EIA-HIV-AGAB-SCREEN"

Specificity of the test "DS-EIA-HIV-AGAB-SCREEN" is shown in Table 6 (results of RPC "Diagnostic Systems").

Table 6

Specificity of the kit "DS-EIA-HIV-AGAB-SCREEN"

№	The category of samples	The number of tested samples	Specificity, %
1	Unselected donors (including 1st time donors)	5640	99.61
2	Pregnant women	364	99.73
3	Clinical patients	273	99.63
4	Samples of patients with rheumatoid factor	182	99.45
5	Samples of patients with hepatitis viruses HBV, HAV, HCV, flu, pneumonia, tonsillitis, herpetic and cytomegalovirus infections, syphilis, chlamydiosis	273	99.63
Total		**6732**	**99.61**

Reproducibility

Within-plate reproducibility of one lot was evaluated by testing 3 positive samples 25 times each. The variation coefficient did not exceed 5%.

Between-plate reproducibility was evaluated by testing 3 positive samples 25 times each using 3 different lots. The variation coefficient did not exceed 5%.

Reproducibility between different lots, operators, days, laboratories was evaluated by testing 3 positive samples 25 times each using 3 different lots. The variation coefficient did not exceed 5%.

XV. LIMITS OF THE TEST

- The "DS-EIA-HIV-AGAB-SCREEN" assay procedure and the interpretation of results must be followed when testing serum, plasma for the presence of anti-HIV-1,2 and antigen p24 HIV-1. The user of this kit is advised to read the Instructions for use carefully before running 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 anti-HIV-1,2 or HIV-1 p24 antigen based on a single reactive test results. The reactive results should be re-tested and then confirmed by supplementary assays.
- A negative result with a screening assay does not exclude the possibility of exposure or infection by Human Immunodeficiency Viruses.
- 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 section "PERFORMANCE CHARACTERISTICS" of this package insert for assay performance characteristics.
- Effect of hyperproteinaemia and hyperbilirubinaemia on test performance of "DS-EIA-HIV-AGAB-SCREEN" was not investigated.
- 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.
- 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. False negative results may also occur in patients on anti-retroviral therapy.
- The variability of HIV virus does not allow excluding the possibility of false negative results. There is no known test method completely ensuring the absence of HIV.
- Performances of the test have not been evaluated on biological fluid other than serum or plasma.

XVI. CONDITIONS OF STORAGE AND TRANSPORTATION

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

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

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XVII. REFERENCES

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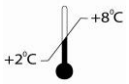
XVIII. EXPLANATION OF SYMBOLS

	For *in vitro* diagnostic use
	Manufacturer
	Authorized representative in the European Community
	Catalog number
	Sufficient for
	Batch code
	Storage temperature limitation
	Expiry date CCYY-MM-DD
	Consult Instruction for use

Annex

Scheme of the assay

1	**Add**	30 µl of Working Solution of Conjugate-1
2	**Add**	70 µl of AG Positive Control, AB Positive Control, Negative Control
3	**Add**	70 µl of tested samples
4	**Incubate**	**Procedure 1:** 45 min at (37.0 ± 1.0) °C, 500 rpm, microplate shaker thermostatically **Procedure 2:** 60 min at (37.0 ± 1.0) °C, microplate incubator
5	**Add**	50 µl of Working Solution of Conjugate-2
6	**Incubate**	**Procedure 1:** 20 min at (37.0 ± 1.0) °C, 500 rpm, microplate shaker thermostatically **Procedure 2:** 30 min at (37.0 ± 1.0) °C, microplate incubator
7	**Wash the plate**	**Working Washing Solution, not less than 400 µl, at least 40 seconds, 4 times**
8	**Add**	100 µl of Substrate Mixture
9	**Incubate**	20 min at 18-24 °C in the dark place or 15 min at (37.0 ± 1.0) °C
10	**Add**	150 µl of Stopping Reagent
11	**Read the optical density**	450 nm/620-680 nm or 450 nm