

Genscreen™ HIV-1/2 Version 2

1 plate - 96 tests

72278

5 plates - 480 tests

72279

**SCREENING KIT FOR THE DETECTION OF ANTIBODIES TO HIV-1
AND HIV-2 IN SERUM/PLASMA BY ENZYME IMMUNOASSAY**

IVD

Manufacturer quality control

All manufactured and commercialised reagents are under complete quality system starting from reception of raw material to the final commercialisation of the product.

Each lot is submitted to a quality control and only is released on the market when conforming to the acceptance criteria.

The records relating to production and control of each single lot are kept within our company.

BIO-RAD

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1 - CLINICAL VALUE

The acquired immunodeficiency syndrome (AIDS) is a virus induced infectious disease characterised by strongly depressed immunity.

Two types of viruses related to the Lentivirus group have been isolated from lymphocytes of patients suffering from AIDS or its prodromes. The first one, named HIV-1, was isolated in France then in the United States. The second one, named VIH-2 or HIV-2 was isolated from two patients living in Africa and has proved to be responsible for a new AIDS focus in West Africa.

Knowledge on genetic variability of the HIV virus strains were acquired by sequencing the GAG, POL, and ENV genes of the representative strains of each subtype. The HIV-1 viruses are divided into 2 groups : M group, including 9 sub-types (A to I) and O group. The HIV-2 virus includes 5 sub-types. The geographical distribution of the different sub-types are now quite well defined. Some HIV-1 variants have only 70% homology for the GAG and POL genes with the main isolates and only 50% for the ENV gene, these differences can explain the failure of the infection diagnosis in some patients.

The various VIH-2 or HIV-2 isolates share common antigens with the SIV simian virus in all proteins (envelope proteins and core proteins: heterology = 30%), but exhibit less than 40% homology with the HIV-1 envelope proteins.

The Genscreen™ HIV-1/2 Version 2 allows the simultaneous detection of anti HIV-1 and anti HIV-2 antibodies.

2 - PRINCIPLE OF THE Genscreen™ HIV-1/2 Version 2 KIT

Genscreen™ HIV-1/2 Version 2 is an enzyme immunoassay based on the principle of the two-step sandwich technique for the detection of the various antibodies associated with HIV-1 and/or HIV-2 virus in human serum or plasma.

Genscreen™ HIV-1/2 Version 2 is based upon the use of a solid phase coated with purified antigens (gp160 and p25 recombinant proteins of HIV-1 and a peptide mimicking the immunodominant epitope of the HIV-2 envelope protein) and of an antigens - peroxidase conjugate (peptides mimicking the immunodominant epitopes of the HIV-1 and HIV-2 envelope glycoproteins, and nucleocapsid recombinant protein).

The assay procedure includes the following reactional steps :

1. The serum samples and control sera to be assayed are pipetted into the microplate wells. HIV-1 and/or HIV-2 antibodies, if any, bind to the antigens immobilized on the solid phase.

Sample deposition is validated through a colour change, from purple to blue (SDP= Sample Deposition Proof).

2. Peroxidase labelled purified HIV-1 and HIV-2 antigens are added. They bind in turn to the IgG, IgM or IgA, captured on the solid phase, from the patient samples.

3. The presence of the enzyme immobilized on the complexes is shown by incubation in the presence of the substrate, after the unbound conjugate fraction has been removed.

4. The reaction is stopped and the absorbances are read using a spectrophotometer at 450/620-700 nm.

The absorbance measured on a sample allows the presence or absence of HIV-1 and/or HIV-2 antibodies to be determined.

3 - CONTENTS OF THE Genscreen™ HIV-1/2 Version 2 KIT

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

LABEL		NATURE OF THE REAGENT	PRESENTATION	
			72278	72279
R1	Microplate	Microplaque 12 strips of 8 wells coated with purified HIV-1 and HIV-2 antigens	1 plate	5 plates
R2	Concentrated Washing Solution (20X)	Concentrated Washing Solution (20X) Tris NaCl Buffer pH 7.4 Preservative: ProClin™ 300 (0.04%)	1 vial 70 ml	1 vial 235 ml
R3	Negative Control	Negative Control Heat inactivated human plasma negative for HBs antigen, HIV antigen, anti HIV-1, anti HIV-2 and anti HCV antibodies Preservative: Sodium azide < 0.1%	1 vial 1 ml	1 vial 1 ml
R4	Cut-off Control	Cut-off Control Heat inactivated human plasma positive for anti HIV antibodies, negative for HBs antigens and anti HCV antibodies Preservative: Sodium azide < 0.1%	1 vial 2.5 ml	1 vial 2.5 ml
R5	Positive Control	Positive Control Heat inactivated human plasma positive for anti HIV antibodies, negative for HBs antigens and anti HCV antibodies Preservative: Sodium azide < 0.1%	1 vial 1 ml	1 vial 1 ml
R6	Sample Diluent	Sample Diluent Calf serum solution (Tris buffer with 0.1% chloroform and ProClin™ 300, and coloured indicator)	1 vial 14 ml	2 vials 2 x 10 ml
R7a	Conjugate	Conjugate Lyophilised peroxidase labelled purifies HIV-1 and HIV-2 antigens	1 vial sqf 12.5 ml	2 vials sqf 2 x 30 ml
R7b	Conjugate Diluent	Conjugate Diluent Skimmed milk solution (Tris buffer with 0.1% chloroform and ProClin™ 300)	1 vial 12.5 ml	2 vials 2 x 30 ml
R8	Peroxidase Substrate Buffer	Peroxidase Substrate Buffer Sodium citrate and sodium acetate solution pH 4.0 containing H ₂ O ₂ (0.015%) and DMSO (4%)	1 vial 60 ml	2 vials 2 x 60 ml
R9	Chromogen	Chromogen Solution containing tetramethylbenzidine (TMB)	1 vial 5 ml	2 vials 2 x 5 ml
R10	Stopping Solution	Stopping Solution 1N sulfuric acid solution	1 vial 28 ml	3 vials 3 x 28 ml

4 - STORAGE CONDITIONS - SHELF LIFE

The kit should be stored at +2-8°C. When stored at this temperature, each reagent contained in the Genscreen™ HIV-1/2 Version 2 kit can be used after a first opening until the expiry date mentioned on the package except specific instruction :

R1: After the vacuum-sealed bag has been opened, the microwell strips stored at +2-8°C in the carefully resealed bag can be used for 1 month.

R2: The diluted washing solution can be stored at +2-30°C during 2 weeks. The concentrated washing solution (R2) can be stored at +2- 30°C.

R7a + R7b: The reagents stored at +2 - 8°C can be used for 4 weeks after the vials have been opened and reconstituted.

R8 + R9: After the reconstitution, the reagent stored in the dark can be used for 6 hours at room temperature (18-30°C).

5 - MATERIAL REQUIRED BUT NOT PROVIDED

- Distilled water.
- Sodium hypochlorite (household bleach) and sodium bicarbonate.
- Automatic or semiautomatic, adjustable or preset pipettes or multipipettes to measure and dispense 25 µl, 75 µl, 80 µl and 200 µl.
- Graduated cylinders of 25 ml; 100 ml; 1000 ml capacity.
- Container for biohazardous waste.
- Water-bath or equiv. microplate incubator, thermostatically set at 37°C ± 1°C (*).
- Manual, semiautomatic or automatic microplate washer (*).
- Microplate reader equipped with 450 nm and 620-700 nm filters (*).
(Contact our technical services for monochromatic procedure).
- Absorbent paper.

(* Consult us for detailed information about the equipment recommended by our technical department.

6 - COLLECTION AND HANDLING OF SPECIMENS

Collect a blood sample according to the current practices. The test should be performed on undiluted serum or plasma (collected with EDTA, heparin, citrate, ACD-based anticoagulants). 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. Specimens with observable particulate matter should be clarified by centrifugation prior testing. Suspended fibrin particles or aggregates may yield falsely positive results.

Do not heat the samples.

The specimens can be stored at +2-8°C if screening is performed within 7 days or they may be deep-frozen at -20°C. The plasma must be quickly thawed by warming for a few minutes in a water bath at 40°C (to avoid fibrin precipitation).

Avoid repeated freeze/thaw cycles. Samples that have been frozen and defrozen more than 3 times cannot be used. If the specimens are to be shipped, they must be packaged in accordance with the regulations in force regarding the transport of aetiological agents.

DO NOT USE CONTAMINATED, HYPERLIPAEMIC OR HYPEHAEMOLYSED SERA OR PLASMA.

REMARK: Samples containing up to 90 g/l albumin, 200 mg/l bilirubin, lipemic samples containing up to the equivalent of 36 g/l triglyceride, and hemolyzed samples containing up to 20 g/l hemoglobin do not affect the results.

7 - PRECAUTIONS

The reliability of the results depends on correct implementation of the following Good Laboratory Practice :

- The name of the test, as well as a specific identification number for the test, are written on the frame of each microtiterplate. This specific identification number is stated on each strip too.

GenSCREEN™ HIV 1/2 Version 2: Specific ID number = 05

Verify the specific identification number before use. If the identification number is missing, or different from the stated number above, the strip should not be used.

- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.

*Remark: For washing solution (R2, label identification : 20X coloured green), peroxidase substrate buffer (R8, label identification: TMB buf, coloured blue), chromogen (R9, label identification: TMB 11X, coloured purple) and stopping solution (R10, label identification: 1N coloured red), it is possible to use other lots than those contained in the kit, provided the same lot is used within a given test run. These reagents can be used with some other products of our company. In addition, the wash solution (R2, label identification: **20X coloured green**) can be mixed with the 2 other wash solutions included in various Bio-Rad Reagent kits (R2, label identifications : 10X coloured blue or 10X coloured orange) when properly reconstituted, provided only one mixture is used within a given test run. Contact our technical service for detailed information.*

- The development solution (substrate buffer + chromogen) must be coloured pink. The modification of this pink colour within a few minutes after reconstitution indicates that the reagent cannot be used and must be replaced. Preparation of the development solution can be made in a clean disposable single use plastic tray or glass container that has first been pre-washed with 1N HCl and rinsed thoroughly with distilled water and dried. This reagent must be stored in the dark.
- Before use, wait 10 minutes for the reagents to stabilize to room temperature.
- Carefully reconstitute the reagents.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay procedure.
- Washing: carefully follow the washing procedures described to obtain maximum test performance.

Health and safety instructions

Positive and cut-off control are heat inactivated.

Human origin material used in the preparation of the negative control has been tested and found non reactive for hepatitis B surface antigen (HBs Ag), antibodies to hepatitis C, and antibodies to human immunodeficiency virus (HIV-1 and HIV-2).

Human origin material used in the preparation of the positive control and cut-off serum has been tested and found non reactive for hepatitis B surface antigen (HBs Ag) and antibodies to hepatitis C.

Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.

Any equipment directly in contact with specimens and reagents as well as the washing solutions should be considered as contaminated products and treated as such.

Autoclaving for at least one hour at 121°C, is the best method to inactivate the HIV viruses and the HB virus.

DO NOT PLACE SOLUTIONS CONTAINING SODIUM HYPOCHLORITE IN THE AUTOCLAVE.

The Safety Data Sheet is available upon request.

The treatment of contaminated solutions and equipment with sodium hypochlorite at the final concentration of 5% for 30 minutes also allows the inactivation of the HIV viruses and of the HB virus.

Avoid any contact of the substrate buffer, the chromogen and the stopping solution with the skin and mucosa (toxicity, irritation or burn hazard).

Chemicals should be handled and disposed of in accordance with Good Laboratory Practice.

Some reagents contain sodium azide as a preservative. Sodium azide may react with laboratory plumbing to form copper or lead azides. Such azides are explosive. To prevent azide build-up, flush the pipes with a large quantity of water if solutions containing azide are disposed of in the sink after inactivation.

Some reagents contain ProCin™ 300 (0.04%, 0.1% and/or 0.5%).



Xi Irritant

R43 : may cause sensitisation by skin contact.

S28-37 : After contact with skin, wash immediately with plenty of soap and water.

Wear suitable gloves.

8 - RECONSTITUTION OF THE REAGENTS

Note : Before use, allow reagents to reach room temperature (18-30°C).

Reagent 1 (R1): Microplate

Each frame support containing 12 strips is wrapped in a sealed foil bag. Cut the bag using scissors or a scalpel 0.5 to 1 cm above the sealing. Open the bag and take out the frame. Put the unused strips back into the bag. Close the bag carefully and put it back into storage at +2-8°C.

Reagent 2 (R2): Concentrated Washing Solution (20X)

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

Reagent (R3): Negative Control

Human serum, negative for HIV-1 and HIV-2 antibodies, HCV antibodies and HBs Ag. Contains 0.1% sodium azide as a preservative.

Reagent (R4): Cut-off Control

Heat inactivated human serum, negative for HBs Ag and HCV antibodies and containing HIV antibodies. Contains 0.1% sodium azide as a preservative.

Reagent 5 (R5): Positive Control

Heat inactivated human serum, negative for HBs Ag and HCV antibodies and containing HIV antibodies. Contains 0.1% sodium azide as a preservative.

Reagent 6 (R6): Sample Diluent

Calf serum solution (Tris buffer with 0.1% chloroform and ProCin™ 300, and coloured indicator).

Reagent 7a (R7a): Conjugate

Purified HIV-1 and HIV-2 antigens labeled with peroxidase.

Contains BSA and 0.1% ProCin™ 300.

Gently tap the vial on the workbench to remove any substance from the rubber cap. Carefully remove the cap and pour the contents of a Conjugate Diluent vial in the Lyophilized Conjugate vial.

Put the cap on and let stand for 10 minutes while gently shaking and inverting from time to time to ease dissolution.

Reagent 7b (R7b) : Conjugate Diluent

Skimmed milk solution (Tris buffer with 0.1% chloroform and ProCin™ 300).

Reagent 8 (R8) : Peroxidase Substrate Buffer

Ready-for-use solution of citric acid and sodium acetate pH 4.0 containing 0.015% hydrogen peroxide, and 4% dimethyl sulfoxide (DMSO).

Reagent 9 (R9): Chromogen

Solution containing tetramethylbenzidine (TMB). Dilute 1:11 the solution in the Substrate Buffer (ex : 1 ml reagent R9+10 ml reagent R8) : Stability for 6 hours in the dark once prepared.

Reagent 10 (R10): Stopping Solution

Ready-for-use 1N sulphuric acid solution.

9 - ASSAY PROCEDURE

Strictly follow the proposed procedure.

Follow the following Good Laboratory Practice:

Use the negative, positive and cut-off controls for each series of determinations to validate the test results.

1. Carefully establish the sample distribution and identification plan
2. Prepare the dilute washing solution,
3. Take the carrier tray and the strips (R1) out of the protective pouch,
4. Apply directly, without prior washing of the plate and in succession:
 - 4.1 25 µl of diluent in each well
 - 4.2 75 µl of negative control serum (R3) in well A1
75 µl of cut-off control serum (R4) in wells B1, C1 and D1.
75 µl of positive control serum (R5) in well E1,
75 µl of specimen 1 in well F1,
75 µl of specimen 2 in well G1, etc...

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

Homogenize the mixture by a minimum of 3 aspirations with 75 µl pipette or by shaking the microplate after the pipeting step.

It is also possible to apply 100 µl of a sample previously diluted 3:4 (ex: 150 µl serum + 50 µl diluent).

N.B.: The sample distribution can be visually controlled at this step of the manipulation : after adding the sample, the diluent turns from purple to blue. (refer to section 12 for automatic verification - SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND conjugate PIPETING).

5. If possible cover the microplate with adhesive film. Press firmly all over the plate to ensure adequate tightness.
6. Incubate the microplate in a thermostat-controlled water-bath or microplate incubator at 37°C ± 1°C for 30 ± 5 minutes.
7. Remove the adhesive film. Aspirate the contents of all wells into a container for biohazardous waste (containing sodium hypochlorite). Add into each well a minimum of 0.370 ml of washing solution. Respect a soak time of a minimum of 30 seconds. Aspirate again. Repeat this procedure at least twice (i.e. a total of a minimum of 3 washes). The residual volume must be lower than 10 µl (if necessary, dry the plate by turning it upside down on absorbent paper). If an automatic washer is used, follow the same procedure (refer to section 10: recommendations).
8. Quickly distribute 100 µl of the conjugate solution into all wells.
The conjugate must be shaken gently before use.

N.B.: The distribution of the conjugate which is coloured green can be visually controlled at this step of the manipulation (refer to section 12 for automatic verification - SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND conjugate PIPETING).

9. If possible cover the microplate with new adhesive film. Incubate for 30 ± 5 minutes at Room temperature (18 - 30°C).
10. Remove the adhesive film, empty all wells by aspiration and wash a minimum of 5 times as described above. The residual volume must be lower than 10 µl (if necessary, dry the strips by turning them upside down on absorbent paper.)
11. Quickly dispense into each well 80 µl of prepared development solution (R8+R9), freshly prepared before use. Allow the reaction to develop in the dark for 30 ± 5 minutes at room temperature (18 - 30°C). Do not use adhesive film during this incubation.

N.B.: The distribution of the development solution, which is coloured pink, can be visually controlled at this step of the manipulation: There is a clear difference of colouration between empty well and well containing the pink substrate solution. (refer to section 12 for automatic verification: SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENT PIPETING).

12. Add 100 µl stopping solution (R10) by using the same sequence and rate of distribution as for the substrate solution. Homogenize the reaction mixture.

N.B.: The distribution of the stopping solution, which is not coloured, can be visually controlled at this step of the manipulation. After the addition of the stopping solution the pink colouration of the substrate disappears (for the negative samples) or turns from blue to yellow (for the positive samples).

13. Carefully wipe the plate bottom. **At least 4 minutes after stopping solution addition and** within 30 minutes of stopping the reaction, read the optical density at 450/620-700 nm using a plate reader.
14. Check all results for agreement between the reading and the plate and sample distribution and identification plan.

10 - RECOMMENDATIONS

CAUTION : AVOID CONTAMINATION WHILST PERFORMING ASSAY IN CASE OF CONTAMINATION (spills, etc)

- Non-acid spills should be wiped up thoroughly with a 5% (minimum) sodium hypochlorite solution.
- Acid spills should be wiped dry. The spilled area should then be cleaned with a 5% (minimum) sodium hypochlorite solution.

The material used for cleaning must be thrown away in the biohazardous waste container and disposed of in accordance with the relevant safety rules (see precautions, §7).

- NEVER USE THE SAME CONTAINER TO DISTRIBUTE THE CONJUGATE AND THE enzymatic development SOLUTION.
- RESPECT THE PRESCRIBED NUMBER OF WASHING CYCLES*.
- CHECK THE ENZYMATIC DEVELOPMENT SOLUTION (buffer substrate and chromogen) BEFORE DISTRIBUTION. It should be colourless.

The appearance of a blue colour within a few minutes after reconstitution indicates that the reagent may be contaminated by metal ions cannot be used and must be replaced.

We recommend the use of plastic containers and distribution equipment, or alternatively glassware previously washed with 1N hydrochloric acid, carefully rinsed with distilled water and dried.

(* Consult us for detailed information about the equipment recommended by our technical department.

11- CALCULATION AND INTERPRETATION OF THE RESULTS

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

1) Calculate the mean absorbance of the cut-off control serum (ODR4)

$$\overline{\text{ODR4}} = \frac{\text{OD (B1)} + \text{OD (C1)} + \text{OD (D1)}}{3}$$

2) Calculate cut-off value

The cut-off value is given by the ratio:

$$\text{C.O} = \frac{\overline{\text{ODR4}}}{10}$$

3) Assay validation

The absorbance of the negative control serum should be less than 70% of the cut-off value :

$$\text{ODR3} < 0.7 \times \text{C.O.}$$

The mean absorbance of cut-off control serum should be greater than 0.80:

$$\overline{\text{ODR4}} > 0.80$$

Optional : The ratio : $\overline{\text{ODR5}} / \overline{\text{ODR4}}$ should be greater than or equal to 1.3 (This optional norm will be applied when the linearity of the utilized reader is over 3.000).

4) Interpretation of the results

Samples with absorbance values less than the cut-off value are considered to be negative by the Genscreen™ HIV-1/2 Version 2 test.

Results just below the cut-off value ($\text{C.O} - 10\% < \text{OD} < \text{C.O}$) should however, be interpreted with caution (it is advise to retest in duplicate the corresponding samples when the utilized systems and laboratory procedures allow it).

Samples with absorbance values equal to or greater than the cut-off value are initially considered to be positive by the Genscreen™ HIV-1/2 Version 2 test. They should be retested in duplicate before final interpretation.

If after retesting of a sample, the absorbance values of the 2 duplicates are less than the cut-off value, the initial result is non repeatable and the sample is declared to be negative with the Genscreen™ HIV-1/2 Version 2 test.

Non repeatable reactions are often caused by :

- inadequate microplate washing,
- contamination of negative samples by serum with a high antibody titre,
- contamination of the development solution by oxidizing agents (bleach, metal ions, etc...),
- contamination of the stopping solution.

If after retesting the absorbance of one of the duplicates is equal to or greater than the cut-off value, the initial result is repeatable and the sample is declared to be positive with the Genscreen™ HIV-1/2 Version 2 test, subject to the limitations of the procedure, described below.

12 - SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND CONJUGATE PIPETING

Sample pipeting verification

After the dispensing of the sample diluent (R6) and of the samples, it is possible to verify the presence of samples to be tested into the wells by a spectrophotometric reading at 620 nm : the optical density of a well containing sample is greater than 0.150 (a lower OD indicates a poor dispensing of the sample).

Conjugate pipeting verification

After the dispensing of the conjugate (R7a + R7b) it is possible to verify its presence into the wells by a spectrophotometric reading at 620 nm : the optical density of a well containing conjugate is greater than 0.100 (a lower OD indicates a poor dispensing of the conjugate).

Development solution pipetting verification :

It is possible to verify the presence of pink development solution into the well by automatic reading at **490 nm** : a well with development solution must have an optical density greater than **0.100** (a lower OD indicates a poor dispensing of the development solution).

13 - PERFORMANCES

Sensitivity studies with Genscreen™ HIV-1/2 Version 2 test have been performed with positive samples from patients diagnosed with AIDS or related complex (ARC) and on sensitivity panels with documented samples from patients recently infected by HIV virus.

- The HIV-1 sensitivity was 100%. It has been evaluated on 413 diagnosed with AIDS or related complex (ARC), positive HIV-1 group M confirmed by Western Blot and 31 HIV-1 group O samples confirmed by Western Blot HIV-1.
- The group O sensitivity has been evaluated too on 4 samples HIV-1 group O indeterminate by Western Blot. It was considered to be satisfactory with 3 samples found positive.
- The HIV-2 sensitivity was 100%. It has been evaluated on 119 diluted or undiluted samples from positive samples confirmed by Western-Blot.
- The sensitivity on HIV-1 group M has been evaluated on 29 commercial seroconversion panels (BBI, NABI, Bioclinical partners) and on INTS sensitivity panel. Results are in agreement with the state of the art.
- From INTS panel, the 45 seroconversion and the 13 per-seroconversion samples are positive.
- 25 additional fresh positive samples (within 1 day after blood collection) were tested and all were found positive.
- At least 42 early seroconversion samples were tested with Genscreen™ HIV-1/2 Version 2.

The specificity of the test on blood bank donors was 99.98% on 5025 tested samples.

3 positives results out of 212 have been observed on patients showing different pathologies or status not linked to the HIV virus (pregnant women, rheumatoid factor, anti-nuclear IgG or other viral infection).

The accuracy of Genscreen™ HIV-1/2 Version 2 test has been determined by the analysis of 4 samples : 1 negative sample (sample 1), 2 low anti HIV-1 positive samples (samples 2 and 3) and 1 high anti HIV-1 positive sample (sample 4). The intra assay reproducibility has been evaluated by testing these 4 samples 30 times in the same run, the inter assay reproducibility has been evaluated by testing these 4 samples 3 times on 2 microplates performed on 2 independent runs each day during 5 days. Results are shown in the following tables :

Tableau 1: Intra assay reproducibility

n = 30	sample 1	sample 2	sample 3	sample 4
mean of ratios*	0.12	3.34	9.05	19.6
standard deviation (SD)	0.04	0.45	0.30	0.73
CV (%) ratios*	31.5%	13.6%	3.3%	3.7%

*ratios = OD/C.O

Tableau 2: Inter assay reproducibility

n = 30	sample 1	sample 2	sample 3	sample 4
mean of ratios*	0.12	3.43	9.27	19.35
standard deviation (SD)	0.02	0.41	0.89	1.93
CV (%) ratios*	19.3%	12.1%	9.65%	10.0%

*ratios = OD/C.O

14 - LIMITS OF THE TEST

Very low titer of antibodies may not be detected during the first stage of the infection, consequently a negative result indicates that the tested sample does not contain detectable anti-HIV antibodies with Genscreen™ HIV-1/2 Version 2 test. However, such a result does not preclude the possibility of exposure to an HIV-1/HIV-2 infection. HIV-1 (group M and group O) and HIV 2 variability does not allow to exclude the possibility of having false negative reactions. No known test method can offer complete assurance that HIV virus is absent. Highly sensitive ELISA technic may produce false positive results.

To verify the specificity of the reaction, every positive result (in accordance with the interpretation criterias of Genscreen™ HIV-1/2 Version 2 test) should be confirmed with an appropriate method (Western-Blot). Heating of samples may affect the quality of the results.

The colorimetric method for the samples, conjugate and development solution deposition verification does not allow to verify the accuracy of the dispensed volumes. This method only shows the presence of sample, conjugate and development solution into wells. The rate of wrong answers with this method is closely linked to the accuracy of the utilized system (cumulated coefficient of variation of dispensing and reading over 10% significantly decrease the quality of the verification).

In case of very poor washing efficiency after the conjugate incubation, the automatic verification of the development solution pipetting (by reading OD of wells at 490 nm) may provide wrong results with OD above 0.100 in the absence of development solution. However this phenomena has not been observed during evaluation on 939 tested samples.

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CE

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

IVD

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

REF

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



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

EC REP

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

LOT

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



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



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



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



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