

# Genscreen™ ULTRA HIV Ag-Ab

1 plate - 96 tests

72386

5 plates - 480 tests

72388

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**SCREENING KIT FOR THE DETECTION OF HIV P24 ANTIGEN  
AND ANTIBODIES TO HIV-1 AND HIV-2 IN HUMAN  
SERUM/PLASMA BY ENZYME IMMUNOASSAY**

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For *In Vitro* Diagnostic Use

### **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. INTENDED USE

The Genscreen™ ULTRA HIV Ag-Ab is a qualitative enzyme immunoassay kit for the detection of HIV p24 antigen and antibodies to HIV-1 (groups M and O) and HIV-2 in human serum or plasma. This kit can be used for both HIV Ag and HIV Ab screening

## 2. CLINICAL VALUE

The acquired immunodeficiency syndrome (AIDS) is a virus inducing 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 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 was 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 : the M group, including 9 sub-types (A to I) and the O group. The HIV-2 virus includes 5 sub-types. The geographical distribution of the different sub-types is 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 diagnosis of infection in some patients.

The various 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.

HIV antigens and antibodies appear and are detectable at different stages of the seroconversion and of the infection. The Genscreen™ ULTRA HIV Ag-Ab allows the simultaneous detection of anti-HIV-1 (M and O groups) and anti-HIV-2 antibodies and antigens (see also limitation of the procedure).

## 3. PRINCIPLE OF THE TEST

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

The solid phase is coated with :

- monoclonal antibodies against p24 HIV-1 antigen
- purified antigens : gp160 recombinant protein, a synthetic peptide mimicking a totally artificial (i.e. encoded by no existing virus) HIV-1 group O-specific epitope and a peptide mimicking the immunodominant epitope of the HIV-2 envelope protein.

The conjugates are based upon the use of :

- biotinylated polyclonal antibodies to HIV Ag (conjugate 1)
- Streptavidin and HIV antigens - peroxidase conjugate (gp41 and gp36 peptides mimicking the immunodominant epitopes of the HIV-1 and HIV-2 envelope glycoproteins, and the same synthetic peptide mimicking a totally artificial HIV-1 group O-specific epitope used for the solid phase) (conjugate 2)

The assay procedure includes the following reaction steps :

1. Conjugate 1 (biotinylated polyclonal antibody to p24 HIV-1 Ag) is added into the microplate wells.
2. Serum samples to be assayed and controls are pipetted into the wells.
  - If present, HIV antigens bind with the monoclonal antibody bound to the solid phase and the conjugate 1
  - HIV-1 and/or HIV-2 antibodies, if any, bind to the antigens immobilised on the solid phase.
  - Deposition of conjugate 1 and sample is validated through a colour change, from yellow-green to blue.
3. After incubation at 37°C then washing, conjugate 2 is added :
  - Streptavidin react with biotinylated Ab-Ag-Ab complexes
  - Peroxidase labelled, purified HIV-1 and HIV-2 antigens bind in turn to the IgG, IgM or IgA antibodies captured on the solid phase.

4. After incubation at 18-30°C the unbound conjugate 2 fraction is removed by washing. After incubation in presence of the substrate at room temperature (18-30°C) the presence of the complexed conjugate is shown by a change of colour.
5. The reaction is stopped and absorbances are read using a spectrophotometer at 450/620-700 nm. The absorbance measured on a sample determines the presence or absence of HIV Ag or HIV-1 and/or HIV-2 antibodies.

#### 4. CONTENTS OF THE KIT

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

LABEL	NATURE OF THE REAGENTS	PRESENTATION	
		72386	72388
<b>R1</b>	<b>Microplate</b> 12 strips of 8 wells coated with monoclonal antibodies to p24 HIV-1 (mouse) and purified HIV-1 and HIV-2 antigens	1 plate	5 plates
<b>R2</b>	<b>Concentrated washing solution (20X)</b> Tris NaCl buffer pH 7.4 Preservative : ProClin™ 300 0.04%	1 vial (70 ml)	1 vial (235 ml)
<b>R3</b>	<b>Negative control</b> 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 (2.5 ml)	1 vial (2.5 ml)
<b>R4</b>	<b>HIV Ab positive control</b> Heat inactivated human plasma positive for anti-HIV antibodies, negative for HIV and HBs antigens and anti-HCV antibodies, in synthetic diluent Preservative : ProClin™ 300 < 0.1%	1 vial (1 ml)	1 vial (1 ml)
<b>R5</b>	<b>HIV Ag positive control</b> Purified HIV 1 antigen inactivated with a chaotropic agent, in synthetic diluent Preservative : ProClin™ 300 < 0.1%	1 vial (1 ml)	1 vial (1 ml)
<b>R6</b>	<b>Conjugate 1</b> biotinylated polyclonal antibodies to p24 HIV 1 (sheep) coloured yellow - green Preservative : ProClin™ 300 0.5%	1 vial (10 ml)	2 vials (2 x 10 ml)
<b>R7a</b>	<b>Conjugate 2</b> Lyophilised peroxidase labelled Streptavidin and purified HIV 1 and HIV 2 antigens	1 vial (sqf 12.5 ml)	2 vials (sqf 2 x 30 ml)
<b>R7b</b>	<b>Conjugate 2 Diluent</b> kimmed milk solution coloured red preservative : ProClin™ 300 0.5%	1 vial (12.5 ml)	2 vials (2 x 30 ml)
<b>R8</b>	<b>Peroxidase substrate buffer</b> Sodium citrate and Sodium acetate solution pH 4.0 containing H <sub>2</sub> O <sub>2</sub> (0.015%) and DMSO (4%)	1 vial (60 ml)	2 vials (2 x 60 ml)
<b>R9</b>	<b>Chromogen</b> solution containing tetramethyl benzidine (TMB)	1 vial (5 ml)	2 vials (2 x 5 ml)
<b>R10</b>	<b>Stopping solution</b> 1N sulphuric acid solution	1 vial (28 ml)	3 vials (3 x 28 ml)

## 5. PRECAUTIONS

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

- 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™ ULTRA HIV Ag-Ab : Specific ID number = 53**

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 Bio-Rad products. 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.*

- Before use, it is necessary to wait 30 minutes for the reagents to stabilise to room temperature.
- 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.
- Waiting time between the dispensing of the conjugate 1 and the samples doesn't have to exceed 10 minutes.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solutions.
- The development solution (substrate buffer + chromogen) must be coloured pink. The modification of this pink colour within a few minutes of 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.
- 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.

## 6. HEALTH AND SAFETY INSTRUCTIONS

- All the reagents included in the kit are intended for "in vitro diagnostic use" and for professional use.
- This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, including lab coat, eye/face protection and disposable gloves (synthetic, non-latex gloves are recommended) and handle reagents and patient samples with the requisite Good Laboratory Practices. Wash hands thoroughly after performing the test.
- Do not pipette by mouth.
- Human origin material used in the preparation of the negative control (R3) has been tested and found non reactive for hepatitis B surface antigen (HBs Ag), HIV antigen, antibodies to hepatitis C, and antibodies to human immunodeficiency virus (HIV-1 and HIV-2).

- Human origin material used in the preparation of HIV-1 antibodies positive control (R4) has been tested and found non reactive for hepatitis B surface antigen (HBs Ag) and antibodies to hepatitis C.
- HIV Ag positive control (R5) has been inactivated using a chaotropic agent.
- 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.
- Avoid spilling samples or solutions containing samples
- Spills must be rinsed with bleach diluted at 10%. If the contaminating fluid is an acid, spills must be initially neutralised with sodium bicarbonate and dried with absorbent paper. The material used for cleaning must be discarded in a contaminated residue container
- Samples and reagent of human origin, as well as, contaminated material and products must be discarded after decontamination :
  - either by immersion in bleach at a final concentration of 5% of sodium hypochlorite (1 volume of bleach for 10 volumes of contaminated fluid or water) for 30 minutes
  - or by autoclaving at 121°C for 2 hours minimum. Autoclaving is the best method to inactivate the HIV and the HBV viruses.
  - DO NOT PLACE SOLUTIONS CONTAINING SODIUM HYPOCHLORITE IN THE AUTOCLAVE
- Do not forget to neutralise and/or autoclave the solutions or washing wastes or any fluid containing biological samples before discarding them into the sink.
- Some reagents contain ProClin™ 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.

- The Safety Data Sheet is available upon request.
- Chemicals should be handled and disposed of in accordance with Good Laboratory Practices.
- Avoid any contact of the substrate buffer, the chromogen and the stopping solution with the skin and mucosa (toxicity, irritation or burn hazard).
- 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.

## 7. 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 100 µl.
- Graduated cylinders of 25 ml; 100 ml; 1 000 ml capacity.
- Container for biohazardous waste.
- Water-bath or equivalent microplate incubator, thermostatically set at 37°C ± 1°C (\*).
- Manual, semiautomatic or automatic microplate washer (\*).
- Microplate reader equipped with 450nm, 490 nm and 620-700 nm filters (\*).
- Absorbent paper.

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

## 8. PREPARATION OF THE REAGENTS

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

### 1) Ready for use reagents

#### 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 3 (R3) : Negative control**

**Reagent 4 (R4) : HIV Ab positive control**

**Reagent 5 (R5) : HIV Ag positive control**

**Reagent 6 (R6) : Conjugate 1**

**Reagent 10 (R10) : Stopping solution**

## **2) Reagents to reconstitute**

**Washing solution (20X concentrate) : Reagent 2 (R2)**

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

**Conjugate 2 working solution : Reagent 7a (R7a) + Reagent 7b (R7b)**

Gently tap the vial of the lyophilised conjugate 2 (R7a) on the workbench to remove any substance from the rubber cap. Carefully remove the cap and pour the contents of Conjugate Diluent vial (R7b) into the Lyophilised Conjugate vial (R7a). Replace the cap and let stand for 10 minutes, whilst gently shaking and inverting from time to time to ease dissolution.

**Enzyme development solution : Reagent 8 (R8) + Reagent 9 (R9)**

Dilute 1:11 the chromogen (R9) in the Substrate Buffer (R8) (ex : 1 ml reagent R9+10 ml reagent R8). Stability is for 6 hours in the dark once prepared.

## **9. STORAGE CONDITIONS - SHELF LIFE**

The kit should be stored at +2-8°C. When stored at this temperature, each reagent contained in the Genscreen™ ULTRA HIV Ag-Ab can be used until the expiry date mentioned on the package (except for specific instructions).

**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 + b** : The reagents stored at +2-8°C can be used for 4 weeks after the vials have been reconstituted. The frozen reconstituted conjugate (R7 a+b) can be used until the expiry date of the kit, it can be frozen then defrozen 11 times.

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

## **10. 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 for 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). Do not repeat more than 3 freeze/thaw cycles.

If the specimens are to be shipped, they must be packaged in accordance with the regulations in force regarding the transport of etiological agents.

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

*REMARK : Samples containing up to 90 g/l albumin, 200 mg/l bilirubin, 50 µg/l biotin, lipemic samples containing up to the equivalent of 36 g/l triglyceride, and hemolyzed samples containing up to 10 g/l haemoglobin do not affect the results.*

## **11. ASSAY PROCEDURE**

**Strictly follow the proposed procedure.**

Use the negative (R3), HIV-1 Ab positive (R4) and HIV Ag positive (R5) controls for each series of determinations to validate the test results.

Follow the following Good Laboratory Practice :

1. Carefully establish the sample distribution and identification plan.
2. Prepare the diluted washing solution (refer to chapter 8).
3. Prepare the conjugate 2 working solution (refer to chapter 8).
4. Take the carrier tray and the strips (R1) out of the protective pouch,
5. Apply directly, without prior washing of the plate and in succession (suggested plate distribution) :
  - 5.1 25 µl of conjugate 1 (R6) in each well
  - 5.2 75 µl of HIV Ag positive control (R5) in well A1  
75 µl of HIV Ab positive control (R4) in well B1,  
75 µl of negative control (R3) in well C1, D1 and E1  
75 µl of specimen 1 in well F1  
75 µl of specimen 2 in well G1, etc. . . .

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

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

*N.B.: The sample and conjugate 1 distribution can be visually controlled at this step of the manipulation : after adding the sample, the conjugate 1 turns from yellow - green to blue (refer to section 14 for automatic verification : SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENTS PIPETING).*

6. When possible, cover the microplate with adhesive film. Press firmly all over the plate to ensure a tight seal.
7. Incubate the microplate in a thermostat-controlled water-bath or microplate incubator at 37°C ± 1°C for 1 hour ± 4 minutes.
8. 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. Allow a soak time of at least 30 seconds. Aspirate again. Repeat this procedure a minimum of two times (i.e. in total of a minimum of three washes). The residual volume must be lower than 10µl (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 12 : SYSTEM ADAPTATION)
9. Quickly dispense 100 µl of conjugate 2 solution (R7a + R7b) into all wells, the conjugate must be shaken before use.

*N.B.: The distribution of the conjugate 2, which is coloured red, can be visually controlled at this step of the manipulation. (refer to section 14 for automatic verification : SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENTS PIPETING)*

10. When possible, cover the plate with new adhesive film and incubate for 30 minutes ± 4 minutes at Room temperature (18 - 30°C).
11. 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.)
12. Quickly dispense into each well 80µl of prepared substrate solution (R8+R9), freshly prepared before use. Allow the reaction to develop in the dark for 30 ± 4 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 14 for automatic verification : SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENTS PIPETING)*

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

*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)*



14. Carefully wipe the plate bottom. **At least 2 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 within 30 minutes of stopping the reaction (the strips must always be kept away from light before reading).
15. Check for agreement between the spectrophotometric and visual readings and against the plate and sample distribution and identification plans.

## 12. SYSTEM ADAPTATION

**WASHING** : Carefully follow the washing procedures described to obtain maximum test performance.

## 13. CALCULATION AND INTERPRETATION OF THE RESULTS

The presence or absence of detectable HIV 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.

### 1) Calculate the mean absorbance of the negative control (OD R3)

$$\overline{OD R3} = \frac{OD (C1) + OD (D1) + OD (E1)}{3}$$

### 2) Calculate the cut-off value

The cut-off value is given by the formula :

$$CO = \overline{OD R3} + 0.200$$

### 3) Assay validation

The absorbance of each negative control (R3) should be less than 0.170 :  $OD R3 < 0.170$

If one negative control does not respect this norm, disregard and recalculate the mean using the two remaining values. Only one value may be eliminated by this way

The mean of the absorbance of the negative controls (R3) should be less than 0.150 :  $\overline{OD R3} < 0.150$

The absorbance of HIV Ab positive control (R4) should be greater than 0.9 :  $OD R4 > 0.9$

The absorbance of HIV Ag positive control (R5) should be greater than 0.9 :  $OD R5 > 0.9$

### 4) Interpretation of the results

Samples with absorbance values less than the cut-off value are considered to be negative by the Genscreen™ ULTRA HIV Ag-Ab test.

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

Samples with absorbance values equal to or greater than the cut-off value are initially considered to be positive by the Genscreen™ ULTRA HIV Ag-Ab 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™ ULTRA HIV Ag-Ab test.

Non repeatable reactions are often caused by :

- inadequate microplate washing,
- contamination of negative samples by serum or plasma with a high antibody titre,
- contamination of the substrate solution by oxidising 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™ ULTRA HIV Ag-Ab test, subject to the limitations of the procedure, described below.

## 14. SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENTS PIPETTING

### Sample and Conjugate 1 pipetting verification

After the dispensing of the conjugate 1 (R6) and of the samples, it is possible to verify the simultaneous presence of the conjugate 1 and the samples to be tested into wells by a spectrophotometric reading at 620 nm : the optical density of a well containing conjugate 1 and sample is greater than 0.600 (a lower OD indicates poor dispensing of the conjugate 1 or of the sample).

## Conjugate 2 working solution pipetting verification

After the dispensing of the conjugate (R7a + R7b) it is possible to verify its presence by a spectrophotometric reading at 450 / 620 nm : the optical density of a well containing conjugate 2 is greater than 0.100 (a lower OD indicates a poor dispensing of the conjugate 2)

## 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 ).

## 15. PERFORMANCES

The performances of Genscreen™ ULTRA HIV Ag-Ab have been determined by testing samples from random blood donors, from patients with HIV infection and commercial seroconversion panels. Moreover the HIV Ag sensitivity limit has been tested using French AFSSAPS Standard.

Patients with diseases unrelated to HIV infection have been tested too.

### Specificity

Specificity has been evaluated by testing :

- 6038 random blood donors from 3 different sites. Specificity on random blood donors was 99.95% (6035 negative samples / 6038 tested samples) with 3 repeated reactive samples which were confirmed negative for HIV by Western Blot and HIV p24 Ag testing.
- 409 clinical samples in 2 hospital clinical laboratories, 14 samples were found initial reactive and 12 of them were repeatedly reactive (positive in a second testing) : 11 were confirmed by HIV Western-Blot, 1 was not confirmed and considered as false positive. Specificity on this population is (397/398) 99.75%.
- 313 patients showing different pathologies or status not linked to the HIV (pregnant women, rheumatoid factor, autoimmune (SLE), cirrhotic, chronic renal failure, dialysis, anti-mouse Ig or other viral or bacterial infections (Hepatitis A, B, C, rubella, Toxoplasmosis, Mumps, Measles, CMV, HSV, EBV, VZV, HTLVI, Malarial, Flu vaccinated patients). Specificity was 98.72% (309/313) with 4 non specific and non significant reactions.

### Sensitivity

Sensitivity has been evaluated by testing confirmed HIV Ab positive samples, specimens from acute infected patients and from commercial seroconversion panels and HIV Ag samples (neat or diluted)

#### 1) Confirmed HIV Ab positive samples

744 positive samples from follow-up of HIV-1 and HIV 2 infected patient have been tested. This study was showing a sensitivity of 100%.

	Types	Number of samples	Number of reactive samples	Sensitivity
HIV 1	A, B, C (CDC classification )	200	200	100%
	HIV-1 WB with complete profiles or with light anti-gag Ab bands	200	200	100%
	VIH 1 group M (18A, 71B, 23C, 9D, 12 E, 4 F)	118	118	100%
	Group O	22	22	100%
	Group N	1	1	100%
	BBI PRZ 204 panel	7	7	100%
HIV 2	HIV-2 WB with complete profiles	196	196	100%

25 additional fresh positive samples (within 1 day after blood collection) were tested and all were found positive.

## 2) Specimens from acute infected patients and from commercial seroconversion panels

- 81 specimens sourced from acute or recently HIV-1 infected patients (35 samples from 28 patients with a Western-Blot seroconversion profile and 46 samples from recent seroconversion) were found positive with Genscreen™ ULTRA HIV Ag-Ab.
- 20 per-seroconversion samples (very early seroconversion samples with negative Western-Blot profile or with very light band for p24 and/ or gp160 on HIV Western-blot) : 19 of them were found positive.
- A total of 90 well documented commercial HIV seroconversion panels were also studied and compared to commercially available EIA assays. From which results were compared on 85 panels to a CE marked Ag-Ab test : Genscreen™ PLUS HIV Ag-Ab.

Genscreen™ ULTRA HIV Ag-Ab Results compared to Genscreen™ PLUS HIV Ag-Ab	Earlier detection (at least one bleed)	Equivalent detection (Same sample recognized as positive)	Later detection
Number of seroconversions	44	41	0

At least 170 early seroconversion samples were tested with Genscreen™ ULTRA HIV Ag-Ab.

## 3) HIV Ag samples

**Analytical sensitivity :** sensitivity limit of the test calculated by interpolation of the curve obtained by testing of dilutions of the AFSSAPS standard (initial concentration 100 pg/ml) was found to be < 25 pg/ml.

During the external evaluations, the limit of detection was established at 13.6 pg/ml by regression of the standard range of the "Ag HIV SFTS 1998" panel (HIV Ag panel from the French Society of Blood Transfusion).

The limit of detection has been estimated less than 2 IU/ml by testing the WHO HIV P24 Antigen 1<sup>st</sup> International Reference NIBSC code 90/636 and found at 0.85 IU/ml CI 95% [0.73 - 1.01 IU/ml] with 4 different batches during internal evaluation.

**Sensitivity on HIV Ag positive samples :** 56 samples were tested : 53 samples containing at least 25 pg/ml of HIV Ag were positive and 3 samples with respectively 13, 16 and 19 pg/ml of HIV Ag had ratios (Optical Density / Cut-off) between 0.9 and 1.00

**Sensitivity on culture cells supernatants :** 83 supernatants from the following genotypes were tested : 76 HIV-1 group M samples (16 A, 16 B, 11 C, 7D, 13 E, 4 F, 4 G, 3 H, 2 J), 4 HIV-1 group O, 1 HIV-1 group N and 2 HIV 2 samples. All of the HIV-1 samples were reactive except one group O sample with a concentration of 29 pg/ml of HIV Ag which was found with a ratio (Optical density / Cut off) of 0.60.

## Assay Reproducibility

The reproducibility of Genscreen™ ULTRA HIV Ag-Ab test has been determined, by the analysis of 10 samples : 1 negative sample, 3 HIV 1 positive samples, 3 HIV 2 positive samples and 3 Antigen positive. The intra assay reproducibility has been evaluated by testing these 10 samples 30 times in the same run. The inter assay reproducibility has been evaluated by testing these 10 samples in duplicate during 20 days on 2 independent runs each days. Results are shown in the following tables :

**Table 1 : Intra assay reproducibility**

Samples		Mean Ratio	SD	CV%
Negative		0.28	0.02	5.37
HIV-1	Low positive	1.62	0.07	4.32
	Medium positive	2.98	0.13	4.33
	High positive	5.37	0.18	3.32
HIV-2	Low positive	2.5	0.18	7.20
	Medium positive	5.35	0.45	8.48
	High positive	11.19	0.58	5.21
HIV Ag	Low positive	1.58	0.06	3.64
	Medium positive	4.19	0.17	4.13
	High positive	9.21	0.34	3.65

**Table 2 : Inter assay reproducibility**

Samples		Mean Ratio	SD	CV%
Negative		0.28	0.04	15.84
HIV-1	Low positive	1.05	0.10	9.44
	Medium positive	2.7	0.22	8.10
	High positive	4.96	0.41	8.37
HIV-2	Low positive	1.91	0.41	21.15
	Medium positive	4.45	0.64	14.29
	High positive	10.93	0.62	5.81
HIV Ag	Low positive	1.29	0.08	6.48
	Medium positive	3.48	0.17	4.99
	High positive	8.91	1.11	12.48

## 16. LIMITS OF THE TEST

Very low titre of HIV antigen or 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 HIV antigen or anti-HIV antibodies with Genscreen™ ULTRA HIV Ag-Ab. However, such a result does not preclude the possibility of exposure to an HIV 1 / HIV 2 infection.

The variability of HIV-1 (group M and group O) and HIV 2 allows the possibility of false negative reactions. No known test method can offer complete assurance that the HIV virus is absent.

Highly sensitive ELISA techniques may produce false positive results.

To verify the specificity of the reaction, every positive result (in accordance with the interpretation criteria of Genscreen™ ULTRA HIV Ag-Ab test) should be confirmed with an appropriate method (with a specific HIV Ag test such as the Genetic System HIV Ag EIA, then neutralisation to prove the presence of HIV Ag - or Western-Blot to prove the presence of anti-HIV antibodies).

Heating of samples may affect the quality of the results.

The spectrophotometric method for verifying the sample, conjugate development solution deposition does allow to verify the accuracy of the dispensed volume of samples and conjugate. This method shows only the presence of sample and conjugate. The error rate with this method is closely linked to the accuracy of the utilised system (a cumulated coefficient of variation of over 10% for dispensing and reading will significantly decrease the quality of this step).

Some icteric hyperlipemic or hyperhemolysed samples may affect the spectrophotometric method for verifying the conjugate 1 deposition. Only the presence of sample can be verified in this case.

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*)  
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 (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) - Katalogové číslo  
 (CZ) - Katalogové číslo  
 (NO) - Katalognummer  
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 (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
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