IVD

PANTEST

MBS N° MI 2008 A 001220

New generation Enzyme Immunoassay for the detection of antibodies to *Treponema p.*, HCV, HIV and Antigen p24-HIV1 in human serum or plasma

CE 1023

96 tests REF 1080KS	96 tests REF 1080 HS
192 tests REF 1080KS.1	192 tests REF 1080.1 HS
480 tests REF 1080KS.3	480 tests REF 1080.3 HS
960 tests REF 1080KS.2	960 tests REF 1080.2 HS

Σ

LOT On the external label 2-8 °C

INTENDED USE

The PANTEST is a *multiscreening* assay of infections for the simultaneous qualitative detection of antibodies to *Treponema p.*, HCV and HIV, HIV1Ag-p24 too in each sample of human serum or plasma.

INTRODUCTION

- *Treponema pallidum* is the causing agent of syphilis. It is a Gram-negative, thin, motile, spiral shaped bacterium in the order of *Spirochaetales* of about 0,25 µm wide and from 5 to 20 µm long. The spiral cellular shape is approximately 16 to 18 bends consisting of an outer sheath, periplasmic space with two flagella and a peptidoglycan layer (1). The complete genome is organized in one circular chromosome containing 1,138,006 base pairs including 1,041 genes (2,3). The *Treponema p.* does not grow on tissue culture (4) and cannot survive for long outside the human body. Several gene products have been specifically associated with virulent strains (5), although their role in pathogenesis remains to be unknown. The most common route of transmission is through contact with an infected person's sore during sexual activity. Other routes are through direct unprotected close contact with an active lesion, and through an infected mother to her unborn child during pregnancy. The first stage of the disease occurs 10-90 days after infection and is characterized by a chancre, usually found on the genitals. The sore is teeming with treponemes and is therefore highly contagious. The second stage of the disease occurs after a period of latency of 2-24 weeks, and is characterized by a rash spreading from the sores of the palms of the feet towards the trunk that may last 2-6 weeks. The period of latency following the second stage may last years, while the disease spreads throughout the body, and into the bones and the cardiovascular and nervous systems. The third stage of the disease can be fatal (6). The diagnosis of syphilis is made by serological testing, since the efforts to culture *Treponema p.* have been generally unsuccessful. Two type of tests can be carried out: non-specific and treponemal specific. The non-treponemal test measure IgG and IgM antibodies developed against lipids from damaged cells during the early stage of the disease. The antigen used is the cardiolipin, derived from beef heart. The two tests used are the Venereal Disease Research Laboratory (VDRL) test and the Rapid Plasma Reagin (RPR). Both tests measure coagulation of cardiolipin antigen by the patient's serum. The treponemal tests are specific antibody tests used to confirm positive reactions with the VDRL or RPR tests. The most commonly used are Fluorescent Treponemal Antibody Absorption (FTA-ABS) and Treponema Pallidum Haemagglutination (TPHA). Recently enzyme immunoassay (EIA) and Western blotting technique (WB) have been also introduced in specific diagnosis using recombinant antigens derived from encoding DNA sequences for membrane lipoprotein (47KDa, 17 KDa and 15 KDa), improving the sensitivity, specificity, and reproducibility of serological tests for syphilis (7-9). These tests, in early syphilis, can be positive even before the non-treponemal tests become positive and may also remain positive when non-specific tests revert to negative, in patients who have late syphilis (10,11).

- The HCV is a member of Flaviviridae family, Hepacivirus genus. It is a spherical enveloped virus with single strand linear RNA of positive sense genome. The RNA of HCV consists of a 5' and 3' non coding regions and of a single large open reading frame (ORF) encoding a large polyprotein precursor of approximately 3000 amino acids (1). Co- or post-traslationally cleavage produce a capsid protein and two envelope glycoproteins (gp33 and gp72). At least five non structural proteins (NS1-NS5) are assayed in the 3' portion of ORF. The 5' end of E2 gene is the most heterogeneous region named "first hypervariable region". Based on genetic heterogeneity, the HCV strains are divided in types (a, b, c, d, e), subtypes (1, 2, 3, 4, 5, 6) and quasispecies indicating the heterogeneous populations of isolate subtype genome (2-4). The HCV infection is spread through sexual intercourse, contaminated needles, blood transfusion, maternal/newborn route (5,6). In the majority of cases, HCV infection gives rise to an acute illness; 80% of such cases develop into chronic hepatitis. Almost all patients develop a vigorous antibody and cell-mediated immune response which fails to clear the infection but may contribute towards liver damage. Spontaneous resolution of chronic liver disease is very rare (<2%) and patients with chronic disease are at risk to developing severe liver damage as cirrhosis and hepatocellular carcinoma (7). Currently, third-generation enzyme immunoassay for antibody to HCV is the most practical screening test for HCV infection. The diagnosis of HCV infection is supported or confirmed by the recombinant immunoblot assay (RIBA) or tests for HCV RNA (RT-PCR) (8-11).

- The HIV is a member of Retroviridae family, Lentivirus genus. It is an enveloped virus with a nucleocapsid containing two molecules of RNA. The RNA template is retrotranscripted to cDNA and integrated into the host genome. The env, pol and gag genes codify for the three major types of structural proteins: gp120 and gp41 envelope proteins, p24 capside protein, p17 matrix protein and an enzymatic protein for transcription of RNA (reverse transcriptase). Two HIV genotypes designed HIV-1 and HIV-2 are originally identified in American and African patients with AIDS and AIDS related complex (1, 2). The HIV-1 is subdivided according to the divergence in the structure of env gene in M (major) group with subtypes A to J and in a further cluster of heterogeneous viruses named group O (outside the classic HIV-1) primary appearing in West Africa (3). The HIV infection is transmitted through sexual intercourse, contaminated needle, blood transfusion, maternal/newborn route. The pathogenesis is characterized by progressive depression of immune system with developing of opportunistic infections (virus, fungi, protozoa) and neoplasms such as Kaposi's sarcoma and malignant lymphoma. Many efforts have been carried out to improve diagnosis of HIV with serological, virological and molecular biology assays. Detection of antibody to HIV using a third generation method able to detect IgM, early antibodies to HIV, in addition to IgG, thus resulting in a reduction of seroconversion window (4, 5).

PRINCIPLE OF THE ASSAY

The kit PANTEST is based on " sandwich Elisa" principle. Each Microplate well is coated with:

- *Treponema p*. recombinant antigens;
- HCV synthetic peptides (Core, and with recombinant antigen (NS3). These antigens are derived from "Core" and "NS" conserved regions encoding for immunodominant antigenic determinants;
- HIV-specific synthetic and recombinant antigens derived from conserved DNA sequences encoding for immune-dominant antigenic determinants (HIV-1 gp41+120, HIV-2 gp36) and Mab1 to HIV1 P24.

The sample and diluted conjugate A, in different volume dispensed into the well, reacts with the solid phase and the antibodies to *Treponema p*., HCV and HIV, as per p24 Ag, if present, are captured by the Antigens / Mab1to p24 Ag, already bound with Mab 2-HRP. So, after further washing, in second incubation bound antibodies , to *Treponema p.* or HIV are detected by the addition of HIV 1/2/O antibodies bound to antigens of solid phase are detected by the addition of ENV HIV antigens and Tp Recombinants, labelled with horse radish peroxidase (HRP).

So, after further washing, in third incubation further antibodies to HCV are detected by the addition of goat anti-human IgA, IgG and IgM antibody, labelled with horse radish peroxidase (HRP), too. The enzyme captured on the solid phase, acting on the Chromogen/Substrate solution, generates an optical signal that is proportional to the amount of antibodies to *Treponema p.*, HCV and HIV, p24-HIVAg too, present in the sample.

CONTENT OF THE KIT

***Strip Microplate* –** (REF PAM01) Microplate of 8 x 12 strips of breakable wells activated each-one with *Treponema p.* recombinant antigens, HCV and HIV synthetic and recombinant antigens, plus Mab1 to p24 Ag-HIV1. The microplates are sealed in an aluminium pouch in presence of desiccant bag.

no. of microplates	REF 1080	no. 1	REF 1080.1	no. 2
	REF 1080.3	no. 5	REF 1080.2	no. 10

Treponema p. Positive Control – *Ready to use.* Buffered solution of serum base highly reactive for antibodies to *Treponema p.*. It contains 0.09% sodium azide 0.09 % Kathon as preservatives and Coomassie brilliant blue as colouring agent.

Volume	REF TTCP01	1.0 ml	REF TTCP01.1	2.0 ml
	REF TTCP01.3	3.0 ml	REF TTCP01.2	6.0 ml

HCV Positive Control – *Ready to use.* Buffered solution of serum base highly reactive for antibodies HCV. It contains 0.09 % sodium azide, 0.09 % Kathon as preservatives and Coomassie brilliant blue as colouring agent.

Volume	REF LCP01	1.0 ml	REF LCP01.1	2.0 ml
	REF LCP01.3	3.0 ml	REF LCP01.2	6.0 ml

HIV Ab Positive Control – *Ready to use*. Buffered solution of human serum base reactive for antibodies to HIV. It contains 0.09 % sodium azide, 0.09 % Kathon as preservatives and Coomassie brillant blue as colouring agent.

Volume	REF SCPB01	1.0 ml	REF SCPB01.1	2.0 ml
	REF SCPB01.3	3.0 ml	REF SCPB01.2	6.0 ml

HIV1 Ag-p24 Positive Control – *Ready to use.* Buffered solution of human serum base reactive for HIV1 Antigen p24. It contains 0.09 % sodium azide, 0.09 % Kathon as preservatives and Coomassie brillant blue as colouring agent.

Volume	REF SCPG01	1.0 ml	REF SCPG01.1	2.0 ml
	REF SCPG01.3	3.0 ml	REF SCPG01.2	6.0 ml

Note – All Positive Controls have been inactivated with 1% tri (n-buthyl) phosphate and 1% Triton X-100 at 30 °C for 4 hours by the manufacturer.

Negative Control – *Ready to use.* Buffered solution of serum base not reactive for antibodies to *Treponema p.*, HCV and HIV that contains 0.09 % sodium azide, 0.09 % Kathon as preservatives and Coomassie brilliant blue as colouring agent.

Volume	REF PACN01	2.0 ml	REFPACN01.1	4.0ml
	REF PACN01.3	6.0 ml	REF PACN01.2	12.0 ml

Sample Diluent – Proteic solution for the dilution of samples that contains stabilizers, 0.09 % sodium azide, 0.09 % Kathon as preservatives and Coomassie brilliant blue as colouring agent.

Volume	REF PADC01	30.0 ml	REF PADC01	60.0 ml
	REF PADC01.3	2x50.0 ml	REF PADC01.3	4x50.0 ml

Washing Solution – *To dilute before use.* Solution 25x concentrated that contains Imidazole buffer and surface-active agent.

Volume	REF SL01	50.0 ml	REF SL01	2x50.0 ml
	REF SL01.3	3x50.0 ml	REF SL01.3	4x50.0ml

Conjugate*- A**, *to dilute before use.* ***Solution of proteic buffer, 20x concentrate, that contains Mab2 to HIV1-p24, labelled with HRP, proteic stabilizers, 0.02% gentamicin sulfate and 0.09% Kathon as preservatives.

Volume	REF PATEM01	0.4 ml	RPATEM01.1	0.8 ml
	REF PATEM01.3	2.0 ml	REFPATEM01.2	4.0 ml

Conjugate Diluent – Buffered proteic solution, for the dilution of the concentrated Conjugate that contains proteic stabilizers, 0.02% gentamicin sulphate, 0.09 % Kathon as preservatives and Ponceau red as colouring agent.

Volume	REF LDT01	10.0 ml	REF LDT01.1	20.0 ml
	REF LDT01.3	50.0 ml	REF LDT01.2	100.0 ml

***Conjugate* B** – *Ready to use.* Buffered proteic solution, that contains synthetic antigens (ENV HIV) and Tp17&Tp47), proteic stabilizers, 0.02% gentamicin sulphate and 0.09 % Kathon as preservatives.

Volume	REF UTEPUB01	11.0 ml	REF UTEPUB01.1	22.0 ml
	REF UTEPUB01.3	55.0 ml	REF UTEPUB01.2	110.0 ml

Conjugate*-C**, *ready to use*. ***Solution of proteic buffer, that contains goat anti-human IgA, IgG and IgM antibodies, labelled with HRP, proteic stabilizers, 0.02% gentamicin sulfate and 0.09% Kathon as preservatives.

Volume	REF PATE01	11.0 ml	REF PATE01.1	22.0 ml
	REF PATE01.3	55.0 ml	REF PATE01.2	110.0 ml

***** Chromogen*** – *To mix with Substrate.* Solution of 3,3',5,5' tetramethylbenzidine (TMB), activators and stabilizers, in a phosphate/citrate buffer.

Warning: Store protected from light.

Volume	REF TA01	8.0 ml	REF TA01.1	15.0 ml
	REF TA01.3	30.0 ml	REF TA01.2	60.0 ml

***** Substrate*** – *To mix with Chromogen.* Solution that contains hydrogen peroxide (H_2O_2), activators and stabilizers, in a phosphate/citrate buffer.

Volume	REF TB01	8.0 ml	REF TB01.1	15.0 ml
	REF TB01.3	30.0 ml	REF TB01.2	60.0 ml

Stop Solution – Solution of 0.3 M sulphuric acid.

Note: handle with care.

Volume	REF SA01	13.0 ml	REF SA01.1	25.0 ml
	REF SA01.3	65.0 ml	REF SA01.2	130.0 ml

Cardboard Sealer (REF 300400) - Transparent plastic sealer to cover microplates during the incubation at 37 °C.

no. of sealers	REF 1080	no. 2	REF 1080.1	no. 4
	REF 1080.3	no. 10	REF 1080.2	no. 20

Package insert (REF INS1080E) **–** The present document.
Symbol information sheet (REF INSYS01) – List of the symbols.

CONTENT OF THE KITS 1080HS

The content of these kits is the same of the *1080KS.x* , except the ***Chromogen* & ***Substrate* that are substituted by the following reagent:

Chromogen/Substrate – *Ready to use.* Solution that contains 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide

(H_2O_2) with activators and stabilizers. *This reagent is stable up the expiration date of the kit.*
Note - Protect from light.

Volume	REF HS01	7.0 ml	REF HS01.1	14.0 ml
	REF HS01.3	35.0 ml	REF HS01.2	70.0 ml

Note - All the materials of human origin have been controlled and certified by the supplier to be negative for HBsAg, HCV Ab and HIV Ab.

PREPARATION OF REAGENTS (ALL KITS)
Washing Solution - The concentrated solution to be diluted 25x with distilled water before use.

For both variants 1080KS/108KS0.X and 1080HS/1080HS.X
ConjugateA is to dilute 1:20 with its Diluent. Mix on vortex before use. *The diluted Conjugate is stable for 1 week at 2 – 8 °C, when stored in a sterile disposable container.*

For 1080KS/1080KS.X only
Chromogen/Substrate - About 5 minutes before use, mix 1 volume of Chromogen with 1 volume of Substrate, in a disposable plastic container, according to needs. *This solution is stable for 4 hours at room temperature protected from light.*

SHELF-LIFE OF THE KIT
The shelf-life of the kit is 15 months from the production date. The validity of the shelf-life is intended for a product stored according to the instructions. The expiration date is indicated on the external label of the package.
Note – Don't use the product after the expiration date.

STORAGE AND STABILITY OF THE REAGENTS
1. The kit has to stored at 2 – 8 °C and used before the expiration date declared on the external label.
2. The pouch containing the microplate has to be brought to room temperature before opening. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag. Close hermetically the pouch and store again at 2 - 8 °C. If stored properly, strips are stable for 2 months from opening.
3. The diluted Washing solution, at room temperature, is stable for 1 week.
4. The Chromogen/Substrate (REF HS) is stable until the expiration of the kit.
5. The other reagents can be used every time, if stored at 2 – 8 °C and handled carefully for avoiding contamination.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Micropipettes of 20, 100, 300 and 1000 µl with disposable tips
2. Vortex mixer and adsorbent papers.
3. Distilled water.
4. Timer.
5. Incubator set at 37 ± 1 °C (dry or moist heat).
6. Automatic or manual microplate washer able to aspirate and dispense volumes of 300 - 400 µl.
7. Photometric microplate reader linear up to at least 2 OD and supplied with filters of 450 nm and 620 - 630 nm.

SAFETY PRECAUTIONS
1. All the reagents contained in the kit are for in vitro diagnostic use only.
2. Do not use the kit or reagents after the expiration date stated on labels.
3. Do not mix reagents of different lots.
4. Procedures should be performed carefully in order to obtain reliable results and clinical interpretations.
5. Bring all the reagents to room temperature for at least 60 minutes, before the test is started.
6. Avoid any contamination of reagents when taking them out of vials. We recommend to use automatic pipettes and disposable tips. When dispensing reagents, do not touch the wall of microplate wells with tips, in order to avoid any cross-contamination.
7. In the washing procedure, use only the Washing Solution provided with the kit and follow carefully the indications reported in the "*Washing Instructions*" section of this insert.
8. Ensure that the Chromogen/Substrate does not come in contact with oxidizing agents or metallic surfaces; avoid any intense light exposure during the incubation step or the reagent preparation.
9. Put the reagents in a glass or plastic disposable container, washed with sulphuric acid 1N, then with deionized water, before use.
10. Samples and materials potentially infective have to be handled with care as they could transmit infection. All objects come in direct contact with samples and all residuals of the assay should be treated or wasted as potentially infective. Best procedures for inactivation are treatments with autoclave at 121 °C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 30 minutes. This last method can be used for the treatment of the liquid waste after that it has been neutralized with NaOH.
11. Avoid any contact of liquids with skin and mucous membrane. Use always protective talk-free gloves, glasses and laboratory coats, according to the safety regulations.
12. Some reagents of the kit contain sodium azide which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water.

TECHNICAL PRECAUTIONS
1. At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag.
2. Distribution and incubation times should be the same for all the wells; avoid long interruptions among the different steps of the assay.
3. It is suggested to eliminate the excess of washing solution from wells by blotting them gently on a paper adsorbent pad.
4. We recommend reading the microplate at 450 nm (reading filter) and subtracting the blank at 620 - 630 nm (blanking filter). Blank the reader on A1 well.

WASHING INSTRUCTIONS
A good washing procedure is essential to get correct and reliable analytical results. 5 cycles of automatic washing of 300 µl/well per cycle, washing solution remaining 20 seconds at least in well, are sufficient to remove false positives and high background values. It is suggested to use an Elisa automatic microplate washer, qualified and properly serviced. Anyhow, we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances. Any case, potentially infective wastes from microplate washing have to be inactivated with Na-hypochlorite at 2.5% final concentration for 30 minutes. All these materials have to be discarded according to the law as potentially infective wastes.

SPECIMEN COLLECTION AND STORAGE
Either fresh sera or plasma (EDTA, Heparin, Citrate) can be used for the assay. If not used immediately, they can be stored at 2 - 8 °C for 1 week. In case of longer storage freeze them at – 20 °C. Samples should be clear. If the samples are turbid, could be contaminated by micro-organism, insofar it recommends to centrifuge them at 2000 rpm x 20 minutes at room temperature or filtrate on 0.22 µm filters. The samples that, after the above said procedure, did not became clear, can not be used.

ASSAY PROCEDURE
At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Do not dilute Controls as they are ready to use.

1 - Leave the A1 well empty for blanking operations. Dispense 100µl of Negative Control in triplicate, then 100µl of Positive Controls in single. Dilute 1:10 all the sample, directly into each microplate well, dispensing 90µl of Sample Diluent and 10µl of sample. Add 50 ul of diluted conjugate A to all the wells, except A1.
2 - Cover the microplate with the plate sealer and incubate strips for 90 minutes at 37 °C.
3. Peel out the plate sealer and wash the microplate according to instructions. Prepare the quantity of ConjugateB you need.
4 - Add 100 µl of the Conjugate B to all the wells, except A1.
5 - Cover the microplate with the plate sealer. Then incubate the microplate sealed for 30 minutes at 37 °C.
6. Peel out the plate sealer and wash the microplate according to instructions. Prepare the quantity of Conjugate C you need.
7 - Add 100 µl of the Conjugate C to all the wells, except A1.
8 - Cover the microplate with the plate sealer. Then incubate the microplate sealed for 30 minutes at 37 °C.
9 - Peel out the plate sealer and wash the microplate according to instructions. Prepare the necessary Chromogen/Substrate solution (1080KS/1080KS.X only).
10 - Add 100 µl of Chromogen/Substrate to all the wells, A1 included.
11 - Incubate the microplate for 15 minutes at room temperature, protected from light.
12 - Block the enzymatic reaction by adding 100 µl Stop Solution to all the wells, A1 included. Read the microplate at 450 nm and 620 - 630 nm blanking the instrument on A1 well.

Note - Read the microplate within 20 minutes after the dispensing of the Stop Solution.

ASSAY SCHEME

At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Do not dilute Controls as they are ready to use.

Position	*Controls/Samples*
A1	Blank
B1+C1+D1	Negative Control
E1	*Treponema p.* Positive Control
F1	HCV Positive Control
G1	HIV Ab Positive Control
H1	HIV1 Ag-p24 Positive Control
A2……..	Diluted samples

Reagents	*Blank (A1)*	*Controls*	*Samples*
Controls	-	**100 µl**	
Sample dil.	-	-	**90 µl**
Samples	-	-	**10 µl**
Conjugate A	-	**50 µl**	**50 µl**

Cover with the sealer and incubate for 90 minutes at 37 °C
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle
Prepare the quantity of ready to use Conjugate B you need (1080/1080.X and 1080HS/1080HS.X only).

Conjugate B	-	**100 µl**	**100 µl**

Cover with the sealer and incubate for 30 minutes at 37 °C
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle.
Prepare the quantity of ready to use Conjugate C you need (1080/1080.X and 1080HS/1080HS.X only).

Conjugate C	-	**100 µl**	**100 µl**

Cover with the sealer and incubate for 30 minutes at 37 °C
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle.
Prepare the necessary Chromogen/Substrate solution (1080KS/1080KS.X only).

Chromogen/ Substrate	**100 µl**	**100 µl**	**100 µl**

Incubate for 15 minutes at room temperature in the dark

Stop Solution	**100 µl**	**100 µl**	**100 µl**

Blank the reader on A1 well. Read at 620 - 630 nm for measuring the microplate background, then at 450 nm.

Note - Read the microplate within 20 minutes after the dispensing of the Stop Solution.

VALIDITY OF THE ASSAY

The assay is considered valid if:
1. the OD 450 nm of the A1 blank well is < 0.100. Higher values are index of Chromogen/Substrate contamination;
2. after blanking on A1, the OD 450 nm mean value of the Negative Control (NC) is < 0.200. Abnormal values may be observed when the washing instrument does not work correctly or the washing procedure has not been adapted to the assay as described in the proper section;
3. the OD 450 nm value of the Positive Controls (PC) is > 0.500. Lower values can be result when the storage temperature was not optimal or with a not correct operative procedure.

In case that the above data do not match the correct values, before repeating the test check carefully the expiration date of the kit, the performances of the instruments used for the assay and the procedure of distribution of Controls and samples.

CALCULATION AND INTERPRETATION OF RESULTS

If the validity of the assay is confirmed, calculate the Cut-off (Co) value through the following formula:

Cut-off = NC mean + 0.200
Grey-zone = ± 10 %

Example of calculation

Negative Controls	0.030 - 0.050 - 0.070	OD 450 nm
Negative Control mean	0.050	OD 450 nm
Treponema p. Positive Control	0.800	OD 450 nm
HCV Positive Control	1.800	OD 450 nm
HIV Positive Control	1.500	OD 450 nm
Cut-off = 0.050 + 0.200 = 0.250		
Grey-zone = 0.225 - 0.275		
Sample 1	0.050	negative
Sample 2	0.198	grey zone
Sample 3	1.250	positive

- Samples with an OD 450 nm value within the range Co -10% and Co +10 % are considered grey zone.
- Samples with OD 450 nm lower than the Co – 10% are considered negative.
- Samples with an OD 450 nm value higher than Co + 10 % are considered positive.
- Positive or indeterminate (grey-zone) samples have to be retested with specific confirmatory test for single marker.

ASSAY PERFORMANCES

All the tests were performed on human sera samples, critical and from routine of laboratory, previously evaluated with traditional referred kits, CE marked, for determination of antibodies to *Treponema p.*, HCV and HIV, separately.

Sensitivity - Preliminary studies were performed in Italy (Milan) at referred Hospital -PMIP- Microbiological Laboratory, Department of Virology. The sensitivity of PANTEST is 100%.

Samples Positive for		**N°**	**PANTEST positive**	**Sensibility**
HIV-1	**No subtype classified**	**366**	**366**	**100%**
	Subtype classified	**49**	**49**	**100%**
HIV-2		**126**	**126**	**100%**
HCV	**No subtype classified**	**98**	**98**	**100%**
	Subtype classified	**315**	**315**	**100%**
T.pallidum		**89**	**89**	**100%**

The analytical sensitivity (for HIV 1 antigen p24) was assessed, in MBS laboratories, examining the HIV 1 Ag Sensitivity Panel PRA801 (BBI):

Panel member ID	**W.H.O. Standard mIU/ml**	**DuPont Standard pg/ml**	**HIV PLUS MBS**

PRA801-01	**>2000**	**>200**	**Reactive**
PRA801-02	**1600**	**140**	**Reactive**
PRA801-03	**970**	**85**	**Reactive**
PRA801-04	**483**	**42**	**B.L.**
PRA801-05	**250**	**21**	**Not Reactive**
PRA801-06	**125**	**10**	**Not Reactive**
PRA801-07	**60**	**5**	**Not Reactive**
PRA801-08	**25**	**2**	**Not Reactive**
PRA801-09	**<10**	**<2**	**Not Reactive**
PRA801-10	**Negative**	**Negative**	**Not Reactive**

Specificity - Specificity studies were performed in Italy (Milan) in the Laboratory of Quality Control of M.B.S. S.R.L.The specificity of PANTEST is more then 99.7%.

Specimen	No. examined	False positive	Specificity
Blood donors sera	**5042**	**15**	**99.7 %**
Hospitalized patients sera	**224**	**0**	**100 %**
Potentially cross-reactive sera	**238**	**0**	**100 %**

Reproducibility – Replicates of negative, three low positive (*w)* and three high positive (h) sera for anti-*Treponema p.* , anti-HCV and anti-HIV respectively, have been examined with the same PANTEST lot and with multiple kit lots on multiple days. The results within and between assays are reported in the table.

Specimen	No. replicates	Intra-assay		No. replicates	Inter-assay	
		SD	CV%		SD	CV%
Negative	**25**	**0.005**	**13.3**	**6**	**0.014**	**27.5**
HIV w+	**32**	**0.038**	**9.4**	**6**	**0.086**	**10.8**
HIV h+	**32**	**0.103**	**4.5**	**6**	**0.168**	**6.0**
HVC w+	**36**	**0.024**	**6.2**	**6**	**0.078**	**8,3**
HCV h+	**36**	**0,129**	**5.0**	**6**	**0,131**	**7.3**
T.p. w+	**36**	**0.029**	**5.2**	**6**	**0.052**	**8.0**
T.p. h+	**36**	**0.091**	**3.4**	**6**	**0.077**	**3.1**

The values of standard deviation (SD) and coefficient variation (CV) were within the acceptability values (< 30% for negative, < 20% for low positive and <10% for high positive).

LIMITATION OF THE PROCEDURE

Highly lipemic, icteric, hemolysed samples or repeatedly defrost samples and therefore subject to contamination, should not be used as they can give false results in the assay.

PROCEDURE AUTOMATION

This procedure can be used with an automatic device under customer's responsibility and providing he validates the results with an adequate method. For more information, please contact the automatic device manufacturer.

PRECAUTIONS IN USE

Refer to the Safety Data Sheet. The use of the laboratory reagents according to Good Laboratory Practice (GLP) is recommended.

WASTE MANAGEMENT

Please, refer to local legal requirements.

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