INSTRUCTIONS FOR USE
HIV Ag/Ab ELISA

REF 790001 IVD CE 0123 2°C 8°C

1. Intended use

The HIV Ag/Ab ELISA produced by apDia is a 4th generation qualitative Enzyme-Linked ImmunoSorbent Assay for the *in vitro* diagnostic screening in human serum and plasma of antibodies to HIV-1, HIV-2 and HIV-1 p24 antigen.

2. Background

Acquired Immune Deficiency Syndrome (AIDS) is a set of symptoms resulting from the incapacitation of the human immune system caused by the Human Immunodeficiency Virus (HIV). HIV infection may progress to a symptomatic phase that is characterized by opportunistic infections and may cause death.

The etiological agent of AIDS, HIV, targets specific types of T-cells causing Lymphopenia and affecting T-cell mediated immunity. HIV is a member of a retrovirus family with two sub-families: HIV-1 and HIV-2. HIV-1 is more virulent and transmittable than HIV-2. HIV-1 is the cause of HIV infections globally, whereas HIV-2 is found predominantly in the countries of West-Africa. As serological cross-reaction between HIV-1 and HIV-2 is highly variable and dependent on the tested sample, antigens for specific detection of both HIV-1 and HIV-2 are included in the assay.

HIV is transmitted through sexual contact with infected persons, sharing needles and syringes with infected people and transfusion of contaminated blood. Enzyme-Linked ImmunoSorbent Assays (such as the HIV Ag/Ab ELISA from apDia, a 4th generation assay) are recommended for screening human blood and plasma for the presence of anti-HIV antibodies and HIV-1 p24 antigen.

3. Test Principle

Antigens representing epitopes of HIV-1 gp41 and HIV-2 gp36 are coated onto microplate wells together with monoclonal antibodies against HIV-1 p24. Serum or plasma sample is added to the well and if antibodies specific for HIV-1 and/or HIV-2 (IgG, IgM or IgA) are present in the sample, stable complexes will be formed with the HIV antigens attached to the well. HIV-1 p24 antigen, if present will bind to the antibodies in the well and to the detector antibodies present in the Sample Diluent. Non-reactive antibodies are removed by washing. Stable antigen-antibody complexes are identified through the successive addition of biotinylated antigens and horseradish peroxidase (HRP) conjugated streptavidin. These antibody-antigen complexes are quantified through the catalytic activity of horseradish peroxidase. Peroxidase substrate solution is added and is converted to a blue-coloured product. A positive sample generates a dark blue colour while faint blue colour or colourless wells indicate a negative sample. Upon adding stopping solution, the colour of the solution will change from blue to yellow. Optical Density (OD) is measured with a spectrophotometer (ELISA reader) at 450 nm with reference wavelength at 600-650 nm and is in proportion to the amount of anti-HIV1/2-antibodies and HIV-1 p24 present in the sample.

4. Kit Components

Component	Name indicated on vial
2 microtiter plates (12 x 8 strips) Coated with anti-HIV-1 p24 monoclonal antibodies, HIV-1 specific gp41 peptide, recombinant gp41 protein and HIV-2 specific gp36 peptide	Precoated Strips MTP
1 vial, 1300 µl, ready to use Pool of negative human serum or plasma. Contains 0.09% NaN_3.	Negative Control CTLNEG
1 vial, 1300 µl, ready to use Positive Control for HIV-1. Contains a pool of inactivated HIV-1 positive serum or plasma diluted in protein matrix. Contains 0.09% NaN_3.	Positive Control 1 CTLPOS1
1 vial, 1300 µl, ready to use Positive Control for HIV-2. Contains a pool of inactivated HIV-2 positive serum or plasma diluted in protein matrix. Contains 0.09% NaN_3.	Positive Control 2 CTLPOS2
1 vial, 1300 µl, ready to use Positive Control for HIV p24. Contains recombinant p24 antigen diluted in protein matrix, concentration about 100 pg/ml. Contains 0.09% NaN_3.	Positive Control 3 CTLPOS3
1 bottle, 25 ml, use to dilute sample Contains anti-HIV-1 p24 biotinylated monoclonal antibodies. Contains 0.05% proclin 300.	Sample Diluent DILSAM
1 bottle, 50 ml, ready to use Contains biotinylated HIV-1 specific gp41 peptide, biotinylated recombinant gp41 protein and biotinylated HIV-2 specific gp36 peptide. Contains 0.05% proclin 300.	Conjugate 1 CONJ1
1 vial, 500 µl, 100x Streptavidin-HRP	Conjugate 2 CONJ2 | 100x
1 bottle, 50 ml, use to dilute conjugate 2 Contains 0.05% Proclin 300.	Conjugate 2 diluent DILCONJ2
1 bottle, 50 ml, use to dilute TMB Contains H_2O_2.	Substrate Buffer DILTMB
1 vial, 500 µl, 100x Contains 0.02% Thiomersal.	TMB TMB | 100x
1 bottle, 100 ml, 25x Contains detergent in phosphate buffered solution. Contains 0.17 % Proclin 300.	Wash solution WASH | 25x
1 bottle, 16 ml, ready to use 1M H_2SO_4	Stop Solution STOP
6 plate sealers	-

5. Materials required but not supplied

- Calibrated precision micropipettes
- Calibrated thermostatic incubator for incubation at 37 °C
- EIA grade water
- Vortex or similar mixing tools
- Absorbent paper tissues
- ELISA microplate reader capable of measuring absorbencies at 450 nm with reference filter at 600-650 nm

6. Warnings and precautions for users

- For *in vitro* diagnostic use only.
- The kit has to be used by properly trained personnel in an appropriate laboratory environment.
- Make sure to properly identify sample wells. Mark data sheet with sample identification.
- Treat controls and samples as if they contain infectious agents. Positive HIV plasma or serum used to prepare the controls are negative for HBsAg and HCV Ab and are inactivated by detergent as described in Horowitz B. et al., 1992, Blood: 826-831.
- Negative human serum or plasma used in the negative control, has been tested and found to be non-reactive for Hepatitis B surface antigen, HCV antibodies and HIV-1 and HIV-2 antibodies. Since no known method can offer complete assurance that products derived from human blood will not transmit Hepatitis or other viral infections, it is recommended to handle this component in the same way as potentially infectious material.
- Dispose patient samples and all materials used to perform this test as if they contain infectious agents.
- Wear disposable gloves and protective clothing when performing a test run.
- Stop Solution is a 1M H_2SO_4 solution which is irritant. In case of contact with eyes or skin, rinse with plenty of water and seek medical advice.
- Do not mix reagents or coated microplates from kits with different lot numbers.
- Some kit components contain sodium azide as preservative. In order to prevent the formation of potentially explosive metal azides in laboratory plumbing, flush drains thoroughly after disposal of these solutions.
- Inform the manufacturer in case broken vials/bottles or improperly sealed/closed components are found.

7. Storage Conditions

- Store the kit and kit components at 2-8 °C. Do not freeze.
- Store the microtiter strips in their original package with the desiccant until all the strips have been used.
- Opened components should be stored at 2-8°C until next use and can be maintained for 1 month.
- For storage of reconstituted components see 9.2.
- Do not use any kit component beyond the expiration date.

8. Specimen collection and handling

- Serum, EDTA plasma and citrate plasma type of samples can be used. Remove serum from clot as soon as possible to avoid haemolysis; haemolysed and/or lipemic samples can cause false results.
- Handle all specimens as if capable of transmitting pathogens.
- Store undiluted samples at -20°C until use. Avoid multiple freeze-thaw cycles.
- Storage of diluted samples is not recommended and can adversely affect test performance.
- Mix the specimens before use.

9. Assay Procedure

9.1. General remarks

- Use a separate disposable tip for each sample transfer to avoid cross-contamination.
- All reagents must be allowed to come to room temperature before use. Microplate packaging may be opened if it has reached room temperature. All reagents must be mixed without foaming.
- Once the assay has been started, all steps should be completed without interruption.

9.2. Reconstitution of the reagents

Prepare only the volumes required to perform the planned test.

- Wash Solution: Dilute the concentrated wash solution 25x in distilled or deionised water. Make sure salt crystals are dissolved before making the dilution.
  Example: Add 40 ml of 25x wash solution to 960 ml of deionised water.
  Diluted Wash Solution may be stored for 1 month at 2-8 °C.
- Conjugate 2: Dilute the concentrated Conjugate 2 (100x) in Conjugate 2 Diluent.
  Example: Add 250 µl of concentrated Conjugate 2 to 25 ml of Conjugate 2 Diluent.
  Diluted Conjugate 2 may be stored for 5 days at 2-8 °C.
- TMB/Substrate Buffer: Dilute the concentrated TMB (100x) in Substrate Buffer. 100xTMB is solid at 4°C. Make sure all TMB is dissolved before making the dilution.
  Example: Add 250 µl of concentrated TMB to 25 ml of Substrate Buffer.
  Diluted TMB/substrate buffer must be used within 8 hr after it has been prepared; keep this solution away from light.

9.3. Washing Procedure

1 wash cycle is done as follows:

- Add 400 µl of diluted Wash Solution to all used wells of the microplate.
- Remove Wash Solution immediately from wells by inverting the microplate and tapping dry on a paper towel.
- An automatic microplate washer may be used but this will require adaptation of the washing procedure.
  If necessary, increase the number of cycles and include a soak time after each wash cycle based on the OD values of the negative and positive controls. It is recommended to increase the soak time in steps of 10 sec, up to 90 sec. Alternatively or simultaneously, the number of wash cycles can be gradually increased, e.g. 7x, 5x, 7x.
  For additional help regarding adaptation of the washing procedure, contact apDia.

9.4. Test procedure

- Take the necessary amount of test strips and return non-used strips in the sachet. In each run, foresee one strip for the 4 controls: 1 negative control and the 3 positive controls should be included in duplo.
- Add 100 µl of Sample Diluent to each well.
- Add 100 µl of sample/controls, pipet up and down for homogenization and seal strips securely with microplate sealer.
- Incubate for 60 minutes at 37°C.
- After incubation, briskly shake out the solution from the wells. Wash the microtiter plate 5 cycles according to the washing procedure (9.3).
- Add 200 µl of ready to use Conjugate 1 solution to the wells. Seal strips securely with a microplate sealer.
- Incubate Conjugate 1 for 30 minutes at 37°C.
- After incubation, Conjugate 1 is removed by inverting the microplate and tapping dry on paper towel. Wash the microtiter plate 3 cycles according to the washing procedure (9.3).
- Add 200 µl of diluted Conjugate 2 solution to the wells. Seal strips securely with microplate sealer.
- Incubate for 30 minutes at 37°C.
- After incubation, remove solution from the wells and wash the microtiter plate 5 cycles according to the washing procedure (9.3).
- Add 200 µl of the diluted TMB solution to the wells of the microtiter plate.
- Incubate for 30 minutes at room temperature (18-30 °C) protected from light.
- After incubation, add 50 µl of Stop Solution and read OD at 450 nm with reference wavelength 600-650 nm in a microplate reader within 30 minutes after stopping.

10. Calculation of results

10.1. Validity

The results for the controls should be within the acceptation criteria before any sample result can be interpreted.

Calculation of the Negative Control Mean NC_{MEAN}:

Example: Absorbance NC
0.021
0.025
0.046

$NC_{MEAN} = 0.046 / 2 = 0.023$

The mean of the absorbance values of the Negative Control must be less than 0.100. If the mean value is greater than or equal to 0.100, the run should be repeated.

Calculation of the Positive Control Mean PC_{MEAN}:

Example: Absorbance HIV-1-PC
1.545
1.239
2.784

HIV-1-$PC_{MEAN} = 2.784 / 2 = 1.392$

Example: Absorbance HIV-2-PC
2.986
> 3.000
cannot be calculated

HIV-2-PC_{MEAN} > 0.500

Example: Absorbance HIV-1 p24-PC
2.223
2.172
4.395

HIV-1 p24-$PC_{MEAN} = 4.395 / 2 = 2.198$

The mean of the absorbance values of each Positive Control (HIV-1 Positive Control; HIV-2 Positive control and HIV-1 p24 Positive Control) must be greater than 0.500. If the mean value is less than or equal to 0.500, the run should be repeated.

10.2. Cut-off Calculation

Cut-off = NC_{MEAN} + 0.170

Example: Cut-off = 0.023 + 0.170 = 0.193

11. Interpretation of results

- Specimens with absorbance values less than the cut-off value are considered not reactive by the criteria of this immunoassay, and may be considered negative for antibodies to HIV-1 and HIV-2 and negative for HIV-1 p24 antigen. Further testing is not required.
- Specimens with absorbance values equal to or greater than the cut-off are considered to be reactive or positive for HIV-1 and/or HIV-2 antibodies or HIV-1 p24 antigen. These specimens (using the original sample) should be re-tested in duplicate before final confirmation of the result.
- Initially reactive specimens, which do not react in either of the duplicate repeat tests, are considered negative for antibodies to HIV-1 and HIV-2 and negative for HIV-1 p24 antigen. Further testing is not required.
- If one of both re-test values is equal to or greater than the cut-off value, the specimen is considered repeatedly reactive. Specimens that have been found repeatedly reactive are interpreted to be positive for the presence of antibodies to HIV-1 and/or HIV-2 or HIV-1 p24 antigen. In most settings it is appropriate to investigate repeatedly reactive specimens by additional, more specific tests.

12. Limitations of the assay

- Strict adherence to the protocol and recommended equipment is necessary to obtain reliable test results. Accurate sample and reagent pipetting and timing of washing and incubation should be respected.
- As with all *in vitro* diagnostic tests, a definitive clinical diagnosis should not be made based only on the results of a single test. A complete evaluation by a physician is needed for a final diagnosis.
- Since no HIV-1 group O peptide is used, group O detection is solely based on cross-reactivity of patient antibodies to HIV-1 gp41 antigens.

13. Performance

Performance evaluation has been conducted in accordance to the Common Technical Specifications 2009/886/EC.

13.1. Example of expected OD values

Controls	*expected OD value**
Negative control, CTLNEG	*0.048*
Positive control 1, CTLPOS1	*2.850*
Positive control 2, CTLPOS2	*>3.000*
Positive control 3, CTLPOS3	*1.980*

*These values are given as an example only.

13.2. Diagnostic Specificity

Normal Human Sera and Plasma

Specificity has been evaluated by testing 5061 unselected donor samples from Belgian and Dutch origin. 22 samples showed a positive result, resulting in a specificity of 99.6%.

13.3. Specificity Interference

145 potentially interfering samples were tested and 204 hospitalized random patients.

Parameter	*# tested*	*# reactive in the HIV Ag/Ab ELISA (apDia)*
HBsAg positive	*14*	*0*
RF positive	*12*	*0*
CMV IgM positive	*27*	*1*
TOXO IgM positive	*8*	*1*
EBV IgM positive	*12*	*1*
Helicobacter pylori positive	*15*	*1*
Malaria positive	*12*	*1*
HAMA positive	*5*	*0*
Lipemic	*5*	*0*
Bilirubin	*5*	*0*
Hyper-protein	*5*	*0*
Hyper-immunoglobulin	*5*	*0*
Hemolysis	*5*	*0*
High biotin	*5*	*0*
Cholesterol	*5*	*0*
1st semester pregnant women	*5*	*0*
Random hospitalized patients	*204*	*2*
Total	***349***	***7***

7 samples tested positive in this panel, resulting in a specificity of 100-(7/349 x 100) = 98.0 %.

13.4. Clinical Sensitivity

Panel 1: HIV-1 positive samples (# 400)

HIV Ag/Ab ELISA (apDia) test result	*Reference results HIV-1 positive*
Reactive	*400*
Non-reactive	*0*
Total	*400*

All HIV-1 positive samples were detected by the HIV Ag/Ab ELISA from apDia, sensitivity: 100/100 X 100 = 100.0 %

Panel 2: Primary HIV infection (pre-seroconversion) samples (# 50)

HIV Ag/Ab ELISA (apDia) test result	*Reference results primary HIV infection*
Reactive	*50*
Non-reactive	*0*
Total	*50*

All primary HIV infection samples were detected by the HIV Ag/Ab ELISA, sensitivity: 50/50 X 100 = 100%.

Panel 3: HIV-1 positive samples belonging different subtypes (non-B)

HIV Ag/Ab ELISA (apDia) test result	*Reference results HIV-1 positive (subtype non-B)*
Reactive	*40*
Non-reactive	*0*
Total	*40*

All HIV-1 non-B specimens were detected by the HIV Ag/Ab ELISA from apDia, sensitivity: 40/40 X 100 = 100%.

In detail:

Sample		*HIV Ag/Ab ELISA result (apDia)*				*Reference method test result*			
						Subtype			*Reference result*
NR	*type*	*OD*	*CO*	*OD/CO*	*result*	*GAG*	*ENV*	*POL*	
1	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*A*	*A*	*NT*	*HIV-1*
2	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*A*	*A*	*NT*	*HIV-1*
3	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*A*	*A*	*NT*	*HIV-1*
4	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*A*	*A*	*NT*	*HIV-1*
5	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*C*	*C*	*NT*	*HIV-1*
6	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*C*	*C*	*NT*	*HIV-1*
7	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*C*	*C*	*NT*	*HIV-1*
8	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*C*	*C*	*NT*	*HIV-1*
9	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*D*	*D*	*NT*	*HIV-1*
10	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*D*	*D*	*NT*	*HIV-1*
11	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*D*	*D*	*NT*	*HIV-1*
12	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*D*	*D*	*NT*	*HIV-1*
13	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*CRF01_AE*	*CRF01_AE*	*NT*	*HIV-1*
14	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*CRF01_AE*	*CRF01_AE*	*NT*	*HIV-1*
15	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*CRF01_AE*	*CRF01_AE*	*NT*	*HIV-1*
16	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*F*	*F*	*NT*	*HIV-1*
17	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*F*	*F*	*NT*	*HIV-1*
18	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*F*	*F*	*NT*	*HIV-1*
19	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*G*	*G*	*NT*	*HIV-1*
20	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*G*	*G*	*NT*	*HIV-1*
21	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*G*	*G*	*NT*	*HIV-1*
22	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*H*	*H*	*NT*	*HIV-1*
23	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*H*	*H*	*NT*	*HIV-1*
24	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*H*	*H*	*NT*	*HIV-1*
25	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*CRF01_AE*	*NT*	*CRF01_AE*	*HIV-1*
26	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*D*	*F*	*NT*	*HIV-1*
27	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*C*	*A*	*NT*	*HIV-1*
28	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*Da*	*CRF01_AE*	*NT*	*HIV-1*
29	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*F*	*D*	*NT*	*HIV-1*
30	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*F*	*D*	*NT*	*HIV-1*
31	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*O*	*O*	*NT*	*HIV-1*
32	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*O*	*O*	*NT*	*HIV-1*
33	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*O*	*O*	*NT*	*HIV-1*
34	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*G*	*A*	*NT*	*HIV-1*
35	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*NT*	*NT*	*J*	*HIV-1*
36	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*NT*	*NT*	*J*	*HIV-1*
37	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*NT*	*NT*	*J*	*HIV-1*
38	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*NT*	*NT*	*K*	*HIV-1*
39	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*NT*	*NT*	*K*	*HIV-1*
40	*p*	*3.000*	*0.247*	*12.15*	*reactive*	*NT*	*NT*	*K*	*HIV-1*

Panel 4: HIV-2 positive samples (# 100)

HIV Ag/Ab ELISA (apDia) test result	Reference results HIV-2 positive specimens
Reactive	100
Non-reactive	0
Total	100

All HIV-2 positive specimens were detected by the HIV Ag/Ab ELISA from apDia, sensitivity: 100/100 X 100 = 100%.

Panel 5: Cell culture supernatants

Type	Subtype	INNOTEST HIV Antigen mAb	Vironostika HIV Uni-Form Ag/Ab	HIV Ag/Ab ELISA apDia
HIV-1	A/A	4/4	4/4	4/4
HIV-1	B/B	4/4	4/4	4/4
HIV-1	C/C	4/4	4/4	4/4
HIV-1	D/D	4/4	4/4	4/4
HIV-1	CRF01_AE	5/5	5/5	5/5
HIV-1	F/F	3/3	3/3	3/3
HIV-1	G/G	2/2	2/2	2/2
HIV-1	CRF02_AG	4/4	4/4	4/4
HIV-1	G/H	1/1	1/1	1/1
HIV-1	H/H	3/3	3/3	3/3
HIV-1	J/J	2/2	2/2	2/2
HIV-1	O/O	4/4	4/4	4/4
HIV-1	G/A	1/1	1/1	1/1
HIV-1	N	1/1	1/1	1/1
HIV-1	K	1/1	1/1	1/1
HIV-2	A	4/4	0/4	4/4
HIV-2	B	3/3	0/3	3/3
Total		50/50	43/50	50/50

The HIV Ag/Ab ELISA test from apDia was able to detect all of the 50 cell culture supernatants. All HIV-1 subtype A, B, C, D, CRF01_AE, F, G, G/H, H, J, G/A, N and K strains were detected at least one dilution higher in comparison to the reference assay (Vironostika HIV Uni-Form Ag/Ab) (higher analytical sensitivity). For subtype CRF02_AG the HIV Ag/Ab ELISA test from apDia detected three strains at one dilution higher and one strain at one dilution lower in comparison to the reference test. One of the four subtype O strains was detected at the same dilution, another one at a dilution higher and two other at a lower dilution. The HIV Ag/Ab ELISA test from apDia detected all HIV-2 subtype A and subtype B strains where the reference test was not able to detect any HIV-2 strain.

Panel 6: Seroconversion

30 seroconversion panels were tested with the HIV Ag/Ab ELISA from apDia, of which 20 were tested in a Belgian WHO reference lab for HIV:

Panel ID	Vironostika HIV Uni-Form II Ag/Ab	HIV Ag/Ab ELISA (apDia)
	Number of positive panel members/ total number tested	
PRB914-N	5/5	5/5
PRB916-P	3/6	3/6
PRB919-S	2/3	3/3
PRB924-X	4/8	4/8
PRB925-Y	2/6	2/6
PRB926-Z	3/6	4/6
PRB927-AB	4/5	4/5
PRB934-AI	3/3	3/3
PRB942-AR	1/4	1/4
PRB947-AW	3/4	3/4
PRB950-AZ	1/4	3/4
PRB951-BA	3/6	4/6
PRB952-BB	2/6	4/6
PRB953-BC	1/4	3/4
PRB954-BD	1/7	2/7
PRB955-BE	3/5	4/5
PRB956-BF	0/5	2/5
PRB957-BG	2/7	2/7
PRB958-BH	2/6	4/6
PRB959-BI	6/7	7/7
Total score	**51/107**	**67/107**

The HIV Ag/Ab ELISA test from apDia detected in total 67 out of the 107 panel members, the Vironostika HIV Uni-Form II Ag/Ab EIA reference test detected 51 samples.
For eleven out of the 20 seroconversion panels the HIV Ag/Ab ELISA test from apDia was able to detect at least one more HIV-1 p24 positive panel member than the reference test. For nine other seroconversion panels the HIV Ag/Ab ELISA from apDia detected as many panel members as the reference test.

13.5. Analytical Sensitivity

The analytical sensitivity for HIV-1 p24 antigen using the HIV Ag/Ab ELISA from apDia was assessed by testing the NIBSC International Standard for HIV-1 p24 antigen 90/636.

NIBSC HIV-1 p24 IU/ml	HIV Ag/Ab ELISA (apDia)				Reference method test results
	OD	CO	OD/CO	Result	OD/CO
12.5	3.000	0.242	12.4	reactive	1.5
6.25	2.189	0.242	9.05	reactive	1.0
3.12	1.146	0.242	4.74	reactive	0.8
0.78	0.294	0.254	1.16	reactive	0.5
0.42	0.188	0.254	0.74	non-reactive	0.3
0.21	0.115	0.254	0.45	non-reactive	0.3

The NIBSC HIV-1 p24 antigen could be detected by the HIV Ag/Ab ELISA test from apDia at three dilutions higher compared to the reference assay (higher analytical sensitivity).

13.6. Precision

Based on the S/CO values from three positive controls and three cut-off controls tested 22 times in one run, following **intra-assay variability** has been calculated:

Mean values (n = 22)	*HIV-1 positive control*	*HIV-1 cut-off control*	*HIV-2 positive control*	*HIV-2 cut-off control*	*p24 positive control*	*p24 cut-off control*
S/CO	*8.2*	*1.2*	*8.2*	*1.2*	*6.8*	*1.0*
% CV	*5.0*	*6.2*	*7.0*	*15.9*	*3.0*	*10.2*

Based on the S/CO values from three positive controls and three cut-off controls tested during 20 days each time in two different runs, following **inter-assay variability** has been calculated:

Mean values (n = 40)	*HIV-1 positive control*	*HIV-1 cut-off control*	*HIV-2 positive control*	*HIV-2 cut-off control*	*p24 positive control*	*p24 cut-off control*
S/CO	*8.4*	*1.2*	*7.6*	*1.3*	*6.8*	*0.9*
% CV	*8.7*	*13.7*	*19.4*	*21.9*	*9.6*	*15.6*

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Summary of Test Procedure

STEP	PROCEDURE
Sample step	Add 100 µl of Sample Diluent. Add 100 µl of sample/control to the diluent and pipet up & down for mixing. Test Negative and Positive controls in duplicate. Incubate 60 minutes at 37°C.
Wash step	Perform wash step 5x.
Conjugate 1	Add 200 µl of ready to use Conjugate 1 to wells of the microtiter plate. Incubate 30 minutes at 37°C.
Wash step	Perform wash step 3x.
Conjugate 2	Add 200 µl of diluted Conjugate 2 to wells of the microtiter plate. Incubate 30 minutes at 37°C
Wash step	Perform wash step 5x.
TMB/Substrate	Add 200 µl of TMB diluted in Substrate Solution to the wells of the microtiter plate.
Colour Development	Incubate 30 minutes at 18-30°C. Protect from light.
Stopping	Add 50 µl of Stop Solution and read OD at 450 nm with reference wavelength at 600-650 nm in an ELISA microplate reader.