DRG® HIV Ab-Ag (EIA-5167 / EIA5180)

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Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTENDED USE

The kit is a solid phase enzyme immunoassay for screening of antibodies to all subtypes of HIV-1 and HIV-2 and HIV-1 antigen (p24) in human serum or plasma.

2 PRINCIPLE OF THE TEST

Synthetic peptides representing immunodominant epitopes of HIV-1 and HIV-2 together with a monoclonal antibody to p24 HIV-1 antigen are coated onto wells of a microplate.

The peptides and the antibody have been carefully selected to ensure the screening of antibody and p24 antigen to all HIV-1 subtypes, including subtype O and HIV-2. Serum or plasma samples are added to these wells and, if antibodies specific to HIV1 and/or HIV-2 (IgG, IgM or IgA) are present in the sample, they will form stable complexes with the HIV peptide antigens in the well. In case HIV-1 p24 is present in the sample, the antigen will be captured by the specific monoclonal antibody.

Antigen-antibody complexes are then identified through the successive addition of: (1) biotinylated peptides, a biotinylated monoclonal antibody to HIV-1 p24, and; (2) horseradish peroxidase HRP Streptavidin conjugate. The hydrolytic activity of horseradish peroxidase allows for the quantification of these antibody-antigen complexes. Peroxidase substrate solution is then added.

During incubation, a blue color will develop in proportion to the amount of anti-HIV-1/2 antibodies or HIV-1 p24 antigen bound to the well, thus establishing their presence or absence in the sample. Wells containing samples negative for anti-HIV antibody and/or p24 antigen remain colorless. A stop solution is added to each well and the resulting yellow color is read on a microplate reader at 450 nm.

3 COMPONENTS

The kit contains reagents for 192 tests [for 96 tests].

1. **Microplate** MICROPLATE
   n°2 microplates. [n°1 microplate]
   12 strips of 8 breakable wells coated with HIV specific gp36, gp41 and gp120 peptides and with a Monoclonal Antibody specific to the HIV-1 p24 Ag. Plates are sealed into a bag with desiccant.

2. **Negative Control** CONTROL -
   1 x 4.0 ml/vial. [1 x 2.0 ml/via] Ready to use control.
   It contains animal serum negative for HIV antibodies and for p24 antigen, and 0.1% Kathon GC as preservatives. The negative control is yellow-brown color coded.

3. **Positive Control HIV-1 Ab**, CONTROL 1+
   1 x 4.0 ml/vial [1 x 2.0 ml/via]. Ready to use control.
   It contains inactivated HIV 1 antibody positive serum, filtered HIV Ab&Ag negative animal serum and 0.1% Kathon GC as preservative. The Positive Control is light green color coded.
   Important Note: The positive control has been inactivated using B-propionolactone BPL/UV. This does not fully ensure the absence of viable pathogens, and therefore, the control should be handled as potentially biohazardous, in accordance with good laboratory practices.

4. **Positive Control HIV-2 Ab**, CONTROL 2+
   1 x 4.0 ml/vial [1 x 2.0 ml/via]. Ready to use control.
   It contains inactivated HIV2 antibody positive serum, filtered HIV Ab&Ag negative animal serum and 0.1% Kathon GC as preservatives. The Positive Control is dark green color coded.
   Important Note: The positive control has been inactivated using B-propionolactone BPL/UV. This does not fully ensure the absence of viable pathogens, and therefore, the control should be handled as potentially biohazardous, in accordance with good laboratory practices.

5. **HIV-1 P24 Ag Standard** CAL Ag,
   2 vials [1 vial]. Lyophilized.
   It contains not infectious recombinant p24 antigen in a 10 mM phosphate buffer pH 7.0+/-0.2 with 0.3 mg/ml Gentamicine Sulphate and 0.1% Kathon GC as stabilizers. This component is calibrated against the NIBSC 1st International HIV-1 p24 Ag reference sample 90/636 (diluted 1:256) as well as the EFS HIV Ag performance panel (30153022).
   Important Notes:
   1) The Calibrator contains p24 recombinant Ag with a concentration of about 100 pg/ml.
   2) The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

6. **Wash buffer concentrate** WASHBUF 20X,
   2 x 60 ml/bottle [1 x 60 ml/bottle]. 20x concentrated solution.
   It contains 0.1% Kathon GC. Once diluted, the wash solution contains 10 mM phosphate buffer saline pH 7.0±0.2 and 0.05% Tween 20.

7. **Conjugate # 1** CONJ 1,
   8 vials [4 vials].
   The vial contains lyophilized biotinylated HIV1&2&0 gp36, gp41 and gp120 peptides and a biotinylated monoclonal antibody specific for HIV 1 p24 antigen.
   Vials are to be resuspended with 6 ml of the Conjugate # 1 diluent.

8. **Conjugate 1 Diluent** CONJ 1 DIL,
   1 x 60 ml/bottle [1 x 30 ml/vial].
   Used to dissolve the lyophilized powder of Conjugate # 1, it contains Tris saline Buffer supplemented with 0.05% Kathon GC, Tween 20 and BSA.

9. **Conjugate # 2** CONJ 2,
   1 x 30 ml/container [1 15 ml/vial].
   The solution contains HRP conjugated with streptavidin in Tris saline Buffer supplemented with 0.05% Kathon GC, Tween 20 and BSA. This component is color coded in red.

10. **Chromogen/Substrate** SUBS TMB,
    1 x 50 ml/container [1 x 25 ml/vial]. Ready-to-use component.
    It contains 50 mM citrate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetramethyl-benzidine or TMB and 0.02% hydrogen peroxide or H_2O_2.
    Note: To be stored protected from light as sensitive to strong illumination.
11. **Sulphuric Acid** H2SO4 0.3M**,**
    1 x 32 ml/container [1 x 15 ml/vial].
    It contains 0.3 M H_2SO_4 solution.
    Attention: Irritant (Xi R36/38; S2/26/30).
12. **Sample Diluent** DILSPE:
    1 x 14 ml/container [1 x 7 ml/vial].
    Contains Tris saline buffer supplemented with 0.05% Kathon GC, anti HAMA blocker, and Tween 20; used for specimen dilution. This component is color coded in light blue.
13. **Plate sealing foils,** n°4; [n°2]
14. **Package insert,** n°1
15. **Conjugate #1 MIX container**: CONJ 1 MIX, 1 empty container.
    Container to be used to unify Conjugate # 1 lyophilise and Conjugate # 1 diluent before starting the automated assay
16. **Container caps,** n°5,
    Plastic cap to be used to firmly seal the container after first use.

4 MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (200ul and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator capable to provide a temperature of +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and possibly with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

5 WARNINGS AND PRECAUTIONS

1. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.

3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2°C - 8°Cinto a tem perature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

6 SPECIMEN: PREPARATION AND RECOMMENDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.

4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°C - 8°Cfor u p to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present filter using 0.2-0.8 µ filters to clean up the sample for testing.
7. Do not use heat inactivated samples as they could give origin to false reactivity.

7 PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not shown any relevant loss of activity up to 2 months.

Microplates:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the pouch is not broken or that some defect is present indicating a problem of storage. In this case call the customer service. Unused strips have to be placed back into the aluminum pouch, in presence of desiccant supplied, firmly zipped and stored at +2°C - 8°C. When opened the first time, residual str ips are stable up to two months.

Negative Control:

Ready to use. Mix well on vortex before use.

Positive Controls Ab:

Positive controls are ready to use. Handle Positive Controls Ab as potentially infective, even if HIV, if present in the control, has been chemically inactivated.

Calibrator Ag

The Lyophilized Calibrator Ag contains a non-infectious recombinant p24 antigen. The volume of EIA grade water to be used for its dissolution and to reach the appropriate p24 concentration (about 100 pg/ml) is written on the vial label. T To help dissolve the lyophilized pellet, vortex a few times, at regular intervals. Complete dissolution should be achieved within 2-5 minutes.

Note: When dissolved the Calibrator is not stable. Store in aliquots at –20°C.

Wash buffer concentrate:

The 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution. In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.

Important Note: Once diluted, the wash solution is stable for 1 week at +2°C - 8°C.

Conjugate # 1:

The Conjugate # 1 mix solution must be prepared immediately before starting the test.
Add 6 ml of Conjugate 1 diluent directly to one Conjugate # 1 vial to dissolve the lyophilized powder. This preparation (a total of 6 ml in one vial) is sufficient for 32 tests, or 4 complete vertical strips of the microplate. To help dissolve the lyophilized powder, vortex a few times, at regular intervals.

Important Note: Any unused portion of this reconstituted Conjugate # 1 Solution may be stored at +2°C - 8°Cfor no more than 12 hours. After this time it has to be discarded and the empty, used container has to be washed with EIA grade water and kept dry for any following re-use.

Conjugate # 2:

Ready to use reagent. Mix well end-over-end before use.

Chromogen/Substrate:

Ready to use. Mix well end-over-end before use.

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong illumination, oxidizing agents and metallic surfaces.

If this component has to be transferred use only plastic, possible sterile disposable container.

Sulphuric Acid:

Ready to use. Mix well end-over-end before use.
Attention: Irritant (Xi R36/38; S2/26/30)
Legend: R 36/38 = Irritating to eyes and skin.
S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Sample Diluent:

Ready to use. Mix well end-over-end before use.

8 INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of ± 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.

3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350 µl/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of +5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450 nm and ideally with a second filter (620-630nm) for blanking purposes. Its standard performances should be (a) bandwidth < 10 nm;
(b) absorbance range from 0 to > 2.0; (c) linearity to > 2.0; repeatability > 1%.
Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended for blood screening when the number of samples to be tested exceed 20-30 units per run.
7. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2°C - 8°C, firmly capped.

9 PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator Ag.
5. Dissolve the Conjugate # 1 vial containing lyophilzed powder with the Conjugate 1 Diluent (1 lyophilized Conjugate # 1 + 6ml Conjugate # 1 Diluent) to obtain the Conjugate # 1 Mix as described in the proper section.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
7. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers' instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.

8. Check that the ELISA reader has been turned on at least 20 minutes before reading.
9. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
10. Check that the micropipettes are set to the required volume.
11. Check that all the other equipment is available and ready to use.
12. In case of problems, do not proceed further with the test and advise the supervisor.

10 ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

10.1 Automated assay

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument dispense 50 µL Sample Diluent first and then 150 µL controls and samples.
Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples or tips have to be changed. For the next operations follow the operative instructions reported below for the Manual Assay. It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

In case the instrument DP1 ScreenEasy is used, follow the indications that the computer provides automatically to the user when the proper assay protocol is launched. Anyway, the correct number of lyophilized Conjugate # 1 must be dissolved each with 6 ml Conjugate # 1 Diluent. Once the lyophilized powders are dissolved and mixed well, they are to be mixed together into the gray plastic container labeled “Conjugate # 1 MIX”. Once the Conjugate # 1 MIX has been transferred to the container the assay may begin.

10.2 Manual assay

1. Dissolve the right number of lyophilized Conjugate # 1 with Conjugate # 1 Diluent before starting to dispense the samples and controls of the test.
2. Place the required number of strips in the microwell holder. Leave the 1st well empty for the operation of blanking.
3. Dispense 50 µL Sample Diluent in all the wells, except A1 used for blanking.
4. Dispense 150 µL of Negative Control in triplicate,
   150 µL HIV1 Positive Control, 150 µL HIV2 Positive Control and 150 µL of Calibrator Ag in duplicate in proper wells.
5. Dispense 150 µL of Sample in each properly identified well. Mix gently the plate on the work surface, avoiding overflowing and contaminating adjacent wells, in order to fully disperse the sample into the diluent.
6. Incubate the microplate for 60 min at +37°C.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

7. Wash the microplate with an automatic washer by delivering and aspirating 350 µL/well of diluted washing solution as reported previously.
8. Pipette 150 µL Conjugate # 1 mix, prepared as described before, into each well, except the 1st blanking well, and cover with the sealer.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Conjugate. Contamination might occur.*

9. Incubate the microplate for **30 min at +37°C.**
10. Pipette 100 µL of Conjugate # 2 in all the wells, except A1, and gently agitate the microplate to mix the two conjugates.

Important Note: *This solution must be added to the bottom of each well to ensure proper performance. Inadequate mixing of the two solutions (Conjugate 1 and Conjugate 2) may reduce the binding of streptavidin HRP (Conjugate 2) to the biotinylated reagents and consequently affect the performance of the assay. Be sure to provide an adequate mixing when the Conjugate # 2 is added, both in the manual and in the automated procedures.*

11. Incubate the microplate sealed for **30 min at +37°C**.
12. Wash as in section 7.
13. Dispense 200 µL of Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-25°C) for 30 minutes**. Start the timing immediately after addition of this component to the first well.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

14. Pipette 100 µL Sulphuric Acid into all the wells using the same pipetting sequence as in step 13 to stop the enzymatic reaction. Addition of acid will turn the positive controls and positive samples from blue to yellow.
15. Measure the color intensity of the solution in each well, as described in section 9.5, at 450 nm filter (reading) and possibly at 620-630 nm (background subtraction), blanking the instrument on A1.

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Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450 nm. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 30 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

11 ASSAY SCHEME

Method	**Operations**
Sample Diluent	50 µL
Controls and calibrator	150 µL
Samples	150 µL
1st incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
Conjugate # 1	150 µL
2nd incubation	**30 min**
Temperature	+37°C
Conjugate # 2	100 µL
3rd incubation	**30 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	200 µL
4th incubation	**30 min**
Temperature	r.t.
Sulphuric Acid	100 µL
Reading OD	450 nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	13
A	BLK	CAL Ag										
B	NC	CAL Ag										
C	NC	S1										
D	NC	S2										
E	POS 1 Ab	S3										
F	POS 1 Ab	S4										
G	POS 2 Ab	S5										
H	POS 2 Ab	S6										

Legenda: BLK = Blank NC = Negative Control POS 1 Ab = HIV -1 Ab Positive Control, POS 2 Ab = HIV -2 Ab Positive, CAL Ag = HIV p24 Ag Calibrator, S = Sample

12 INTERNAL QUALITY CONTROL

A check is carried out on the controls and the calibrator any time the kit is used in order to verify whether their OD450nm values are as expected and reported in the table below.

Check	Requirements
Blank well	< 0.100 OD 450 nm value
Negative Control (NC)	≤ 0.200 mean OD450nm value after blanking Absorbance of individual negative control values must be less than or equal to 0.200. If one value is outside this range, discard this value and recalculate mean. If two values are outside this range the run should be repeated.
HIV-1 Ab Positive Control	Mean OD450nm ≥ 0.700.
HIV-2 Ab Positive Control	Mean OD450nm ≥ 0.700.
HIV Ag Calibrator	S/Co > 1

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Blank well > 0.100 OD 450 nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Negative Control (NC) > 0.200 OD 450 nm after blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of their wells has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Controls < 0.700 OD 450 nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of positive control. In this case, the negative control will have an OD450nm value > 0.200, too. 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.
HIV Ag Calibrator S/Co < 1	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of Calibrator Ag. In this case, the negative control will have an OD450nm value > 0.200, too. 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred. 5. that the lyophilize powder was dissolved correctly with the correct volume of water written on the vial label.

Should these problems happen, after checking, report any residual problem to the supervisor for further actions.

13 CALCULATION OF THE CUT-OFF

The tests results are calculated by means of a cut-off value determined with the following formula on the mean OD 450 nm value of the Negative Control (NC):

NC + 0.125 = Cut-Off (Co)

The value found for the test is used for the interpretation of results as described in the next paragraph.

Important note: *When the calculation of results is done by the operative system of an ELISA automated work station be sure that the proper formulation is used to calculate the cut-off value and generate the right interpretations of results.*

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