fortress diagnostics

ISO 13485 accredited company

BXE0793A

96 TESTS

STORE AT 2-8ºC

FOR IN-VITRO DIAGNOSTICS USE ONLY

anti-HIV 1+2 ELISA

Read the package insert carefully and completely before performing the assay. Follow the instructions and do not modify them. Only by strict adherence to these instructions, the erroneous results can be avoided and the optimal performance of anti-HIV 1+2 ELISA achieved.

INTENDED USE

anti-HIV 1+2 ELISA is an enzyme-linked immunosorbent assay (ELISA) intended for qualitative detection of antibodies to Human Immunodeficiency Viruses (HIV) type 1 (group M - O) or type 2 in human serum or plasma samples. The assay can be utilized for screening of blood donors and/or as an aid in the diagnosis of clinical conditions related to infection with HIV-1 and /or HIV-2 – the etiological agents of the acquired immunodeficiency syndrome (AIDS).

SUMMARY

Serological evidence of infection with HIV may be obtained by testing for presence of HIV antigens or antibodies in serum of individuals suspected for HIV infection. Antigen can generally be detected during both acute phase and the symptomatic phase of AIDS only. The antibodies to HIV-1 and/or HIV-2 can be detected throughout virtually the whole infection period, starting at or shortly after the acute phase and lasting till the end stage of AIDS[1]. Therefore, the use of highly sensitive antibody assays is the primary approach in serodiagnosis of HIV infection. Apart from sexual transmission, the principal route of infection with HIV is blood transfusion. HIV can present both in cellular and cell-free fractions of human blood. Therefore, all donations of blood or plasma should be tested due to the risk of HIV transmission through contaminated blood[2]. This can be effectively achieved by testing for the antibodies to HIV-1 and HIV-2 by using a highly sensitive ELISA tests[3].

PRINCIPLE OF THE TEST

anti-HIV 1+2 ELISA is a two step incubation antigen "sandwich" enzyme immunoassay kit, which uses polystyrene microwell strips pre-coated with recombinant HIV antigens expressed in E.coli (recombinant HIV-1gp41, gp120, and recombinant HIV-2 gp-36). Patient's serum or plasma sample is added, and during the first incubation step, the specific HIV1/2 antibodies will be captured inside the wells if present. The microwells are then washed to remove unbound serum proteins. A second set of recombinant antigens conjugated to the enzyme Horseradish Peroxidase (HRP-Conjugate) and expressing the same epitopes as the pre-coated antigens is added, and during the second incubation, they will bind to the captured antibody. The microwells are washed to remove unbound conjugate, and Chromogen solutions are added into the wells. In wells containing the antigen-antibody-antigen(HRP) "sandwich" immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after the reaction is stopped with sulphuric acid. The amount of color intensity can be measured and it is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HIV 1/2 remain colorless.

In Vitro Diagnostic Use Only

This kit contains reagents sufficient for testing of maximum of 91 specimens in a test run.

Code / Size	Component / Description
CODE 5 (1x96 wells) 8×12/12×8 well per plate	**MICROWELL PLATE:** Blank microwell strips fixed on white strip holder. The plate is sealed in aluminium pouch with desiccant. Each well contains recombinant HIV 1/2 antigens (recombinant HIV-1 gp41, gp120, and recombinant HIV-2 gp36). The microwell strips can be broken to be used separately. Place unused strips in the provided plastic storage bag together with the desiccant and return to 2-8°C. Once open, the plate strips are stable for one month when stored at 2-8°C together with the desiccant. The microwell strips are for SINGLE USE only. Do not use if the vacuum sealing has been damaged when first time taken of out the box.
CODE 8 (1x1ml per vial) preserv.0.1% ProClinTM 300	**NEGATIVE CONTROL:** Yellow-colored liquid filled in a vial with green screw cap. Protein-stabilized buffer tested non reactive for non reactive for HIV 1/2, HBsAg, HCV and TP antibodies. Ready to use as supplied. Once open, stable for one month at 2-8°C.
CODE 7 (1x1ml per vial) preserv.0.1% ProClinTM 300	**POSITIVE CONTROL -1:** Red-colored liquid filled in a vial with red screw cap. Protein-stabilized buffer solution tested positive for antibodies to HIV-1. Ready to use as supplied. Once open, stable for one month at 2-8°C.
CODE 7a (1x1ml per vial) preserv.0.1% ProClinTM 300	**POSITIVE CONTROL -2:** Red-colored liquid filled in a vial with yellow screw cap. Protein-stabilized buffer solution tested positive for antibodies to HIV-2. Ready to use as supplied. Once open, stable for one month at 2-8°C.
CODE 6 (1x12ml per vial) preserv.0.1% ProClinTM 300	**HRP-CONJUGATE:** Red-colored liquid in a white vial with red screw cap. Horseradish peroxidase-conjugated recombinant HIV 1+2 antigens. Ready to use as supplied. Once open, stable for one month at 2-8°C.
CODE 1 (1x50ml per bottle) ***DILUTE BEFORE USE!*** detergent Tween-20	**WASH BUFFER:** Colorless liquid filled in a clear bottle with white screw cap. PH 7.4, 20 × PBS The concentrate must be diluted 1 to 20 with distilled/ deionized water before use. Once diluted, stable for one week at room temperature, or for two weeks when stored at 2-8°C.
CODE 2 (1x8ml per vial)	**CHROMOGEN SOLUTION A:** Colorless liquid filled in a white vial with green screw cap. Urea peroxide solution. Ready to use as supplied. Once open, stable for one month at 2-8°C.
CODE 3 (1x8ml per vial)	**CHROMOGEN SOLUTION B:** Colorless liquid filled in a black vial with black screw cap.TMB solution (Tetramethyl benzidine). Ready to use as supplied. Once open, stable for one month at 2-8°C.
CODE 4 (1x8ml per vial)	**STOP SOLUTION:** Colorless liquid in a white vial with yellow screw cap. Diluted sulfuric acid solution (0.5M H_2SO_4). Ready to use as supplied. Once open, stable for one month at 2-8°C.

- PLASTIC SEALABLE BAG: For enclosing the strips not in use (1 unit)
- PACKAGE INSERT (1 copy)
- CARDBOARD PLATE COVER (3 sheets)

To cover the plates during incubation and prevent evaporation or contamination of the wells.

MATERIALS REQUIRED BUT NOT PROVIDED

Freshly distilled or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, dispensing system and/or pipette, disposable pipette tips, absorbent tissue or clean towel, dry incubator or water bath, 37±0.5°C, plate reader, single wavelength 450nm or dual wavelength 450/630nm, microwell aspiration/wash system.

SPECIMEN COLLECTION, TRANSPORTING AND STORAGE

1. Specimen Collection: No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particulate matters in the specimen should be removed by centrifugation at 3000 RPM (round per minutes) for 20 minutes at room temperature or by filtration.
2. Plasma specimens collected into EDTA, sodium citrate or heparin may be tested, but **highly lipaemic, icteric, or hemolytic specimens should not be used** as they can give false results in the assay. **Do not heat inactivate specimens.** This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.
3. anti-HIV 1+2 ELISA is intended ONLY for testing of individual serum or plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
4. **Transportation and Storage:** Store specimens at 2-8°C. Specimens not required for assaying within 3 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, do not freeze. To assure maximum performance of anti HIV 1+2 ELISA, during storage, protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

TO BE USED ONLY FROM QUALIFIED PROFESSIONALS

The ELISA assays are time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.
7. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
8. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.
9. Assure that the incubation temperature is 37°C inside the incubator.
10. When adding specimens, do not touch the well's bottom with the pipette tip.
11. When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.
12. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.
13. If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.
14. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.
15. **WARNING:** Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
16. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
17. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
18. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.
19. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.
20. The Stop solution 0.5M H_2SO_4 is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.
21. ProClinTM 300 0.1% used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

INDICATIONS OF INSTABILITY DETERIORATION OF THE REAGENT:

Values of the Positive or Negative controls, which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results and proven deterioration or instability of the reagents, immediately substitute the reagents with new one or contact Fortress technical support for further assistance.

ProClin™ 300
S phrases:
S26-28-36/37/39-45-60-61
R phrases: 43

Do not eat and drink at the laboratory

Ware protective clothing
Ware eye protection

Biohazard
Danger

PROCEDURE

Reagents preparation: Allow the reagents to reach room temperature (18-30°C). Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash buffer (20X) as indicated in the washing step instructions. Use distilled or

deionized water and only clean vessels to dilute the buffer. All other reagents are **READY TO USE AS SUPPLIED**.

STEP 1	Preparation: Mark three wells as Negative control (e.g. B1, C1, D1), two wells as Positive control (e.g. E1 for HIV-1 and F1 for HIV-2) and one Blank (e.g. A1, neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.
STEP 2	Adding Sample: Add 100µl of Positive control, Negative control, and Specimen into their respective wells except the Blank. Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination.
STEP 3	Incubating: Cover the plate with the plate cover and incubate for 30 minutes at 37°C.
STEP 4	Washing: At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remainders.
STEP 5	Adding HRP-Conjugate: Add 100µl HRP-Conjugate into each well except the Blank.
STEP 6	Incubating: Cover the plate with the plate cover and incubate for 30 minutes at 37°C.
STEP 7	Washing: At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Washing buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
STEP 8	Coloring: Add 50µl of Chromogen A and 50µl Chromogen B solutions into each well including the Blank. Incubate the plate at 37°C for 15 minutes avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and anti-HIV ½ positive sample wells.
STEP 9	Stopping Reaction: Using a multichannel pipette or manually, add 50µl Stop solution into each well and mix gently. Intensive yellow color develops in Positive control and anti-HIV 1/2 positive sample wells.
STEP 10	Measuring the Absorbance: Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 10 minutes after stopping the reaction).

INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential in order to obtain correct and precise analytical data.
2. It is therefore, recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles of 350-400µl/well are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with specimen or HRP-conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to carry out 5 washing cycles, dispensing 350-400µl/well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before they are wasted in an appropriate way.
7. The concentrated Wash buffer should be diluted 1:20 before use. If less than a whole plate is used, prepare the proportional volume of solution.

QUALITY CONTROL AND CALCULATION OF THE RESULTS

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well A value from the print report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well A value from the print report values of specimens and controls.

Calculation of the Cut-off value (C.O.) = Nc + 0.12 (Nc = the mean absorbance value for three negative controls).

Quality control (assay validation): The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

- The A value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450 nm.
- The A values of the Positive control must be ≥ 0.800 at 450/630nm or at 450nm after blanking.
- The A values of the Negative control must be < 0.100 at 450/630nm or at 450nm after blanking.

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded and the mean value calculated again using the remaining two values. If more than one Negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Example:

1. Quality Control

Blank well A value: A1= 0.025 at 450nm (Note: blanking is required only when reading with single filter at 450nm)

Well No.:	**B1**	**C1**	**D1**
Negative control A values after blanking:	0.020	0.012	0.016
Well No.:	**E1**	**F1**	
Positive control A values after blanking:	2.421	2.369	

All control values are within the stated quality control range

2. Calculation of Nc: = $\frac{(0.020+0.012+0.016)}{3}$ = 0.016

3. Calculation of the Cut-off: (C.O.) = 0.016 +0.12 = 0.136

INTERPRETATIONS OF THE RESULTS

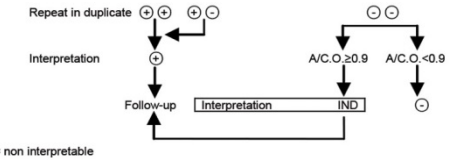
Negative Results (A / C.O. < 1): Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no anti-HIV 1/2 antibodies have been detected with anti-HIV 1+2 ELISA, therefore the patient is probably not infected with HIV 1/2 and the blood unit do not contain antibodies to HIV 1/2 and could be transfused in case that other infectious diseases markers are also absent.

Positive Results (A / C.O. ≥ 1): Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that anti-HIV 1/2 antibodies have probably been detected using anti-HIV 1+2 ELISA. All initially reactive specimens should be retested in duplicates using anti-HIV 1+2 ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for antibodies to HIV 1/2 with anti-HIV 1+2 ELISA.

Borderline (A / C.O. = 0.9-1.1): Specimens with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results.

Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system (e.g. WB, PCR) is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

INITIAL RESULTS INTERPRETATION AND FOLLOW-UP
ALL INITIALY REACTIVE OR BORDERLINE SAMPLES



IND = non interpretable

- If, after retesting of the initially reactive samples, both wells are negative results (A/C.O.<0.9), these samples should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step. For more information regarding Fortress ELISA Troubleshooting, please refer to Fortress's "ELISAs and Troubleshooting Guide".
- If after retesting in duplicates, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for antibodies to HIV 1/2 and therefore the patient is probably infected with HIV 1/2 and the blood unit must be discarded.
- After retesting in duplicates, samples with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone sample, or uninterpretable for the time of testing.

PERFORMANCE CHARACTERISTICS

Evaluation study carried in Alkmaar, the Netherlands, between April and November 2005, demonstrated the following performance characteristics of anti-HIV 1+2 ELISA: The diagnostic specificity of the kit was 99.85% as determined on all negative samples (5471) that were investigated. When examined on the unselected donors only (random and first time donors), the specificity was 99.92% (95% CI 99.84-100%).

anti-HIV 1+2 ELISA test results on unselected donors:

Panel	No tested	Positive (A/C.O. ≥ 1)		Negative (A/C.O. <1)	
		No	%	No	%
Radom serum donor	2654	2	0.08	2652	99.92
Random plasma donor	1400	1	0.07	1399	99.93
First time donor	989	1	0.10	988	99.90
Total	5043	4	0.08	5039	99.92

All panels of HIV-1, HIV-1 subtype O and HIV-2 confirmed antibody positive samples that were used in this study were also tested reactive with anti-HIV 1+2 ELISA which resulted in diagnostic sensitivity of 100%.

A total of 32 seroconversion panels, which represent 210 samples tested. 13 samples not classified from PRB918 and PRB917 because there are not data of Antigen or RNA determination required for the classification. 41 samples classified as negative. RNA and or Antigen negative. 61 samples classified as early-seroconversion. 95 samples classified as seroconversion.

The testing results also show that anti-HIV 1+2 ELISA is a state-of-the-art compare to most of the currently available on the market CE-marked tests.

The analytical sensitivity was evaluated on PeliCheck anti-HIV panels. The analytical sensitivity of anti-HIV 1+2
ELISA on the PeliCheck anti-HIV standard dilutions was comparable to other anti-HIV assays.

Analytical specificity: anti-HIV 1+2 ELISA test results on samples from hospitalized patients and samples containing potentially cross-reacting blood-specimens.

Type of sample	No tested	Positive (A/C.O. ≥ 1)		Negative (A/C.O. <1)	
		No	%	No	%
Mononucleosis	296	4	1.35	292	98.65
Pregnant woman	101	0	0	101	100
RF+	17	0	0	17	100
Anti-TPO	5	0	0	5	100
Anti-smooth muscle	5	0	0	5	100
Elevated IgG levels	4	0	0	4	100
Total	428	4	0.93	424	99.07

In a separate study, the following specificity results were obtained:

- Possible high dose hook effect is eliminated due to the implementation of two-step procedure.
- Frozen positive/negative specimens have been tested to check for interferences due to collection and storage. The performance characteristics of anti-HIV 1+2 ELISA were not affected for at least 3 freeze/thaw cycles.
- Samples from patents infected with hepatitis A, B, C as well as samples from patients infected with Treponema pallidum were tested with no cross-reactive reactions observed.
- 25 positive fresh serum samples tested in INSTITUTE FOR TROPICAL MEDICINE, BELGIUM have been tested with anti-HIV 1+2 ELISA. All 25 positive fresh serum samples have been positive with anti-HIV 1+2 ELISA.

Accuracy: The below tables represent the results of analytical sensitivity and reproducibility of anti-HIV 1+2 ELISA as controlled with PeliSpyMulti-Marker run control and with Fortress QC sample tested in every plate – the 1:2048 dilution of the anti-HIV standard in this PeliSpy sample was consistently detected in all plates. Fortress's QC sample was always detected in all plates.

PeliSpyMulti-Marker results:

Dilution	n	Average	Percentiles 5th	Percentiles 95th	Measured Min.	Measured Max.
1:2048	80	3.08	1.76	4.39	1.70	4.96

Wantai's QC sample results:

n	Average	Percentiles 5th	Percentiles 95th	Measured Min.	Measured Max.
160	7.71	5.32	10.10	4.37	10.89

Intra-batch variations:

Serum Sample	Max.	Min.	Average	CV
Strong positive	2.785	2.315	2.469	≤15%
Strong positive	2.789	2.302	2.625	≤15%
Moderate positive	1.302	1.011	1.161	≤15%
Moderate positive	1.305	1.044	1.228	≤15%
Weak positive	0.638	0.415	0.464	≤15%
Weak positive	0.601	0.418	0.511	≤15%

Inter-batch variations:

Serum Sample	Max.	Min.	Average	CV
Strong positive	2.755	2.355	2.574	≤15%
Strong positive	2.579	2.324	2.425	≤15%
Moderate positive	1.122	1.087	1.098	≤15%
Moderate positive	1.255	1.087	1.124	≤15%
Weak positive	0.602	0.457	0.498	≤15%
Weak positive	0.581	0.433	0.458	≤15%

Operator variations:

Serum Sample	Max.	Min.	Average	CV
Strong positive	2.865	2.401	2.554	≤15%
Strong positive	2.579	2.287	2.333	≤15%
Moderate positive	1.232	1.054	1.101	≤15%
Moderate positive	1.241	1.066	1.101	≤15%
Weak positive	0.587	0.498	0.524	≤15%
Weak positive	0.615	0.521	0.531	≤15%

LIMITATIONS

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antibodies may be undetectable during the early stage of the disease and in some immunosuppressed individuals. Therefore, negative results obtained with anti-HIV 1+2 ELISA are only indication that the sample does not contain detectable level of anti-HIV 1/2 antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with HIV 1/2 or the blood unit is not infected with HIV 1/2.
3. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step. For more information regarding Fortress ELISA Troubleshooting, please refer to Fortress's "ELISAs and Troubleshooting Guide", or contact Fortress technical support for further assistance.
5. The most common assay mistakes are: using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.
6. The prevalence of the marker will affect the assay's predictive values.
7. This assay cannot be utilized to test pooled (mixed) plasma. anti-HIV 1+2 ELISA has been evaluated only with individual serum or plasma specimens.
8. anti-HIV 1+2 ELISA is a qualitative assay and the results cannot be use to measure antibodies concentrations. This assay cannot distinguish between infections with HIV-1 and HIV-2.

REFERENCES

1. Barre-Sinoussi, F et al., (1984) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immunodeficiency syndrome (AIDS), Science, 220: 868-871.
2. Barbe, F.et al., (1994) Early detection of anti bodies to HIV-1 by a third generation enzyme immunoassay. Ann. Biol. Clin. (Paris), 52: 341-345.
3. Constantine,. N., T. et al., (1993) Serologic test for the retroviruses: approaching a decade of evolution. AIDS, 7: 1-13Gnann JW et al. (1987) Science; 237: 1346-1349.

SUMMARY OF THE MAJOR COMPONENTS OF THE KIT:

Use this summary only as a reference and always follow the comprehensive method sheet when performing the assay. Note: the components of individual kits are not lot- interchangeable.

1	Microwell plate	Code 5	one
2	Negative Control	Code 8	1x1ml
3	Positive Control (HIV-1)	Code 7	1x1ml
4	Positive Control (HIV-1)	Code 7a	1x1ml
5	HRP-Conjugate	Code 6	1x12ml
6	Wash Buffer	Code 1	1x50ml
7	Chromogen Solution A	Code 2	1x8ml
8	Chromogen Solution B	Code 3	1x8ml
9	Stop Solution	Code 4	1x8ml

SUMMARY OF THE ASSAY PROCEDURE:

Use this summary only as a reference and always follow the detailed method sheet when performing the assay.

Add Samples	100µl
Incubate	30 minutes
Wash	5 times
Add HPR-Conjugate	100µl
Incubate	30 minutes
Wash	5 times
Coloring	50µl A + 50µl B
Incubate	15 minutes
Stop the reaction	50µl stop solution

Read the absorbance	450nm or 450/630 nm

EXAMPLE SCHEME OF CONTROLS / SAMPLES DISPENSING:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	S3										
B	Neg.	…										
C	Neg.	…										
D	Neg.											
E	Pos.											
F	Pos.											
G	S1											
H	S2											

IVD For In Vitro Diagnostics Use Only

LOT Batch

REF Catalogue Number

Storage Temperature Conditions

Use By / Expiry Date (Year / Month)

Warning, Read Enclosed Documents

Instructions For Use

Manufactured By

Fortress Diagnostics Limited,
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