Size : 274 x 218 mm

Retrolisa 3.0®
3rd Generation ELISA to detect antibodies to HIV-1& 2

Third Generation Double Antigen Sandwich Enzyme Linked Immunosorbent Assay (ELISA) for the detection of Total Antibodies to HIV-1 & HIV-2 including HIV-1 group "O" in human serum or plasma

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

RETROLISA® 3.0 is intended to be used for the detection of total antibodies (i.e. IgG, IgM, IgA etc) to both HIV-1 & HIV-2 including HIV-1 GROUP "O" in human serum or plasma.

SUMMARY AND EXPLANATION

Present evidence indicates that Acquired Immunodeficiency Syndrome is caused by HIV-1 and HIV-2. The viruses are transmitted by sexual contact, exposure to blood (including sharing contaminated needles and syringes) or certain blood products, or transmitted from an infected mother to her fetus or child during the prenatal period. Presence of antibodies to the virus in the serum of a patient indicates viral infection.

RETROLISA® 3.0 is a third generation solid phase Enzyme Linked Immunosorbent Assay (ELISA) which employs highly purified recombinant antigens representing envelope glycoprotein gp41 and core protein p24-O subtype fusion polypeptide of HIV-1 and envelope glycoprotein gp36 of HIV-2. The use of HIV antigens to sandwich specific antibodies enables detection of IgG, IgM and IgA antibodies thus reducing window period and earlier detection of seroconverters. Some reports suggests that third generation ELISA's like **RETROLISA® 3.0** can detect HIV antibodies at 2-3 weeks earlier than second generation ELISA's that detects only IgG.

PRINCIPLE OF THE ASSAY

Microwell strips are coated with recombinant antigens, gp41, p 24-O subtype fusion polypeptide and gp36 representing both HIV-1 and HIV-2. Samples along with positive and negative controls are added in the coated wells and incubated. The wells are washed to remove unbound components. The presence of bound antibodies is detected by adding Antigen- HRP conjugate. After washing wells to remove unbound enzyme, substrate is added. The reaction is stopped after specified time with acid and absorbance is determined for each well at 450 nm with an ELISA reader. The cutoff value is calculated by the given formula and absorbance of all the wells are compared with the cutoff value. Any sample having absorbance more than the cutoff value is considered reactive.

KIT COMPONENTS

RETROLISA® 3.0 has following components:

1. Coated microwells : Microwells coated with recombinant antigens representing both HIV-1 and HIV -2. Ready to use.
   96 Wells: (3x8) x 4 pouches
   192 Wells: (3x8) x 8 pouches
   480 Wells: (3x8) x 20 pouches
2. Positive control: Inactivated and stabilized human serum reactive for HIV -1 with preservatives.
3. Negative control: Inactivated and stabilized human serum non reactive for HIV-1 and HIV-2, HBsAg and HCV.
4. Conjugate: Antigen-HRP conjugate (50 X). To be diluted 50 times with conjugate diluent.
5. Conjugate diluent: Buffered solution containing stabilizing proteins and preservatives.
6. Sample diluent: Buffered solution containing stabilizing proteins and preservatives.
7. Substrate: Solution containing Tetramethyl benzidine (TMB) and hydrogen peroxide. Ready to use.
8. Wash buffer: Buffer containing surfactants (20 X). To be diluted 20 times with distilled or deionized water.
9. Stop solution:Diluted Sulphuric acid.
10. Microwell holder.
11. Instruction for use.
12. ELISA protocol sheet.
13. Plate sealer.

STORAGE AND STABILITY

(1). **RETROLISA® 3.0** kit is stable at 2-8°C up to the expiry date printed on the label. (2).Coated microwells should be used within two months of opening the pouch. Once opened, the pouch must be sealed properly to protect from moisture. (3). Diluted conjugate must be used immediately. (4).Diluted wash buffer is stable upto one week.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Manual or automatic pipette.
2. Pipette tips.
3. Incubator.
4. Absorbent sheets.
5. ELISA washer.
6. ELISA reader
7. Pipetting troughs or boats
8. Disinfectant
9. Reagent grade water
10. Disposable gloves
11. Timer
12. Biohazard waste container
13. Serological pipettes.

Size : 137 x 218 mm

SAMPLE COLLECTION

(1). No prior preparation of the patient is required. (2). Collect blood specimen by venipuncture according to the standard procedure. (3).Serum or plasma can be used. (4). Specimen should be free of particulate matter and microbial contamination. (5). Preferably use fresh sample. However, specimen can be stored refrigerated for short duration. For long storage, freeze at -20^{0}C or below. Do not freeze samples in frost-free freezer. (6). Specimen should not be frozen and thawed repeatedly. (7). Do not heat inactivate before use. (8). Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.

PRECAUTIONS

(1). Bring all reagents and specimen to room temperature before use. (2). Do not pipette any material by mouth. (3).Do not eat, drink or smoke in the area where testing is done. (4).Use protective clothing and wear gloves when handling samples. (5). Use absorbent sheet to cover the working area. (6). Immediately clean up any spills with sodium hypochlorite. (7).Dispose off all the reagents and material used as if they contain infectious agent. (8).Neutralize acid containing waste before adding hypochlorite. (9).Do not use kit after the expiration date. (10).Do not mix components of one kit with another. (11). Always use new tip for each specimen and reagent. (12). Do not let the dispensing tip of ELISA washer touch liquid in the wells. (13). Do not allow liquid from one well to mix with other wells. (14).Do not let the strips dry in between steps. (15).Incubation times and temperatures other than those specified may give erroneous results. (16). Cross contamination of reagents or samples may give false results. Samples must remain confined to microwells during testing. (17).Inadequate removal of residual wash buffer can cause inconsistent color development. Microwell strips should be tapped vigorously and blotted on absorbent paper or towels to minimize residual wash buffer. (18).Reusable glassware must be washed and thoroughly rinsed free of detergents prior to use. All glassware must be clean and dry before use. (19).Adequate washing of wells is extremely important. Inadequately washed wells will exhibit high background values, and may show false positive values. For manual washing, aspirate the contents of the wells, then fill each well with wash buffer solution. Avoid cross- contamination of the wells, particularly in the first wash after aspiration. Drain all of the wash buffer from the wells by inverting, then shaking residual wash buffer from the wells with a sharp “snapping” motion of the wrist. Repeat these steps for at least six washes. The wells should then be tapped vigorously on a paper towel or other absorbent material to remove all traces of residual wash buffer. The use of an automated microwell washing system will assure consistent washing of the wells, and is recommended.

REAGENT PREPARATION

1. Dilute wash buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water).
2. Dilute conjugate 50 times (for example add 20 µl concentrated conjugate to 980 µl conjugate diluent).

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
50 x Conjugate	20µl	40µl	60µl	80µl	100µl	120µl	140µl	160µl	180µl	200µl	220µl	240µl
Conjugate Diluent	980µl	1960µl	2940µl	3920µl	4900µl	5800µl	6860µl	7840µl	8820µl	9800µl	10780µl	11760µl

TEST PROCEDURE

1. Bring all the reagents and specimen to room temperature before use.
2. Take out required number of strips and immediately close the pouch.
3. Prepare data sheet indicating the location of controls and specimen.
4. Use controls in duplicate.
5. Add **100 µl** Sample diluent in each well.
6. Add **50 µl** control or specimen in separate wells.
7. Apply plate sealer and incubate for 30 minutes at 37^{0} C.
8. Wash each well by filling approximately 350 ml diluted wash buffer, giving 30 seconds soak time for each wash and aspirating/flicking off six times. Blot dry.
9. Add **100 µl** diluted conjugate in each well and incubate for 30 minutes at room temperature(20-28^{0}C)
10. Wash six times as in step 8. Blot dry.
11. Add **100 µl** substrate in each well and incubate at room temperature (20-28^{0} C) away from light for 30 minutes.
12. Stop reaction by adding **100 µl** stop solution. The stop solution should be added in the same sequence as substrate addition.
13. Read the absorbance at 450 nm with 600-700 nm as reference within 30 minutes of stopping the reaction.

RUN CRITERIA

1. The individual absorbance value of negative controls should be less than 0.1.
2. The individual absorbance value of positive controls should be more than 1.0.

CALCULATIONS

The cutoff value (COV) is calculated by adding 0.2 to average absorbance value of negative control.

EXAMPLE: COV = Av.Nc+0.2

Absorbance of negative control	
1	0.02
2	0.015
Avg NC	0.017

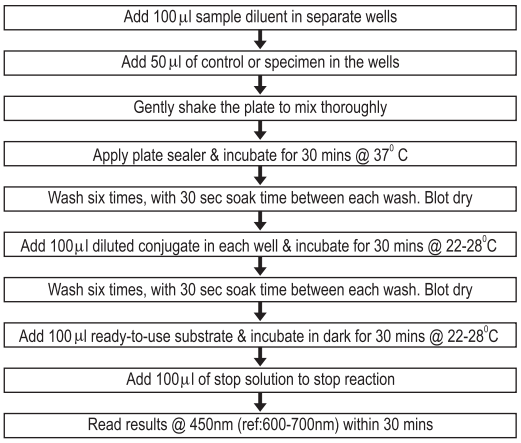
Absorbance of positive control	
1	2.723
2	2.703
Avg PC	2.713

Cutoff Value = Av.Nc + 0.2 = 0.017 + 0.2 = 0.217

SAMPLE DATA

Well	Absorbance	Mean	Cutoff	Result
NC	0.02	0.017	0.228	
NC	0.015			
PC	2.723	2.713	0.217	
PC	2.703			
Sample 1	3.487			Reactive
Sample 2	0.654			Reactive
Sample 3	0.056			Non-Reactive

RETROLISA®3.0 procedure flow-chart



INTERPRETATION OF RESULTS

1. Samples with absorbance value less than the cutoff value are considered non-reactive by **RETROLISA® 3.0** ELISA kit and are considered negative for HIV 1 / 2
2. Samples with absorbance value equal to or greater than cutoff value are considered reactive by **RETROLISA® 3.0** ELISA kit. The original sample should be retested in duplicate. Initially reactive sample that do not react in either of duplicate are considered negative for antibodies to HIV 1 / 2. Initially reactive sample that reacts in either or both duplicates are considered repeatedly reactive.
3. If a sample is repeatedly reactive the probability of antibodies to HIV 1 / 2 are high, especially with patients at high risk or high absorbance values. Such samples should be retested with supplemental third generation tests. Specimens that are repeatedly reactive in ELISA but not reactive in additional testing are considered indeterminate and a further sample after 3 to 6 months should be tested.
4. In case of samples with high OD, there are possibilities of black precipitate formation after the addition of stop solution. This will not interfere with the interpretation of results.

NOTE

All ELISA readers have a linear measuring range (approx. 2.5Å). Beyond this range the OD values are non-linear. That is why some instruments can be pre-programmed to show "out of range" values if the OD exceeds 2.5 or 3.00. **RETROLISA® 3.0** positive control may show "out of range" values in such instruments even after dilution. Please note that this indicate a valid run (provided the negative control value is < 0.1) and does not interfere with assay results..

PERFORMANCE CHARACTERISTICS

Four hundred and sixty four samples-out of which one hundred and fourteen HIV positive specimen and three hundred and fifty HIV negative specimen were tested with **RETROLISA®3.0** and compared with commercially available ELISA. The results are as shown below.

Specimen Data	Total	RETROLISA® 3.0	Commercial ELISA
Total Number	464	464	464
HIV Positive	114	114	114
HIV Negative	350	349	350

Sensitivity of **RETROLISA® 3.0** : 100 %

Specificity of **RETROLISA® 3.0** : 99.71 %

Evaluation with Seroconversion panel

RETROLISA® 3.0 was evaluated with anti-HIV-1 serconversion Panel D (PRB904) obtained from Boston Biomedica Inc., USA. The results are as follows:

PRB 904-01	0	0.20	0.5	No Bands	BLD**
PRB 904-02	21	0.10	0.4	No Bands	BLD
PRB 904-03	49	0.40	0.5	No Bands	Positive
PRB 904-04	92	11.62	5.1	18,24, f41, 55, f65, 120, 160	Positive
PRB 904-05	99	14.41	4.9	18,24, 41, 51, 55, 65, 120, 160	Positive

Evaluation with Anti-HIV 1 Low Titre Performance Panel

RETROLISA® 3.0 was evaluated with anti-HIV-1 Low Titre Performance Panel (Modified) PRB 107M obtained from Boston Biomedica Inc., USA. The results are as follows :

Panel ID #	Gen Sys HIV 1/2*	Org. Tek Uni-Form II+O*	RETROLISA®3.0*	Bio Rad Western Blot	Roche RNA PCR
PRB 107-02	2.4	2.5	6.75	24,55	Positive
PRB 107-03	0.3	3.7	5.14	No Bands	Positive
PRB 107-05	0.1	0.6	0.10	No Bands	BLD**
PRB 107-06	3.2	3.0	12.55	No Bands	Positive
PRB 107-07	1.1	2.8	3.04	No Bands	Positive
PRB 107-08	0.6	2.4	10.20	No Bands	Positive
PRB 107-10	1.7	3.5	14.41	No Bands	Positive
PRB 107-12	0.2	2.6	11.90	No Bands	Positive
PRB 107-13	1.8	3.9	11.15	24	Positive
PRB 107-15	2.2	5.1	5.34	24	Positive

Values are specimen to cut-off ratios. Ratios = 1.0 is considered reactive. Data other than that for **RETROLISA® 3.0** was provided by BBI

**BLD Below Detection limit

REMARKS

(1). Though **RETROLISA®3.0** is a reliable screening assay, it should not be used as a sole criterion for diagnosis of HIV infection. (2). Absence of antibodies to HIV does not indicate that an individual is absolutely free of HIV infection as the collection of sample and its timing vis-à-vis seroconversion will influence the test outcome. (3).Since various tests for HIV differ in their performance characteristics and antigenic composition, their reactivity patterns may differ. (4).Testing of pooled samples is not recommended. (5). As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated. (6). **RETROLISA® 3.0** should only be used as a screening test and its results should be confirmed by other supplemental method before taking clinical decisions.

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(1) Centers for Disease Control, Update on Acquired Immune Deficiency Syndrome (AIDS) MMWR 1982; 31;507-508. (2) Popovic. M., et.al., Detection Isolation and continuous production of Cytopathic Retroviruses (HTLV-III) from patients with AIDS and pre-AIDS, Science 19834:224:497-500. (3) Harshida .S. et.al., Earlier detection of Human immunodeficiency Virus Type 1 p24 antigen and immunoglobulin G and M antibodies to p17 antigen in seroconversion serum panels by immune complex transfer enzyme immunoassays, Clinical and Diagnostic Laboratory Immunology, Nov 2000 p872-881, Vol 7, No.6. (4) Dorn et.al., Analysis of Genetic Variability within the Immunodominant Epitopes of Envelope gp41 from Human Immunodeficiency Virus Type 1 (HIV-1) Group M and Its Impact on HIV-1 Antibody Detection, Journal of Clinical Microbiology, Feb 2000, p.773-780. Vol.38. No.2. (5) Vanhems P. et.al., HIV seroconversion interval and demographic characteristics ; no evidence of selection bias: Sex Transm inf. 2002:77;446-448. (6) Cecilia C. L. Ngan et.al., Alternative Strategies for Confirmation of Human Immunodeficiency Virus Infection Require Judicious Use, Journal of Clinical Microbiology, Jan 2002, p 314-315, Vol 40, No.1. (7) Persaud D et.al., Latency in Human Immunodeficiency Virus Type 1 Infection: No Easy Answers, Journal of Virology, Feb 2003, p.1659-1665. Vol. 77, No.3. (8) Data on file: Qualpro Diagnostics.

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