Size : 274 x 218 mm

Qualisa™

Microwell Enzyme Immunoassay | **HIV 4.0**

Fourth Generation Enzyme Linked Immunosorbent Assay (ELISA) for the detection of Antibodies to HIV-1/2 & "O" subtypes and HIV-1 p24 antigen in human serum or plasma

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C.

INTENDED USE

QUALISA™ HIV 4.0 is intended to be used for the detection of antibodies to HIV 1/2 & "O" subtype virus and HIV-1 p24 antigen in human serum or plasma.

SUMMARY AND EXPLANATION

QUALISA™ HIV 4.0 is a fourth generation solid phase Enzyme Linked Immunosorbent Assay (ELISA) which employs Agglutinating sera for p24 antigen and highly purified recombinant antigens representing envelope glycoprotein gp 41 of HIV 1 and envelope glycoprotein gp 36 of HIV 2. The use of HIV antigens to sandwich specific antibodies enables detection of both IgG and IgM antibodies. Detection of p24 antigen also reduces window period

PRINCIPLE OF THE ASSAY

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 antigen/antibodies to the virus in the serum of a patient indicates viral infection.

Microwell strips are coated with Agglutinating sera for p24 antigen along with the recombinant antigens, gp41 and gp36 representing both HIV 1 and HIV 2. Samples along with positive and negative controls are simultaneously incubated with biotinylated Agglutinating sera for p24 antigen. The wells are washed to remove unbound components. The presence of bound antigen/Agglutinating sera is detected by adding antigen-HRP/ streptavidin-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 value equal to or more than the cutoff value is considered reactive.

KIT COMPONENTS

QUALISA™ HIV 4.0 has following components:

(1). Coated microwells: 96 microwells (3 x 8 wells X 4) are coated with Agglutinating sera for p24 antigen and recombinant antigens representing both HIV 1 and HIV 2. (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/ Streptavidin- HRP conjugate (50 x). To be diluted 50 times with conjugate diluent. (5).Antibody Reagent: Biotinylated Agglutinating sera for p24 antigen. (6). Conjugate diluent: Buffered solution containing stabilizing proteins and preservatives. (7).Activator : Buffered solution containing activator and preservatives. (8). Substrate: Solution containing Tetramethyl benzidine (TMB) and hydrogen peroxide. Ready to use. (9).Wash buffer: Buffer containing surfactants (20 x). To be diluted 20 times with distilled or deionized water. (10). Stop solution: Diluted Sulphuric acid. (11). Microwell holder. (12). Instruction for use. (13).ELISA protocol sheet. (14). Plate sealer.

STORAGE AND STABILITY

(1). **QUALISA™ HIV 4.0** kit is stable at 2-8°C upto the expiry date printed on the label. (2). Coated microwells should be used within one month of opening the pouch. Once opened, the pouch must be sealed properly to protect from moisture. If the colour of the desiccant has changed from blue to white at the time of opening the pouch, another coated microwell pouch should be used. (3).Diluted conjugate must be used immediately.(4).Diluted wash buffer is stable upto one week.(5). Activated antibody reagent should used immediately

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

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°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.

Size : 137 x 218 mm

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 expiry date. (10). Do not mix components of one kit with another. (11). Always use new tip for each specimen and reagent. (12). Do not allow liquid from one well to mix with other wells. (13). Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. Mix activator and antibody reagent as shown

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Activator	25µl	50µl	75µl	100µl	125µl	150µl	175µl	200µl	225µl	250µl	275µl	300µl
Antibody reagent	475µl	950µl	1425µl	1900 µl	2375µl	2850µl	3325µl	3800µl	4275µl	4750 µl	5225µl	5700µl

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

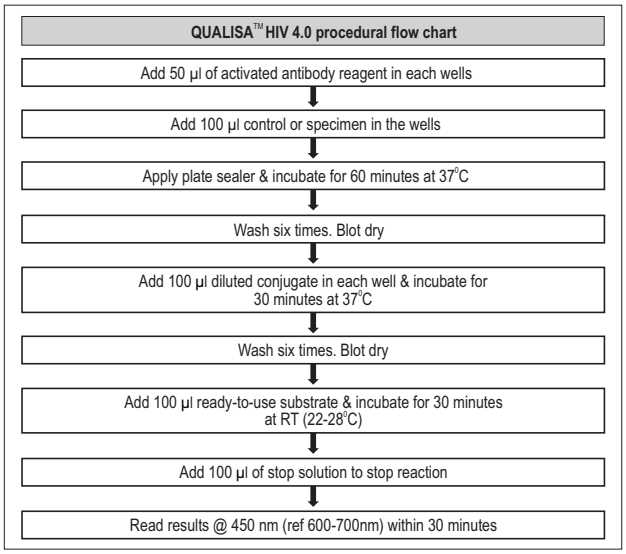
No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
50 x Conjugate	20 µl	40 µl	60µl	80 µl	100 µl	120µl	140 µl	160µl	180 µl	200 µl	220µl	240µl
Conjugate Diluent	980 µl	1960 µl	2940µl	3920 µl	4900 µl	5880µl	6860 µl	7840µl	8820 µl	9800 µl	10780µl	11760µl

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 50 µl activated antibody reagent in each well .
6. Add 100 µl control or specimen in separate wells.
7. Apply plate sealer and incubate for 60 minutes at 37^{0}C .
8. Wash each well by filling approximately 350µl 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 37^{0}C.
10. Wash each well by filling approximately 350µl diluted wash buffer, giving 30 seconds soak time for each and aspirating/flicking off six times. Blot dry.
11. Add 100µl substrate in each well and incubate for 30 minutes at room temperature (22-28^{0}C) away from direct light.
12. Stop reaction by adding 100µl 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.
3. If the test run does not meet above criteria, test run is invalid and should be repeated.



CALCULATIONS

The cut-off 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 (NC)	
Reading - 1	0.02
Reading - 2	0.015
Average NC reading	0.017

Absorbance of Positive Control (PC)	
Reading - 1	2.840
Reading - 2	2.884
Average PC reading	2.862

Cutoff value = Av. NC + 0.2 = 0.017 + 0.2 = 0.217

SAMPLE DATA

Well	Absorbance	Mean	Cutoff	Result
NC	0.02	0.017	0.217	
NC	0.015			
PC	2.840	2.862		
PC	2.884			
Sample 1	3.487			Reactive
Sample 2	0.654			Reactive
Sample 3	0.056			Non-Reactive

INTERPRETATION OF RESULTS

(1). Samples with absorbance value less than the cutoff value are considered non-reactive by **QUALISA™ HIV 4.0** ELISA kit and are considered negative for HIV 1 / 2 antibodies / antigen.(2). Samples with absorbance value equal to or greater than cutoff value are considered reactive by **QUALISA™ HIV 4.0** ELISA kit. The original sample should be retested in duplicate. Initially reactive sample that do not react in either of duplicate are considered negative. Initially reactive sample that reacts in either or both duplicates are considered repeatedly reactive.(3). If a sample is repeatedly reactive the probability of antigen/antibodies to HIV 1 / 2 are high, especially with patients at high risk or high absorbance values. Such samples should be retested with supplemental fourth generation tests or PCR. 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.

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 **QUALISA™ HIV 4.0** and compared with commercially available ELISA. The results are as shown below.

Specimen Data	Total	QUALISA™ HIV 4.0	Commercial ELISA
Total Number	464	464	464
HIV Positive	114	114	114
HIV Negative	350	349	350

Sensitivity : 100%
Specificity : 99.71%

Evaluation with Seroconversion panels:

QUALISA™ HIV 4.0 results with Anti-HIV 1 seroconversion Panel D (PRB904) obtained from Boston Biomedica Inc.

Panel ID #	Days Since 1st bleed	QUALISA ™ HIV 4.0*	Org. Tek. HIV*	Dupont Western Blot	Roche RNA PCR
PRB 904-01	0	0.08	0.5	No Bands	BLD**
PRB 904-02	21	0.08	0.4	No Bands	BLD
PRB 904-03	49	0.06	0.5	No Bands	Positive
PRB 904-04	92	15.8	5.1	18,24,f41,55,f65,120,160	Positive
PRB 904-05	99	15.8	4.9	18,24,41,51,55,65,120,160	Positive

QUALISA™ HIV 4.0 results with Anti-HIV 1 seroconversion Panel AN (PRB939 E) obtained from Boston Biomedica Inc.

Panel ID #	Days Since 1st bleed	QUALISA™ HIV 4.0*	HIV Combo Ag/Ab Assay 1*	HIV Combo Ag/Ab Assay 2*	Org. Tek. HIV*	Biorad Western Blot	Roche RNA PCR
PRB 939-01	0	0.33	-	0.431	0.5	No Bands	BLD
PRB 939-02	2	0.4	-	0.486	0.5	No Bands	BLD
PRB 939-03	7	0.33	-	0.459	0.5	No Bands	BLD
PRB 939-04	9	0.33	-	0.459	0.5	No Bands	BLD
PRB 939-05	14	0.46	0.27 (0)	0.557	0.5	No Bands	Positive
PRB 939-06	16	0.86	0.98 (2)	0.894	0.4	No Bands	Positive
PRB 939-07	21	19.86	16.30 (7)	11.635	0.5	No Bands	Positive
PRB 939-08	23	19.53	16.30 (9)	12.694	0.5	No Bands	Positive
PRB 939-09	103	19.4	16.30 (89)	13.361	8.6	18,24,31,41,51,55,65, 120,160	Positive

QUALISA™ HIV 4.0 results with Anti-HIV 1 seroconversion Panel Z (PRB926) obtained from Boston Biomedica Inc.

Panel ID #	Days Since 1st bleed	Qualisa™ HIV 4.0*	HIV Combo Ag/Ab Assay 1*	Org. Tek. HIV*	Biorad Western Blot	Roche RNA PCR
PRB 926-01	0	0.46	0.21	0.3	No Bands	BLD
PRB 926-02	2	0.46	0.22	0.9	No Bands	Positive
PRB 926-03	7	7.33	5.00	0.9	No Bands	Positive
PRB 926-04	9	19.66	16.48	0.2	No Bands	Positive
PRB 926-05	27	16.4	16.48	9.0	24,55,120,160	Positive
PRB 926-06	32	12.2	16.48	9.0	24,55,120,160	Positive

Evaluation with Anti-HIV 1 Low Titer Performance Panel

QUALISA™ HIV 4.0 was evaluated with Anti-HIV 1 Low Titer Performance Panel (Modified) PRB 107M obtained from Boston Biomedica Inc.

Panel ID #	Gen Sys HIV 1/2*	Org. Tek. Uni- Form II+O*	Qualisa HIV™ 4.0*	Biorad 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 **QUALISA™ HIV 4.0** was provided by BBI.

** BLD Below Detection limit

LIMITATION OF THE PROCEDURE

QUALISA™ HIV 4.0 ELISA kit alone cannot be used to diagnose infection with HIV even if the sample is repeatedly reactive or has high absorbance value. A physician can only establish clinical diagnosis. A negative result does not preclude the possibility of exposure to or infection with HIV.

The user of this kit is advised to carefully read and understand the instructions for use. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of incubation steps is essential for accurate, reproducible detection of HIV -1 and HIV-2 antibodies and p24 antigens.

If possible, use fresh serum or plasma samples. Sample degradation as well as multiple freeze-thaw cycles may cause erroneous results.

Do not use heat-inactivated samples.

Falsely reactive test results can be expected with a test kit of this nature. The proportion of reactives will depend on the sensitivity and specificity of the test kit and on the prevalence of HIV-1 and HIV-2 antibodies in the population to be screened. After the **QUALISA™ HIV 4.0** is performed, repeatedly reactive specimens should be submitted for additional testing using Western Blot (WB), Indirect Immunofluorescense Assay (IFA) or Radioimmunoprecipitation Assay (RIPA) tests.

The determination that a person's specimen contains antibodies to HIV and/or HIV antigens has extensive medical, social, psychological and economic implications. It is recommended that confidentiality, appropriate counselling and medical evaluation be considered an essential aspect of the testing sequence.

BIBLIOGRAPHY

(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). 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. (4). Data on File : Qualpro Diagnostics.

Manufactured by:

Qualpro Diagnostics

A Division of Tulip Diagnostics (P) Ltd.

88/89, Phase II C, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.

Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

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