Panel Member	Day Since 1st Bleed	ELECTRA™ HIV Ag/Ab 4.0 CLIA (S/Co)	Abbott ARCHITECT HIV Ag/Ab Combo (S/Co)	Abbott AxSym HIV Ag/Ab Combo (S/Co)	Abbott PRISM HIV Ag/Ab Combo (S/Co)	Dade Behring Enzygnost Integral II	Roche Elecsys Combination HIV Ag/Ab (S/Co)	Abbott Murex Combination HIV Ag/Ab (S/Co)
1	0	0.4	0.1	0.4	0.1	0.1	0.3	0.3
2	2	0.4	0.2	0.4	0.1	0.1	0.3	0.3
3	20	0.3	0.2	0.5	0.1	0.1	0.3	0.3
4	22	0.4	0.1	0.4	0.1	0.1	0.3	0.3
5	30	0.4	0.2	0.4	0.1	0.1	0.3	0.3
6	35	0.4	0.2	0.4	0.1	0.1	0.3	0.3
7	37	0.3	0.3	0.4	0.1	0.1	0.3	0.3
8	44	1.7	2.1	1.0	1.1	2.0	0.6	1.9
9	48	5.3	2.1	2.5	1.4	0.8	2.0	4.0
10	51	9.4	9.6	12.4	3.5	6.2	89.6	15.4

6. **ELECTRA Ag/Ab HIV 4.0 CLIA** was also evaluated using the serial dilution of 15 HIV-1 Positive samples, 3 HIV-2 Positive samples and 1 p24 antigen (IDP.P/ P24/E-01B).

Precision : % CV from Inter and Intra-assay studies = 10±2

REMARKS

1. Though **ELECTRA Ag/Ab HIV 4.0 CLIA** is a reliable screening assay, it should not be used as a sole criterion for diagnosis of HIV infection.
2. As with all immunoassays, the **ELECTRA Ag/Ab HIV 4.0 CLIA** may yield nonspecific reactions due to other causes, particularly when testing in low prevalence populations. A repeatedly reactive specimen should be tested further with supplemental confirmatory HIV-specific tests, such as Immunoblots and HIV nucleic acid tests.
3. 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.

BIBLIOGRAPHY

(1) Alonso R, Roa LP, Suarez M, Bouza E. 2014. New automated chemiluminescence immunoassay for simultaneous but seperate detection of Human Immunodeficiency Virus antigens and antibodies. *J. Clin. Microbiol.* 52:1467–1470. 10.1128/JCM.03486-13. (2) Kwon JA, Yoon SY, Lee CK, Lim CS, Lee KN, Sung HJ, Brennan CA, Devare SG. 2006. Performance evaluation of three automated human immunodeficiency virus antigen-antibody combination immunoassays. *J. Virol. Methods* 133:20–26. 10.1016/j.jviromet.2005.10.013. (3) Masciotra S, McDougal JS, Fieldman J, Sprinkle P, Wesolowski L, Owen SO. 2011. Evaluation of an alternative HIV diagnostic algorithm using specimen from seroconversion panels and persons with established HIV infections. *J. Clin. Virol.* 52:S17–S22. (4) Muhlbacher A, Schennach H, van Helden J, Hebell T, Pantaleo G, Burgisser P, Cellerai C, Permpikul P, Rodriguez MI, Eiras A, Alborino F, Cunningham P, Axelsson M, Andersson S, Wetlitzky O, Kaiser C, Moller P, de Sousa G. 2013. Performance evaluation of a new fourth-generation HIV combination antigen-antibody assay. *Med. Microbiol. Immunol.* 202:77–86. 10.1007/s00430-012-0250-5. (5) Salmona M, Delarue S, Delaugerre C, Simon F, Maylin S. 2014. Clinical evaluation of BioPlex(R) 2200 HIV Ag-Ab: an automated screening method providing discrete detection of HIV-1 p24 antigen, HIV-1 antibody, and HIV-2 antibody. *J. Clin. Microbiol*. 52:103–107. 10.1128/JCM.02460-13. (6) Tavakoli A, Niya MHK, Kashavarz M, Ghaffari H, Asoodeh A, Monavari SH, Keyvani H. 2017. Current diagnostic methods for HIV. *Future Virol*. 12: 141-155. 10.2217/fvl.2016-0096. (7) Weber B, Orazi B, Raineri A, Thorstensson R, Burgisser P, Muhlbacher A, Areal C, Eiras A, Villaescusa R, Camacho R, Diogo I, Roth HJ, Zahn I, Bartel J, Bossi V, Piro F, Atamasirikul K, Permpikul P, Webber L, Singh S. 2006. Multicenter evaluation of a new 4th generation HIV screening assay Elecsys HIV combi. *Clin. Lab*. 52:463–473.

SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	LOT Batch Number / Lot Number
Manufacturer	IVD *In vitro* Diagnostic Medical Device	This side up	Σ Contains sufficient for <n> tests
Use by	REF Catalogue Number	Do not reuse	

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.

An ISO 13485 Certified Company

1222/VER-07

electra™

HIV Ag/Ab 4.0 CLIA

4th Generation Chemiluminescence Assay for the Detection of Antigens and Antibodies to HIV 1 & 2 Virus in Human Serum or Plasma.

FOR IN VITRO DIAGNOSTIC USE ONLY

INTENDED USE

ELECTRA™ Ag/Ab HIV 4.0 CLIA 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

ELECTRA™ Ag/Ab HIV 4.0 CLIA is a fourth generation micro-well Chemiluminescence assay (CLIA) 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

The **ELECTRA™ Ag/Ab HIV 4.0 CLIA** assay is for use on **ELECTRA™ SA** and **ELECTRA™ FA** analyzers. **ELECTRA™ Ag/Ab HIV 4.0 CLIA** works on the principle of chemiluminescence wherein light is produced by a chemical reaction from a substance as it returns from an electronically excited state to the ground state. When catalysed by HRP,the oxidation of luminol by hydrogen peroxide produces an electronically excited form of 3-aminophthalate which on relaxation emits light with maximum intensity at λ=425nm.

ELECTRA Ag/Ab HIV 4.0 CLIA micro-well strips are coated with agglutinating sera for p24 antigen along with purified recombinant antigens, gp41and 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 antigen/agglutinating sera is detected by adding biotinylated agglutinating sera for p24 antigen followed by antigen-HRP/streptavidin-HRP conjugate. After washing wells to remove unbound enzyme, chemiluminescent substrate is added. The bound enzyme converts the substrate to a reaction product that emits a photon of light.

The Chemiluminescence thus produced is measured in Relative Light Units (RLU) that are typically proportionate to the amount of analyte present in the sample. The presence or absence of analyte in the sample is determined by comparing the sample RLU with Cutoff which is calculated by **ELECTRA™ SA** and **ELECTRA™ FA** analyzers and expressed as Electra Cutoff Index (E.C.I.). E.C.I. is equivalent to S/Co ratio which is calculated by using sample RLU and calculated Cutoff of specific testing batch. Samples with E.C.I. values greater than or equal to 1.00 are considered reactive and samples with E.C.I. values less than 1.00 are considered non-reactive.

KIT COMPONENTS

ELECTRA™ Ag/Ab HIV 4.0 CLIA has following components:

1. Coated micro-wells: Micro-wells (3 x 8 wells) are coated with agglutinating sera for p24 antigen and recombinant antigens representing both HIV-1 and HIV-2. Ready to use.
   96 Wells : (3x8) x 4 pouches, 192 Wells: (3x8) x 8 pouches.
2. Positive control: Inactivated and stabilized human serum reactive for HIV with preservatives.
3. Negative control: Inactivated and stabilized human serum non-reactive for HIV-1 and HIV-2, HBsAg and HCV.
4. Ab. Activator: Buffered solution containing activator and preservatives.
5. Conjugate: Antigen-HRP/streptavidin-HRP conjugate (50 X). To be diluted 50 times with conjugate diluent.
6. Antibody Reagent: Biotinylated agglutinating sera for p24 antigen. Ready to use.
7. Conjugate diluent: Buffered solution containing stabilizing proteins and preservatives.
8. Substrate A: Chemiluminescent substrate containing enhanced luminol solution
9. Substrate B: Chemiluminescent substrate containing stabilized peroxide solution
10. Wash buffer: Buffer containing surfactants (20 X). To be diluted 20 times with distilled or deionized water.
11. Microwell holder.
12. Instructions for use.
13. Plate sealer.
14. Protocol sheet.

REF	407010096	407010192
Σ	96 Tests	192 Tests

STORAGE AND STABILITY

1. **ELECTRA™ Ag/Ab HIV 4.0 CLIA** kit is stable at 2-8°C up to the expiry date printed on the label.
2. Coated micro-wells should be used within one month of opening the pouch. Once opened, the pouch must be sealed properly to protect from moisture. In case the desiccant pouch changes color from blue to white, the microwell strips should not be used.
3. Diluted conjugate must be used immediately.
4. Diluted wash buffer is stable up to one week at 2-8°C.

5. Working Substrate (A+B) must be used immediately.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Manual or automatic pipettor.
2. Pipettor tips.
3. Incubator.
4. Micro-well washer.
5. **ELECTRA™ SA** or **ELECTRA™ FA**.
6. Distilled water.
7. Disposable gloves.
8. Timer/ Stop Watch.

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. Specimen containing precipitate or particulate matter should be centrifuged prior to use.
6. Use of fresh sample is preferred. However, specimen samples can be stored refrigerated for short duration. For long term storage, freeze at -20°C or below. Do not freeze samples in frost-free freezer.
7. Specimen should not be frozen and thawed repeatedly.
8. Do not heat inactivate before use.

PRECAUTIONS

1. Do not pipette any material by mouth.
2. Do not eat, drink or smoke in the area where testing is done.
3. Use protective clothing and wear gloves when handling samples.
4. Immediately clean up any spills with sodium hypochlorite.
5. Dispose off all the reagents and material used as they contain infectious agent.
6. Neutralize acid containing waste before adding hypochlorite.
7. Do not use kit after the expiry date.
8. Do not mix components of one kit with another.
9. Always use new tip for each specimen and reagent.
10. Do not allow liquid from one well to mix with other wells.
11. Do not let the microwell strips dry in between the steps.
12. Do not expose the working substrate to direct light.

REAGENT PREPARATION

1. Mix Activator and Antibody Reagent according to requirement as shown below:

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Activator	15µl	30µl	45µl	60µl	75µl	90µl	105 µl	120 µl	135 µl	150 µl	165 µl	180 µl
Antibody Reagent	285 µl	570 µl	855 µl	1140 µl	1425 µl	1710 µl	1995 µl	2280 µl	2565 µl	2850 µl	3135 µl	3420 µl

2. Dilute wash buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water).
3. Dilute conjugate with Conjugate diluent according to the requirement as shown below. Prepare a fresh dilution for each assay in a clean glass tube.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Conjugate	10µl	20µl	30µl	40µl	50µl	60µl	70µl	80µl	90µl	100 µl	110 µl	120 µl
Conjugate Diluent	490 µl	980 µl	1470 µl	1960 µl	2450 µl	2940 µl	3430 µl	3920 µl	4410 µl	4900 µl	5390 µl	5880 µl

4. Working Substrate: Mix Substrate A and Substrate B (1:1 ratio) in equal volume before addition to the micro-wells.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Substrate A	250 µl	450 µl	650 µl	850 µl	1050µl	1250µl	1450µl	1650µl	1850µl	2050µl	2250µl	2450µl
Substrate B	250 µl	450 µl	650 µl	850 µl	1050µl	1250µl	1450µl	1650µl	1850µl	2050µl	2250µl	2450µl

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. Well A1 must be used as blank.
4. Add 25 µl Activated Antibody Reagent in each well except well A1.
5. Add 75 µl of NC's in well B1 & C1 and PC's in wells D1 & E1 respectively.
6. Add 75 µl of samples to the remaining wells.
7. Apply plate sealer and incubate for 45 minutes at 37°C.
8. Wash each well using a semi-automated micro-plate washer (Preferably LisaWash models) with diluted wash buffer for 6 wash cycles giving 30 seconds soak time for each wash cycle and Blot dry.
9. Add 50 µl Diluted Conjugate in each well except well A1 and incubate for 15 minutes at 37°C.
10. Wash six times as described in step 8 and Blot dry.
11. Add 50 µl of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
12. Cover the Electra microplate and incubate for 10 mins at room temperature (18-25°C) in dark.
13. Read the Electra micro-plate exactly at 10 mins in **ELECTRA™ SA** or **ELECTRA™ FA.** If Electra micro-plate is not read between 10- 15 mins, the test results should be considered as invalid.

TEST VALIDATION CRITERIA

1. The individual RLU of negative controls should be less than 200000 for **ELECTRA™ SA** and **ELECTRA™ FA.**
2. The individual RLU of positive controls should be more than 7500000 for **ELECTRA™ SA** and **ELECTRA™ FA.**

CALCULATIONS

The cutoff value is calculated by the software of **ELECTRA™ SA** and **ELECTRA™ FA** using the mean of negative control RLU and a factor.
Factor for **ELECTRA™ SA** and **ELECTRA™ FA** is 700000

SAMPLE DATA

For **ELECTRA™ SA** and **ELECTRA™ FA:**
Considering the Cutoff for this batch is 707418.

Well	RLU	E.C.I.	Interpretation
Blank	88		
NC	7278		
NC	7558		
PC	15097162		
PC	15087017		
Sample 1	1910725	2.7	Reactive
Sample 2	9361	0.01	Non-reactive
Sample 3	9939857	14.05	Reactive
Sample 4	155378	0.21	Non-reactive

INTERPRETATION OF RESULTS

1. Results are interpreted in E.C.I. This is determined by dividing the RLU of the sample by the Cutoff value calculated for that specific run.
2. Samples with E.C.I. values greater than or equal to 1.00 are considered Reactive and samples with E.C.I. values less than 1.00 are considered Nonreactive.
3. Samples that are initially reactive in **ELECTRA™ Ag/Ab HIV 4.0 CLIA** should be retested in duplicate. Repeat reactivity is highly predictive of the presence of HIV-1 p24 antigen and/or HIV-1/HIV-2 antibodies.
4. A grey zone of ± 10% is recommended.

PERFORMANCE CHARACTERISTICS

1. **ELECTRA™ Ag/Ab HIV 4.0 CLIA** was evaluated with 672 samples out of which 654 were negative samples and 18 were HIV positive samples. The results were compared with commercially available 4th Generation HIV EIA.

Specimen Data	Total	ELECTRA™ Ag/Ab HIV 4.0 CLIA	Other HIV EIA
Total Specimens	672	672	672
HIV Reactive	18	18	18
HIV Non-reactive	654	654	654

Sensitivity : 100%
Specificity : 100%

2. **ELECTRA™ Ag/Ab HIV 4.0 CLIA** was also evaluated against Reactive and Non-reactive panels provided by national Institute of Biologicals (NIB), India. In this evaluation, HIV reactive panel ID A1-A100 consisting of 100 HIV positive samples were tested and showed 100% correlation. HIV Non-reactive panel ID N1-N300 consisting of 300 negative samples was also evaluated and showed 100% correlation.
3. For sensitivity of p24 antigen, **ELECTRA™ Ag/Ab HIV 4.0 CLIA** was evaluated using WHO International Standard HIV-1 p24 Antigen from NIBSC (Code 90/636) and found to have a sensitivity of 8IU/ml.
4. **ELECTRA™ Ag/Ab HIV 4.0 CLIA** was also evaluated using WHO International Standard, HIV (Antibody), 1st International Reference Panel, NIBSC code: 02/210. The reference panel contains 1:40 dilutions of HIV positive plasma samples corresponding to:
   - Anti-HIV-1 subtype A (Group M)
   - Anti-HIV-1 subtype B (Group M)
   - Anti-HIV-1 subtype C (Group M)
   - Anti-HIV-1 subtype E (Now referred as CRF01_AE) (Group M)
   - Anti-HIV-1 Group O
   - Anti-HIV-2
5. **ELECTRA™ Ag/Ab HIV 4.0 CLIA** was also evaluated using HIV-1 AccuVert Seroconversion panel (PRB966) from Seracare.

electra™ Chemiluminescence assay
electra™ Chemiluminescence assay
electra™ Chemiluminescence assay