Panel Member	Day Since 1st Bleed	ELECTRA™ HBsAg (S/Co) E.C.I.	Abbott ARCHITECT HBsAg Qualitative (S/Co)	Bio-Rad genetic Systems HBsAg EIA 3.0 (S/Co)	DiaSorin ETI-MAK-2 PLUS HBsAg (S/Co)	Ortho VITROS HBsAg (S/Co)	Siemens ADVIA Centaur HBsAg (S/Co)
1	0	0.4	0.7	0.3	0.2	0.1	0.2
2	2	0.5	0.8	0.5	0.3	0.1	0.3
3	9	1.2	1.9	1.4	1.0	1.2	1.1
4	13	1.1	2.9	3.0	2.1	3.2	3.0
5	16	2.0	6.3	6.1	5.0	8.6	6.3

Analytical Sensitivity = 0.02ng/ml; **Precision :** % CV from Inter and Intra-assay studies = 10±2

REMARKS

1. Though **ELECTRA™ HBsAg CLIA** is a reliable screening assay, it should not be used as a sole criterion for diagnosis of HBV infection. Reactive sample should be retested with confirmatory assays like Neutralization assays, HBV DNA by PCR etc.
2. Absence of HBsAg does not indicate that an individual is absolutely free of HBV infection.
3. Since various tests for HBV differ in their performance characteristics and antibody composition, their reactivity patterns may differ.
4. 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.
5. Interferences due to heterophile antibodies, Rheumatoid Factors and other non-analyte substances in patient's serum, capable of binding antibodies multivalently and providing erroneous analyte detection in immunoassays, has been reported in various studies. Though **ELECTRA™ HBsAg CLIA** uses sufficient amounts of HETEROPHILE BLOCKING REAGENT (HBR) to inhibit the majority of this interference; nevertheless, some samples with high titers may still express clinically important assay interference. Both laboratory professionals and clinicians must be vigilant to this possibility of antibody interference. Results that appear to be internally inconsistent or incompatible with the clinical presentation should invoke suspicion of the presence of an endogenous artefact and lead to appropriate in vitro investigative action.

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SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	Batch Number / Lot Number
Manufacturer	*In vitro* Diagnostic Medical Device	This side up	Contains sufficient for <n> tests
Use by	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.

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An ISO 13485 Certified Company

1222/VER-08

electra™ HBsAg CLIA

Chemiluminescence Assay for the Detection of Hepatitis B Surface Antigen (HBsAg) in Human Serum or Plasma.

FOR IN VITRO DIAGNOSTIC USE ONLY

INTENDED USE

ELECTRA™ HBsAg CLIA is intended to be used for the detection of Hepatitis B Surface Antigen (HBsAg) in Human Serum or Plasma.

SUMMARY

ELECTRA™ HBsAg CLIA is a fourth generation micro-well Chemiluminescence assay (CLIA) which employs highly purified, high affinity agglutinating sera for HBsAg having reactivity for both *ad* and *ay* subtypes. **ELECTRA™ HBsAg CLIA** is categorised as fourth generation HBsAg CLIA, based on high sensitivity as compared with licensed third generation EIA kits.

PRINCIPLE

The **ELECTRA™ HBsAg CLIA** assay is for use on **ELECTRA™ SA** and **ELECTRA™ FA** analyzers. **ELECTRA™ HBsAg CLIA** works on the principle of chemiluminescence where, in a chemical reaction a substance emits light as it returns from a temporary electronically excited state to the stable 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™ HBsAg CLIA micro-well strips are coated with agglutinating sera for HBsAg. Agglutinating sera for HBsAg is conjugated to horseradish peroxidase (HRPO) enzyme. Samples along with positive and negative controls are added in the coated wells along with the conjugate and incubated. The wells are then washed to remove unbound components following which the chemiluminescent substrate is added. 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™ HBsAg CLIA has following components:

1. Coated micro-wells: Microwells coated with agglutinating sera for HBsAg. 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 HBsAg with preservatives.
3. Negative control: Inactivated and stabilized human serum non-reactive for HIV-1 and HIV-2, HBsAg and HCV.
4. Conjugate: Agglutinating sera for HBsAg - HRPO conjugate.
5. Conjugate Activator: Buffered solution containing activator and preservatives.
6. Substrate A: Chemiluminescent substrate containing enhanced luminol solution
7. Substrate B: Chemiluminescent substrate containing stabilized peroxide solution
8. Wash buffer: Buffer containing surfactants (20 X). To be diluted 20 times with distilled or deionized water.
9. Microwell holder.
10. Instructions for use.
11. Plate sealer.
12. Protocol sheet.

REF	407030096	407030192
Σ	96 Tests	192 Tests

STORAGE AND STABILITY

1. **ELECTRA™ HBsAg 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 strips should not be used.
3. Diluted wash buffer is stable up to one week at 2-8°C.
4. 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 strips dry in between the steps.
12. Do not expose the working substrate to direct light.

REAGENT PREPARATION

1. Dilute wash buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water).
2. Mix conjugate activator and conjugate 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
Activator	10 µl	20 µl	30 µl	40 µl	50 µl	60 µl	70 µl	80 µl	90µl	100 µl	110 µl	120 µl
Conjugate	500 µl	1000µl	1500µl	2000 µl	2500 µl	3000µl	3500µl	4000µl	4500 µl	5000µl	5500µl	6000µl

3. 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 100 µl of NC's to well B1 & C1 and PC's to D1 & E1 respectively.
5. Add 100 µl of samples to remaining wells.
6. Add 50 µl Activated Conjugate in each well except well A1.
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 of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
10. Cover the Electra microplate with plate sealer and incubate for 10 mins at room temperature (18-25°C) in dark.
11. 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 400000

SAMPLE DATA

For **ELECTRA™ SA** and **ELECTRA™ FA:**
Considering the Cutoff for this batch is 402392.

Well	RLU	E.C.I.	Interpretation
Blank	46		
NC	2269		
NC	2515		
PC	12157164		
PC	12130666		
Sample 1	10376929	25.78	Reactive
Sample 2	6694	0.016	Non-reactive
Sample 3	1021864	2.53	Reactive
Sample 4	18516	0.046	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™ HBsAg CLIA** should be retested in duplicate. Repeat reactivity is highly predictive of the presence of HBsAg.
4. As with all immunoassays, the **ELECTRA™ HBsAg 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 supplementary confirmatory assays.
5, A grey zone of ± 10% is recommended.

PERFORMANCE CHARACTERISTICS

1. **ELECTRA™ HBsAg CLIA** was evaluated with 670 samples out of which 653 were negative samples and 17 were HBsAg positive samples. The results were compared with commercially available HBsAg EIA.

Specimen Data	Total	ELECTRA™ HBsAg	Other HBsAg EIA
Total Specimens	670	670	670
HBsAg Reactive	17	17	17
HBsAg Non-reactive	653	653	653

Sensitivity : 100%
Specificity : 100%

2. **ELECTRA™ HBsAg CLIA** was also evaluated against Reactive and Non-reactive panels provided by national Institute of Biologicals (NIB), India. In this evaluation, HBsAg reactive panel consisting of 100 HBsAg positive samples were tested and showed 100% correlation. HBsAg Non-reactive panel ID N1-N300 consisting of 300 negative samples was also evaluated and showed 100% correlation.
3. **ELECTRA™ HBsAg CLIA** was also evaluated using the serial dilution of 17 HBsAg Positive samples.
4. **ELECTRA™ HBsAg CLIA** was also evaluated using
   - British Working Standard for HBsAg 0.2IU/ml, NIBSC code 07/288
   - WHO Reference Reagent, WHO HBsAg subtype adw2, genotype A Reference Panel, NIBSC code 03/262
   - WHO International Standard, 3rd International Standard for HBsAg (HBV genotype B4, HBsAg subtypes ayw1/adw2), NIBSC code 12/226.
5. **ELECTRA™ HBsAg CLIA** was also evaluated using HBV AccuVert Seroconversion panel (PHM937) from Seracare.