Chemiluminescence assay

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**PERFORMANCE CHARACTERISTICS**

**A) Internal Evaluation:**

1. **Accuracy:** In an internal study ELECTRA™ AFP was evaluated against commercially available licensed kit with 90 random clinical samples, & ELECTRA™ AFP has demonstrated 100% clinical correlation with the commercially available licensed kit.

2. **Precision:** ELECTRA™ AFP was evaluated with licensed external Quality controls for Precision Studies & following is the data:

<table>
<thead>
<tr>
<th>Controls</th>
<th>No. of tests</th>
<th>Mean Control values with ELECTRA™ AFP</th>
<th>Coefficient of Variation (CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>10</td>
<td>8.32</td>
<td>4.23</td>
</tr>
<tr>
<td>Level 2</td>
<td>10</td>
<td>69.19</td>
<td>2.76</td>
</tr>
<tr>
<td>Level 3</td>
<td>10</td>
<td>196.02</td>
<td>1.44</td>
</tr>
</tbody>
</table>

**B) External Evaluation:**

Electra™ AFP CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation ELECTRA™ AFP has demonstrated 100% correlation with the reference method.

*Data file: Zephyr Biomedicals (A Division of Tulip Diagnostics Pvt. Ltd.)*

**Important Note:**

1. The ELECTRA™ AFP assay is a temperature sensitive assay. The best temperature condition for this assay is from 18°C to 25°C.

2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

3. It is recommended to use the multiple channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipette is available.

**Duplication of Standards & samples is not mandatory but may provide information on reproducibility & application errors.**

**LIMITATIONS OF THE ASSAY**

(1). As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated. (2). The activity of the enzyme used is temperature-dependent and the RLU values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the RLU values. Corresponding variations apply also to the incubation times. However, the Standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result. (3). Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits. (4). Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect results.

**BIBLIOGRAPHY**


**INTRODUCTION**

Alpha-fetoprotein (AFP) is normally produced during fetal and neonatal development by the liver, yolk sac, and in small concentrations by the gastrointestinal tract. After birth, serum AFP concentrations decrease rapidly, and by the second year of life and thereafter only trace amounts are normally detected in serum. Elevation of serum AFP to abnormally high values occurs in several malignant diseases, most notably nonsemionomatous testicular cancer and primary hepatocellular carcinoma. In the case of nonsemionomatous testicular cancer, a direct relationship has been observed between the incidence of elevated AFP levels and the stage of disease. Elevated AFP levels have also been observed in patients diagnosed with seminoma with nonsemionomatous elements, but not in patients with pure seminoma. In addition, elevated serum AFP concentrations have been measured in patients with other noncancerous diseases, including ataxia telangiectasia, hereditary tylosisemia, neonatal hyperbilirubinemia, acute viral hepatitis, chronic active hepatitis, and cirrhosis. Elevated serum AFP concentrations are also observed in pregnant women. Therefore, AFP measurements are not recommended for use as a screening procedure to detect the presence of cancer in the general population.

**PRINCIPLE**

ELECTRA™ AFP Quantitative CLIA assay is for use on ELECTRA™ analyzer. ELECTRA™ AFP 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-aminoanthphate which on relaxation emits light with maximum intensity at λ=425nm. In ELECTRA™ AFP, the assay system utilizes one Anti-AFP antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal Anti-AFP antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the AFP antibody coated microtiter wells and incubated with the zero buffer. If human AFP is present in the specimen, it will combine with the antibody in the well. The well is then washed to remove any residual test specimen, and AFP antibody labeled with horseradish peroxidase (conjugate) is added. The conjugate will bind immunologically to the AFP on the wells, resulting in the AFP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation at room temperature, the wells are washed with wash buffer and bound enzyme is detected by adding the chemiluminescent substrate. The bound enzyme converts substrate to a reaction product that emits a photon of light. Chemiluminescence is measured in Relative Light Units (RLU). The amount of light emitted is proportional to the amount of enzyme present and is directly related to the amount of AFP in the sample. By reference to a series of AFP standards assayed in the same way, the concentration of AFP in the unknown sample is quantified.

**MATERIALS & COMPONENTS**

**Materials provided with the test kits:**

- Coated Microwells: Microwells coated with Anti-AFP antibody.
- AFP Zero Buffer. Ready to use.
- AFP Enzyme Conjugate. Ready to use.
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- AFP Standard: Set of 6 standards labeled as A to F in liquid form. Ready to use. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X).

**Materials required but not provided:**

- Precision pipettes: 10-100μl, 20-200μl, 100-1000μl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELECTRA™ Analyzer

**STORAGE AND STABILITY**

1. ELECTRA™AFP 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.

SPECIMEN COLLECTION
1. Collect blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination

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. Do not eat, drink or smoke in the area where testing is done.

REAGENT PREPARATION
13. Do not let the strips dry in between the steps.
12. Do not allow liquid from one well to mix with other wells.
11. Always use new tip for each specimen and reagent.
10. Do not mix components of one kit with another.
9. Do not use kit after the expiry date.
8. Neutralize acid containing waste before adding hypochlorite.
7. Use absorbent sheet to cover the working area.
4. Use protective clothing and wear gloves when handling samples.

TEST PROCEDURE
1. Secure the desired number of coated wells in the holder. Dispense 25µl of Standards & sera into the appropriate wells.
2. Dispense 100µl of Zero Buffer into each well. Incubate at room temperature (18-25°C) for 15 minutes.
3. After incubation, empty the microtitre wells and wash the plate 5 times with 350µl of diluted wash buffer. Strike the microtitre plate sharply onto absorbent paper towel to remove all residual droplets.
4. Dispense 100µl of Enzyme Conjugate into each well. Incubate at room temperature (18-25°C) for 15 minutes.
5. After incubation, empty the microtitre wells and wash the plate 5 times with 350µl of diluted wash buffer. Strike the microtitre plate sharply onto absorbent paper towel to remove all residual droplets.
6. Add 50µl substrate in each well. Incubate for 10 minutes at 18-25°C in dark.
7. Cover the ELECTRA™ microplate and incubate for 10 minutes at room temperature (18-25°C) in dark.
8. Read the ELECTRA™ micro-plate exactly at 10 minutes in ELECTRA™ Analyzer.

CALCULATIONS
Construct a standard curve by plotting the RLU obtained from each reference standards against its concentration in ng/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding concentration of AFP in ng/ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Standard curve
Results of a typical standard run with RLU's shown in the Y axis against AFP concentrations shown in the X axis. Suggest: Use 4-Parameter Standard curve to calculate sample values.

<table>
<thead>
<tr>
<th>AFP Values (ng/ml)</th>
<th>RLU's</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>462</td>
</tr>
<tr>
<td>B</td>
<td>94120</td>
</tr>
<tr>
<td>C</td>
<td>502859</td>
</tr>
<tr>
<td>D</td>
<td>1448474</td>
</tr>
<tr>
<td>E</td>
<td>4214755</td>
</tr>
<tr>
<td>F</td>
<td>6655116</td>
</tr>
</tbody>
</table>

This Standard curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain their own Standard curve and data.

Expected Ranges of values
In high-risk patients, AFP values between 100 and 350 ng/ml suggest a diagnosis of hepatocellular carcinoma, and levels over 350 ng/ml usually indicate the disease. Approximately 97% of the healthy subjects have AFP levels less than 8.5 ng/ml. It is recommended that each laboratory establish its own normal range. The minimum detectable concentration of AFP by this assay is estimated to be 2.0 ng/ml.