Chemiluminescence assay

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INTENDED USE

ELECTRA™ IPSA CLIA test is intended for the quantitative determination of Free Prostate Specific Antigen (fPSA) in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

Human Prostate Specific Antigen (PSA) is a 33KD serine proteinase which, in human serum, is predominantly bound to alpha 1-antichymotrypsin (PSA-AC) and alpha 2-macroglobulin (PSA-AMG). Since early studies had suggested that the percentage of free PSA is lower in patients with prostate cancer than with benign prostatic hyperplasia, efforts have been made to develop a test that could discriminate between these two conditions. This assay involves the measurement of free PSA levels. Preliminary studies have suggested that the percentage of free PSA is lower in patients with prostate cancer than with benign prostatic hyperplasia. Thus, the measurement of free PSA in conjunction with total PSA, can improve specificity of prostate cancer screening in selected men with elevated total serum PSA levels, which would subsequently reduce unnecessary prostate biopsies with minimal effects on cancer detection rates.

ELECTRA™ IPSA Quantitative CLIA assay is for use on ELECTRA™ analyzer. ELECTRA™ IPSA 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 catalyzed by HRP, the oxidation of lumino by hydrogen peroxide produces an electronically excited form of 3-aminophthalate which on relaxation emits light with maximum intensity at λ=425nm in ECL. In ELECTRA™ IPSA a certain amount of anti-PSA antibody is coated on microtiter wells. A measured volume of patient serum, and a constant volume of PSA conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, IPSA antibody in the samples and conjugated IPSA compete for the limited binding sites on the anti-PSA antibody of the wells. After incubation the wells are washed 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 inversely related to the amount of unbound IPSA in the sample. By reference to a series of IPSA standards assayed in the same way, the concentration of IPSAIN the unknown sample is quantified.

MATERIALS & COMPONENTS

Materials provided with the test kits:
- Coated Microwells: Microwells coated with Anti-PSA antibody.
- Sample Diluent: Ready to use.
- Enzyme Conjugate: Ready to use.
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxidase solution.
- IPSA Standard set of 6 standards labeled as A to F in linear 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™ IPSA 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 pink, the strip 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 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. Do not use kit after the expiry date.
7. All specimens and standards should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not mix components of one kit with another.
10. Do not let the strips dry in between the steps.
11. Use absorbent sheet to cover the working area.
12. Do not allow liquid from one well to mix with other wells.
13. Do not use kit after the expiry date.

**REAGENT PREPARATION**
- All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
- Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
- Prepare a Working Substrate by mixing Substrate A and Substrate B in equal volume (1:1 ratio) before addition to the micro-wells.

<table>
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<th>No. of Strips</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Substrate-A µl</td>
<td>250</td>
<td>450</td>
<td>650</td>
<td>850</td>
<td>1050</td>
<td>1250</td>
<td>1450</td>
<td>1650</td>
<td>1850</td>
<td>2050</td>
<td>2250</td>
<td>2450</td>
</tr>
<tr>
<td>Substrate-B µl</td>
<td>250</td>
<td>450</td>
<td>650</td>
<td>850</td>
<td>1050</td>
<td>1250</td>
<td>1450</td>
<td>1650</td>
<td>1850</td>
<td>2050</td>
<td>2250</td>
<td>2450</td>
</tr>
</tbody>
</table>

**TEST PROCEDURE**
1. Secure the desired number of coated wells in the holder. Dispense 100 µl of Standards & sera into the appropriate wells.
2. Dispense 100 µl of Sample diluent into each well. Incubate at 37°C for 15 minutes.
3. After incubation, empty the microtitre wells and wash the plate 5 times with 350 µl of diluted wash buffer. Strike the microtitre plate sharply onto absorbent paper towel to remove all residual droplets.
4. Dispense 150 µl of Enzyme Conjugate into each well. Incubate at 37°C for 15 minutes.
5. After incubation, empty the microtitre wells and wash the plate 5 times with 350 µl of diluted wash buffer. Strike the microtitre plate sharply onto absorbent paper towel to remove all residual droplets.
6. Add 50 µl of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
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 fPSA 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 fPSA concentrations shown in the X axis.
Suggest: Use 4-Parameter Standard curve to calculate sample values.

<table>
<thead>
<tr>
<th>fPSA (ng/ml)</th>
<th>RLU’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>55</td>
</tr>
<tr>
<td>B</td>
<td>34133</td>
</tr>
<tr>
<td>C</td>
<td>353788</td>
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<tr>
<td>D</td>
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<td>E</td>
<td>3709640</td>
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<tr>
<td>F</td>
<td>6287156</td>
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</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 values:**
The relationship between fPSA/t-PSA ratio and risk of prostate cancer is also age related. When Total PSA is in the range of 4.0-10.0 ng/ml the following risk interpretation is indicated:
- fPSA/t-PSA ratio ≤ 0.10 indicates 49% - 65% risk of prostate cancer
- fPSA/t-PSA ratio > 0.25 indicates 9% - 16% risk of prostate cancer

Multiple factors such as population, age, specificity of test method may affect interpretation of f-PSA and t-PSA values. These ranges should be used as guidelines only. Each laboratory should establish its own reference values.

**PERFORMANCE CHARACTERISTICS**
A) Internal Evaluation:
1. Accuracy: In an internal study Electra™ fPSA was evaluated against commercially available licensed kit with 90 random clinical samples. Electra™ fPSA demonstrated 100% clinical correlation with the commercially available licensed kit.
2. Precision: Electra™ fPSA was evaluated with licensed external Quality controls for Precision Studies & following is the data: