1. NAME AND INTENDED USE

ELSA2-CEA is an immunoradiometric assay for the quantitative determination of the carcinoembryonic antigen in human serum or plasma.

2. INTRODUCTION

The carcinoembryonic antigen (CEA), first described by Gold and Freedman in 1965, is a glycoprotein with a molecular weight of approximately 180,000 Da. Carcinoembryonic antigen is mainly secreted and excreted by digestive tract glandular cancers (of the colon, rectum, pancreas, stomach) and their metastases. It is also found in other types of cancer (breast, lung, ovary, bladder, thyroid...).

A high level of CEA is present in fetal colic mucosa, and a low level in normal adult colic mucosa. This lack of specificity is demonstrated by increased levels in benign digestive inflammatory diseases and in hepatobiliary system diseases. At present, the CEA assay's clinical interest is mainly demonstrated in the field of colorectal cancers:
- It helps determine the disease's stage and it prognosis, with a particularly significant correlation in the last 2 stages of Dukes classification.
- It allows monitoring of therapeutic efficacy. In particular, postoperative persistence of CEA elevation indicates an incomplete resection.
- It is of undoubtable interest in relapse diagnosis and consequent early second-look surgery decision-taking.

In the breast and lung-cancer fields, CEA level is related to metastatic dissemination, and its variations are considered useful indicators when monitoring patient response to therapy.

Finally, the association of CEA with thyrocalcitonin is helpful in diagnosing thyroid medullary cancer and in following up any relapse.

3. PRINCIPLE

ELSA2-CEA is a solid phase two-site immunoradiometric assay. Monoclonal antibodies were prepared against sterically remote antigenic sites on the CEA molecule: the first is coated on the ELSA solid phase, while the second, radiolabelled with iodine 125, is used as a tracer.

The CEA molecules present in the standards or the samples to be tested are "sandwiched". Following the formation of the coated antibody/antigen/iodinated antibody sandwich, the unbound tracer is easily removed by a washing step.

The radioactivity bound to the ELSA is proportional to the concentration of CEA present in the sample.

4. REAGENTS

Each kit contains enough reagents for 96 tubes. The expiry date is marked on the external label.

<table>
<thead>
<tr>
<th>REAGENTS</th>
<th>QUANTITY</th>
<th>STORAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELSA: ready for use. Anti-CEA monoclonal antibody coated on ELSA fixed to the bottom of the tube.</td>
<td>4 traypacks of 24 tubes</td>
<td>2-8°C until the expiry date. Tubes removed from their packs must be stored in the bag supplied with the kit.</td>
</tr>
<tr>
<td>ANTI-CEA 125I: ready for use. 125I anti-CEA monoclonal antibody, buffer, bovine albumin, sodium azide, non-immunized mouse immunoglobulins, red dye. ≤ 296 kBq (≤ 8 µCi).</td>
<td>1 30 ml vial</td>
<td>2-8°C until the expiry date. After opening, 15 days at 2-8°C.</td>
</tr>
<tr>
<td>STANDARD 0: ready for use. Bovine serum, sodium azide.</td>
<td>1 0.8 ml vial</td>
<td>2-8°C until the expiry date. After opening and first use, 15 days at 2-8°C.</td>
</tr>
<tr>
<td>STANDARDS: ready for use. Highly purified CEA (human), normal human serum, sodium azide. 4 - 20 - 80 - 140 - 200 ng/ml*</td>
<td>5 0.8 ml vials</td>
<td>2-8°C until the expiry date. After opening and first use, 15 days at 2-8°C.</td>
</tr>
<tr>
<td>CONTROL: ready for use. Highly purified CEA (human)**, normal human serum, sodium azide.</td>
<td>1 0.8 ml vial</td>
<td>2-8°C until the expiry date. After opening and first use, 15 days at 2-8°C.</td>
</tr>
<tr>
<td>DILUENT: ready for use. Bovine serum, sodium azide.</td>
<td>1 10 ml vial</td>
<td>2-8°C until the expiry date.</td>
</tr>
<tr>
<td>TWEEN 20: concentrated solution. Dilute 9 ml of TWEEN 20 in 3 liters of distilled water. Shake gently.</td>
<td>1 10 ml vial</td>
<td>2-8°C until the expiry date. After dilution, store in a capped container for 15 days maximum.</td>
</tr>
<tr>
<td>PLASTIC BAG</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* The values shown above are only target values; the true value of each standard is shown on its label.
** The acceptance range true values are printed on the vial label.
5. PRECAUTIONS FOR USE

5.1. Safety measures

Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

Do not pipette by mouth.
Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.
Avoid splashing.
Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.
Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.
The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user’s country.
The enforcement of the basic rules for handling radioactive products ensures adequate security.
A summary of these is given below:
Radioactive products must be stored in their original containers in a suitable area.
A record of the reception and storage of radioactive products must be kept up to date.
Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).
Do not eat, drink, smoke or apply cosmetics in a controlled zone.
Do not mouth-pipette radioactive solutions.
Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.
Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.
Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.
All radioactive waste disposal must be carried out according to the regulations in force.

5.3. Handling precautions

Do not use kit components beyond their expiry date.
Do not mix reagents from different batches.
Avoid any microbic contamination of the reagents or of the water used for washing.
Fully respect the incubation conditions and the washing instructions indicated.

6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed directly on serum or plasma. If the test is to be carried out within 24 hours, the samples should be stored refrigerated at 2-8°C. Otherwise, they should be divided into aliquots and deep frozen (-20°C) until needed.
Dilution
Should elevated CEA levels be suspected, dilution is performed with the diluent found in the kit.
It is recommended that disposable plastic tubes be used when carrying out dilutions.

7. ASSAY PROCEDURE

7.1. Material required

Precision micropipettes or similar with disposable tips, capable of dispensing 100 µl and 300 µl (±1%). Their calibration should be checked regularly.
Distilled water.
Disposable plastic tubes.
Vortex-type mixer.
Water bath or incubator (45 ± 1°C). To avoid condensation, do not cover the water bath.
Gamma scintillation counter calibrated for 125 iodine measurement.
Equipment suitable for this assay is available from CIS bio international; information on request.
7.2. Protocol

All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use. Dispensing of the reagent into the ELSA tubes is carried out at room temperature (18-25°C).

The assay requires the following groups of tubes:
- O standard group for the determination of non-specific binding.
- Standard groups to establish the standard curve.
- Control group for the control.
- Sx groups for the samples to be assayed.

It is recommended to perform the assay in triplicate for the standards and in duplicate for the samples.

Check that the water-bath or dry heat incubator temperature is 45 ± 1°C.

Respect the order in which reagents are to be added:
- Dispense 300 μl of 125I anti-CEA monoclonal antibody into all ELSA tubes.
- Add 100 μl of standards, control or unknown samples into the corresponding groups of tubes.
- Gently mix each tube with a Vortex type mixer.
- Incubate for 3 hours ± 5 minutes at 45 ± 1°C.
- Wash the ELSA tubes as follows:
  - Aspirate the content of the tubes as completely as possible.
  - Add 3.0 ml of washing solution to each tube.
  - Wait at least 2 minutes and empty them again.
  - Repeat the process twice more.

To obtain reliable and reproducible results, the different washing steps have to be correctly performed. As much as possible of the incubation and washing solutions must be removed. If the washing is carried out manually, the tip of the aspirating device must be placed right at the bottom of the ELSA tube.

Measure the radioactivity bound to the ELSA with a gamma scintillation counter.

8. QUALITY CONTROL

Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

9. RESULTS

For each group of tubes, calculate the mean counts after subtracting the background.

Draw up the standard curve by plotting the standard’s cpm against their concentrations.

Read the sample values directly from the curve, correcting the read value for the dilution factor, if necessary.

**Typical standard curve** (example only): this data must under no circumstances be substituted for results obtained in the laboratory.

<table>
<thead>
<tr>
<th>Groups of tubes</th>
<th>Mean cpm</th>
<th>Concentration ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 0</td>
<td>156</td>
<td>0</td>
</tr>
<tr>
<td>Standard 1</td>
<td>853</td>
<td>4</td>
</tr>
<tr>
<td>Standard 2</td>
<td>3 906</td>
<td>20</td>
</tr>
<tr>
<td>Standard 3</td>
<td>19 518</td>
<td>80</td>
</tr>
<tr>
<td>Standard 4</td>
<td>34 473</td>
<td>140</td>
</tr>
<tr>
<td>Standard 5</td>
<td>46 425</td>
<td>200</td>
</tr>
<tr>
<td>Control serum</td>
<td>1 324</td>
<td>7</td>
</tr>
<tr>
<td>Sample A</td>
<td>12 808</td>
<td>54.6</td>
</tr>
<tr>
<td>Sample B</td>
<td>21 063</td>
<td>85.8</td>
</tr>
<tr>
<td>Sample C</td>
<td>34 295</td>
<td>142.4</td>
</tr>
</tbody>
</table>

10. PROCEDURAL LIMITATIONS

Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results.

Do not extrapolate sample values beyond the last standard. Dilute the samples concerned and re-assay.

For assays when the antigen and labelled antibodies are incubated simultaneously with the solid phase undiluted specimens with extremely elevated antigen concentrations may give reading lower than the highest standard. For ELSA2-CEA assay this generally occurs at concentrations higher than 20,000 ng/ml of CEA.
11. EXPECTED VALUES

Each laboratory should establish its own range of normal values. The expected values shown below may only serve as a guide.

**Distribution of normal values**

These values have been obtained from presumably healthy subjects.

<table>
<thead>
<tr>
<th>Concentration range</th>
<th>Smokers and non-smokers mixed</th>
<th>Smokers</th>
<th>Non smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 3 ng/ml</td>
<td>86 %</td>
<td>76 %</td>
<td>86 %</td>
</tr>
<tr>
<td>3 - 5 ng/ml</td>
<td>13 %</td>
<td>19 %</td>
<td>14 %</td>
</tr>
<tr>
<td>5 - 7 ng/ml</td>
<td>1 %</td>
<td>5 %</td>
<td>0 %</td>
</tr>
</tbody>
</table>

12. SPECIFIC CHARACTERISTICS OF THE ASSAY

12.1. Imprecision

This has been assessed using 4 samples with different concentrations. They were tested either 30 times in the same series of assays or in duplicate in 15 different series.

<table>
<thead>
<tr>
<th>Samples</th>
<th>X ng/ml</th>
<th>Within-run CV %</th>
<th>Samples</th>
<th>X ng/ml</th>
<th>Between-run CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>3.4</td>
<td>A</td>
<td>13.0</td>
<td>6.9</td>
</tr>
<tr>
<td>2</td>
<td>58.5</td>
<td>2.6</td>
<td>B</td>
<td>26.9</td>
<td>3.0</td>
</tr>
<tr>
<td>3</td>
<td>106</td>
<td>1.9</td>
<td>C</td>
<td>57.9</td>
<td>3.5</td>
</tr>
<tr>
<td>4</td>
<td>148</td>
<td>2.0</td>
<td>D</td>
<td>148.5</td>
<td>5.3</td>
</tr>
</tbody>
</table>

12.2. Recovery test

Known quantities of CEA were added to human sera. The recovery percentages of CEA in the samples ranged from 95.7% to 103.7%.

12.3. Specificity

This assay does not present any cross-reaction with other normal related substances, especially NCA.

12.4. Detection limit

The detection limit is defined as being the smallest detectable concentration different from zero with a probability of 95%. It has been assessed as being 0.3 ng/ml.

12.5. Hook effect

No hook effect is observable up to 20 000 ng/ml.

**ASSAY FLOW CHART**

<table>
<thead>
<tr>
<th>Tubes</th>
<th>125I anti-CEA μl</th>
<th>Standards Control Samples μl</th>
<th>Mix gently Incubate 3 h (± 5 mn) at 45°C (± 1°C)</th>
<th>Wash 3 times</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards</td>
<td>300</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>300</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples</td>
<td>300</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>