Intended Use
In vitro assay for the quantitative determination of carcinoembryonic antigen (CEA) in serum during the follow-up of cancer patients.

Summary and Explanation of the Test
CEA was first isolated from colon tumours (4). It belongs to a family of glycoproteins with a molecular weight of approx. 180,000 – 200,000 and a carbohydrate content varying between 50 and 60% (7).
Elevated CEA serum concentrations are found in patients with carcinoma of the lung, colon or breast (1, 2, 3). CEA measurement, alone or in combination with other markers, is intended for the early diagnosis of relapses and for therapy monitoring (5, 8).

Principles of the Procedure
Two-site immunoradiometric assay (sandwich principle) using two highly specific monoclonal antibodies for coating of the solid phase (coated tubes) and the tracer. The tracer antibody and the coated antibody react simultaneously with the CEA present in patient samples or standards. Excess tracer is removed by a washing step and the radioactivity bound to the tube wall is measured in a gamma scintillation counter.

CONTENTS

<table>
<thead>
<tr>
<th>Determinations</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹²⁵I-anti-CEA, monoclonal (mouse), red radioactive content (kBq / μCi)</td>
<td>&lt; 705 /19</td>
</tr>
<tr>
<td>6 standards A-F (1.0 mL) in buffer</td>
<td>1 set</td>
</tr>
<tr>
<td>(The exact concentration is indicated on each vial label)</td>
<td></td>
</tr>
<tr>
<td>Diluent (0 ng/mL) in buffer</td>
<td>11 mL</td>
</tr>
<tr>
<td>Test tubes, coated with anti-CEA, monoclonal (mouse)</td>
<td>2 x 50</td>
</tr>
<tr>
<td>Control serum, A and B, human, lyophilic.</td>
<td>2 x 1mL</td>
</tr>
<tr>
<td>(For concentration: see Quality Control report)</td>
<td></td>
</tr>
<tr>
<td>Quality control report</td>
<td>1</td>
</tr>
</tbody>
</table>
Material Required but not Provided
- Micropipets (100 µL) with disposable plastic tips
- Vortex mixer
- Manual or automatic washer with aspiration device
- Horizontal shaker
- Gamma scintillation counter
- Alternatively an appropriate automated analyser system, if available
- Uncoated polystyrene tubes for the dilution of sera and controls
- 0.9 % NaCl solution for washing steps
- Distilled or deionised water (in further text named dist. water)

Warnings and Precautions for Users
- This kit contains radioactive material. During handling and disposal of radioactive materials, the existing legal regulations must be observed.
- Do not pipet by mouth.
- Do not smoke, eat or drink while handling the reagents of the kit.
- Wear gloves throughout the testing procedure and carefully wash your hands afterwards.
- Avoid spillage and forming of aerosol.
- All spills should be wiped up thoroughly and immediately and contaminated materials disposed of or decontaminated in accordance with the regulations.

Preparation of Reagents
Allow test components to reach room temperature (18–25°C) prior to testing and mix thoroughly (avoid foam formation).
Controls must be opened carefully and reconstituted with 1 mL of dist. water. Avoid heavy shaking when dissolving (foaming). Make sure that lyophilised material adherent to the screw cap is also dissolved.

Storage of Reagents
Reconstituted controls: 1 week at 2-8°C or 4 weeks at –20°C.
Store all reagents at 2-8°C until the expiry date printed on the package label.
- Keep upright for storage.
- Keep away from direct light.

Sample Collection and Storage
- Collect samples using standard procedures.
- Sample material: serum; disturbances with plasma are not known.
- Storage at 2-8°C: 24 h.
- For longer storage periods: freeze to below -20°C.
- Freezing and thawing samples once does not affect the test results.
- Stored samples should be thoroughly mixed prior to use (vortex mixer).
- Do not use samples which are agglutinated, lipemic, hemolysed, icteric or contaminated.
Interfering Substances
No interference with test results is seen by concentrations of bilirubin < 0.125 mg/mL, haemoglobin < 500 mg/dL or triglycerides < 12.5 mg/mL.

Procedural Notes
- The single components of each kit are carefully matched. In case of exchange or mixture of any components from different lots the manufacturer does not guarantee reliable results. See bottom of the kit for the lot numbers of all components.
- Strictly adhere to the procedures.
- The measuring time at the gamma counter must be adjusted for counting at least 1 minute.
- This kit must not be used after the expiry date printed on the package label.
- Observe quality control guidelines for medical laboratories.
- Avoid microbial contamination of the reagents.

Test Procedure
It is recommended that standards and samples are assayed in duplicate.
If values are expected above the highest standard concentration, samples should be further diluted with the diluent (e.g. by factors 10, 100, 1000).
Alternatively to the manual performance and evaluation an appropriate automated analyser system can be used on responsibility of the laboratory.
1. Pipet 100 µL each of standard, control or patient sample onto the bottom of the corresponding coated tube.
2. Add 100 µL ¹²⁵I-anti-CEA, mix gently (do not vortex).
3. Incubate for 4 hours (± 5 min) at room temperature (18–25°C) on a horizontal shaker.*
4. Aspirate the liquid.
5. Wash all tubes 3 x with 2 mL of 0.9% NaCl solution.
6. Measure radioactivity (CPM) in all tubes (at least 1 min).

| 100 µL | Pipet standard, control or patient sample |
| 100 µL | Add ¹²⁵I-anti-CEA |
| 4 h (± 5 min) | Incubate at room temperature (18–25°C) on a shaker* |
| 3 x 2 mL | Wash with 0.9% dist. water |
| 1 min | Measure (gamma scintillation counter) |

* Keep shaking conditions constant!
Optimum:
Amplitude 20 mm = 150 rpm
Amplitude 10 mm = 220 rpm
Amplitude < 8 mm = 300 rpm

According to experience an incubation time of 2 h at room temperature is sufficient to perform the test automatically. This should be checked in individual cases.
Calculation of Results
The standard curve is established manually as follows:

1. Determine the mean CPM for each pair of duplicate tubes.

2. Divide the mean CPM of each standard \( (B) \) by the mean CPM of the highest standard \( (B_{\text{max}}) \) and multiply by 100 in order to obtain the percentage of relative binding \( (%B/B_{\text{max}}) \) for each standard.

3. On semi-log paper, plot the relative binding of each standard \( (%B/B_{\text{max}}) \) on the Y-axis versus the corresponding concentrations (ng/mL) on the X-axis.

4. Read sample concentrations (ng/mL) directly off the standard curve by their corresponding relative binding \( (%B/B_{\text{max}}) \).

Samples with counts above the highest standard concentration must be diluted with the kit diluent and re-assayed. For the final concentration of such samples, the appropriate dilution factor has to be considered. An example of a standard curve is given in the QC-certificate. This curve must not be used for the calculation of unknown samples.

The instrumental calculation of radio-immunological measuring values is based on a spline approximation.

Quality Control
Observe quality control guidelines for medical laboratories.

Validity and precision of the results should be checked with control sera or pool sera prepared by the laboratory.

The control included with the kit is well suited for in-house quality control in the laboratory. This control should be simultaneously tested with each test run and treated like patient samples.

Compare your results with those given in the quality control report listing the expected value for the control serum and the corresponding tolerances.

Expected Values
The reference range of CEA described in the literature is method dependent and varies between 1.5 – 5 ng/mL (9).

The upper reference range of IRMA – coat® CEA in apparently healthy patients \( (n=60) \) was found to be 4.4 ng/mL (mean ± 2 standard deviation). A CEA value of 2.1 ng/mL (mean ± 2 standard deviation) was found for non-smokers \( (n=30) \) and 5.8 ng/mL was found for smokers (mean ± 2 standard deviation).

Since CEA values may vary depending on the laboratory method used, each laboratory should establish its own reference range.

Limitations of the Procedure
Patients with malignancies may exhibit CEA values within the reference range. Elevated CEA values may also be observed in patients with benign diseases such as liver cirrhosis, viral hepatitis, pancreatic or gastrointestinal disorders. Smoking and alcohol consumption may also lead to an increase in CEA concentrations (6).

Therefore, CEA serum levels may only be interpreted in context with the clinical picture and other diagnostic procedures.

All tests, in which antigen is incubated together with labelled antibodies and immobilised antibodies in a liquid phase, bear the risk that undiluted samples containing extremely high concentrations of the antigen, will give measuring values below those of the highest standard. In case of the IRMA-coat® CEA, this phenomenon is observed at concentrations exceeding 10 300 ng/mL. If such values are suspected, measurement should be repeated after further dilution (e.g. by factors 10, 100, 1000) of the specimen.

HAMA
Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralising agents are added, extremely high HAMA serum concentrations may occasionally influence results. These samples should not be used for the IRMA-coat® CEA assay.
Analytical Data

Calibration
The test has been calibrated using an internal reference standard.

Measuring range
IRMA-coat® CEA allows to measure concentrations between 0.6 and 100 ng/mL.

High-dose hook
A high-dose hook effect was observed for CEA concentrations > 10 300 ng/mL.

Precision

<table>
<thead>
<tr>
<th>Intra-assay variation</th>
<th>Inter-assay variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean value (ng/mL)</td>
<td>CV (%)</td>
</tr>
<tr>
<td></td>
<td>n=</td>
</tr>
<tr>
<td>4.5</td>
<td>4.3</td>
</tr>
<tr>
<td>20.2</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Analytical sensitivity
The detection limit of the IRMA-mat® CEA assay is < 0.6 ng CEA/mL. This limit is defined as a value exceeding the zero standard by three standard deviations; it is the lowest CEA concentration that can be differentiated from zero with statistical significance.

Analytical specificity
No cross-reactivity is observed due to the presence of AFP (10000 ng/mL), Ferritin (8000 ng/mL) and PSA (1000 ng/mL).

Dilution
A patient’s serum was diluted with diluent and then measured. The measured values were compared with expected values obtained from linear regression.
Original concentration: 70.5 ng/mL

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Measured value (ng/mL)</th>
<th>Expected value (ng/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: 1.25</td>
<td>54.9</td>
<td>56.1</td>
<td>98</td>
</tr>
<tr>
<td>1: 2.5</td>
<td>28.8</td>
<td>28.5</td>
<td>101</td>
</tr>
<tr>
<td>1: 5</td>
<td>16.4</td>
<td>14.7</td>
<td>112</td>
</tr>
<tr>
<td>1: 10</td>
<td>7.8</td>
<td>7.8</td>
<td>100</td>
</tr>
</tbody>
</table>
Recovery

A patient’s serum with low CEA content was spiked with different amounts of CEA and then measured. Original concentration: 2.2 ng/mL

<table>
<thead>
<tr>
<th>Measured value (ng/mL)</th>
<th>Expected value (ng/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>47.0</td>
<td>47.8</td>
<td>98</td>
</tr>
<tr>
<td>25.5</td>
<td>25.0</td>
<td>102</td>
</tr>
<tr>
<td>13.4</td>
<td>13.6</td>
<td>98</td>
</tr>
<tr>
<td>8.3</td>
<td>7.9</td>
<td>105</td>
</tr>
</tbody>
</table>
References – Bibliografía – Références – References – Bibliografía – Βιβλιογραφία


SYMBOLS USED WITH IVD DEVICES
SIMBOLI USATI CON I DIAGNOSTICI IN VITRO
SYMBOLES UTILISÉS AVEC LES DIAGNOSTICS IN VITRO
MIT IN-VITRO-DIAGNOSTIKA GEBRAUCHTE SYMBOLE
SÍMBOLOS USADOS CON LOS DIAGNÓSTICOS IN VITRO
SÍMBOLOS UTILIZADOS COM OS DIAGNÓSTICOS IN VITRO
SYMBOLER SOM ANVÅNDS MED IN VITRO-DIAGNOSTIK
SYMBOLOX XRHΣΙΜΟΠΟΙΟΥΜΕΝΑ ΜΕ ΤΙΣ ΣΥΣΚΕΥΕΣ ΙΕΩD

CONT. Kit contents / Contenuto del kit / Contenu de la trousse / Inhalt des Kits
Inhalt des Kits / Contenido del kit / Conteúdo do dispositivo / Satınnehâll
Kittets indhold / Περιεχόμενα συσκευασίας.

Ab $^{125}$I Tracer: antibody labelled with $^{125}$I / Tracciante: anticorpi marcati con $^{125}$I
Traceur: anticorps marqués à l'$^{125}$I / Tracer: mit $^{125}$J markierte Antikörper
Trazador: anticuerpos marcados con $^{125}$I
Marcador: anticorpos marcados com $^{125}$I
Spårämne: antikroppar märkta med $^{125}$I
Tracer: antistoffer mærket med $^{125}$I
Ιχνηθέτης: αντίσωμα σημασμένο με $^{125}$I.

SORB Solid phase (Coated tubes / Coated beads).
Fase solida (Provette sensibilizzate / Sferette sensibilizzate).
Phase solide (Tubes revêtues / Billes revêtues).
Feste Phase (Beschichtete Röhrchen / Beschichtete Kugeln).
Fase sólida (Tubos recubiertos / Bolas recubiertas).
Fase sólida (Tubos revestidos / Bolas revestidas).
Fast stadium (Belagda rör / Belagda kulor).
Fast stadium (Sensibiliserede rør / sensibiliserede kugler).
Στερεά φάση (επικαλυμμένοι δοκιμαστικοί σωλήνες / επικαλυμμένα σφαιρίδια).

DIL Sample diluent / Diluente campioni / Diluant pour échantillons
Probenverdünnungslösung / Diluyente de muestras / Diluente das amostras
Provsämpning / Fortyndingsmiddel til prøver / Διαλύτης δειγμάτων.

CAL Calibrator / Calibratore / Etalon / Kalibrator / Calibrador / Kalibrator
Calibrator / Μέσο βαθμονόμησης.

CONTROL Control serum / Siero di controllo / Sérum de contrôle / Kontrollserum
Suero de control / Soro de controlo / Kontrollserum / Controlserum
Όρος ελέγχου.

St XX For XX tests / Per XX dosaggi / Pour XX dosages / Für XX
Bestimmungen / Para XX ensayos / Para XX testes / För XX dosering
Til XX test / Περιεχόμενο επαρκές για XX εξέτασης.

RCNS X mL Reconstitute with X mL / Ricostitui con X mL / Reconstituer avec X mL
Mit X mL auflösen / Reconstituya con X mL / Reconstituia com X mL
Återställ med X mL / Rekonstruer med X mL / Ανασύσταση με X mL.