**Intended Use**

*In vitro* test for the quantitative determination of alpha fetoprotein (AFP) in human serum and amniotic fluid for pregnancy monitoring and during the follow-up of tumour patients.

**Summary and Explanation of the Test**

Alpha fetoprotein is a macromolecular glycoprotein (with a molecular weight of approx. 68,000) consisting of a single polypeptide chain. AFP, which belongs to the group of oncofetal proteins, is produced by the yolk sac and in the foetal liver.

In oncology, AFP is determined in patients with liver-cell carcinoma (1, 5) or germ-cell tumours (non-seminomatous tumours of the testes; endodermal sinus tumour of the ovaries) (3, 6, 7) and in pregnancy monitoring (2, 4). During pregnancy, AFP levels in maternal blood continuously increase. After reaching a maximum between weeks 28 to 32 of gestation, the level again falls until delivery. In the amniotic fluid, the maximum is already achieved between the 13th and 15th week of gestation. Elevated AFP levels in early pregnancy indicate neural tube defects (spina bifida, anencephaly). Lower AFP concentrations in maternal serum are indicative of Down’s syndrome (trisomy 21).

The determination of serum AFP during monitoring of therapy and the progression of patients with carcinoma provides valuable information about the success of treatment and whether recidivation occurs.

**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 AFP 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>100</th>
</tr>
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<tbody>
<tr>
<td>¹²⁵I-anti-AFP, monoclonal (mouse), red radioactive content (kBq / µCi)</td>
<td>33 mL</td>
</tr>
<tr>
<td>7 standards A-G (0.6 mL) in human serum albumin</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 IU/mL) in phosphate buffer (concentrate)</td>
<td>2 x 11 mL</td>
</tr>
<tr>
<td>Test tubes, coated with anti-AFP, monoclonal (mouse)</td>
<td>2 x 50</td>
</tr>
<tr>
<td>Control serum, human, lyophilic</td>
<td>0.6 mL</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
- Micropipettes (25 µL, 300 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
- 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).
Open the control carefully and reconstitute with 0.6 mL of dist. water. (Avoid foam formation). Make sure that lyophilised material adhering to the cap is also dissolved.
Dilute 20 mL of diluent concentrate with 100 mL of dist. water.

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, amniotic fluid; 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 has been observed with 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 above the highest standard are expected, samples should be further diluted (e.g. by dilution factor 10, 100, 1000).

Amniotic fluid must always be diluted. During the diagnostically relevant weeks of pregnancy (between 16th and 19th week) 1:100 dilution is sufficient. In pathological cases a higher dilution may be necessary (parallel testing of 1:100 and 1:1000 dilutions).

As an alternative to manual performance and evaluation, an appropriate automated analyser system can be used at the responsibility of the laboratory.

1. Pipette 25 µL of standard, control or patient sample onto the bottom of a coated tube.
2. Add 300 µL 125I-anti-AFP, mix (vortex mixer).
3. Incubate the tubes for 3 hours (± 5 min) at room temperature (18–25°C) on a horizontal shaker.*
4. Aspirate the liquid.
5. Wash all tubes 3 times with 2 mL of dist. water.
6. Measure radioactivity (CPM) in all tubes (at least 1 min.)

<table>
<thead>
<tr>
<th>25 µL</th>
<th>Pipette standard, control or patient sample onto the bottom of a coated tube.</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 µL</td>
<td>Add 125I-anti-AFP</td>
</tr>
<tr>
<td>3 hrs (± 5 min)</td>
<td>Incubate at room temperature (18–25°C) on a shaker*</td>
</tr>
<tr>
<td>3 x 2 mL</td>
<td>Wash with dist. water</td>
</tr>
<tr>
<td>1 min</td>
<td>Measure (gamma scintillation counter)</td>
</tr>
</tbody>
</table>

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

The standard curve can be established manually as follows:

1. Determine the mean CPM for each pair of tubes (double determination).
2. Divide the mean CPM of each standard (B) by the mean CPM of the highest standard (B_{max}) and multiply by 100 in order to obtain the percentage of relative binding (%B/B_{max}) for each standard.
3. On semi-log paper, plot the relative binding of each standard (%B/B_{max}) on the Y-axis versus the corresponding concentrations (IU/mL) on the X-axis.
4. Sample concentrations (IU/mL) can be read directly off the standard curve from their corresponding relative binding (%B/B_{max}).

If the radioactivity measured is above that of the highest standard, the samples must be diluted with the diluent and tested again. With diluted samples the actual serum concentration and the appropriate dilution factor have to be established. An example of a standard curve is given in the QC report. This curve must not be used for the calculation of unknown samples.

The instrumental calculation of radio-immunological measured values is performed using a spline approximation.

Quality Control

Observe the 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. Here the expected value for the control serum and the corresponding tolerances are given.

Interpretation of the Results

In healthy men and non-pregnant women (n=63) AFP values do not rise above 6 IU/mL (95th percentile). Elevated levels point to neoplastic activity.

During pregnancy, the AFP concentrations in the maternal serum rise until they reach a maximum between the 28th and 32nd week of pregnancy. After week 32 a fall in values is observed.

AFP levels outside the reference range are indicative of fetal damage. Elevated AFP concentrations indicate a neural tube defect. The serum findings are verified by AFP determination in the amniotic fluid. Lower AFP concentrations in maternal serum are indicative of Down’s syndrome (trisomy 21).

Based on healthy, non-pregnant women, the following values for serum and amniotic fluid** have been evaluated for the IRMA - mat® AFP assay:

<table>
<thead>
<tr>
<th>Week of gestation</th>
<th>AFP in maternal serum (Median IU/mL) (n*)</th>
<th>AFP in amniotic fluid** (Median IU/mL) (n*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>26.7, 236</td>
<td>17,083, 15</td>
</tr>
<tr>
<td>16</td>
<td>28.0, 236</td>
<td>14,679, 107</td>
</tr>
<tr>
<td>17</td>
<td>34.1, 236</td>
<td>12,532, 135</td>
</tr>
<tr>
<td>18</td>
<td>38.4, 236</td>
<td>10,075, 106</td>
</tr>
<tr>
<td>19</td>
<td>41.9, 236</td>
<td>8,381, 37</td>
</tr>
<tr>
<td>20</td>
<td>60.0, 236</td>
<td>6,877, 24</td>
</tr>
</tbody>
</table>

** Values for amniotic fluid have been evaluated with the Liaison® AFP assay. As both assays use the same antibodies, the values determined using the Liaison® AFP can be considered standard values.
Since AFP values may vary depending on the laboratory method used, each laboratory should establish its own reference range.

**Limitations of the Procedure**

Patients with carcinomas may also exhibit AFP values within the normal range. AFP concentrations may in comparison be elevated in the case of benign diseases such as cirrhosis of the liver, hepatitis or tyrosinaemia (8). For this reason AFP determination is most suitable for therapeutic monitoring or during follow-up and for confirmation of histological results. The AFP assay should not be used as the only criterion for tumour screening.

Since no data on the diagnosis of Down’s syndrome has been ascertained with IRMA-mat® AFP, the assay is not recommended for use in triple screening.

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-mat® AFP, this phenomenon is observed at concentrations exceeding 30,000 IU/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-mat® AFP assay.
Analytical Data

Calibration
IRMA-mat® AFP has been calibrated using the Reference Standard MRC 72/225.

\[ 1 \text{ng AFP} = 0.83 \text{ IU AFP (MRC 72/225)} \]
\[ 1 \text{IU AFP (MRC 72/225)} = 1.21 \text{ ng AFP} \]

Measuring range
Measuring range is 2 - 600 IU/mL.

High-dose hook
No high-dose hook effect was observed for AFP concentrations up to 30,000 IU/mL.

Precision

<table>
<thead>
<tr>
<th>Intra-assay</th>
<th>Inter-assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean value (IU/mL)</td>
<td>CV (%)</td>
</tr>
<tr>
<td>19</td>
<td>2.6</td>
</tr>
<tr>
<td>99</td>
<td>2.8</td>
</tr>
<tr>
<td>338</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Analytical sensitivity
The lower detection limit is < 2.0 IU AFP/mL. This detection limit is defined as a value exceeding the zero standard by three standard deviations; it is the lowest AFP concentration that can be differentiated from zero with statistical significance.

Specificity
No cross-reactivities with mitomycin-C, doxorubicin or fluorouracil in therapeutic ranges were found.

Dilution
A patient’s serum was diluted with diluent and then measured. The measured values were compared with expected values obtained from linear regression.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Measured value (IU/mL)</th>
<th>Expected value (IU/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 : 1.25</td>
<td>368</td>
<td>366</td>
<td>101</td>
</tr>
<tr>
<td>1 : 2.5</td>
<td>202</td>
<td>183</td>
<td>110</td>
</tr>
<tr>
<td>1 : 5</td>
<td>100</td>
<td>92</td>
<td>109</td>
</tr>
<tr>
<td>1 : 10</td>
<td>50</td>
<td>46</td>
<td>109</td>
</tr>
</tbody>
</table>

Recovery
A patient’s serum with low AFP content was spiked with different amounts of AFP and then measured.

<table>
<thead>
<tr>
<th>Measured value (IU/mL)</th>
<th>Expected value (IU/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>253</td>
<td>252</td>
<td>100</td>
</tr>
<tr>
<td>197</td>
<td>202</td>
<td>97</td>
</tr>
<tr>
<td>154</td>
<td>153</td>
<td>101</td>
</tr>
<tr>
<td>52</td>
<td>53</td>
<td>98</td>
</tr>
</tbody>
</table>
References – Bibliografia – Références – References – Bibliografía – Βιβλιογραφία
8. Stránsky J et al. Assessment of Alpha-Fetoprotein in chronic HBsAg Carriers and in HBsAg Negative Cirrhosis of the Liver. *Sborník lékarsky* 1993;
SYMBOLS USED WITH IVD DEVICES

CONT. Kit contents / Contenuto del kit / Contenu de la trousse / Inhalt des Kits
Inhalt des Kits / Contenido del kit / Conteúdo do dispositivo / Satsinnehåll
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 $^{125}I$ markierte Antikörper
Trazador: anticuerpos marcados con $^{125}I$
Markador: 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 kolor).
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
Provspädning / Fortyndingsmiddel til prøver / Διαλύτης δειγμάτων.

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

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

$\Sigma XX$ 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 εξετάσεις.

Radioactive / Radioattivo / Radioactif / Radioaktiv / Radioactivo / Radioaktiv
Radioaktiv / Ραδιενεργός.

RCNS $X \text{ mL}$ Reconstitute with $X \text{ mL}$ / Ricostitui re con $X \text{ mL}$ / Reconstituer avec $X \text{ mL}$
Mit $X \text{ mL}$ auflösen / Reconstituya con $X \text{ mL}$ / Reconstitua com $X \text{ mL}$
Återställ med $X \text{ mL}$ / Rekonstruer med $X \text{ mL}$ / Ανασύσταση με $X \text{ mL}$.