Kit for the radioimmunological determination of alpha fetoprotein (AFP)
The kit comprises:
1. vial of \(^{125}\text{I}\)-AFP antibody (monoclonal, mouse) < 300 kBq, 22 ml human serum with bovine albumin, rat serum, sodium azide and a red dye
2. 2 x 50 test tubes coated with anti-AFP antibodies (monoclonal, mouse).
5. vials of AFP standards, with 0.3 ml human serum and sodium azide, concentration in the nominal range of 4 - 800 IU AFP/ml (the standards are calibrated against WHO 72/225).
2. vials of AFP control serum, 0.3 ml human serum and sodium azide, concentration stated.
1. vial of diluent/zero standard, antigen free dilution and assay medium, 30 ml, solution with buffer, bovine albumin, sodium azide and a blue dye.
1. tube of wash reagent, 5 buffer tablets.
1. plastic bag.
1. instruction for use

The dissolved reagents contain sodium azide as preservative. Avoid swallowing and contact with the skin or mucous membranes. 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.

1. Introduction
Alpha-foetoprotein (AFP) is a glycoprotein with a molecular weight of about 70000. It is formed during pregnancy in the foetal liver and the yolk-sac. It is detectable in the embryonic serum from the sixth week of pregnancy onwards, rising to a maximum concentration between the 13\textsuperscript{th} and 15\textsuperscript{th} weeks of pregnancy and then decreasing to lower values until birth. After birth the AFP concentrations in the serum of the newborn infant fall very rapidly and are usually within the range of the adult values when the infant is four to six weeks old. The pattern followed by the concentrations in the amniotic fluid is similar to that in the foetal serum (max. 14\textsuperscript{th} to 16\textsuperscript{th} week of pregnancy), but is lower by a factor of \(\approx 100\). The foetal AFP passes into the maternal circulation from the amniotic fluid and is metabolized in the liver.

The physiological significance of AFP is not clearly understood as yet. It is thought that AFP may be a foetal form of albumin. The maternal serum concentration of AFP increases during pregnancy, reaching its peak during the last trimester (34\textsuperscript{th}-35\textsuperscript{th} week of pregnancy).

2. Clinical results with RIA-gnost®AFP

2.1. Clinical significance of quantitative measurement of AFP

2.1.1 Diagnosis of AFP-producing tumours in the testes
As circulating AFP has a half-life of between four and five days, it is usually possible, just two to three weeks after the surgical removal of an AFP-producing tumour, to determine whether it has been completely excised and whether there are any metastases. If an AFP-producing tumour has been totally removed without metastases, the serum AFP concentration falls to the normal range. After surgery it is advisable first to carry out a screening check so as to detect any increase in serum AFP at an early stage. It is not so much an increase in serum AFP to above a specific limit that governs later therapy, as a continuous rise within the observation period. It is best to measure the seric hCG at the same time.

2.1.2 Serum AFP in liver disorders
Raised serum AFP concentrations are also found in patients with liver disorders such as hepatitis, cirrhosis of the liver and liver cell carcinoma.

2.1.3 Screening during pregnancy
Measurement of the AFP concentration in maternal serum between the 14\textsuperscript{th} and 21\textsuperscript{st} week of pregnancy is carried out mainly as a screening test for the presence of an open neural tube defect or anencephaly. AFP serum concentrations that are higher than the standard level for the stage of pregnancy in question can indicate the presence of conditions that put the foetus at risk, in some cases causing its death in utero, such as spina bifida or anencephaly, or even in some cases omphalocele and congenital nephrosis. Other causes can be hepatic disease in the mother or multiple pregnancy. Raised AFP levels are also encountered when the stage of pregnancy has not been correctly determined. This parameter must be taken into account when measuring AFP. If the serum AFP level still exceeds the 2.5-fold median when the stage of pregnancy has been correctly determined and the test has been repeated, and the ultrasound examination does not suggest any other reason for raised serum AFP concentration, it is advisable to measure the AFP concentration in the amniotic fluid obtained by amniocentesis. These samples are diluted with the diluent so that they come within the measuring range.

2.2 Normal values

2.2.1 Tumour diagnosis
The upper limit of the normal range has been determined by \(n = 130\) serum samples from healthy men and \(n = 168\) serum samples from healthy, non-pregnant women using the 95\textsuperscript{th} percentile. An upper limit of 5 IU AFP/ml results for both groups.

Seric AFP values above these limits can be regarded as an indication of a tumour. The AFP tumour marker is particularly suitable for the follow-up of patients with AFP-producing tumours.
2.2.2 Screening during pregnancy

The determination of the normal range for RIA-gnost® AFP was carried out by measuring 12,264 sera of normal pregnancies between the 15th and 21st current weeks of pregnancy. The median (50th percentile) and the 2.5-fold median were calculated to establish the normal range (see Table 1).

Table 1: Standard values for maternal serum AFP concentrations between the 14th and 21st week of pregnancy

<table>
<thead>
<tr>
<th>Weeks of gestation</th>
<th>Completed Current</th>
<th>14th</th>
<th>15th</th>
<th>16th</th>
<th>17th</th>
<th>18th</th>
<th>19th</th>
<th>20th</th>
<th>21st</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>116</td>
<td>1587</td>
<td>4112</td>
<td>3092</td>
<td>2038</td>
<td>1065</td>
<td>254</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IU/ml)</td>
<td>21</td>
<td>27.5</td>
<td>31</td>
<td>37</td>
<td>42</td>
<td>48</td>
<td>56.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5-fold Median (IU/ml)</td>
<td>52.5</td>
<td>69</td>
<td>77.5</td>
<td>92.5</td>
<td>105</td>
<td>120</td>
<td>141</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The definition of gestational age varies for different countries

The range given in Table 2 was determined for the AFP concentration in the amniotic fluid in normal pregnancy with RIA-gnost® AFP after appropriate dilution. It is important to ensure that the amniotic fluid sample is free from foetal erythrocytes.

Table 2: AFP concentration in the amniotic fluid of women between 15th and 21st week of pregnancy

<table>
<thead>
<tr>
<th>Current week of pregnancy</th>
<th>15th</th>
<th>16th</th>
<th>17th</th>
<th>18th</th>
<th>19th</th>
<th>20th</th>
<th>21st</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>61</td>
<td>119</td>
<td>154</td>
<td>88</td>
<td>49</td>
<td>43</td>
<td>38</td>
</tr>
<tr>
<td>Median (IU/ml)</td>
<td>10620</td>
<td>9440</td>
<td>8220</td>
<td>7750</td>
<td>5310</td>
<td>5530</td>
<td>4780</td>
</tr>
<tr>
<td>3-fold Median (IU/ml)</td>
<td>31860</td>
<td>28320</td>
<td>24660</td>
<td>23250</td>
<td>15930</td>
<td>16590</td>
<td>14340</td>
</tr>
</tbody>
</table>

It is recommended to control the standard values in your own laboratory.

3. Principle of measurement and characteristic data of the RIA-gnost® AFP

3.1 Principle

RIA-gnost® AFP enables the in vitro determination of alpha-fetoprotein in human serum (or plasma) and amniotic fluid by the “sandwich” assay principle. A complex of anti-AFP antibodies (monoclonal, mouse) bound to the tube wall, AFP in the sample and 125I-labelled anti-AFP antibodies (monoclonal, mouse) are formed during this process. The amount of tracer specifically bound to the coated test tubes is measured with a gamma counter. The evaluation of the unknown samples is carried out by reading from a standard curve constructed under identical conditions. All the samples outside the measuring range, as well as all amniotic fluid samples, are diluted 1:100 with the diluent contained in the kit. The monoclonal antibodies used in the kit are highly specific for AFP. There is virtually no risk of a cross reaction with other serum proteins occurring in the physiologically relevant concentration ranges.

3.2 Specific characteristics of the assay

This was evaluated with 2 samples assayed 8 times in the same series and in 30 different series.

<table>
<thead>
<tr>
<th>Within-run</th>
<th>Samples</th>
<th>Mean (IU/ml)</th>
<th>CV (%)</th>
<th>Between-run</th>
<th>Samples</th>
<th>Mean (IU/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.8</td>
<td>4.6</td>
<td></td>
<td>3</td>
<td>24.0</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>105</td>
<td>2.2</td>
<td></td>
<td>4</td>
<td>69.9</td>
<td>5.7</td>
<td></td>
</tr>
</tbody>
</table>

The detection limit is defined as being the smallest concentration different from 0 with a confidence interval of 95%. It has been determined as being 0.3 IU/ml.

Note:
The high sensitivity of the assay can only be achieved if the following recommendations are followed:

a) Avoid external contamination of the test tubes.

b) Ensure that the unbound tracer fraction is completely removed (decantation/aspiration). During aspiration the capillary tubes must not become blocked; after decantation tap the test tubes well onto an absorbent surface.

c) Check the measuring device and any associated equipment that may be used regularly, and if necessary decontaminate.

d) Exclude any interference effect from external sources of radiation.

e) 4. Working procedure

4.1. Apparatus required

Precision micropipettes or similar with disposable tips, permitting the dispensing of 50 and 200 µl, measuring cylinders, horizontal shakers, gamma scintillator counter calibrated for 125 iodine measurement.
4.2. Preparation of the reagents

The kit components, which have been stored at +2 et +8°C, are brought up to room temperature (17 - 27°C) before use. The washing buffer is prepared by dissolving five buffer tablets in 500 ml distilled water. All unused reagents should be stored at 2-8 °C. The remaining test tubes are stored in the original package, resealed.

4.3. Preparation of the serum samples

When blood samples have been taken, serum or plasma is obtained by the usual methods. The serum or plasma is used directly in the assay or stored for up to 3 days at 2-8 °C. If stored for a longer period, this should be at -20 °C. The serum samples must be carefully mixed after thawing.

4.4. Warnings and Precautions

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-HVC 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.

4.5. Assay procedure (see Table 1)

1. Number sufficient coated test tubes as shown in Table 3 (1 diluent D = S0, 5 standards S1 - S5, 2 control sera C1, C2, up to 42 serum samples).
2. Pipette 50 µl standard (or patient sample) into the bottom of the coated tubes. Use a new pipette tip for each sample.
3. Dispense 200 µl diluent into each test tube.
4. Shake the test tubes on a horizontal shaker for 15 minutes at 17 - 27°C.
5. Then introduce 1 ml washing buffer into each test tube, decant (aspirate) and wash again with 1 ml.
6. Dispense 200 µl 125I-anti-AFP solution into the bottom of each incubation tube.
7. Shake for 15 minutes, as described in 4.
8. Wash, as described in 5.
9. Measure the tubes for 1 minute in the gamma scintillation counter.

Note

It is important to ensure that this procedure is carried out rapidly (e. g. rack by rack). The total batch assayed should not exceed 200 tubes.

4.6. Evaluation of the results

The radioactivity measured in counts per minute (cpm) for all individual tubes of the standards S0 - S5 (diluent = standard S0), is plotted against the appropriate AFP concentration on prepared graph paper (see "Example of a standard curve"). The "best fit" standard curve is drawn through these points. For reading very low AFP concentrations the first part of the curve can be extended. The measured values of the patients' sera and the test serum are marked on the graph and the desired AFP content is read from the standard curve.

**Tab. 3 : AFP assay procedure**

<table>
<thead>
<tr>
<th>Labelling of the tubes</th>
<th>Standards (µl)</th>
<th>Control sera (µl)</th>
<th>Samples (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S0 (diluent)</td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td>Standard S0 = Diluent</td>
<td>50/50</td>
<td>50/50</td>
<td>50/50</td>
</tr>
<tr>
<td>Control sera C1, C2</td>
<td>50/50</td>
<td>50/50</td>
<td></td>
</tr>
<tr>
<td>Samples</td>
<td>200 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diluent</td>
<td>1 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Washing buffer</td>
<td>200 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-AFP tracer</td>
<td>1 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Washing buffer</td>
<td>1 ml</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Example of a standard curve

- Measure
5. 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 safety.

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.