Contents of the kit
1. 1 vial TRACER (8.0 ml), ready for use after thawing, containing <190 kBq. 5.1 µCi \[^{125}\text{I} \] C3a des Arg in phosphate buffered saline containing gelatin and 0.01 % sodium azide. Store at -15°C to -30°C.
2. 5 vials STANDARD (5 x 0.9 ml), ready for use after thawing, containing (A-E) 25, 10, 5, 2.5, 1 ng/50 µl human C3a des Arg in buffer with 0.01% sodium azide. Store at -15°C to -30°C.
3. 1 vial ANTISERUM (8.0 ml), ready for use after thawing, containing rabbit anti-C3a des Arg antiseraum solution in phosphate buffered saline with porcine gelatin and 0.01% sodium azide. Store at -15°C to -30°C.
4. 1 vial ASSAY BUFFER (9.0 ml), ready for use, containing gelatin and 0.01 % sodium azide. Store at 2°C to 8°C after thawing.
5. 1 vial SECOND ANTIBODY (8.0 ml), ready for use after thawing, containing goat anti-rabbit antiseraum solution in phosphate buffered saline with gelatin and 0.01% sodium azide. Store at -15°C to -30°C.
6. 1 bottle PRECIPITATING REAGENT (80 ml), ready for use, diluted in distilled water. Store at 2°C to 8°C after thawing.

Materials, tools and equipment required
Pipettes or pipetting equipment with disposable tips (50, 100, 450 and 2000 µl); disposable polypropylene, polystyrene or glass tubes (12 x 75 mm) or conical shaped test tubes capable of withstanding centrifugation speeds of up to 5000 xg.

Sample preparation
1. Label 2 sets of 12 x 75 mm disposable tubes. These tubes should be capable of withstanding centrifugation speeds of up to 5000 xg.
2. Pipette 450 µl of each sample into one of the sets of labelled tubes (sample tubes).
3. Pipette 450 µl precipitating reagent into each sample tube. (This creates a 1:1 dilution of the sample).
4. Mix each tube well.
5. Incubate for 5–30 minutes at room temperature.
6. Centrifuge at a minimum of 2500 xg for 15 minutes at 4°C. (Microcentrifuges should not be used unless they are refrigerated).
7. Transfer the supernatants to the second set of labelled tubes. Discard the first tube. The supernatant should be processed immediately, not stored for later use.

Note: If, prior to the assay, it is anticipated that sample C3a concentration will lie above the upper limit of the assay range, it is recommended that the following dilution procedure be performed. Assays can then be performed on both diluted and undiluted samples:

Dilute by mixing the supernatant in step 7 with isotonic saline containing disodium EDTA and Futhan. The most convenient method for preparation of the diluent is to add isotonic saline to a sample collection tube specified in the previous section. The resultant solution is then used as the diluent. Investigations have shown that samples containing elevated levels of C3a des Arg can be diluted a maximum of 1:30 after addition of the precipitation reagent.

Preparation of reagents, storage
Storage: see Contents of the kit. At these temperatures each reagent is stable until expiry date. The actual expiry date is given on the package label and on the quality certificate.

CAUTION! Equilibrate all reagents and samples to room temperature prior to use.
Mix all reagents and samples thoroughly prior to use except for standards. Standards should be given a brief gentle mix only.

Assay procedure
(For a quick guide, refer to Table 1.)

1. Label 12x75 mm disposable tubes in duplicate for each total count (TC), non-specific binding (NSB), Bo (0 standard), standards (A-E) and each sample.
2. Pipette 50 µl assay buffer into all tubes.
3. Vortex mix all tubes well to coat tube walls with buffer.
4. Pipette 50 µl isotonic saline into Bo tubes and 100 µl isotonic saline into NSB tubes.
5. Pipette 50 µl of each standard (SA-E) directly into the bottom of appropriately labelled tubes.
6. Vortex mix each treated plasma sample (see step 7 of Sample preparation). Pipette 50 µl of each sample (Mx) directly into the bottom of appropriately labelled tubes.
The average Bo/TC(%) should be in the range 30–60%. Calculate the percent bound for each standard and sample using the following relationship:

\[ \text{B/Bo(\%)} = \frac{\text{SA-E/M}_c \text{ (cpm)} - \text{NSB (cpm)}}{\text{Bo (cpm) – NSB (cpm)}} \times 100 \]

A standard curve can be generated by plotting the percent B/Bo as a function of the log C3a des Arg concentration.

Plot B/Bo(%) (y-axis) against concentration (ng) C3a des Arg per tube (x-axis). The concentration (ng per tube) value of the samples can be read directly from the graph (see table 2).

The concentration ng per tube value can be converted to ng/ml by multiplying by 40 as described below:

\[ \text{ng/tube} \times 2 (1:2 \text{ dilution, step 3}) \times 20 (50 \text{ microlitres sample volume}) = \text{ng/ml} \]

Note: If samples were further diluted, this dilution factor must be taken into account.

Note: Curve-fitting algorithms available for data reduction may be used. It should be noted that whilst results may differ by up to 5–10% depending on the algorithm used, this should not affect assay precision.

Table 2. Typical assay data

<table>
<thead>
<tr>
<th>Tube</th>
<th>Conc. ng/50 µl</th>
<th>Mean counts (cpm)</th>
<th>B/TC (%)</th>
<th>B/Bo (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>43038</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NSB</td>
<td>1020</td>
<td>24.4</td>
<td>40.2</td>
<td>100</td>
</tr>
<tr>
<td>SE</td>
<td>15857</td>
<td>-</td>
<td>-</td>
<td>81.0</td>
</tr>
<tr>
<td>SD</td>
<td>12425</td>
<td>-</td>
<td>-</td>
<td>62.2</td>
</tr>
<tr>
<td>SC</td>
<td>9887</td>
<td>-</td>
<td>-</td>
<td>48.4</td>
</tr>
<tr>
<td>SB</td>
<td>7114</td>
<td>-</td>
<td>-</td>
<td>33.3</td>
</tr>
<tr>
<td>SA</td>
<td>3552</td>
<td>-</td>
<td>-</td>
<td>13.8</td>
</tr>
</tbody>
</table>

Figure 1: A typical standard curve (Do not use to calculate unknown samples)

Calibration

The assay standards are prepared from highly purified human C3a des Arg. The assay system is calibrated against a reference standard prepared from purified C3a des Arg.

Sample stability

The high sensitivity of RIA for complement activation products such as C3a means that in vitro complement activation in the sample must be kept to a minimum. With this in mind, the stability of samples during collection, processing and storage in tubes containing disodium EDTA and the serine protease inhibitor, Futhan, and disodium EDTA alone, has been investigated. The results are shown below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>EDTA+Futhan</th>
<th>EDTA only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/ml)</td>
<td>157</td>
<td>283</td>
</tr>
<tr>
<td>Std dev</td>
<td>27</td>
<td>104</td>
</tr>
</tbody>
</table>

These results were obtained from 50 blood samples collected into tubes containing EDTA+Futhan, and EDTA only. (It is recommended that individual laboratories determine levels for their own reference population).

Sensitivity

The sensitivity of the assay is defined as the C3a des Arg concentration at two standard deviations below the mean zero dose binding (n=20). This was calculated as <1 ng/50 µl (<40 ng/ml) and is below the lowest standard concentration.

Specificity

The antisera cross-reactivities with related peptides are shown below:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>100</td>
</tr>
<tr>
<td>C3a</td>
<td>100</td>
</tr>
<tr>
<td>C3a des Arg</td>
<td>100</td>
</tr>
<tr>
<td>C4a des Arg</td>
<td>0.89</td>
</tr>
<tr>
<td>C5a des Arg</td>
<td>&lt;0.22</td>
</tr>
</tbody>
</table>

Note: The assay procedure is selective for C3a and C3a des Arg, eliminating interference from C3.

Reproducibility

The intra-assay variation (expressed as coefficient of variation) ranges from 3% to 9% depending on the C3a standard concentration. Further data on assay variation is given below.

Coefficient of variation is defined as:

\[ \text{CV}(\%) = \frac{\text{Standard deviation of mean}}{\text{Mean value}} \times 100 \]

The inter-assay precision values are shown below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of replicates</th>
<th>Mean value ng/ml</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>70</td>
<td>20.5</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>137</td>
<td>16.5</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>213</td>
<td>8.7</td>
</tr>
</tbody>
</table>

The inter-assay precision values are shown below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of replicates</th>
<th>Mean value ng/ml</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>68</td>
<td>7.6</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>91</td>
<td>5.0</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>213</td>
<td>8.7</td>
</tr>
</tbody>
</table>

Dilution

Samples may be diluted up to 1:30 with isotonic saline containing Futhan without affecting the accuracy of determination. The diluent is prepared by adding isotonic saline.
to an evacuated sample collection tube containing Futhan.

Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.
Do not use lipaemic, haemolyzed or turbid specimens. The assay system is designed for use with sample collection tubes containing Futhan.

Precautions

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of both Human Immunodeficiency Virus antibody (Anti-HIV-1) and Hepatitis B surface Antigen (HBsAg). Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV-1), or other infectious agents are absent. Human blood samples should therefore be handled as potentially infectious materials.

Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 3.8 mg.

Safety data sheet

Product name:
Sodium azide
CAS No. 26628-22-8
R: 22-32 Toxic if swallowed. Contact with acids liberates very toxic gas.
S: (1/2)-28-45 (Keep locked up and out of the reach of children). After contact with skin, wash immediately with plenty of water. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).
Composition:
Sodium azide solution.
Hazard identification:
Toxic if swallowed, inhaled, or absorbed through skin. May cause eye and skin irritation.
First aid measures:
In case of contact, immediately flush eyes or skin with copious amounts of water. If inhaled remove to fresh air. In severe cases seek medical attention.
Fire fighting measures:
Dry chemical powder. Do not use water.

Accidental release:
Wear suitable protective clothing including laboratory overalls, safety glasses and gloves. Mop up spill area, place waste in a bag and hold for waste disposal. Wash spill site area after material pick-up is complete.

Handling and storage:
Wear suitable protective clothing including overalls, safety glasses and gloves. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling.

Personal protection:
See above instructions for handling and storage.

Physical and chemical properties:

Stability and reactivity:
Avoid contact with metals and acid chlorides. This yields a very toxic gas.

Toxicological information:
LD50: 27 mg/kg oral, rat
LD50: 20 mg/kg skin, rabbit

Ecological information:
Not applicable

Disposal consideration:
Up to 5 vials worth of material may be disposed of directly down the sink with water. If 6 or more vials are to be disposed of they should pass through a chemical waste route.

Transport information:
No special considerations applicable.

Regulatory information:
The information contained in this safety data sheet is based on published sources and is believed to be correct. It should be used as a guide only. It is the responsibility of the user of this product to carry out an assessment of workplace risks, as may be required under national legislation.