**Biotest Lymphoscreen ABC 60**

For the detection of HLA-ABC antibodies

**Intended Use**

The microtest cell plate MZP. Lymphoscreen ABC 60 is used for screening and differentiation of complement-dependent cytotoxic HLA-ABC antibodies (1,5). Each MZP contains a panel of deep-frozen HLA-ABC typed lymphocytes (6).

**Summary and Explanation**

Cytoxic HLA-ABC antibodies can be formed after blood transfusions, after organ transplantsations and during pregnancy (2,3,4). Using Lymphoscreen ABC 60, sera from pregnant women, from transfused or transplanted patients can be screened rapidly. The determination of cytotoxic HLA-ABC antibodies against a panel of HLA typed lymphocytes is a method which can be carried out in any HLA laboratory.

**Principle of the Microlymphocytotoxicity Test**

Lymphocytes with known antigens are incubated with an unknown serum and rabbit complement. If the corresponding antigens are present, the addition of the serum result in the lysis of the lymphocytes. This is rendered visible by staining (e.g. Eosin Y). The lysed and vital lymphocytes are assessed using an inverted phase contrast microscope.

**Reagent Description**

In Lymphoscreen ABC 60, peripheral blood lymphocytes from various, healthy blood donors with defined HLA antigens are deep-frozen in a microtest plate MZP. The membrane of the frozen lymphocytes is stabilized using DMSO (dimethyl sulphoxide). To perform the microlymphocytotoxicity test, Lymphoscreen ABC 60 is thawed at room temperature. The DMSO is removed using a washing medium, as it is highly cytotoxic at room temperature. One package of Lymphoscreen ABC 60 consists of 6 MZP. Each MZP contains a deep-frozen panel of 56 HLA-ABC typed lymphocytes for screening of one sample and 2 lymphocyte pools (each 2 wells) provided for positive and negative double controls. The control positions are marked on the plate. Each well of the plate contains 2 μl of lymphocyte suspension in 13 μl freezing medium. The cell count is approx. 7000 lymphocytes per well.

**Statement of Precautions**

For In Vitro Diagnostic Use

The test must be performed by well-trained and authorised laboratory technicians. All materials of human origin used in this product have been tested and found to be non-reactive for HBsAg, anti-HCV and anti-HIV-1/2 using FDA licensed test kits. However, all products of human origin should be considered to be potential transmitters of hepatitis, HIV or other infectious agents. Appropriate safety measures are recommended.

**Storage**

Lymphoscreen ABC 60 is stable up to the date stated on its label. Lymphoscreen ABC 60 must be stored at -70°C or below. The packages, which are delivered in dry ice, must be transferred immediately into storage at -70°C. Opened Lymphoscreen ABC 60 packages must not be stored together with dry ice.

**Indication of Deterioration**

If the negative control shows over 10% lysed lymphocytes and the background toxicity is >10%, the test should be repeated.

If the positive control shows less than 80% lysed lymphocytes, the test must be repeated.

**Specimen Collection and Preparation**

Obtain the serum sample from:

- approx. 3 ml native blood or
- approx. 3 ml heparinised blood (convert plasma to serum by adding thrombin) or
- approx. 3 ml EDTA or ACD blood (convert plasma to serum by adding calcium chloride)

The serum sample should either be used fresh or stored frozen at -20°C or below.

**Procedure**

Materials Provided:

- Lymphoscreen ABC 60 (1 microtest plate MZP for 1 sample)
- lyophilised rabbit complement COMP (3x1 ml are added to each package)
- worksheet (6 are added to each package)

Additional Materials Required:

1. Lymphostab (McCoy’s medium 5A modified according to Park and Terasaki, Ca²⁺ and Mg²⁺ free)
2. Fetal calf serum (FCS), heat-inactivated, e.g. Sigma
3. **Control HLA (pos)**
4. **Control HLA (neg)**
5. Eosin Y (dissolved 5% in distilled water and filtered), e.g. Merck
6. Formaldehyde for histology, 37%, acid-free (filtered and adjusted to pH 7.2 ± 0.2 with 0.1 N sodium hydroxide solution), e.g. Merck
7. **Cover oil**
8. Absorbent cellulose
9. **Cover slips 50x75 mm**
10. Microtitre syringe, e.g. Hamilton no. 710 (0.100 ml syringe)
11. Volume dispenser, e.g. Hamilton PB 600-1
12. Terasaki dispenser, e.g. Hamilton no. 1725 (6 x 0.250 ml syringes)
13. Microdispenser for microtest plates, e.g. Greiner
14. Inverted phase contrast microscope
15. Water-jet pump

**Preparation of the Reagents**

- Prepare the washing medium: 10% (V/V) FCS in Lymphostab.
- Reconstitute the control HLA (pos.) in the volume of distilled water as stated on the label = positive control.
- Reconstitute the control HLA (neg.) in the volume of distilled water as stated on the label = negative control.

**Washing Procedure**

1. **Washing procedure by using of absorbent cellulose**:  
   - Allow the Lymphoscreen ABC 60 to thaw briefly at room temperature (18...22°C).
   - Reconstitute the complement in the volume of distilled water as stated on the label = positive control.
   - Reconstitute the control HLA (neg.) in the volume of distilled water as stated on the label = negative control.

2. **Washing procedure by using of microplate centrifuge**:  
   - Place the lymphocytes ABC 60 with cover oil (approx. 5 μl per well), or alternatively

**Microlymphocytotoxicity Test Procedure**

- **Pipeette 2μl of the negative control into each of the wells 1A and 1B**
- **Pipeette 2μl of the positive control into each of the wells 10A and 10B**. The positive controls are in position 10A and 10B and are used to check the complement activity. The positive control should yield more than 80% lysed lymphocytes.
- **Allow the Lymphoscreen ABC 60 to stand for 15 minutes at room temperature (18...22°C)**.
- **Using the absorbent cellulose, soak up the washing medium from one corner of the**  
  - With a second piece of cellulose remove the remaining washing medium row by row.
  - Note: If the washing medium is not completely removed weakened reactions may occur owing to dilution of the sera being tested.
  - **Cover the Lymphoscreen ABC 60 with cover oil (approx. 5 μl per well)**.

- **Washing procedure by using of absorbent cellulose**

- **Pipeette 20μl washing medium immediately into each well, using the Terasaki dispenser**.
- **Allow the MZP to stand for 15 minutes at room temperature (18...22°C)**.
- **Using the absorbent cellulose, soak up the washing medium from one corner of the**
  - With a second piece of cellulose remove the remaining washing medium row by row.
  - **Care**: If the washing medium is not completely removed weakened reactions may occur owing to dilution of the sera being tested.
  - **Cover the Lymphoscreen ABC 60 with cover oil (approx. 5 μl per well)**, or alternatively

- **Washing procedure by using of microplate centrifuge**

- **Pipeette 2μl of the negative control into each of the wells 1A and 1B**
- **Pipeette 2μl of the positive control into each of the wells 10A and 10B**. The positive controls are in position 10A and 10B and are used to check the complement activity. The positive control should yield more than 80% lysed lymphocytes.
- **Allow the Lymphoscreen ABC 60 to stand for 15 minutes at room temperature (18...22°C)**.
- **Centrifuge MZP immediately at 1000 x g for 30 seconds (without braking)**.
- **Carefully aspirate off the washing medium using the 6-channel aspiration head attached to the water-jet pump**.

**Warning**: Place manifold tips at the edge of the wells, so that only the washing medium is aspirated off, and not the sedimented cells.
- **Cover Lymphoscreen ABC 60 with cover oil (approx. 5 μl per well)**.

Lymphoscreen ABC 60 is now ready to be used for the microlymphocytotoxicity test.

- **Add 5-μl complement COMP per well** and incubate for 60 minutes at room temperature (18...22°C).
- **Stain the cells for approx. 3 minutes with 3-4 μl of 5% Eosin Y per well**.
- **Fix the reactions by adding of 6-7 μl of buffered formaldehyde per well**.
- If necessary, oil should be added to the microtest plates.
- **Cover the completed plate with a cover slip and read the results at least 30 minutes** after completion of the test. If stored at 2...8°C evaluation will still be possible for up to 3 days. Read the tray in the following serpentine pattern which corresponds to the worksheet. 1A through 1F, 2F through 2A, 3A through 3F, etc.

**Quality Control**

The negative controls are in positions 1A and 1B and are used to test the viability of the lymphocytes. The viability should be higher than 90%.

The positive controls are in position 10A and 10B and are used to check the complement activity. The positive control should yield more than 80% lysed lymphocytes.

* available from Biotest
Results
The reactions are read under an inverted phase contrast microscope. Viable lymphocytes are bright and shining (= negative reaction); lysed lymphocytes are dark and larger (= positive reaction). The percentage of lysed lymphocytes in the total lymphocyte count is expressed as score values.

<table>
<thead>
<tr>
<th>% lysed cells</th>
<th>evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 10%</td>
<td>score 1</td>
</tr>
<tr>
<td>11 - 20%</td>
<td>score 2</td>
</tr>
<tr>
<td>21 - 50%</td>
<td>score 4</td>
</tr>
<tr>
<td>51 - 80%</td>
<td>score 6</td>
</tr>
<tr>
<td>81 - 100%</td>
<td>score 8</td>
</tr>
</tbody>
</table>

The reactions read off must be entered in the appropriate column of the worksheet. The antigens of the positively reacting cells can be marked in the antigen table with a coloured marking pen. By selection of the antigens which are completely marked, the HLA specificity of the antibody is defined.

If the specificity cannot be determined, the total of the positive reactions is expressed as a percentage of the size of the panel (% PRA = % Panel Reactive Antibodies).

\[
\text{Number of positive reactions} \times 100
\]

\[
\text{Number of cells in the panel}
\]

Limitations
If the negative control shows over 10% lysed lymphocytes and the background toxicity is >10%, the test should be repeated. Possible reasons are:
1. Lymphoscreen ABC 60 was exposed to room temperature too long during unpacking.
2. Lymphoscreen ABC 60 was stored at a temperature less than -70°C.
3. Lymphoscreen ABC 60 was completely thawed before adding the washing medium.

If the positive control shows less than 80% lysed lymphocytes, the test must be repeated. Possible reasons are:
1. Complement activity inadequate.
2. Dilution of the antibody in question through inadequate removal of the washing medium.
3. Opened packages or individual MZP have been stored together with dry ice.

Specific Performance Characteristics
Lymphoscreen ABC 60 for screening and differentiation of complement-dependent cytotoxic HLA-ABC antibodies is prepared using lymphocytes from donors whose cells have been tested with appropriate antisera and shown to possess or lack the antigens listed on the accompanying worksheet. Each lot is tested with at least 10 well-known selected anti-HLA-ABC test sera, whereby the proportion of 10% false positive and 5% false negative reactions must not be exceeded. On testing with a negative control serum, the viability of the lymphocytes must be at least 90%. On testing with a positive control serum, at least 90% of the cells in each well must be lysed.

Comparison data of Lymphoscreen ABC 60 and cell panel trays produced under guidelines and regulations of ASHI:

- 78/101 were non-reactive for both assays
- 22/101 were positive in both assays
- 1/101 were positive only in Lymphoscreen ABC 60

References