ISBT Working Party on Platelet Immunobiology
Second training course on platelet immunology

May 7 – 9, 2013 Guangzhou Blood Centre, Guangzhou, GuangDong China

Organisers:
- Dr. Fu YongShui (Guangzhou blood centre, Guangzhou)
- Dr. Wu GuoGuang (Nanning blood centre, Nanning)

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Programme

Tuesday May 07, 2013
8.30 – 8.45 : Opening (Dr. Fu)
8.45 – 9.15 : Platelet Immunology: Overview (Dr. Santoso)
9.15 – 9.45 : Genotyping of Platelet Polymorphism (Dr. Ye)
9.45 – 10.15 : Detection of Platelet Antibodies (Dr. Tsuno)
10.15 – 10.30 : Organization of Training Course (Dr. Xia)
10.30 – 11.00 : Break
11.00 – 12.30 : Genotyping I; PCR-SSP (Dr. Ye)
12.30 – 14.00 : Lunch
14.00 – 17.00 : Demonstration Antibody Testing by MPHA (Mika Matsuhashi)
17.00 – 18.00 : Genotyping II; Analysis of PCR-SSP (Dr. Ye)
19.00 – 21.00 : Dinner

Wednesday May 8, 2013
8.30 – 12.30 : Antibody Testing Flow cytometry (Dr. Xia)
- MAIPA (Dr. Santoso)
12.30 – 14.00 : Lunch
14.00 – 17.00 : Antibody Testing
- Flow cytometry (Dr. Xia)
- MAIPA (Dr. Santoso)
19.00 – 21.00 : Dinner
Thursday May 9, 2013
8.30 – 12.30 : Analysis of Antibody Testing and Discussion
12.30 – 13.30 : Lunch
14.30 – 15.00 : Closing (Dr. Fu)

Registration fee : Free

**Training Course Exercises**

**Workshop 1: Genotyping**
DNA Typing for HPAs (HPA-1, HPA-2, HPA-3, HPA-4, HPA-5 and HPA-15) of three samples (Sample 1; Sample 2; Sample 3)
Platelets of these samples will be used for the Workshop 2

**Aim of the exercise:**
Participants should learn to type platelets for the most important HPAs and used such platelet panels to identify platelet antibodies

**Workshop 2: Antibody Testing**

1) **MAIPA**
Testing of 3 sera against platelets from sample 1, sample 2 and sample 3 for three different glycoprotein specificities IIb/IIIa, Ia/IIa and HLA

**Aim of the exercise:**
Participants should learn to characterise platelet specific alloantibodies in a complex mixture of serum using their own platelets panel by the use of antigen capture assay (MAIPA). Three different mabs against platelet glycoproteins IIb/IIIa, Ia/IIa and HLA class I should be used.

<table>
<thead>
<tr>
<th>Platelets</th>
<th>Serum 1</th>
<th>Serum 2</th>
<th>Serum 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>IIb/IIIa; Ia/IIa; HLA</td>
<td>IIb/IIIa, Ia/IIa; HLA</td>
<td>IIb/IIIa, Ia/IIa; HLA</td>
</tr>
<tr>
<td>Sample 2</td>
<td>IIb/IIIa; Ia/IIa; HLA</td>
<td>IIb/IIIa, Ia/IIa; HLA</td>
<td>IIb/IIIa, Ia/IIa; HLA</td>
</tr>
<tr>
<td>Sample 3</td>
<td>IIb/IIIa; Ia/IIa; HLA</td>
<td>IIb/IIIa, Ia/IIa; HLA</td>
<td>IIb/IIIa, Ia/IIa; HLA</td>
</tr>
</tbody>
</table>

2) **Flow cytometry**

Testing of mab against CD36 (direct labelled) and sera containing anti-Nak(a) against two different phenotyped platelets (CD36 positive and CD36 negative)

**Aim of the exercise:**
Participants should learn to type platelets for CD36 deficiency and to identify anti-Naka antibody.
<table>
<thead>
<tr>
<th>Platelets</th>
<th>Isotype control FITC</th>
<th>Anti-CD36 FITC</th>
<th>Serum 1* Control</th>
<th>Serum 2* anti-Nak(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1 CD36 neg.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 2 CD36 pos.</td>
<td></td>
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</tbody>
</table>

*) indirect; plus FITC labelled anti-human IgG

3) **MPHA (mixed passive hemagglutination assay)**

The MPHA assay, developed by Prof. Yoichi Shibata represents the standard method for platelet antibody detection in Japan. MPHA is a direct binding assay, in which platelets (or extracts of platelet membrane) are coated on microtiter-wells. After addition of test sera, the reaction is developed by adding of indicator cells (sheep red cells or magnetic beads) coated with anti-human IgG. In case of positive reaction, the indicator cells are “trapped” by platelet antibodies (no sediment). In case of negative reaction, the indicator cells form spontaneously sediment, visible as a “ring”. Two kits are commercialized in Japan; the screening kit and the antibody specificity determination kit (panel kit).

In this session, 6 human sera containing anti-HPA antibodies will be provided, and the participants will be requested to screen the sera in the screening kit, and then determine the antibody specificity in the panel kit. The performance of MPHA test will be demonstrated.

**Aim of the exercise:**
Participants should learn the principle of MPHA, and detect and identify the specificity of alloantibodies in human sera using this kit.

First, the 4 human sera will be tested, together with the positive and negative controls, by the MPHA screening kit.

<table>
<thead>
<tr>
<th>Screening</th>
<th>Positive or Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td></td>
</tr>
<tr>
<td>Sample 2</td>
<td></td>
</tr>
<tr>
<td>Sample 3</td>
<td></td>
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<tr>
<td>Sample 4</td>
<td></td>
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<tr>
<td>Sample 5</td>
<td></td>
</tr>
<tr>
<td>Sample 6</td>
<td></td>
</tr>
</tbody>
</table>

After the screening test, each group will choose one of the 6 sera, and has to determine the antibody specificity, using the panel kit.

<table>
<thead>
<tr>
<th>Identification</th>
<th>Specificities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample [ ]</td>
<td></td>
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</table>
**Hotels**

Guangzhou blood centre will book the rooms on behalf of the participants of the second training course on platelet immunology. The hotel expenses for three nights will be paid by Guangzhou blood centre (200-300 RMB/night).

If you would like to book the hotel yourself, please check the following websites

Please note that if you book the room yourself, Guangzhou blood centre will not pay your accommodation fee.