Antigens on platelets include:
1. Human platelet antigens (HPA)
2. Human Leukocyte antigens (HLA)
3. ABO

HPA
- HPA are carried on membrane glycoproteins.
- HPA are often called “platelet specific antigen,” but the term is a misnomer because some of these markers have been found on other types of cells, especially leukocytes.
- Molecular typing by polymerase chain reaction (PCR) is available for many platelet antigens. PCR genotyping with sequence-specific primers (SSP) appears to be the most common and reliable method of determining platelet antigens, making it feasible for genotyping HPA independent of the patient’s platelet count and of rare typing sera.
- Anti-HPA-1a is the most commonly implicated antibody in fetal/neonatal alloimmune thrombocytopenia (FNAIT) and posttransfusion purpura (PTP).
  - PTP is a rare condition characterized by concomitant destruction of autologous platelets. The lag time between transfusion and onset of thrombocytopenia is approximately 9 days.
  - FNAIT involves the destruction of fetal platelets by maternal antibody.
- Autoimmune thrombocytopenia may result in idiopathic thrombocytopenic purpura (ITP) is characterized by an insidious onset and moderate thrombocytopenia.

HLA
- HLA is the human major histocompatibility complex (MHC). This group of genes resides on chromosome 6, and encodes cell-surface antigen-presenting proteins.
- HLA are found on the surfaces of both platelets and nucleated cells.
- HLA loci are classified as MHC Class I and Class II. Class I antigens are further categorized into HLA-A, -B, -C, and others.
- Platelets are the major source of Class I HLA antigens in whole blood.
- HLA sensitization is the most common immune cause of platelet refractoriness
- Nomenclature:

<table>
<thead>
<tr>
<th>Serologic</th>
<th>Example</th>
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<tbody>
<tr>
<td>Letter denotes the HLA series followed by a number</td>
<td>A2</td>
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<tr>
<td>Molecular</td>
<td>Letter denotes the locus, followed by an asterisk, and then several digits separated by colons</td>
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ABO
- ABO antigens on platelets are intrinsic as well as adsorbed from the plasma.
- Lewis, II, P, P^k and Cromer are also found on platelets, but antibodies to these antigens do not seem to affect platelet survival.
- Platelets do not have Rh. However, residual red cells in a platelet unit do carry Rh antigens.

PLATELET ANTIGEN/ANTIBODY TESTING AND PLATELET CROSSMATCH

ISBT has determined multiple test methods is needed for platelet antigen and antibodies:
1. Glycoprotein-specific assay: most sensitive and specific in identifying HPA antibodies
2. Test using intact/whole platelet: can detect antibodies that are missed by the glycoprotein-specific assay.
3. HPA genotyping: helpful to confirm the HPA specificity of the antibody and for prenatal typing of a fetus in suspected FNAIT cases.

HLA/HPA antibody screening/identification methods
- ELISA
- Flow cytometry
- Platelet genotyping

Platelet crossmatch using intact platelets
- Solid Phase Red Cell Adherence (SPRCA) is the most widely used. Intact platelets bound to microtiter plate, antibody detection by AHG binding to anti-IgG coated cells.
- Flow cytometry (washed intact platelets are incubated with the patient’s serum, antibody detection by FITC labeled anti-IgG) and Modified Antigen Capture ELISA (MACE, washed platelets incubated with serum, platelets lysed and bound to antibody-coated microtiter plate, antibody detection by enzyme-labeled AHG and addition of substrate).

CCI AND PLATELET REFRACTORINESS

Response to platelet transfusion is commonly measured by the corrected count increment (CCI) using a 1-hour post-infusion platelet count.

CCI is determined by the platelet increment (post-pre platelet count), multiplied by the body surface area (BSA) in \(\text{m}^2\), and divided by the number of platelets \((\times 10^{11})\) transfused.

Platelet transfusion is successful if the CCI is \(\geq 7500\).
A patient is considered refractory when two or more transfusions with CCI of less than 7500.

Example calculation:
Patient BSA: 2 m^2
Pre-platelet count: 5,000/µL
Post-platelet count: 35,000/µL
Platelet content in unit: 6 \((\times 10^{11})\)

Count increment (CI) = 35,000 – 5,000 = 30,000

\[
CCI = \frac{30,000 \times 2}{6} = 10,000 \quad \text{(Interpretation: acceptable response, successful transfusion)}
\]
Patients who repeatedly fail to achieve a therapeutic platelet count increment after transfusion are said to be refractory. While both human platelet antigens (HPA) and human leukocyte antigens (HLA) and the antibodies produced by them have been implicated, the most common alloantibodies are directed against Class I antigens in the HLA system.

HLA and HPA antibodies develop after exposure to foreign antigens whose source may be pregnancy, organ or tissue transplantation, or transfusion. Antibodies to specific HPA have been linked to neonatal alloimmune thrombocytopenia (NAIT) and posttransfusion purpura (PTP).

Non-Immune Cause
When a patient is refractory to platelet transfusion, first review the patient history to rule out non-immune causes, which include:
- Massive bleeding
- Fever
- Sepsis
- Splenomegaly (splenic sequestration)
- DIC
- Allogeneic transplantation

Immune Cause
Next, investigate for immune causes of platelet refractoriness by testing for antibodies. The most common antibodies associated with platelet refractoriness are directed against HLA Class I antigens. The diagnosis of alloimmunization is supported by a positive test for HLA antibodies or by a positive platelet crossmatch.

1. HLA antibody screening/identification
2. Platelet antibody screening/identification
3. Platelet crossmatch

SELECTION OF PLATELETS FOR PATIENTS WITH ALLOIMMUNE REFRACTORINESS

| Platelet crossmatch | - Platelet crossmatch is the optimal method. |
| - Platelets that are crossmatch compatible will be negative for both the HLA antigens as well as platelet specific antigens corresponding to the antibodies in the patient. |
| - Crossmatching can be more cost effective. |
| - Solid-phase red cell adherence (SPRCA) is a common method for crossmatching. |

| Antibody specificity prediction | - Select platelet donors negative for the HLA antigens to the antibodies present in the patient are selected. |
| - This method requires HLA typing of the donors and HLA antibody testing of the patient. |

| HLA-matching | - Apheresis platelets that are closely matched for Class I HLA are selected. The best choice is 4 antigen match (Grade A) of all antigens for which the recipient and donor share. The next best choices are B1U or B2U, where one or more antigens are unknown or blank. |
| - Disadvantages include the need of a large pool of HLA-typed platelet donors to find sufficient HLA compatible donors, and the method may exclude those donors with different HLA type but still may be suitable for transfusion. |