

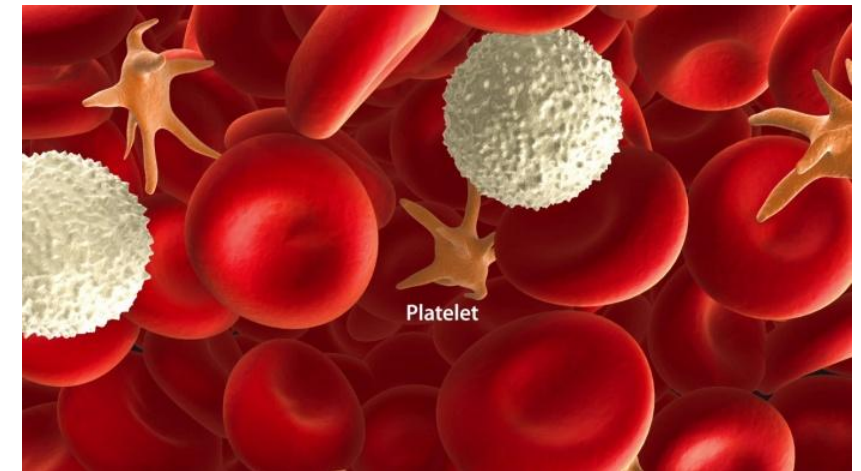
Platelet Transfusion Refractoriness: Clinical Significance and Laboratory Diagnostics



ASTA SEMENE

PLATELETS

- First described in **1882** by the Italian scientist *Giulio Bizzozero* as small, nucleus-free blood cells.
- **Normal count:** $150\text{--}400 \times 10^9/\text{L}$ in healthy blood.
- **Lifespan:** short-lived – only **7–9 days**.
- **Clearance (destruction):**
 - Primarily in the **spleen**.
 - In **asplenia**, the clearance function shifts to the liver.



PLATELET FUNCTIONS

Primary hemostasis – platelets rush to the site of injury, adhere, activate, and clump together to stop bleeding.

Supporting coagulation – they trigger and amplify the activation of clotting factors.

Secreting growth factors – they release signals that drive tissue regeneration and accelerate wound healing.

Shaping immune responses – they interact with leukocytes and help identify pathogens.

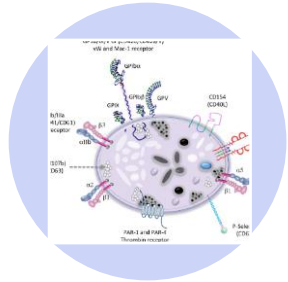
Defending against infections – they bind to pathogens, release signaling molecules, and activate other immune cells.

Key structures and molecules on the platelet surface – and why they matter:

◆ Glycoproteins – the connectors

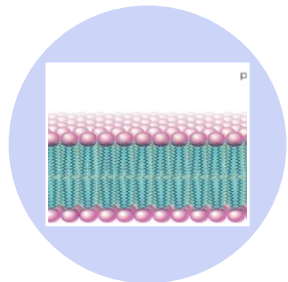
These proteins are crucial for platelet activation and adhesion to injured vessel walls.

Example: Glycoprotein IIb/IIIa acts like a “molecular glue,” driving platelet aggregation and clot formation.



◆ Phospholipids – the activators

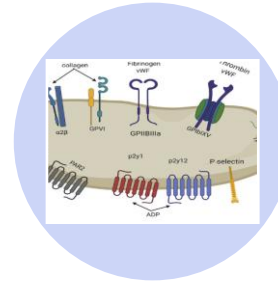
Located in the platelet membrane, phospholipids switch on when platelets respond to injury – triggering the powerful coagulation cascade.



◆ Receptors – the sensors

Platelets are equipped with receptors that allow them to “listen” and respond to signals from vessel walls and other blood components.

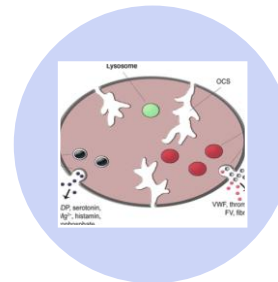
Example: Platelet receptors detect collagen, fibrinogen, and other key molecules.



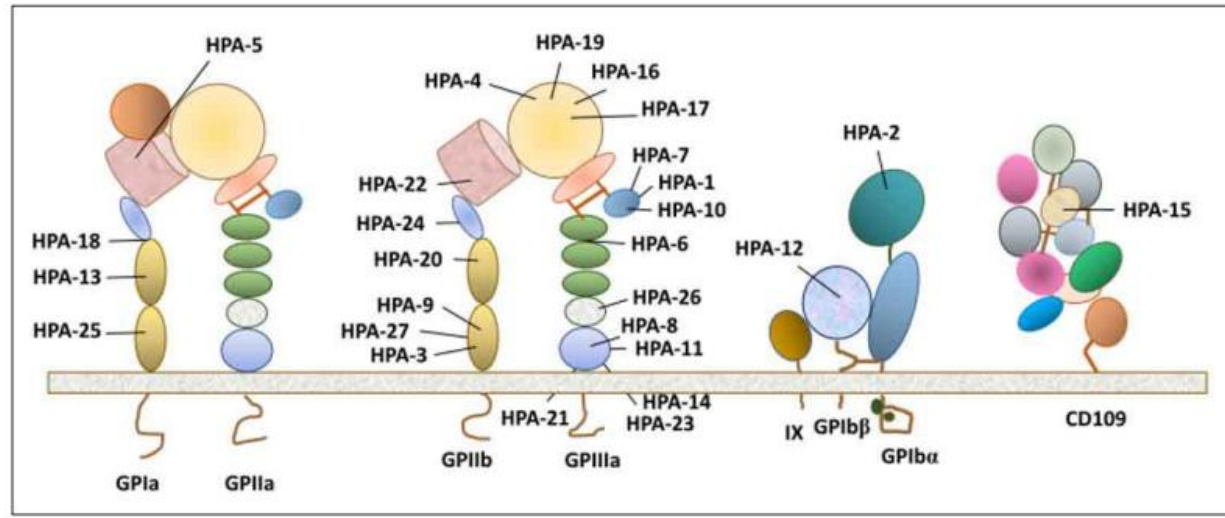
◆ Granules – the messengers

Platelet granules release chemical substances essential for clotting and defense.

For example: ADP, serotonin, and platelet activation factors act as chemical signals to amplify the response.



HUMAN PLATELET ANTIGENS



Distribution of human platelet antigens on platelet glycoproteins

- HPAs = unique antigens on the platelet surface.
- Located on glycoproteins or glycolipids.
- Clinical importance: immune reactions after transfusion or pregnancy.
- Most clinically relevant: [HPA-1](#), [HPA-2](#), [HPA-3](#), [HPA-4](#), [HPA-5](#), [HPA-15](#).

HUMAN PLATELET ANTIGENS

To date, **41 human platelet antigen (HPA) systems** have been identified.

HPAs are classified **from HPA-1 to HPA-41**.

Traditionally, they are grouped into **six major biallelic systems**:
HPA-1, -2, -3, -4, -5, and -15.

The numbering of HPA systems follows the **chronological order of their discovery**.

Nomenclature:

- **a** = more frequent allele.
 - **b** = less frequent allele.
 - **w** = provisional assignment until molecular definition is confirmed.
- The differences between the *a* and *b* alleles are determined by **single nucleotide polymorphisms (SNPs)**, most often located within platelet **glycoproteins**.

System	Antigen	Original names	Glycoprotein	CD
HPA-1	HPA-1a	Zw ^a , Pl ^{Al}	GPIIIa	CD61
	HPA-1b	Zw ^b , Pl ^{As}		
HPA-2	HPA-2a	Ko ^b	GPIb ^α	CD42b
	HPA-2b	Ko ^a , Sib ^a		
HPA-3	HPA-3a	Bak ^a , Lek ^a	GPIIb	CD41
	HPA-3b	Bak ^b		
HPA-4	HPA-4a	Yuk ^b , Pen ^a	GPIIIa	CD61
	HPA-4b	Yuk ^a , Pen ^b		
HPA-5	HPA-5a	Br ^b , Zav ^b	GPIa	CD49b
	HPA-5b	Br ^a , Zav ^a , Hc ^a		
HPA-6	HPA-6bw	Ca ^a , Tu ^a	GPIIIa	CD61
HPA-7	HPA-7bw	Mo ^a	GPIIIa	CD61
HPA-8	HPA-8bw	Sr ^a	GPIIIa	CD61
HPA-9	HPA-9bw	Max ^a	GPIIb	CD41
HPA-10	HPA-10bw	La ^a	GPIIIa	CD61
HPA-11	HPA-11bw	Gro ^a	GPIIIa	CD61
HPA-12	HPA-12bw	Iy ^a	GPIb ^β	CD42c
HPA-13	HPA-13bw	Sit ^a	GPIa	CD49b
HPA-14	HPA-14bw	Oe ^a	GPIIIa	CD61
HPA-15	HPA-15a	Gov ^b	CD109	CD109
	HPA-15b	Gov ^a	CD109	CD109
	HPA-16bw	Duv ^a	GPIIIa	CD61

Distribution of Platelet Antigens

- HPA allele frequencies vary significantly between populations.
- Genotyping confirms earlier serological findings.

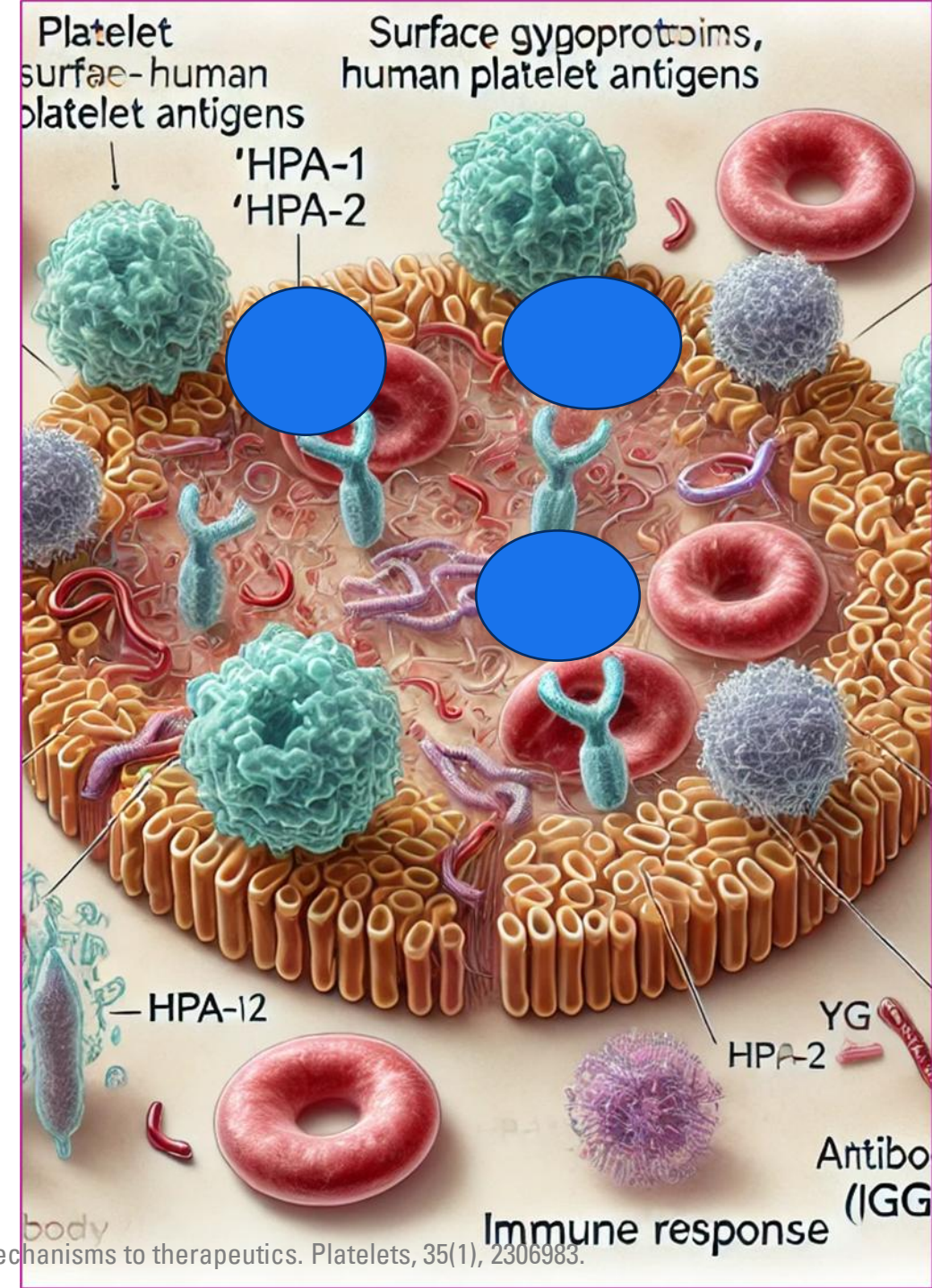
Data on HPA distribution across populations are essential for:

- Diagnosing immune thrombocytopenias
- Determining platelet transfusion refractoriness (PTR)
- Planning screening programs for women at risk of **neonatal alloimmune thrombocytopenia (NAIT)**.

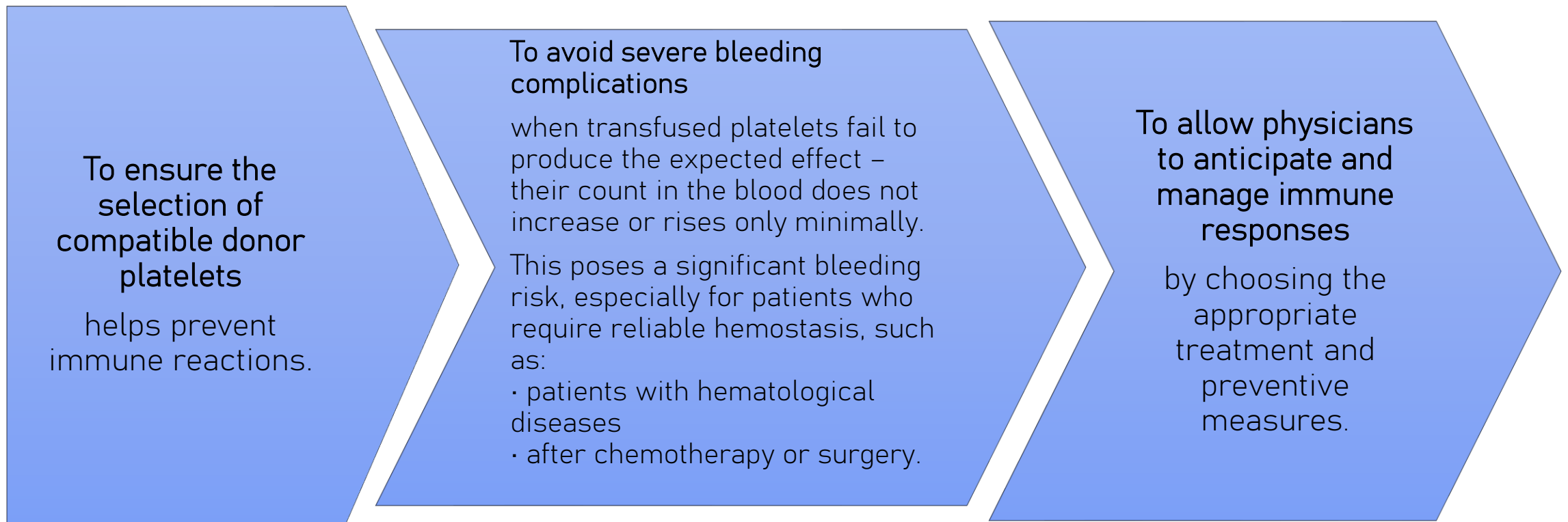
HPA System	a allele	b allele	Notes
HPA-1	HPA-1a (~98% in Europe)	HPA-1b (~2-3%)	HPA-1a most often associated with alloimmunization (NAIT, PTR)
HPA-2	HPA-2a	HPA-2b	HPA-2b more common in African populations
HPA-3	Similar distribution	-	In Europe, HPA-3a and HPA-3b ~50/50
HPA-4	Very rare in Europe	HPA-4b common in Asia	In Japan, HPA-4b reaches up to 90%
HPA-5	HPA-5a	HPA-5b	HPA-5b more frequent in South American populations
HPA-15	HPA-15a and HPA-15b equally common	-	Both alleles may trigger immune responses if mismatched between donor and recipient

Antibodies against platelet antigens

- are immunoglobulins that recognize, bind to, and target specific antigens on the platelet surface – sometimes leading to their destruction.



Why is it important to detect antibodies against platelet antigens?



When do antibodies against HPA or HLA develop?

01

During pregnancy

If the mother's immune system recognizes fetal platelets as foreign (e.g., due to HPA or HLA incompatibility), **neonatal alloimmune thrombocytopenia (NAIT)** may develop.

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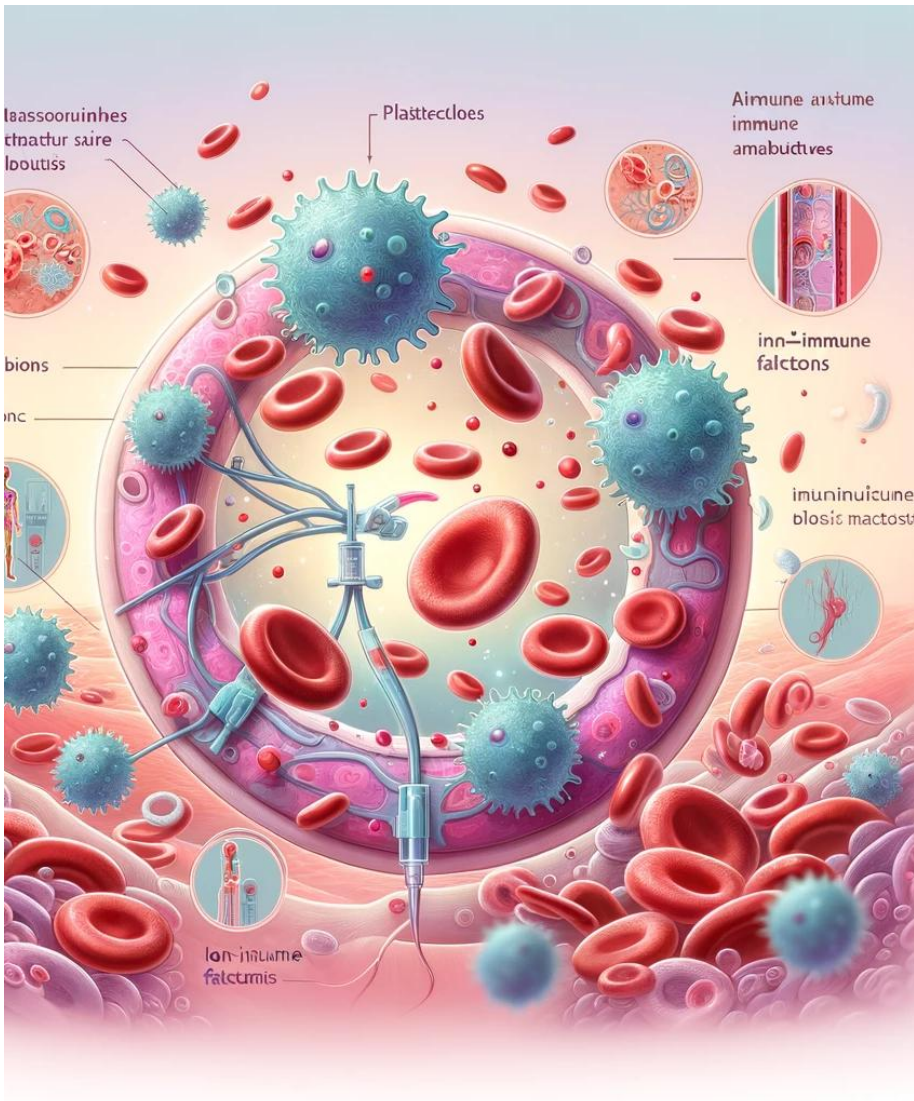
After platelet transfusions

When transfused donor platelets carry foreign antigens, antibodies may form, leading to **platelet transfusion refractoriness** (poor response to transfusions).

03

After pregnancy or transfusion

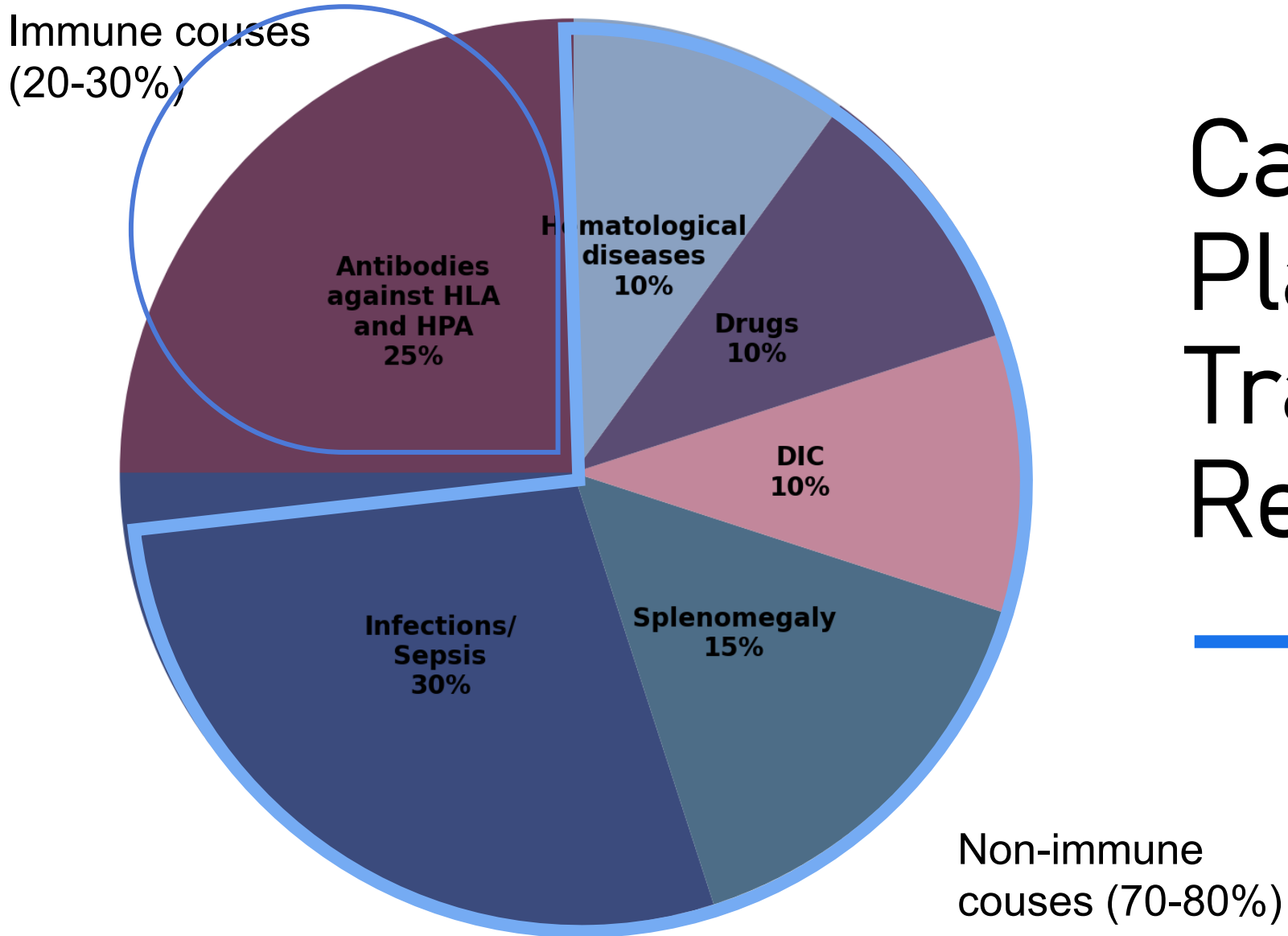
In rare cases, **post-transfusion purpura (PTP)** may occur – an autoimmune condition characterized by severe **thrombocytopenia**, typically developing **5–14 days** after a platelet transfusion.



PLATELET TRANSFUSION REFRACTORINESS

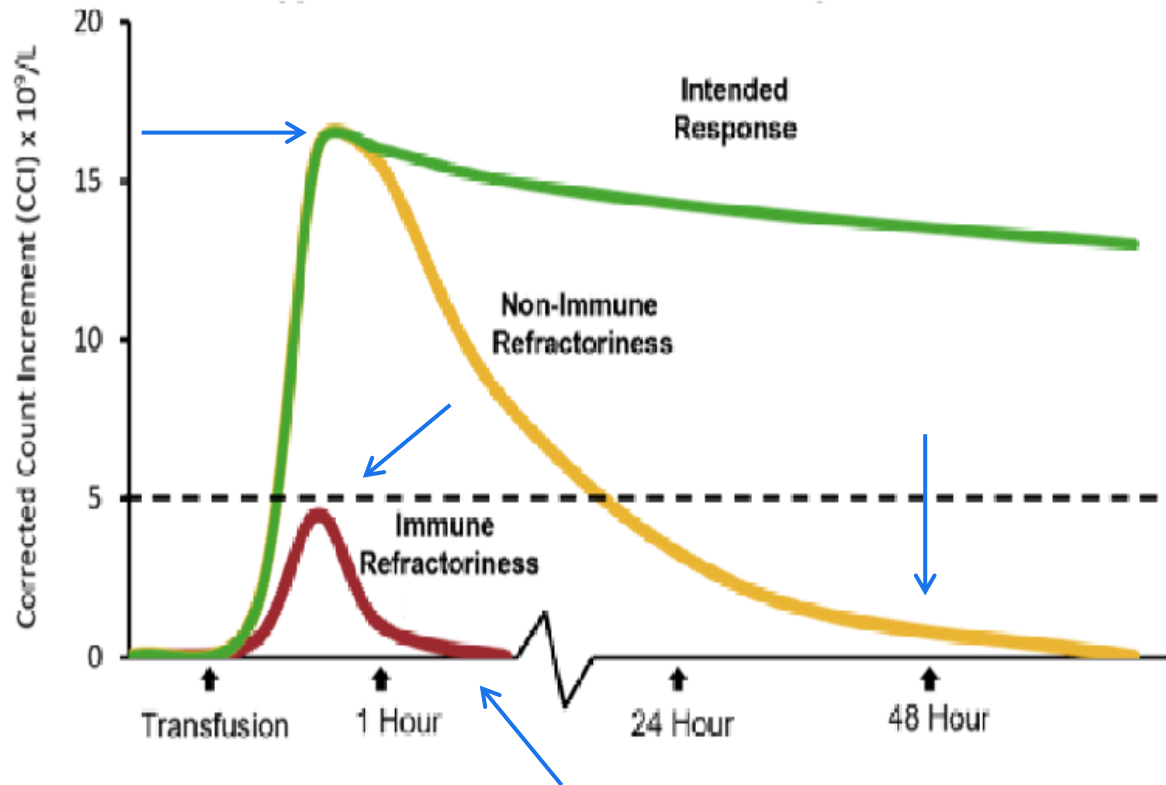
- Defined as insufficient platelet count increase after ≥ 2 ABO-compatible transfusions.
- Expected response: rise in platelet count, improved hemostasis, reduced bleeding risk.
- Failure to respond → platelets destroyed or removed due to immune or non-immune factors

Causes of Platelet Transfusion Refractoriness



Platelet transfusion refractoriness

- Non-immune causes → normal 1h increment, but return to baseline within 24h.
- Immune-mediated PTR → minimal 1h increment.
- 1h platelet count test is essential to distinguish immune vs non-immune PTR.



Corrected Count Increment (CCI)

- CCI is the key laboratory index used to assess how many transfused platelets remain in circulation after transfusion, taking into account the patient's body surface area and the number of transfused platelets.

$$CCI = \frac{(Post - transfusionplateletcount - Pre - transfusionplateletcount) \times Bodysurfacearea(m^2)}{Numberoftransfusedplatelets \times 10^{11}}$$

Example calculation:

$$CCI = \frac{(15 \times 10^9 - 5 \times 10^9) \times 1.8}{2.25 \times 10^{11}} = 4.0$$

If CCI after 1 hour is < 7.5 or after 24 hours < 4.5 , the patient is considered to be refractory to platelet transfusions.

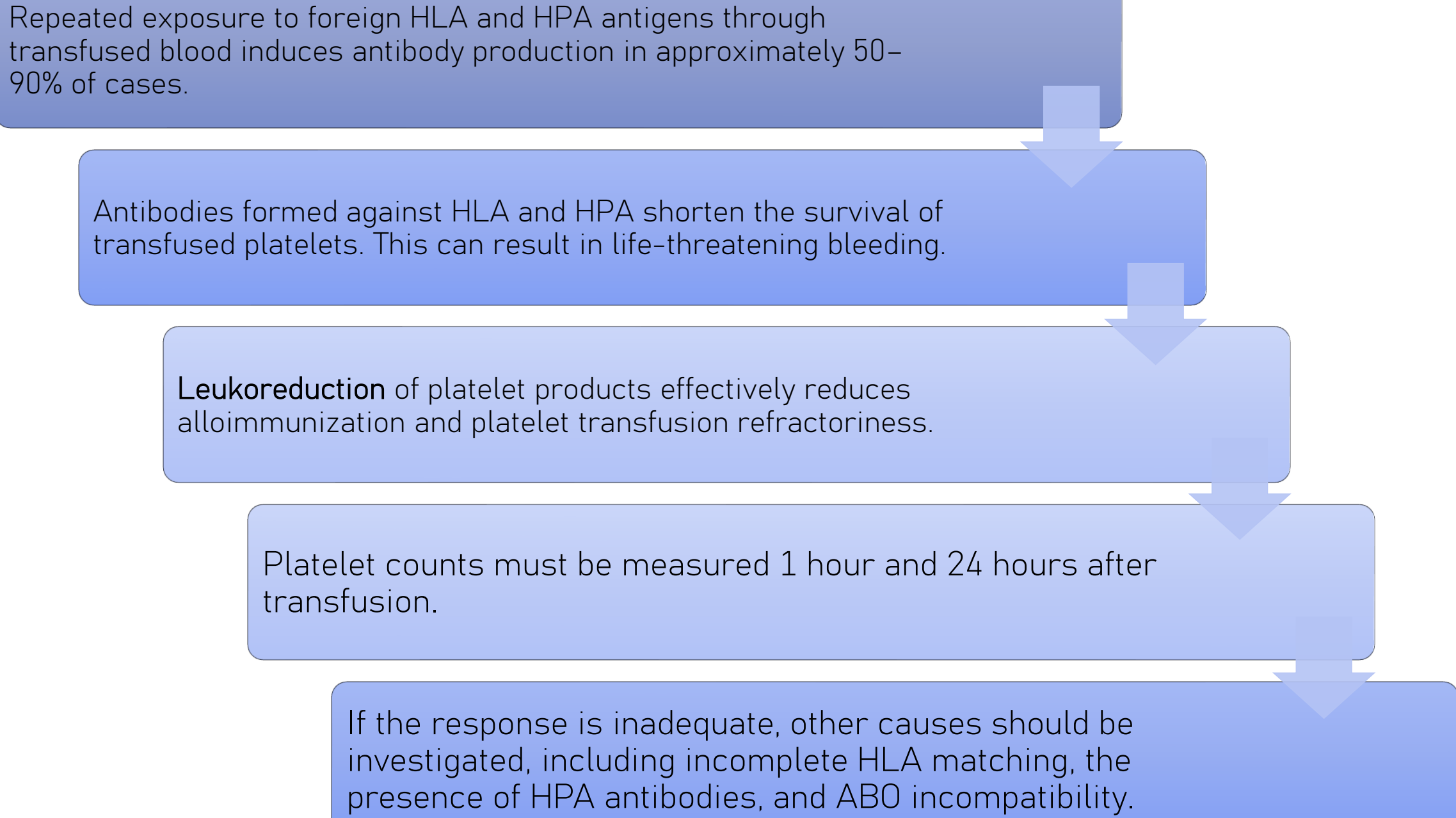
— Absolute Platelet Count Increment

Absolute Platelet Increment	Interpretation
$\geq 20-30 \times 10^9 /L$	Good response – transfusion effective
$10-20 \times 10^9 /L$	Partial response – possible mild refractoriness to platelet transfusion
$< 10 \times 10^9 /L$	Platelet transfusion refractoriness – transfusion ineffective

To evaluate the effectiveness of platelet transfusions, a simpler parameter can also be used – the **absolute platelet count increment** after transfusion:

Platelets = Post-transfusion platelet count – Pre-transfusion platelet count

Repeated exposure to foreign HLA and HPA antigens through transfused blood induces antibody production in approximately 50–90% of cases.



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graph TD; A[Repeated exposure to foreign HLA and HPA antigens through transfused blood induces antibody production in approximately 50–90% of cases.] --> B[Antibodies formed against HLA and HPA shorten the survival of transfused platelets. This can result in life-threatening bleeding.]; B --> C[Leukoreduction of platelet products effectively reduces alloimmunization and platelet transfusion refractoriness.]; C --> D[Platelet counts must be measured 1 hour and 24 hours after transfusion.]; D --> E[If the response is inadequate, other causes should be investigated, including incomplete HLA matching, the presence of HPA antibodies, and ABO incompatibility.];
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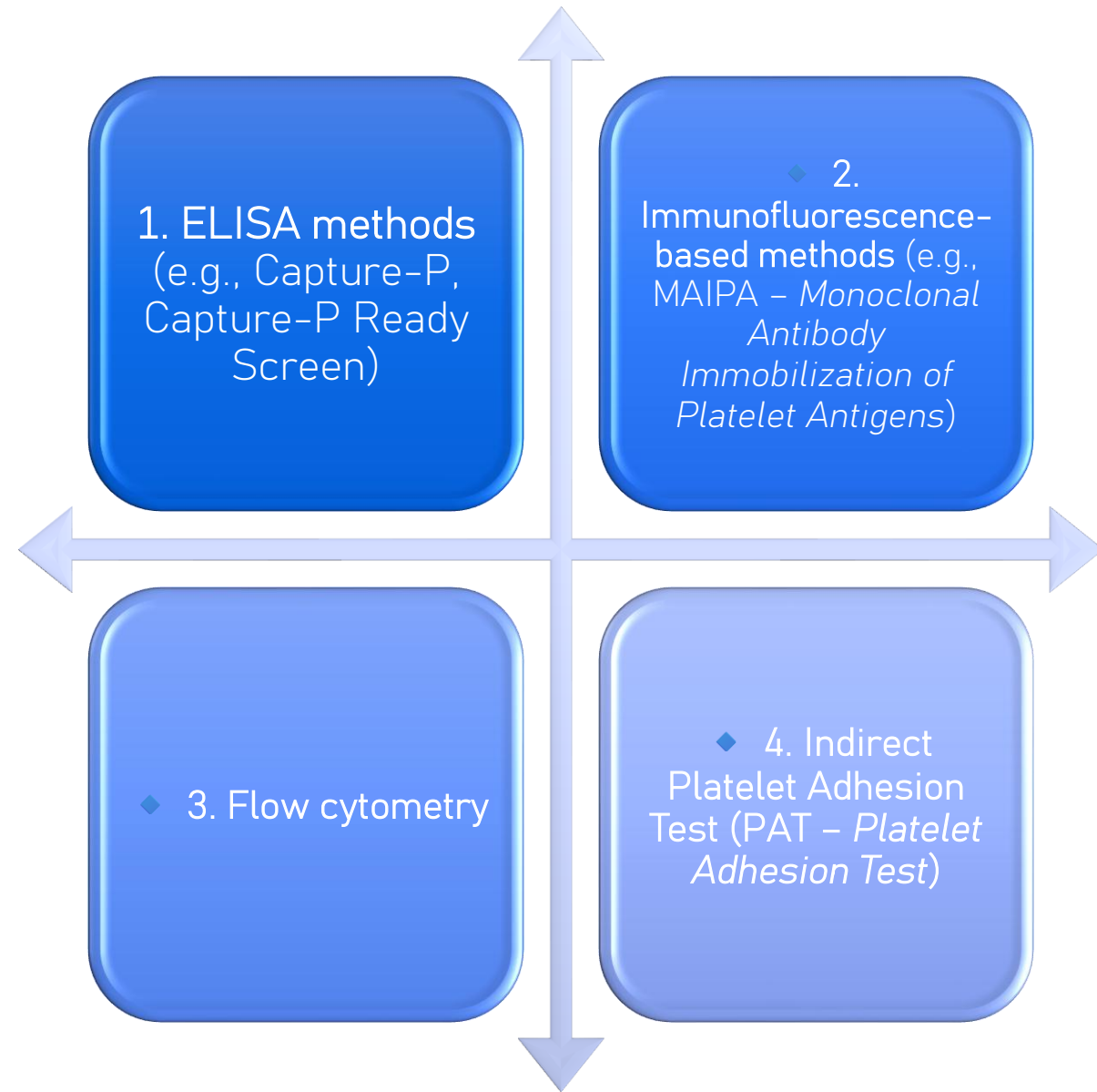
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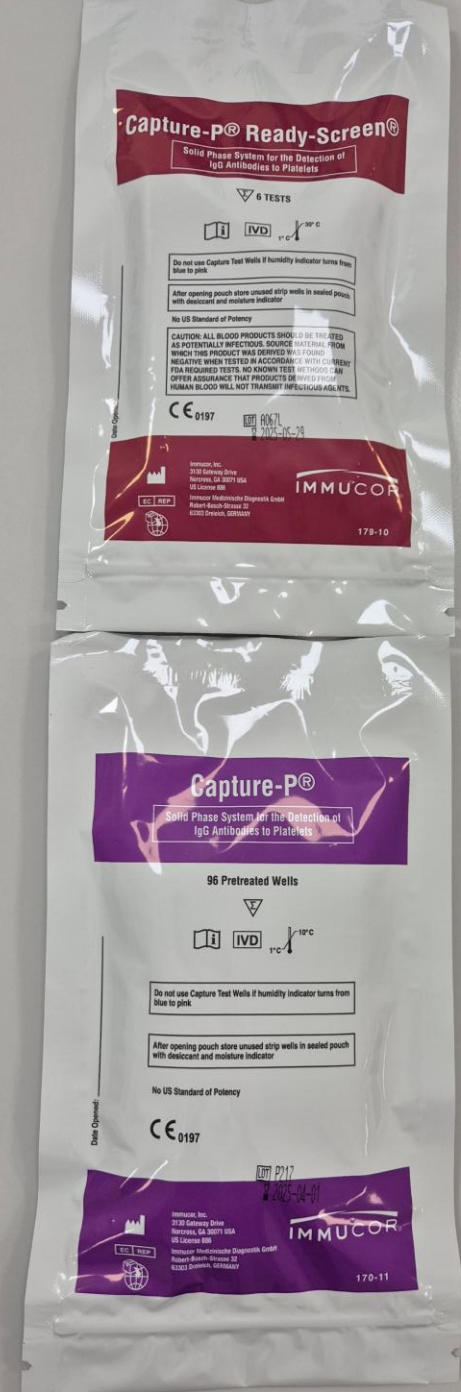
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Methods Used to Detect Antibodies Against Platelet Antigens



Screening and Identification Tests

- Solid-phase **immunochemical methods** are used for the detection and determination of platelet antibodies.
- The principle of the method – the interaction between antigens and antibodies, which is visualized by an **agglutination reaction**.
- In the **screening microwell plate**, antibodies against HPA and HLA can be detected, as well as crossmatching with donor platelets. In the **identification microwell plate**, antibodies against HPA and HLA can be specifically identified.



Steps of Antibody Detection Against Platelet Antigens



Preparation of platelet antigens

- In the screening plate wells, the surface proteins (antigens) of platelets are coated.
- This allows the capture of antibodies, if present in the patient's serum.

Identification wells

- The identification plate wells are pre-coated with specific antigens (e.g., HPA antigens such as HPA-1a, HPA-5b, HPA-15a, or HLA class I antigens).

Antibody binding

- When patient serum is added to the wells, antibodies (if present) specifically bind to the corresponding antigens in the wells.

Washing and incubation

- During washing, unbound antibodies and proteins are removed.

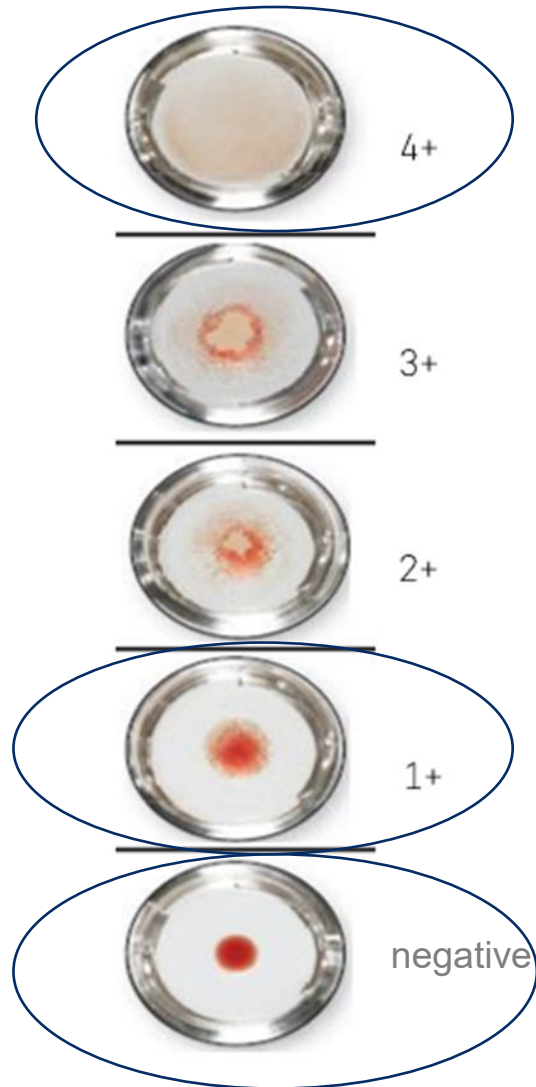
Antigen-antibody optimization

- The antigen-antibody complexes are incubated at 37 °C in the microwells to stabilize the reaction.

Addition of indicator cells

- Indicator cells are **anti-human globulin (AHG)-coated red cells**, which bind to human antibodies if they are attached to the antigens at the bottom of the well.

Why are screening (Capture-P) and identification (Capture-P Ready Screen) tests convenient to use?



◆ High sensitivity and accuracy:

Antigen-coated plates are used. With this method, even a small number of antibodies can be detected. Indicator cells enhance the visualization of the reaction, thus reducing the risk of false-negative results.

◆ Fast and reliable results:

The reactions occur relatively quickly, and the results are often clearly visible as agglutination reactions, making interpretation easier.

◆ Lower risk of false-positive and false-negative results:

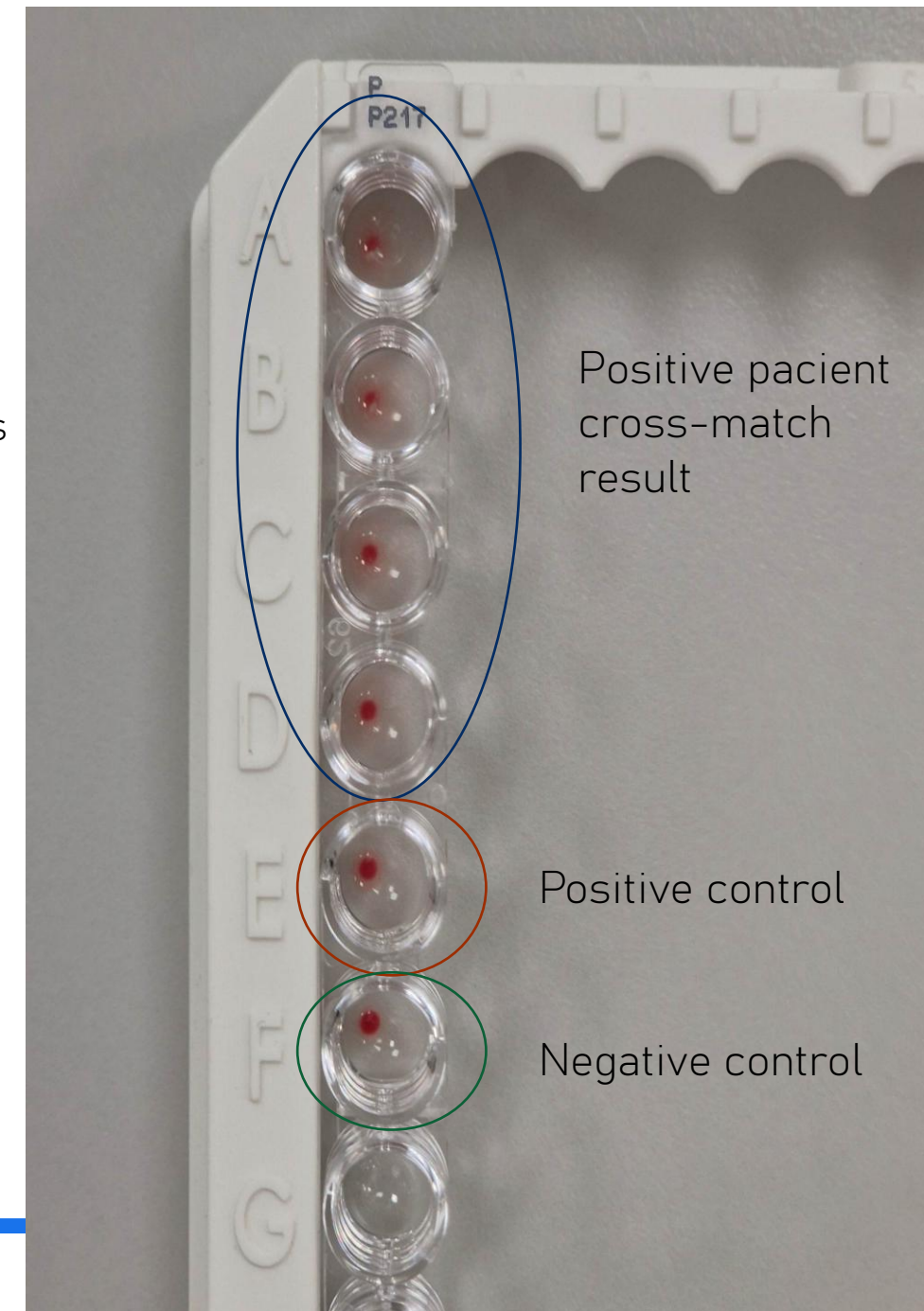
During the washing step, serum proteins are removed, which reduces the likelihood of nonspecific reactions.

Screening Antibody Detection Test

The **screening antibody detection test** is an important first step in evaluating whether a patient's serum contains **antibodies against human leukocyte antigens (HLA)** or **human platelet antigens (HPA)**.

The purpose of the cross-match test is to determine whether the antibodies present in the patient's serum react with donor platelets.

Result	Explanation
Positive cross-match	Antibodies are present in the patient's serum against donor platelets → transfusion incompatible .
Negative cross-match	No antibodies are present in the patient's serum against donor platelets → transfusion compatible .



Identification Antibody Detection Test

- The identification test includes:
- **HPA antigens in wells** (e.g., HPA-1a, HPA-1b, HPA-2a, HPA-2b, HPA-5b, HPA-15a, etc.) – used to detect antibodies against **human platelet antigens (HPA)**.
- **HLA class I antigens** – allow detection of **antibodies against leukocyte antigens**, which are also expressed on the platelet surface.
- The identification test is performed using a **micro-well plate**, designed to **identify antibodies against HPA and HLA**.
- The identification test not only determines whether antibodies are present, but also reveals **the specific antigen they target**.



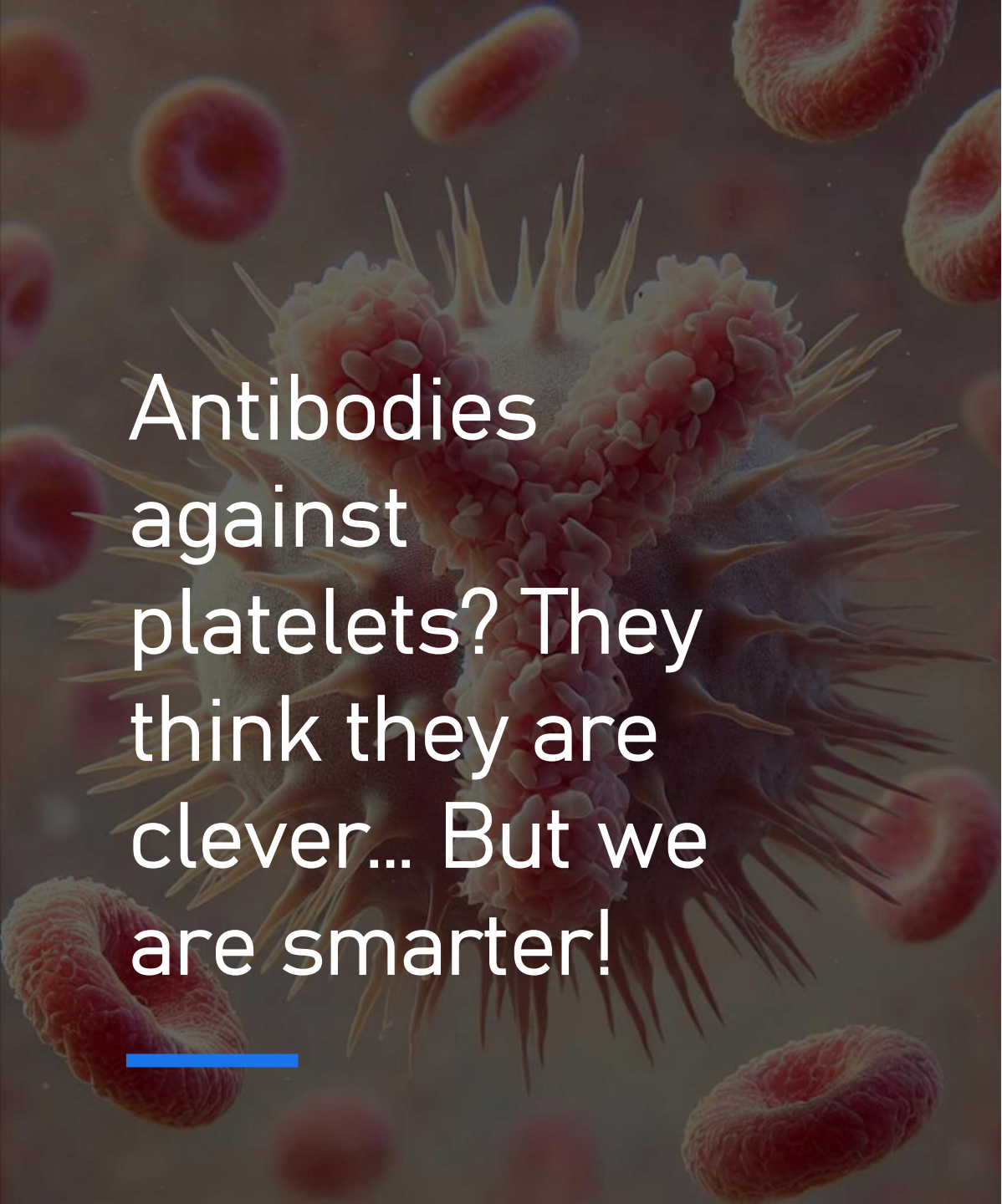
Empty well

Positive control

Negative control

Overview of Tests

Characteristic	Screening (Capture-P)	Identification (Capture-P Ready Screen)
Purpose	Detection of antibodies against HPA and HLA	Identification of antibodies against HPA and HLA
Procedure	Wells must be coated with antigens	Wells are pre-coated with antigens by the manufacturer
Duration	Longer: 2–3 hours	Shorter: 1–2 hours
Application	Screening test	Detailed specific diagnostics
When to choose?	Suitable when rapid detection of antibodies against platelets (HPA or HLA) is needed	Used when identification of specific antigens is required



Antibodies against platelets? They think they are clever... But we are smarter!

- Antibodies may believe they can sneak past unnoticed through the immune system's filters.
- ...but the laboratory works like a detective with pipettes, and with the help of [Capture-P](#) and [Capture-P Ready Screen](#), unmask every suspect!
- And when antibodies try to hide – the color reaction gives them away!



Platelet Transfusion Refractoriness: Clinical Significance and Laboratory Diagnostics

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