ImmunoPlatelet MAIPA ID Kit Identification

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The ImmunoPlatelet MAIPA ID Kit is an advanced diagnostic tool specifically designed for anti-platelet antibody identification in clinical settings. Utilizing the MAIPA (Monoclonal Antibody-specific Immobilization of Platelet Antigen) technique, it provides a highly sensitive and specific method for detecting and identifying antibodies related to platelet-related immune disorders.

How It Works:

The ImmunoPlatelet MAIPA ID Kit functions through two primary steps:

1. Screening – Indirect MAIPA (MAIPAI): This initial step screens serum or plasma samples for the presence of anti-platelet antibodies. The test identifies both alloantibodies and autoantibodies.
2. Identification – Direct MAIPA (MAIPAD): For positive results in the screening step, Direct MAIPA is used to identify specific antibodies bound to the patient's platelets, providing a thorough analysis of the immune response.

The kit employs monoclonal antibodies that bind to specific platelet glycoproteins, ensuring a comprehensive assessment of anti-platelet antibodies and their specificities.

What It Detects:

The ImmunoPlatelet MAIPA ID Kit identifies the following:

- Anti-Platelet Antibodies: These include alloantibodies (antibodies generated against foreign platelet antigens) and autoantibodies (self-targeting antibodies).
- Human Platelet Antigen (HPA): The kit identifies which platelet antigens the antibodies target, such as GPIIbIIIa, GPIaIIa, GPIbIX, and β2-microglobulin/HLA.

A positive screening result allows the identification of the specific HPA-antigen involved, enabling precise diagnosis and treatment strategies.

Applications in Diagnosis:

1. Platelet Disorder Diagnosis: The kit is essential in diagnosing immune thrombocytopenia, FNAIT, and PTP by detecting anti-platelet antibodies.
2. Platelet Refractoriness: It helps identify platelet refractoriness, ensuring compatibility in platelet transfusions.
3. Targeted Treatment: By identifying the specific platelet antigen (HPA) targeted by the anti-platelet antibody, it provides critical data for personalized patient care.

Why Choose the ImmunoPlatelet MAIPA ID Kit?

- High Sensitivity: Offers accurate detection of even low levels of anti-platelet antibodies, ensuring early diagnosis.
- Specificity: Allows for precise identification of autoantibodies and alloantibodies, improving diagnostic precision.
- Reliable for Cross-Matching: Provides crucial data for platelet transfusion compatibility, helping avoid complications.
- Manual Use: Ideal for clinical laboratories, offering a trusted method for diagnosing and managing platelet-related disorders.

Principle of Detection and Identification Using the ImmunoPlatelet MAIPA ID Kit Detection

The ImmunoPlatelet MAIPA ID Kit Detection operates on the principle of capturing platelet antigens using mouse monoclonal antibodies that specifically bind to a single platelet membrane glycoprotein. This process is followed by the binding of human antibodies to the platelet antigen and the subsequent analysis of bound human IgG using an ELISA immuno-assay.

Method Overview :

1. **Platelet Antigen Capture**:
   - Mouse monoclonal antibodies specifically designed to target platelet glycoproteins bind to these proteins on the platelet surface.
   - These antigens are then captured by the monoclonal antibody.
2. **Screening Step (Indirect MAIPA and Direct MAIPA)**:
   - Indirect MAIPA: Platelets from a pool of 6-12 blood group O donors, each selected for a specific platelet genotype, are incubated with patient serum. The monoclonal

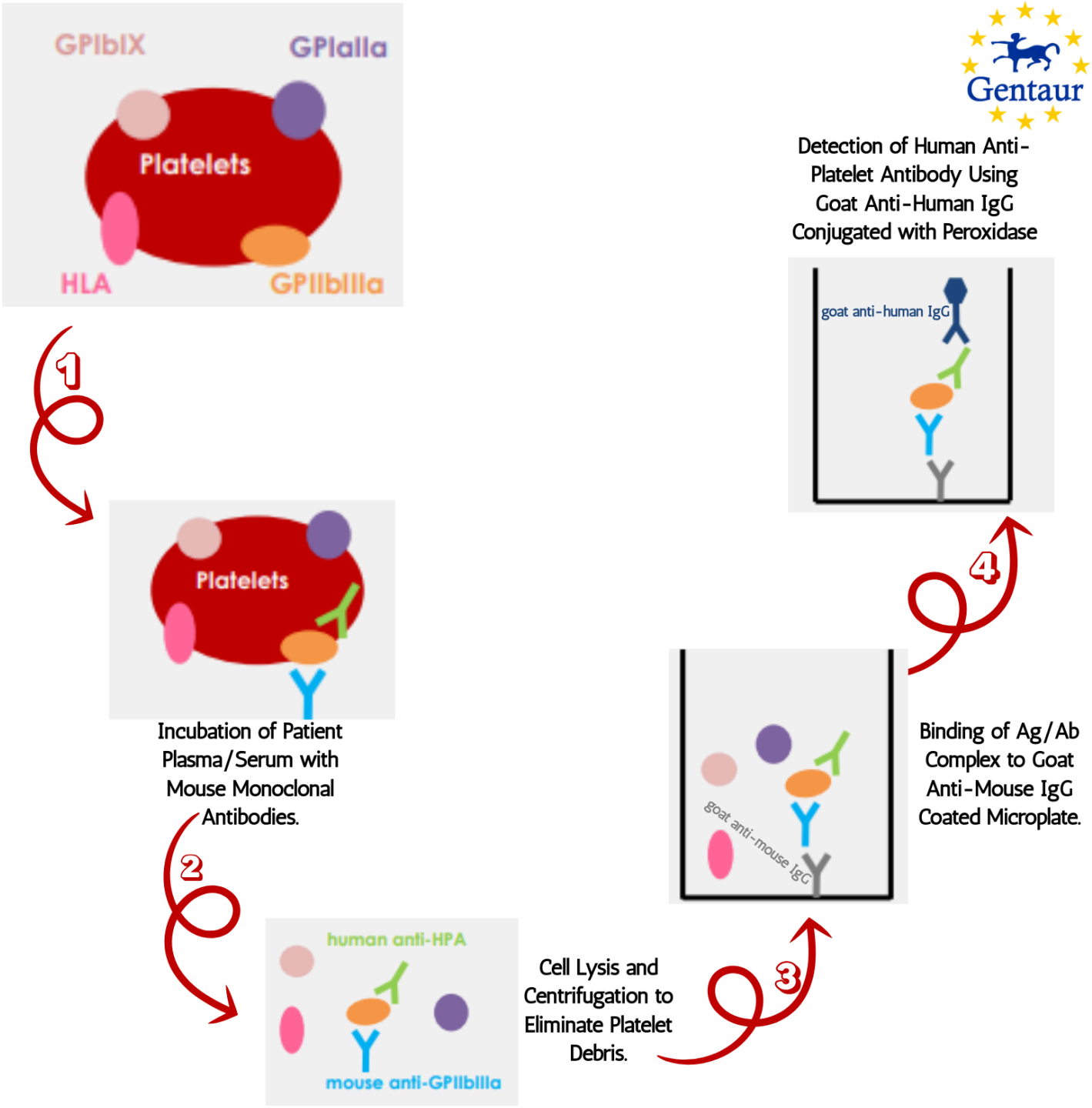
antibodies used target four key platelet glycoproteins: GPIIbIIIa, GPIaIIa, GPIbIX, and β2-microglobulin/HLA.
    - Direct MAIPA: If the patient's platelets already have IgG antibodies bound to them, these platelets are incubated directly with the same set of monoclonal antibodies.
3. **Detection of Antibody Binding**:
    - After incubation, platelets are lysed, and the lysates are cleared using centrifugation.
    - The lysates are added to a microplate pre-coated with goat anti-mouse IgG antibodies, which capture the antibody-platelet complex.
4. **Colorimetric Detection**:
    - The binding of the antibody-platelet complex is detected by adding a goat peroxidase-coupled anti-human IgG, followed by the TMB substrate.
    - A blue color appears, indicating the presence of an anti-platelet antibody. This color change is stopped by the addition of H_2SO_4, converting the blue color to yellow, measurable at 450 nm.

Identification Step (for Indirect MAIPA Only):

1. **Confirmation**:
    - If the **indirect MAIPA** screening produces a positive result, the **antibody** detected in the serum is identified using **genotyped platelets**.
    - **Serum** is incubated with the **monoclonal antibody** that tested positive during the **screening** step, and **known genotype platelets** are used to confirm which **platelet antigen (HPA)** the antibody targets.
2. **Direct MAIPA**:
    - If **direct MAIPA** shows **platelet-bound antibodies**, further identification of the **specific platelet antigen** is generally not required, unless **exceptional cases** call for further analysis.

Why the ImmunoPlatelet MAIPA ID Kit Detection Works

This method provides high **sensitivity** and **specificity** for detecting **anti-platelet antibodies** in patient samples. By combining the **monoclonal antibody binding** technique with **colorimetric ELISA detection**, it ensures accurate identification of **alloantibodies** and **autoantibodies**, allowing for better diagnosis and management of **platelet-related immune disorders**.



Revised MAIPA Assay Procedure

The ImmunoPlatelet MAIPA ID Kit Detection follows a series of structured steps for both screening and identification of anti-platelet antibodies. The procedure consists of Indirect Screening, Direct Screening, and Identification Assays, each involving careful handling of platelet samples and patient serum/plasma for accurate antibody detection.

☐ 1. Indirect Screening Assay

Objective: Screening serum or plasma for the presence of anti-platelet antibodies.

1. **Prepare Platelets**:
   a. Add 50 µL of screening platelets to the microplate.
   b. Centrifuge for 3 minutes at 1050 g and decant.

c. Shake for 2 x 10 seconds at 700-1000 rpm.

2. **Cell Wash**:
   a. Add 200 µL of Cell Wash Buffer 1x to each well.
   b. Centrifuge for 3 minutes at 1050 g and decant.
   c. Shake for 2 x 10 seconds at 700-1000 rpm.
3. **Add Patient Serum/Plasma**:
   a. Add 50 µL of patient serum/plasma to each well.
   b. Incubate for 30 minutes at 36 ± 1°C.
   c. Centrifuge for 3 minutes at 1050 g and decant.
4. **Add Monoclonal Antibodies**:
   a. Add 50 µL of monoclonal antibody MAB to the wells.
   b. Incubate for 30 minutes at 36 ± 1°C.
   c. Centrifuge for 3 minutes at 1050 g and decant.
5. **Cell Wash Again**:
   a. Add 200 µL of Cell Wash Buffer 1x and centrifuge for 3 minutes at 1050 g.
   b. Shake for 2 x 10 seconds at 700-1000 rpm.
6. **Lyse Platelets**:
   a. Add 130 µL of Lysis Buffer and mix vigorously.
   b. Incubate for 15 minutes at 2-8°C.
7. **Add TMB Substrate**:
   a. Add 200 µL of TMB substrate and incubate for 15 minutes at 36 ± 1°C.
   b. Stop the reaction by adding H2SO4 and measure absorbance at 450 nm.

▢ Direct Screening Assay

Objective: Testing for antibodies bound directly to platelets from the patient.

1. **Prepare Platelets**:
   a. Add 50 µL of patient platelets to the microplate.
   b. Centrifuge for 3 minutes at 1050 g and decant.
   c. Shake for 2 x 10 seconds at 700-1000 rpm.
2. **Cell Wash**:
   a. Add 200 µL of Cell Wash Buffer 1x and centrifuge for 3 minutes at 1050 g.
   b. Shake for 2 x 10 seconds at 700-1000 rpm.
3. **Add Serum and Monoclonal Antibodies**:
   a. Add 50 µL of serum and incubate for 30 minutes at 36 ± 1°C.
   b. Add 50 µL of monoclonal antibody MAB to the plate and incubate for 30 minutes at 36 ± 1°C.
   c. Centrifuge for 3 minutes at 1050 g and decant.
4. **Lyse Platelets**:
   a. Add 130 µL of Lysis Buffer and mix vigorously for 15 minutes at 2-8**°C**.
5. **Add TMB Substrate**:
   a. Add 200 µL of TMB substrate and incubate for 15 minutes at 36 ± 1°C.
   b. Stop the reaction by adding H2SO4 and measure absorbance at 450 nm.