IVD

OBJECTIVE

This procedure is intended to give technical instructions for performing the screening and identification of anti-platelet IgG antibodies by using the MAIPA (Monoclonal Antibody-specific Immobilization of Platelet Antigen) technique. This test, described by Kiefel et al (Blood 70: 1722-1726, 1987) can detect with high sensitivity and specificity anti-platelet antibodies and allows identification of the specificity of the antibody.

DEFINITION AND SCOPE

The MAIPA technique is a qualitative assay used for screening serum or plasma for anti-platelet antibodies (Indirect MAIPA or MAIPAI) and/or testing for antibodies bound to the patient's platelets (Direct MAIPA or MAIPAD). A positive test result in the indirect MAIPA requires subsequent identification of antibodies using the same method.

The screening test permits to detect both the presence of antibodies (allo- and/or circulating auto-antibodies) in serum or plasma samples, and the presence of antibodies bound to platelets. This test is performed in patients with defined hematological diseases or viral diseases or in case feto-maternal allo-immunization is suspected. The test is also useful to examine clinical cases of post-transfusion purpura or platelet refractoriness. If positive, antibody identification will be performed by the same technique.

The identification test applies for:
- patient samples tested positive for anti-platelet antibodies.
- samples of pregnant women whatever the outcome of the antibody screening test.
- samples of patients where clinical diagnosis points to platelet immunization.

The MAIPA technique is also used for cross-match reactions of:
- donor platelet antigens and serum of the recipient (Platelet Refractoriness).
- paternal platelet antigens (and/or antigens of the baby) and serum of the mother (Neonatal-Fetal-Maternal Allo-Immunization).

PRINCIPLE OF THE METHOD OF DETECTION AND IDENTIFICATION

The test principle is based on the capture of a platelet antigen using a mouse monoclonal antibody that reacts specifically with a single human platelet membrane glycoprotein. This is followed by binding of human antibodies to antigens on this glycoprotein and analysis of bound human IgG by an ELISA immuno-assay.

The technique is performed in two steps:
- Screening of the glycoprotein complex implicated in immunization (MAIPAI and MAIPAD)
- Identification using genotyped platelets (MAIPAI only)

For screening, platelets from a pool comprising 6 erythrocyte group O donors selected for their particular platelet genotype are incubated with the serum to be tested (indirect MAIPA) and mouse monoclonal antibodies specific for each of the four relevant platelet glycoproteins: GpIIbIIIa, GpIaIIa, GpIbIX and β-2-microglobulin/HLA. For a direct MAIPA, platelets of patients which already have an IgG bound, are directly incubated with the same set of monoclonal antibodies.

Currently, 4 murine monoclonal antibodies, anti-GpIIbIIIa, anti-GpIaIIa, anti-GpIbIX and anti-β-2-microglobuline/HLA are tested in parallel in 4 individual reactions.

After incubation platelets are lysed. The lysates are then cleared by centrifugation and placed in the wells of a microplate pre-coated with goat anti-mouse IgG antibodies.

The mouse monoclonal antibody – platelet glycoprotein complex specific to the potential anti-human platelet antibody is thereby attached to the bottom of the plate. The binding of this complex is detected in a second step by a goat peroxidase-coupled anti-human IgG and revealed by the peroxidase substrate TMB (3,3',5,5'-Tetramethylbenzidine). A blue color appears indicating the presence of an anti-Gp (corresponding to the specificity of the monoclonal antibody present for this reaction). The reaction is stopped by adding H_2SO_4 and the blue color is converted into an equivalent amount of yellow color measurable at 450 nm.

The second step consists of identifying the antibody detected in the screening test and follows the same technical protocol. Individual platelets of known genotype are selected and serum is tested with the mouse monoclonal antibody that gave a positive result in the first screening assay stage. This second step is generally performed only for the indirect test. If the direct test shows platelet bound antibodies to one or more glycoproteins, further examination to find the specific platelet antigen (HPA) is performed only in exceptional cases but not routinely.

MAIPA PROCEDURE

1. Reagents

Platelet-Antibody Screening Cells Ref. 900001
(set of 5 identical tubes)

Platelet-Antibody Identification Panel Cells Kit Ref. 900002
(set of 6 different cells)

Platelet-Antibody Control Plasma/Serum Kit Ref. 900003
(set of 4 controls)

MAIPA Reagents Kit Ref. 900004
(Monoclonal Antibodies, Cell Wash Buffer, Platelet Lysis Buffer and Microplate)

MAIPA ELISA Detection Kit Ref. 900005
(Coated Microplate, Antibody Conjugate, ELISA Wash Buffer, Chromogen and Stop Solution)

Complete MAIPA Kit Ref. 900006
Consists of a combination of 5 kits: 900001, 900002, 900003, 900004 and 900005
(Contains all cells, control plasma/serum, reagents and materials to perform a compete MAIPA test)

All the above listed reagents are offered as individual kits and can be ordered separately.

2. Additional reagents required

Distilled water (for buffer dilution)

3. Further materials required

Micropipettes 10-100 µl and tips

Multi-channel pipette100-250 µl and tips

Tubes and vials for reagents dilution

Reagent reservoirs

Vortex mixer

Microplate shaker

Microplate reader for measurement of optical densities at 450 nm with reference filter 600-650 nm

Microplate centrifuge

Incubator at 37 °C

Refrigerator at 4°C

Absorbent paper

4. Sample material

Serum or plasma can be used for indirect MAIPA.

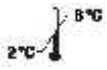
Platelets isolated from EDTA whole blood can be used for direct MAIPA. Whole blood is centrifuged at 200g for 10 minutes to form platelet-rich plasma (PRP). The PRP is then centrifuged at 2050g for 10 minutes to pellet the platelets. After removal of the plasma, the cells are washed twice with PBS / 1% BSA / 0.33% EDTA and adjusted to 500.10^6/ml in the same buffer (the recommended minimum amount of platelets for one autologous test is 5.10^6).

5. Controls

Negative and positive control samples should be included in each determination to validate the results. Two blank reagent control wells should be included in all test procedures.

apDia offers a set of 4 control Plasma/Sera (Platelet-Antibody Control Plasma/Serum Kit, Ref. 900003).

6. Reagent preparation and storage



All reagents must be kept at 2-8°C.
All reagents may be stored at 2-8°C until the expiry date indicated on the label.

The complete MAIPA procedure takes about 6 hours: the required amount of all reagents will be removed from the refrigerator immediately before use. An environment temperature in the lab of 19-25°C is advised. The remaining unused reagents should be re-stored at 2-8°C as soon as possible. Reagents should be used within two months after the first opening.

Both 10x and 20x concentrated wash buffers can be diluted and kept at room temperature during the time of the test. Store the unused buffers at 2-8°C after the test.

10x concentrated Cell Wash Buffer can contain phosphate crystals after storage at 4°C. These crystals will disappear at room temperature. Wait for diluting the Cell Wash buffer until no crystals remain.

7. Test procedure: general information

MICROPLATE METHOD for platelet incubation and washings!

Samples may be tested single or in duplicate, it is however recommended to test in duplicate.

A microplate washer may be used but in this case the washing steps should be validated. If necessary increase the number of washing cycles.

All centrifugation steps are done at room temperature: 19-25°C.

Besides the use of either screening or identification cells, the assay procedure is identical for both screening and identification of anti-platelet antibodies. As such screening and identification can be performed simultaneously on the same plate.

The entire MAIPA procedure is given below. Slight differences in this procedure for either screening or identification are described in detail in the respective assay steps (Steps 8.2, 8.3 and 8.5). All other steps are identical for both screening and identification.

8. Test procedure: detailed description

8.1. Reagent preparation

8.1.1. Dilute (1/10) Cell Wash Buffer in distilled water (MAIPA Reagents Kit, Ref. 900004) and keep it at room temperature (19-25°C).

8.1.2. Dilute (1/20) ELISA Wash Buffer in distilled water (MAIPA ELISA Detection Kit, Ref. 900005) and keep it at room temperature (19-25°C).

8.2. Preparation of the platelets

FOR SCREENING OF ANTI-PLATELET ANTIBODIES

8.2.1. s Remove the uncoated microtiter plate from the box (MAIPA Reagents Kit, Ref. 900004). Push the 8 micro-well rows out of the frame according to the necessary number of wells (don't forget the controls). Place these micro-wells into an empty frame. Put the remaining micro-wells/plate back in the box.
Provide as many wells as patients and targeted glycoproteins to be tested.
Generally 8 wells are required per patient for an anti-platelet antibody screening test, which consists of both a direct and an indirect MAIPA. For both of these assays 4 glycoproteins (Gp) are tested in 4 separate wells.
For each screening assay, provide 6 extra wells for the controls: 2 blank reagent wells on position A1 and B1; 3 positive controls plasma/serum in wells C1 (anti-HPA-1a), D1 (anti-HPA-5b) and E1 (anti-HLA); and 1 negative control plasma/serum in well F1.

8.2.2. s For an indirect MAIPA add 50 µl of screening cells (Platelet-Antibody Screening Cells, Ref. 900001) in the sample and control wells. The Platelet-Antibody Screening Cells contain about 500.000 cells/µl, therefore 25 x 10^6 platelets are added into each well.
For a direct MAIPA add 50 µl of patient platelets previously washed (2 times) and adjusted in PBS / 1% BSA / 0.33% EDTA (minimum 5 x 10^6 platelets per test). The necessary quantity of platelets to add is normally 25 x 10^6, however, 5 x 10^6 cells might be sufficient for thrombopenic samples.
The patient platelets could also be added at the next step (when adding serum or plasma). They have been washed previously and it is not necessary to wash again.

8.2.3. s Centrifuge the microplate at 1050g ± 50g for 3 minutes. Empty the wells by inverting the microplate taking care to keep the cells at the bottom of the wells. Mix the platelets gently using a microplate shaker (2 x 10 seconds at 700-1000 rpm; first 10 seconds in one direction, followed by 10 seconds in the opposite direction).

8.2.4. s Wash the microplate as follows: add 200 µl of diluted Cell Wash Buffer in each well and centrifuge at 1050g ± 50g for 3 minutes. Empty the wells by inverting the microplate taking care to keep the cells at the bottom of the wells. Mix the platelets gently using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).

8.2.5. s Repeat step 8.2.4.s once (total of 2 washes).

FOR IDENTIFICATION OF ANTI-PLATELET ANTIBODIES

8.2.1. i Remove the uncoated microtiter plate from the box (MAIPA Reagents Kit, Ref. 900004). Push the 8 micro-well rows out of the frame according to the necessary number of wells (don't forget the controls). Place these micro-wells into an empty frame. Put the remaining micro-wells/plate back in the box.
Provide as many wells as patients and targeted glycoproteins to be tested.
For an anti-platelet antibody identification MAIPA (always indirect) 6 wells are required per positive test result obtained in the preceding screening assay. One sample may react with several monoclonal antibodies. Generally, identification is done with anti-GpIaIIa and/or GpIIbIIIa.
For each identification assay, provide 6 extra wells for the controls: 2 blank reagent wells on position A1 and B1; 3 positive controls plasma/serum in wells C1 (anti-HPA-1a), D1 (anti-HPA-5b) and E1 (anti-HLA); and 1 negative control plasma/serum in well F1.
Low volume laboratories might prefer to limit the number of controls used. In this case, we would advise to use only 3 control wells: 1 blank reagent well on position A1; 1 positive control in well B1; and 1 negative control in well C1. For the positive control it is suggested to use the control that reacts with the monoclonal antibody giving rise to the positive test result in screening step.

8.2.2. i For an anti-platelet antibody identification (indirect) MAIPA add 50 µl of each of the six identification cells (Platelet-Antibody Identification Panel Cells Kit, Ref. 900002) in the respective sample wells.
Add 50 µl of screening cells (Platelet-Antibody Screening Cells, Ref. 900001) in the control wells. The Platelet-Antibody Screening Cells and Platelet-Antibody Identification Panel Cells contain about 500.000 cells/µl, therefore 25 x 10^6 platelets are added into each well.

8.2.3. i Centrifuge the microplate at 1050g ± 50g for 3 minutes. Empty the wells by inverting the microplate taking care to keep the cells at the bottom of the wells. Mix the platelets gently using a microplate shaker (2 x 10 seconds at 700-1000 rpm; first 10 seconds in one direction, followed by 10 seconds in the opposite direction).

8.2.4. i Wash the microplate as follows: add 200 µl of diluted Cell Wash Buffer in each well and centrifuge at 1050g ± 50g for 3 minutes. Empty the wells by inverting the microplate taking care to keep the cells at the bottom of the wells. Mix the platelets gently using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).

8.2.5. i Repeat step 8.2.4.i once (total of 2 washes).

8.3. Incubation of platelets with serum

FOR SCREENING OF ANTI-PLATELET ANTIBODIES

8.3.1. s For an anti-platelet antibody screening assay (indirect MAIPA) add 50 µl of patient plasma/serum in 4 wells containing screening platelets. A direct MAIPA doesn't require the addition of plasma/serum (antibodies are already bound), but 50 µl of Cell Wash Buffer should be added to protect the cells from drying. Or patient platelets can be added at this step if it wasn't done before (step 8.2.2.s).
Add 50 µl of the control plasma/serum in the respective wells (Control Plasma/serum Kit, Ref. 900003).

8.3.2. s Mix gently using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).

8.3.3. s Incubate for 30 ± 5 minutes at 37 °C.

8.3.4. s Centrifuge the microplate at 1050g ± 50g for 3 minutes. Empty the wells by inverting the microplate taking care to keep the cells at the bottom of the wells. Mix the platelets gently using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).

FOR IDENTIFICATION OF ANTI-PLATELET ANTIBODIES

8.3.1. i For an anti-platelet antibody identification assay (indirect MAIPA) add 50 µl of patient plasma/serum in 6 wells containing the different identification platelets.
Add 50 µl of the control plasma/serum in the respective wells (Control Plasma/serum Kit, Ref. 900003).

8.3.2. i Mix gently using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).

8.3.3. i Incubate for 30 ± 5 minutes at 37 °C.

8.3.4. i Centrifuge the microplate at 1050g ± 50g for 3 minutes. Empty the wells by inverting the microplate taking care to keep the cells at the bottom of the wells. Mix the platelets gently using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).

8.4. Removal of unbound immunoglobulins

8.4.1. Wash the microplate as follows: add 200 µl of diluted Cell Wash Buffer in each well and centrifuge at 1050g ± 50g for 3 minutes. Empty the wells by inverting the microplate taking care to keep the cells at the bottom of the wells. Mix the platelets gently using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).

8.4.2. Repeat step 8.4.1. once (total of 2 washes).

8.5. Incubation of platelets with monoclonal antibody

FOR SCREENING OF ANTI-PLATELET ANTIBODIES

8.5.1. s For an anti-platelet antibody screening assay add 50 µl of each monoclonal antibody (MAIPA Reagents Kit, Ref. 900004) in the 4 respective wells containing screening platelets (indirect MAIPA) or in each 4 wells containing patient platelets (direct MAIPA).
For controls: add 50 µl of anti-GpIIbIIIa in well C1 (anti-HPA-1a), 50 µl of anti-GpIaIIa in well D1 (anti-HPA-5b), 50 µl of anti-HLA/β2-microglobulin in well E1 (anti-HLA) and 50 µl of anti-GpIbIX in well F1 (negative control).

8.5.2. s Mix gently using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).

8.5.3. s Incubate for 30 ± 5 minutes at 37 °C.

8.5.4. s Centrifuge the microplate at 1050g ± 50g for 3 minutes. Empty the wells by inverting the microplate taking care to keep the cells at the bottom of the wells. Mix the platelets gently using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).

FOR IDENTIFICATION OF ANTI-PLATELET ANTIBODIES

8.5.1. i For an anti-platelet antibody identification assay add 50 µl of the monoclonal antibody (giving rise to a positive result in a screening test) in each 6 wells containing identification platelets.
For controls: add 50 µl of anti-GpIIbIIIa in well C1 (anti-HPA-1a), 50 µl of anti-GpIaIIa in well D1 (anti-HPA-5b), 50 µl of anti-HLA/β2-microglobulin in well E1 (anti-HLA) and 50 µl of anti-GpIbIX in well F1 (negative control).
For low volume laboratories that use a reduced number of controls (see 8.2.1):
Add 50 µl of the monoclonal antibody corresponding to the chosen positive control in well B1 (e.g. anti-GpIIbIIIa for the anti-HPA-1a control; anti-GpIaIIa for the anti-HPA-5b control).
Add 50 µl of the same monoclonal antibody in well C1 for the negative control.

8.5.2. i Mix gently using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).

8.5.3. i Incubate for 30 ± 5 minutes at 37 °C.

8.5.4. i Centrifuge the microplate at 1050g ± 50g for 3 minutes. Empty the wells by inverting the microplate taking care to keep the cells at the bottom of the wells. Mix the platelets gently using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).

8.6. Removal of unbound monoclonal antibody

8.6.1. Wash the microplate as follows: add 200 µl of diluted Cell Wash Buffer in each well and centrifuge at 1050g ± 50g for 3 minutes. Empty the wells by inverting the microplate taking care to keep the cells at the bottom of the wells. Mix the platelets gently using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).

8.6.2. Repeat step 8.6.1. three times (total of 4 washes).

8.7. Solubilisation of platelet membranes

8.7.1. Add 130 µl of Platelet Lysis Buffer (MAIPA Reagents Kit, Ref. 900004) in all wells and mix the platelet pellet vigorously by pipetting up and down. This handling induces foaming, which is necessary for efficient lysis of the platelets. Take good care not to cross-contaminate wells.

8.7.2. Incubate for 15 minutes at 4 °C.

8.7.3. Centrifuge at 1050g ± 50g for 15 minutes.
Complexes (mab, Gp and human Ab) remain in the supernatant, while cell debris sediments to the bottom of the well.
Remark : before or after centrifugation, lysates can be stored at 4 °C for one night if necessary.

8.8. Transfer of the platelet lysates to the goat anti-mouse IgG coated microplate

8.8.1. Remove the goat anti-mouse IgG coated microplate from the aluminium pouch (MAIPA ELISA Detection Kit, Ref. 900005). Push the 8 micro-well rows out of the frame and cut to break off the number of wells corresponding to the number of lysates to be analysed. Place these micro-wells into an empty frame. Put the remaining micro-wells/plate back in the aluminium pouch and re-seal.

8.8.2. Transfer 100 µl of the cell lysis supernatant into the goat anti-mouse IgG coated wells without touching or aspirating the debris at the bottom of the uncoated plate.

8.8.3. Incubate for 30 ± 5 minutes at 37 °C.

8.9. Removal of unbound lysate proteins

8.9.1. Empty the wells by inverting the microplate and tap the plate on absorbent paper.

8.9.2. Wash the microplate as follows: add 200 µl of diluted ELISA Wash Buffer in each well, empty the wells by inverting the microplate and tap the plate on absorbent paper.
Remark: It is recommended to use reverse pipetting for the washing steps in order to avoid bubbling of the washing solution (risk for cross-contamination).

8.9.3. Repeat step 8.9.2. five times (total of 6 washes).

8.9.4. Make sure the wells are dry after the final wash step.

8.10. Addition of peroxidase labeled goat anti-human IgG

8.10.1. Add 100 µl of the goat anti-human IgG HRP conjugate in each well.

8.10.2. Incubate for 30 ± 5 minutes at 37 °C.

8.11. Removal of unbound peroxidase labeled goat anti-human IgG

8.11.1. Empty the wells by inverting the microplate and tap the plate on absorbent paper.

8.11.2. Wash the microplate as follows: add 200 µl of diluted ELISA Wash Buffer in each well, empty the wells by inverting the microplate and tap the plate on absorbent paper.
Remark: It is recommended to use reverse pipetting for the washing steps in order to avoid bubbling of the washing solution (risk for cross-contamination).

8.11.3. Repeat step 8.11.2. five times (total of 6 washes).

8.11.4. Make sure the wells are dry after the final wash step.

8.12. Addition of TMB substrate

8.12.1. Add 100 µl of the TMB solution in each well.
Remark: Keep the solution strictly protected from light.

8.12.2. Incubate for 15 minutes at 37°C in the dark.

8.13. Addition of acid to stop color development

8.13.1. Add 100 µl of stop solution (H_2SO_4) in each well.

8.14. Reading of microtiter plate

8.14.1. Measure the optical density at 450 m with reference filter 600-650 nm in a microplate reader and record the results.

INTERPRETATION AND VALIDATION OF THE TEST RESULTS

Assay validation is based on the OD values of the control samples. In general it can be said that (after subtraction of the blank value):

- The OD value for the negative control should be below 0,1
- The OD value for the positive controls should be above 0,5

In our hands assay interpretation is relatively straightforward with a cut-off of OD = 0,2 (after subtraction of the blank value):

- OD values above 0,2 are considered positive
- OD values below 0,2 are considered negative

It is however strongly advised for each laboratory to determine its own cut-off using a panel of negative samples, as well as the acceptance OD criteria for the negative and positive controls. Where homemade controls are used, each laboratory should define the corresponding OD values for assay validation.

1. For a platelet-antibody screening test, one pool of cells is used and 4 reactions with four different monoclonal antibodies are performed. If these reactions are negative no further action is required. If

one of the reactions yields a positive result, a platelet-antibody identification assay is performed using the monoclonal antibody that resulted in a positive screening test.

2. For a platelet-antibody identification assay, at least 4 cells from the platelet cell panel showing a positive platelet-antibody screening phenotype and the glycoprotein-specific monoclonal antibody yielding a positive result in the preceding screening assay are used.

PERFORMANCE CHARACTERISTICS

In a validation study in a French Reference Lab for Platelet Immunology 29 platelet-antibody positive samples were analyzed 3 times (triplicates) by the MAIPA technology using the apDia reagents (ref. 900001, 900002, 900003, 900004 and 900005). In this study a **diagnostic sensitivity** of 97.8 % was obtained (2/(29*3) tests were found negative).

In the same study using the apDia reagents described above, 326 true platelet-antibody negative samples were analyzed of which 325 were found negative resulting in a **specificity** of 99,7 %.

LIMITATIONS

MAIPA is considered as the gold standard method for platelet antibody detection and identification.
False positive or false negative results may occur in case of bacterial or other contamination.
In case of spurious or even inconsistent results we recommend to have the sample examined by another laboratory specialized in platelet diagnostics or in a platelet reference laboratory.

MAIPA sensitivity and specificity is high but not 100%. Furthermore to obtain reliable test results it is necessary that the given protocol is strictly followed. The test is designed to detect IgG-type anti-platelet antibodies only.

Inherent to the microplate handling is the risk of cross-contamination. It should be kept in mind that false positive results could originate here from and all precautions to avoid this should be respected.

The apDia platelet products (Ref. 900001 and Ref. 900002) are not useful for screening and identification of the HPA-15 system. This group is included for information purposes only. For HPA-15a and HPA-15b some specific platelets are required to identify the antibodies. Additionally, a specific monoclonal antibody is required.

BIBLIOGRAPHY

1. Monoclonal antibody-specific immobilization of platelet antigens (MAIPA): a new tool for the identification of platelet-reactive antibodies. Kiefel V, Santoso S, Weisheit M, Müeller-Eckhardt C. Blood. 1987 Dec; 70(6):1722-6.
2. A modified rapid monoclonal antibody-specific immobilization of platelet antigen assay for the detection of human platelet antigen (HPA) antibodies: a multicentre evaluation. Campbell K, Rishi K, Howkins G, Gilby D, Mushens R, Ghevaert C, Metcalfe P, Ouwehand WH, Lucas G. Vox Sang. 2007 Nov; 93(4):289-97.
3. Report on the 13th International Society of Blood Transfusion Platelet Immunology Workshop. Foxcroft Z, Campbell K, Mérieux Y, Urbaniak S, Brierley M, Rigal D, Ouwehand WH, Metcalfe P. Vox Sang. 2007 Nov; 93(4):300-5.
4. The detection of platelet antibodies by simultaneous analysis of specific platelet antibodies and the monoclonal antibody-specific immobilization of platelet. Nguyen XD, Goebel M, Schober M, Klüter H, Panzer S. Transfusion. 2010 Jul; 50(7):1429-34. Epub 2010 Apr 23.
5. Human platelet antigen frequencies of platelet donors in the French population determined by polymerase chain reaction with sequence-specific primers. Mérieux Y, Debost M, Bernaud J, Raffin A, Meyer F, Rigal D. Pathol. Biol. (Paris) 1997 Nov; 45(9):697-700.

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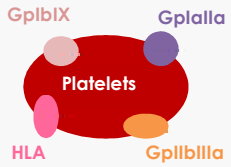
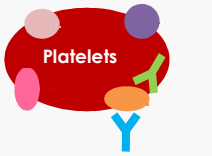


Platelets

Platelet-Antibody Screening Cells, Ref. 900001

Platelet-Antibody Identification Panel Cells Kit, Ref. 900002

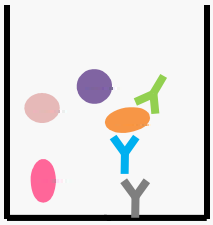
Platelet-Antibody Control Plasma/Serum Kit, Ref. 900003



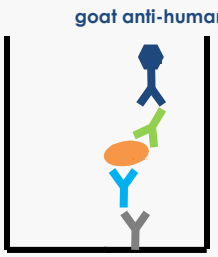
Incubation with patient plasma/serum and mouse monoclonal antibodies

Lysis and centrifugation to remove platelet debris

MAIPA Reagents Kit, Ref. 900004



Immobilisation of Ag/Ab complex on goat anti-mouse IgG coated microplate



Detection of human anti-platelet antibody through goat anti-human IgG coupled to peroxidase

MAIPA ELISA Detection Kit, Ref. 900005

Complete MAIPA Kit, Ref. 900006

Examples of MAIPA Worksheets

MAIPA – Worksheet: 5 patients direct & indirect screening

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	PLTL PATIENT 1 no serum MAB IIbIIIa	PLTL PATIENT 2 no serum MAB IIbIIIa	PLTL PATIENT 3 no serum MAB IIbIIIa	PLTL PATIENT 4 no serum MAB IIbIIIa	PLTL PATIENT 5 no serum MAB IIbIIIa						
B	BLANK	PLTL PATIENT 1 no serum MAB Ia/Ia	PLTL PATIENT 2 no serum MAB Ia/Ia	PLTL PATIENT 3 no serum MAB Ia/Ia	PLTL PATIENT 4 no serum MAB Ia/Ia	PLTL PATIENT 5 no serum MAB Ia/Ia						
C	SCREEN PLTL CONTR 1a MAB IIbIIIa	PLTL PATIENT 1 no serum MAB HLA	PLTL PATIENT 2 no serum MAB HLA	PLTL PATIENT 3 no serum MAB HLA	PLTL PATIENT 4 no serum MAB HLA	PLTL PATIENT 5 no serum MAB HLA						
D	SCREEN PLTL CONTR 5b MAB Ia/Ia	PLTL PATIENT 1 no serum MAB Ib/IX	PLTL PATIENT 2 no serum MAB Ib/IX	PLTL PATIENT 3 no serum MAB Ib/IX	PLTL PATIENT 4 no serum MAB Ib/IX	PLTL PATIENT 5 no serum MAB Ib/IX						
E	SCREEN PLTL CONTR HLA MAB HLA	SCREEN PLTL SERUM PATIENT 1 MAB IIbIIIa	SCREEN PLTL SERUM PATIENT 2 MAB IIbIIIa	SCREEN PLTL SERUM PATIENT 3 MAB IIbIIIa	SCREEN PLTL SERUM PATIENT 4 MAB IIbIIIa	SCREEN PLTL SERUM PATIENT 5 MAB IIbIIIa						
F	SCREEN PLTL CONTR NEG MAB Ib/IX	SCREEN PLTL SERUM PATIENT 1 MAB Ia/Ia	SCREEN PLTL SERUM PATIENT 2 MAB Ia/Ia	SCREEN PLTL SERUM PATIENT 3 MAB Ia/Ia	SCREEN PLTL SERUM PATIENT 4 MAB Ia/Ia	SCREEN PLTL SERUM PATIENT 5 MAB Ia/Ia						
G		SCREEN PLTL SERUM PATIENT 1 MAB HLA	SCREEN PLTL SERUM PATIENT 2 MAB HLA	SCREEN PLTL SERUM PATIENT 3 MAB HLA	SCREEN PLTL SERUM PATIENT 4 MAB HLA	SCREEN PLTL SERUM PATIENT 5 MAB HLA						
H		SCREEN PLTL SERUM PATIENT 1 MAB Ib/IX	SCREEN PLTL SERUM PATIENT 2 MAB Ib/IX	SCREEN PLTL SERUM PATIENT 3 MAB Ib/IX	SCREEN PLTL SERUM PATIENT 4 MAB Ib/IX	SCREEN PLTL SERUM PATIENT 5 MAB Ib/IX						

MAIPA – Worksheet: 7 patients identification

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	ID PLTL 1 SERUM PATIENT 1 MAB IIbIIIa	ID PLTL 1 SERUM PATIENT 2 MAB IIbIIIa	ID PLTL 1 SERUM PATIENT 3 MAB IIbIIIa	ID PLTL 1 SERUM PATIENT 4 MAB IIbIIIa	ID PLTL 1 SERUM PATIENT 5 MAB Ia/Ia						
B	BLANK	ID PLTL 2 SERUM PATIENT 1 MAB IIbIIIa	ID PLTL 2 SERUM PATIENT 2 MAB IIbIIIa	ID PLTL 2 SERUM PATIENT 3 MAB IIbIIIa	ID PLTL 2 SERUM PATIENT 4 MAB IIbIIIa	ID PLTL 2 SERUM PATIENT 5 MAB Ia/Ia						
C	SCREEN PLTL CONTR 1a MAB IIbIIIa	ID PLTL 3 SERUM PATIENT 1 MAB IIbIIIa	ID PLTL 3 SERUM PATIENT 2 MAB IIbIIIa	ID PLTL 3 SERUM PATIENT 3 MAB IIbIIIa	ID PLTL 3 SERUM PATIENT 4 MAB IIbIIIa	ID PLTL 3 SERUM PATIENT 5 MAB Ia/Ia						
D	SCREEN PLTL CONTR 5b MAB Ia/Ia	ID PLTL 4 SERUM PATIENT 1 MAB IIbIIIa	ID PLTL 4 SERUM PATIENT 2 MAB IIbIIIa	ID PLTL 4 SERUM PATIENT 3 MAB IIbIIIa	ID PLTL 4 SERUM PATIENT 4 MAB IIbIIIa	ID PLTL 4 SERUM PATIENT 5 MAB Ia/Ia						
E	SCREEN PLTL CONTR HLA MAB HLA	ID PLTL 5 SERUM PATIENT 1 MAB IIbIIIa	ID PLTL 5 SERUM PATIENT 2 MAB IIbIIIa	ID PLTL 5 SERUM PATIENT 3 MAB IIbIIIa	ID PLTL 5 SERUM PATIENT 4 MAB IIbIIIa	ID PLTL 5 SERUM PATIENT 5 MAB Ia/Ia						
F	SCREEN PLTL CONTR NEG MAB Ib/IX	ID PLTL 6 SERUM PATIENT 1 MAB IIbIIIa	ID PLTL 6 SERUM PATIENT 2 MAB IIbIIIa	ID PLTL 6 SERUM PATIENT 3 MAB IIbIIIa	ID PLTL 6 SERUM PATIENT 4 MAB IIbIIIa	ID PLTL 6 SERUM PATIENT 5 MAB Ia/Ia						
G	ID PLTL 1 SERUM PATIENT 6 MAB Ia/Ia	ID PLTL 2 SERUM PATIENT 6 MAB Ia/Ia	ID PLTL 3 SERUM PATIENT 6 MAB Ia/Ia	ID PLTL 4 SERUM PATIENT 6 MAB Ia/Ia	ID PLTL 5 SERUM PATIENT 6 MAB Ia/Ia	ID PLTL 6 SERUM PATIENT 6 MAB Ia/Ia						
H	ID PLTL 1 SERUM PATIENT 7 MAB Ia/Ia	ID PLTL 2 SERUM PATIENT 7 MAB Ia/Ia	ID PLTL 3 SERUM PATIENT 7 MAB Ia/Ia	ID PLTL 4 SERUM PATIENT 7 MAB Ia/Ia	ID PLTL 5 SERUM PATIENT 7 MAB Ia/Ia	ID PLTL 6 SERUM PATIENT 7 MAB Ia/Ia						

MAIPA – Worksheet: 3 patients identification and 3 patients direct & indirect screening

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	ID PLTL 1 SERUM PATIENT 1 MAB IIbIIIa	ID PLTL 1 SERUM PATIENT 2 MAB IIbIIIa	PLTL PATIENT 4 no serum MAB IIbIIIa	PLTL PATIENT 5 no serum MAB IIbIIIa	PLTL PATIENT 6 no serum MAB IIbIIIa						
B	BLANK	ID PLTL 2 SERUM PATIENT 1 MAB IIbIIIa	ID PLTL 2 SERUM PATIENT 2 MAB IIbIIIa	PLTL PATIENT 4 no serum MAB Ia IIa	PLTL PATIENT 5 no serum MAB Ia IIa	PLTL PATIENT 6 no serum MAB Ia IIa						
C	SCREEN PLTL CONTR 1a MAB IIbIIIa	ID PLTL 3 SERUM PATIENT 1 MAB IIbIIIa	ID PLTL 3 SERUM PATIENT 2 MAB IIbIIIa	PLTL PATIENT 4 no serum MAB HLA	PLTL PATIENT 5 no serum MAB HLA	PLTL PATIENT 6 no serum MAB HLA						
D	SCREEN PLTL CONTR 5b MAB Ia IIa	ID PLTL 4 SERUM PATIENT 1 MAB IIbIIIa	ID PLTL 4 SERUM PATIENT 2 MAB IIbIIIa	PLTL PATIENT 4 no serum MAB Ib IX	PLTL PATIENT 5 no serum MAB Ib IX	PLTL PATIENT 6 no serum MAB Ib IX						
E	SCREEN PLTL CONTR HLA MAB HLA	ID PLTL 5 SERUM PATIENT 1 MAB IIbIIIa	ID PLTL 5 SERUM PATIENT 2 MAB IIbIIIa	SCREEN PLTL SERUM PATIENT 4 MAB IIbIIIa	SCREEN PLTL SERUM PATIENT 5 MAB IIbIIIa	SCREEN PLTL SERUM PATIENT 6 MAB IIbIIIa						
F	SCREEN PLTL CONTR NEG MAB Ib IX	ID PLTL 6 SERUM PATIENT 1 MAB IIbIIIa	ID PLTL 6 SERUM PATIENT 2 MAB IIbIIIa	SCREEN PLTL SERUM PATIENT 4 MAB Ia IIa	SCREEN PLTL SERUM PATIENT 5 MAB Ia IIa	SCREEN PLTL SERUM PATIENT 6 MAB Ia IIa						
G	ID PLTL 1 SERUM PATIENT 3 MAB Ia IIa	ID PLTL 3 SERUM PATIENT 3 MAB Ia IIa	ID PLTL 5 SERUM PATIENT 3 MAB Ia IIa	SCREEN PLTL SERUM PATIENT 4 MAB HLA	SCREEN PLTL SERUM PATIENT 5 MAB HLA	SCREEN PLTL SERUM PATIENT 6 MAB HLA						
H	ID PLTL 2 SERUM PATIENT 3 MAB Ia IIa	ID PLTL 4 SERUM PATIENT 3 MAB Ia IIa	ID PLTL 6 SERUM PATIENT 3 MAB Ia IIa	SCREEN PLTL SERUM PATIENT 4 MAB Ib IX	SCREEN PLTL SERUM PATIENT 5 MAB Ib IX	SCREEN PLTL SERUM PATIENT 6 MAB Ib IX						

MAIPA Worksheet Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK											
B	BLANK											
C	SCREEN PLTL CONTR 1a MAB IIbIIIa											
D	SCREEN PLTL CONTR 5b MAB Ia IIa											
E	SCREEN PLTL CONTR HLA MAB HLA											
F	SCREEN PLTL CONTR NEG MAB Ib IX											
G												
H												