



MAIPA PROCEDURE

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 (FNAIT) is suspected. The test is also useful to examine clinical cases of post-transfusion purpura (PTP) or platelet refractoriness (PR). 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 (PR).
- paternal platelet antigens (and/or antigens of the baby) and serum of the mother (FNAIT: 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 to 12 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: GPIIb/IIIa, GPIIb/IIIa, GPIb/IX 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-GPIIb/IIIa, anti-GPIIb/IIIa, anti-GPIb/IX and anti- β -2-microglobulin/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 antibody (corresponding to the specificity of the monoclonal antibody present for this reaction). The reaction is stopped by adding H₂SO₄ and the blue colour is converted into an equivalent amount of yellow colour 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 Complete MAIPA Assay kit (REF 900016)

Platelet Antibody Screening Cells

Component	Description	Quantity, Volume	Volume needed for one test	Format
Screening cells SCREEN PLTL	Platelet pool of 6 to 12 blood group O donors, typed for HPA-1, -2, -3, -4, -5, -6, -15	2 vials, 2 x 12,5 ml	50 μ l	RTU

Platelet Antibody Identification Panel Cells

Component	Description	Quantity, Volume	Volume needed for one test	Format
Identification platelet 1 IDENT PLTL 1	Single donor (blood group O) platelet cells, typed for HPA-1, -2, -3, -4, -5, -6, -15; batch dependent	1 vial, 3,25 ml	50 μ l	RTU
Identification platelet 2 IDENT PLTL 2	Single donor (blood group O) platelet cells, typed for HPA-1, -2, -3, -4, -5, -6, -15; batch dependent	1 vial, 3,25 ml	50 μ l	RTU
Identification platelet 3 IDENT PLTL 3	Single donor (blood group O) platelet cells, typed for HPA-1, -2, -3, -4, -5, -6, -15; batch dependent	1 vial, 3,25 ml	50 μ l	RTU

Identification platelet 4 IDENT PLTL 4	Single donor (blood group O) platelet cells, typed for HPA-1, -2, -3, -4, -5, -6, -15; batch dependent	1 vial, 3,25 ml	50 µl	RTU
Identification platelet 5 IDENT PLTL 5	Single donor (blood group O) platelet cells, typed for HPA-1, -2, -3, -4, -5, -6, -15; batch dependent	1 vial, 3,25 ml	50 µl	RTU
Identification platelet 6 IDENT PLTL 6	Single donor (blood group O) platelet cells, typed for HPA-1, -2, -3, -4, -5, -6, -15; batch dependent	1 vial, 3,25 ml	50 µl	RTU

Kit Controls

Component	Description	Quantity, Volume	Volume needed for one test	Format
Control serum/plasma CONTR 1a	Anti-HPA-1a positive	1 vial, 2 ml	50 µl	RTU
Control serum/plasma CONTR 5b	Anti-HPA-5b positive	1 vial, 2 ml	50 µl	RTU
Control serum/plasma CONTR HLA	Anti-HLA positive	1 vial, 2 ml	50 µl	RTU
Control serum/plasma CONTR NEG	Negative	2 vials, 2 x 3 ml	50 µl	RTU

MAIPA Reagents

Component	Description	Quantity, Volume	Volume needed for one test	Format
Microplate MTP	12 individual strips	5 plates, 5 x 96 wells	N/A	RTU
Antibody MAB IIbIIIa	Anti-GPIIbIIIa, 10 µg/ml	1 vial, 12 ml	50 µl	RTU
Antibody MAB IaIIa	Anti-GPIaIIa, 10 µg/ml	1 vial, 9 ml	50 µl	RTU
Antibody MAB IbIX	Anti-GPIbIX, 10 µg/ml	1 vial, 7,5 ml	50 µl	RTU
Antibody MAB HLA	Anti-HLA (B2M), 10 µg/ml	1 vial, 7,5 ml	50 µl	RTU
Cell Wash Buffer CELLWASHBUF 10x	MAIPA Platelet Wash Buffer	1 vial, 100 ml	(2+2+4) x 200 µl	10x
Platelet Lysis buffer LYSBUF	MAIPA Platelet Lysis Buffer	1 vial, 75 ml	130 µl	RTU

Component	Description	Quantity, Volume	Volume needed for one test	Format
Coated microtiter plate COATMTP	Goat anti-mouse IgG coated microtiter plate	5 plates, 5 x 96 wells	N/A	RTU
Conjugate CONJ	Goat anti-Human IgG-HRP	1 vial, 60 ml	100 µl	RTU
ELISA Wash buffer ELISAWASHBUF 20x	TRIS buffered Triton X-100 / Tween 20	2 vials, 2 x 125 ml	12 x 200 µl	20x
Chromogen TMB TMB	TMB	2 vials, 2 x 30 ml	100 µl	RTU
Stop Solution STOPSOL	0.5 M H ₂ SO ₄	1 vial, 60 ml	100 µl	RTU

2. Additional reagents required

Distilled water (for buffer dilution)

PBS 1x w/1 % BSA, w/ 0,33 % EDTA (for platelets preparation in Direct MAIPA)

3. Further materials required

Micropipettes 10-100 µl and tips

Multi-channel pipette 100-250 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 validated for MAIPA (see section 8)

Incubator at 36 ± 1 °C

Refrigerator at 2 – 8 °C

Absorbent paper

4. Warnings and precautions for users

4.1. For *in vitro* diagnostic use only.

4.2. For professional laboratory use.

4.3. Do not mix reagents or coated microtiter strips from kits with different lot numbers.

4.4. Treat controls and samples as if they contain infectious agents.

4.5. Dispose of patients samples and all materials used to perform the test as if contaminated with potentially infectious substances.

4.6. Stop Solution is a 0,5 M H₂SO₄ solution which is irritant. In case of contact with eyes or skin, rinse with plenty of water and seek medical advice.

4.7. The Lysis Buffer and the ELISA Wash Buffer 20x contain the substance Triton X-100. Triton X-100 is a surfactant belonging to the group of OPEs (octylphenol ethoxylates) that have been included in Annex XIV (Authorisation list) of REACH Regulation (EC) N° 1907/2006.

These substances have endocrine disrupting properties through their degradation, for which it is scientifically proven that they can have serious effects on the environment.

Classification for Lysis Buffer :

Signal word : Warning

Following hazard statements are applicable :

H317 : May cause an allergic skin reaction.

H411 : Toxic to aquatic life with long lasting effects.

Following precautionary statements are applicable :

P273 : Avoid release to the environment.

P280 : Wear protective gloves/protective clothing/eye protection/face protection.

P302+P352 : IF ON SKIN : Wash with plenty of water/...

P391 : Collect spillage.

Classification for ELISA Wash Buffer 20x:

Signal word : Danger

Following hazard statements are applicable :

H317 : May cause an allergic skin reaction.

H318 : Causes serious eye damage.

H315 : Causes skin irritation.

H410 : Very toxic to aquatic life with long lasting effects.

Following precautionary statements are applicable :

P273 : Avoid release to the environment.

P280 : Wear protective gloves/protective clothing/eye protection/face protection.

P302+P352 : IF ON SKIN : Wash with plenty of water/...

P310 : Immediately call a POISON CENTER/doctor/...

P391 : Collect spillage.

- 4.8.** Chromogen Solution contains the hazardous ingredient N-Methyl-2-pyrrolidone at a concentration > 0,3 %. It is classified as a Reproductive Toxicant Category 1B.

Signal word: Danger

Following hazard statements are applicable:

H360D: May damage the unborn child.

Following precautionary statements are applicable:

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P308+P313: IF exposed or concerned: Get medical advice/attention.

- 4.9.** Some kit components contain sodium azide as a preservative. In order to prevent the formation of potentially explosive metal azides in laboratory plumbing, flush drains thoroughly after disposal of these solutions.
- 4.10.** After obtaining the results, no decisions should be made without first consulting the medical relevance of these results.
- 4.11.** Serious incidents related to the MAIPA Assay must be reported to the manufacturer.

5. Sample material

Serum or plasma can be used for indirect MAIPA.

Specimens can be stored at 2 – 8 °C for 3 to 4 days or they can be stored frozen at – 20 °C for at least one year.

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 500.10⁶ cells/ml in the same buffer (the recommended minimum amount of platelets for one autologous test is 5.10⁶).

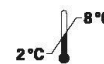
The prepared patient platelet suspension can be stored at 2 – 8 °C for maximum 5 days.

6. 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.

A set of 4 control Plasma/Sera (CONTR 1a, CONTR 5b, CONTR HLA, CONTR NEG) is included in the complete MAIPA kit.

7. Reagent preparation and storage conditions



All reagents must be kept at 2 – 8 °C.

All reagents can be stored at 2 – 8 °C until the expiry date indicated on the label.

Never use any kit components beyond the indicated expiry date.

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 six months after the first opening.

Both 10x and 20x concentrated wash buffers have to be diluted to 1x strength before starting the assay. Diluted wash buffers can be kept at room temperature during the time of the test. Store remaining volume of the 1x diluted buffers at 2 – 8 °C after the test. They can be reused for maximum 1 month when intermittently stored at 2 – 8 °C.

10x concentrated Cell Wash Buffer can contain phosphate crystals after storage at 2 - 8 °C. These crystals will disappear at room temperature. Wait for diluting the Cell Wash buffer until no crystals remain.

8. Test procedure: general information

MICROPLATE METHOD for platelet incubation and washings!

Samples may be tested in singlicate 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.

It is important to validate your centrifuge for usability with regard of the MAIPA procedure.

A centrifuge equipped with a swing-out rotor and four microplate holders positioned in the rotor cross at an angle of 45 °C to the rotor arms is preferred.

Example: Eppendorf 5810 with rotor A-4-81 and MTP buckets or with rotor A-4-62 with MTP buckets.

We do not recommend a centrifuge equipped with a swing-out rotor and two microplate holders positioned in parallel with the rotor arm.

Example: Eppendorf 5430 with rotor A-2-MTP.

Beside 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.

9. Test procedure Indirect MAIPA (MAIPA I): detailed description

9.1. Reagent preparation

9.1.1. Dilute (1/10) CELL Wash Buffer (CELLWASHBUF 10x) in distilled water and keep it at room temperature (19-25°C).

9.1.2. Dilute (1/20) ELISA Wash Buffer (ELISAWASHBUF 20x) in distilled water and keep it at room temperature (19-25°C).

9.2. Preparation of the platelets

FOR SCREENING OF ANTI-PLATELET ANTIBODIES

- 9.2.1. s Remove the uncoated MICROPLATE (MTP) from the box. 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 (GPs) are tested in 4 separate wells. For each screening assay, provide 6 extra wells for the controls in order to validate the run: 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.
- 9.2.2. s Add 50 µl of SCREENING CELLS (SCREEN PLTL) in the sample and control wells. [The Platelet Antibody Screening Cells are ready-to-use.](#)
- 9.2.3. s Centrifuge the MICROPLATE (MTP) 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).
- 9.2.4. s Wash the MICROPLATE (MTP) 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).
- 9.2.5. s Repeat step 9.2.4. s once (total of 2 washes).

FOR IDENTIFICATION OF ANTI-PLATELET ANTIBODIES

- 9.2.1. i Remove the uncoated MICROPLATE (MTP) from the box and proceed as described in 9.2.1.s. 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-GPIIb/IIIa and/or GPIIb/IIIa. Identification is not performed for samples that reacted positive for anti-HLA antibodies in the screening assay. If the identification is performed in a separate assay, provide 6 extra wells for the controls in each run for validation: 2 blank reagent wells on position A1 and B1; 3 positive control plasma/sera 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.](#)
- 9.2.2. i For an anti-platelet antibody identification (indirect) MAIPA add 50 µl of each of the six IDENTIFICATION PLATELETS (IDENT PLTL) in the respective sample wells. Add 50 µl of SCREENING CELLS (SCREEN PLTL) in the control wells. [The Platelet Antibody Screening Cells and Platelet Antibody Identification Panel Cells are ready-to-use.](#)
- 9.2.3. i Proceed with washing of the IDENTIFICATION PLATELETS (IDENT PLTL) according to 9.2.3.s till 9.2.5.s.

9.3. Incubation of platelets with serum

FOR SCREENING OF ANTI-PLATELET ANTIBODIES

- 9.3.1. s For an anti-platelet antibody screening assay (indirect MAIPA) add 50 µl of patient plasma/serum in 4 wells containing SCREENING CELLS (SCREEN PLTL). Add 50 µl of the CONTROL PLASMA/SERA (CONTR) in the respective wells.
- 9.3.2. s Mix gently manually or by using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).
- 9.3.3. s Incubate for 30 ± 5 minutes at 36 ± 1 °C.
- 9.3.4. s Centrifuge the MICROPLATE (MTP) 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

- 9.3.1. 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 (IDENT PLTL). Add 50 µl of the CONTROL PLASMA/SERA (CONTR) in the respective wells.
- 9.3.2. i Mix gently manually or by using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).
- 9.3.3. i Incubate for 30 ± 5 minutes at 36 ± 1 °C.
- 9.3.4. i Centrifuge the MICROPLATE (MTP) 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).

9.4. Removal of unbound immunoglobulins

- 9.4.1. Wash the MICROPLATE (MTP) 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).
- 9.4.2. Repeat step 9.4.1. once (total of 2 washes).

9.5. Incubation of platelets with monoclonal antibody

FOR SCREENING OF ANTI-PLATELET ANTIBODIES

- 9.5.1. s For an anti-platelet antibody screening assay add 50 µl of each MONOCLONAL ANTIBODY (MAB) in the 4 respective wells containing SCREENING CELLS (SCREEN PLTL).
For controls: add 50 µl of anti-GPIIbIIIa (MAB IIbIIIa) in well C1 (CONTR 1a), 50 µl of anti-GPIIa (MAB Ialla) in well D1 (CONTR 5b), 50 µl of anti-HLA/β2-microglobulin (MAB HLA) in well E1 (CONTR HLA). In well F1 (CONTR NEG), add 50 µL of anti-GPIbIX (MAB IbIX) or 50 µl of anti-GPIIbIIIa (MAB IIbIIIa) or 50 µl of anti-GPIIa (MAB Ialla).
- 9.5.2. s Mix gently manually or by using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).
- 9.5.3. s Incubate for 30 ± 5 minutes at 36 ± 1 °C.
- 9.5.4. s Centrifuge the MICROPLATE (MTP) 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

- 9.5.1. i For an anti-platelet antibody identification assay add 50 µl of the MONOCLONAL ANTIBODY (MAB) (giving rise to a positive result in a screening test) in each of 6 wells containing IDENTIFICATION PLATELETS (IDENT PLTL).
For controls: add 50 µl of anti-GPIIbIIIa (MAB IIbIIIa) in well C1 (CONTR 1a), 50 µl of anti-GPIIa (MAB Ialla) in well D1 (CONTR 5b), 50 µl of anti-HLA/β2-microglobulin (MAB HLA) in well E1 (CONTR HLA). In well F1 (CONTR NEG), add 50 µL of anti-GPIbIX (MAB IbIX) or 50 µl of anti-GPIIbIIIa (MAB IIbIIIa) or 50 µl of anti-GPIIa (MAB Ialla).
[For low volume laboratories that use a reduced number of controls \(see 9.2.1\):](#)
[Add 50 µl of the MONOCLONAL ANTIBODY \(MAB\) corresponding to the chosen positive control in well B1 \(e.g. anti-GPIIbIIIa for the CONTR 1a; anti-GPIIa for the CONTR 5b\). Add 50 µl of the same MONOCLONAL ANTIBODY \(MAB\) in well C1 for the negative control.](#)
- 9.5.2. i Mix gently manually or by using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).
- 9.5.3. i Incubate for 30 ± 5 minutes at 36 ± 1 °C.
- 9.5.4. i Centrifuge the MICROPLATE (MTP) 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).

9.6. Removal of unbound monoclonal antibody

- 9.6.1. Wash the MICROPLATE (MTP) 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 (MTP) [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).

9.6.2. Repeat step 9.6.1. three times (total of 4 washes).

9.7. Solubilisation of platelet membranes

9.7.1. Add 130 µl of PLATELET LYSIS BUFFER (LYSBUF) in all wells and resuspend the platelet pellet first by gently pipetting up and down. Next, pipet the platelet suspension 10 times vigorously up and down. This handling induces foaming, which is necessary for efficient lysis of the platelets. Take good care not to cross-contaminate wells.

9.7.2. Incubate for at least 15 minutes at 2 - 8 °C.

9.7.3. Centrifuge at 1050g ± 50g for 15 minutes.

Immune 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 2 – 8 °C °C for one night if necessary.

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

9.8.1. Remove the goat anti-mouse IgG COATED MICROPLATE (COATMTP) from the aluminium pouch. 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.

9.8.2. 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](#).

9.8.3. Incubate for 30 ± 5 minutes at 36 ± 1 °C.

9.9. Removal of unbound lysate proteins

9.9.1. Empty the wells by inverting the MICROPLATE (COATMTP) and tap the plate on absorbent paper.

9.9.2. Wash the MICROPLATE (COATMTP) 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).

9.9.3. Repeat step 9.9.2. five times (total of 6 washes).

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

9.10. Addition of peroxidase labeled goat anti-human IgG

9.10.1. Add 100 µl of the goat anti-human IgG HRP CONJUGATE (CONJ) in each well.

9.10.2. Incubate for 30 ± 5 minutes at 36 ± 1 °C.

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

9.11.1. Empty the wells by inverting the MICROPLATE (COATMTP) and tap the plate on absorbent paper.

9.11.2. 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).

9.11.3. Repeat step 9.11.2. five times (total of 6 washes).

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

9.12. Addition of TMB substrate

9.12.1. Add 100 µl of the TMB solution (CHROM) in each well.

[Remark](#): Keep the solution strictly protected from light.

9.12.2. Incubate for 15 minutes at 36 ± 1°C in the dark.

9.13. Addition of acid to stop colour development

9.13.1. Add 100 µl of STOP SOLUTION (STOPSOL) (H₂SO₄) in each well.

9.14. Reading of microtiter plate

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

10. Test procedure Direct MAIPA (MAIPAD)

The test procedure for a direct MAIPA is generally the same as the procedure for an indirect MAIPA as described in section 9.

In the first part of the MAIPA, a prepared suspension of the patient's platelets are used in stead of the screening platelets of the kit.

Antibodies detected during the direct MAIPA are autoantibodies directed against the glycoproteins (GPIIb/IIIa, GPIIb/IIIa or GPIIb/IX) on the patient's platelets, targeting epitopes which are commonly present on the various platelet glycoproteins and not a specific HPA platelet antigen.

Therefore, further identification with the identification panel cells of the MAIPA kit is not deployed.

10.1. Preparation of the platelets

- 10.1.1. Remove the uncoated MICROPLATE (MTP) from the box. 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 (GPs) are tested in 4 separate wells. For each screening assay, provide 6 extra wells for the controls in order to validate the run: 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.
- 10.1.2. Add 50 µl of SCREENING CELLS (SCREEN PLTL) in the control wells. [The Platelet Antibody Screening Cells are ready-to-use.](#) Add 50 µl of patient platelets previously washed (2 times) and adjusted in PBS / 1% BSA / 0.33% EDTA (minimum 5×10^6 platelets per test) in the sample wells. [The necessary quantity of platelets to add is normally \$25 \times 10^6\$, however, \$5 \times 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.](#)
- 10.1.3. Centrifuge the MICROPLATE (MTP) at $1050g \pm 50g$ 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).
- 10.1.4. Wash the MICROPLATE (MTP) as follows: add 200 µl of diluted CELL WASH BUFFER in each well and centrifuge at $1050g \pm 50g$ 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).
- 10.1.5. Repeat step 10.1.4. once (total of 2 washes).

10.2. Incubation of platelets with serum

- 10.2.1. A direct MAIPA doesn't require the addition of plasma/serum (antibodies are already bound to the platelets), but 50 µl of diluted CELL WASH BUFFER should be added to the sample wells to protect the cells from drying. [Or patient platelets can be added at this step if it wasn't done before \(step 10.1.2.\).](#) Add 50 µl of the CONTROL PLASMA/SERA (CONTR) in the respective wells.
- 10.2.2. Mix gently manually or by using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).
- 10.2.3. Incubate for 30 ± 5 minutes at 36 ± 1 °C.
- 10.2.4. Centrifuge the MICROPLATE (MTP) at $1050g \pm 50g$ 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).

10.3. Removal of unbound immunoglobulins

- 10.3.1. Wash the MICROPLATE (MTP) as follows: add 200 µl of diluted CELL WASH BUFFER in each well and centrifuge at $1050g \pm 50g$ 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).

10.3.2. Repeat step 10.3.1. once (total of 2 washes).

10.4. Incubation of platelets with monoclonal antibody

10.4.1. For an anti-platelet antibody screening assay add 50 µl of each MONOCLONAL ANTIBODY (MAB) in the 4 respective wells containing patient platelets.

For controls: add 50 µl of anti-GPIIb/IIIa (MAB IIb/IIIa) in well C1 (CONTR 1a), 50 µl of anti-GPIIa (MAB Ialla) in well D1 (CONTR 5b), 50 µl of anti-HLA/β2-microglobulin (MAB HLA) in well E1 (CONTR HLA). In well F1 (CONTR NEG), add 50 µl of anti-GPIbIX (MAB IbIX) or 50 µl of anti-GPIIb/IIIa (MAB IIb/IIIa) or 50 µl of anti-GPIIa (MAB Ialla).

10.4.2. Mix gently manually or by using a microplate shaker (2 x 10 seconds at 700-1000 rpm as described previously).

10.4.3. Incubate for 30 ± 5 minutes at 36 ± 1 °C.

10.4.4. Centrifuge the MICROPLATE (MTP) 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).

10.5. Removal of unbound monoclonal antibody

10.5.1. Wash the MICROPLATE (MTP) 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 (MTP) [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).

10.5.2. Repeat step 10.5.1. three times (total of 4 washes).

10.6. Solubilisation of platelet membranes

10.6.1. Add 130 µl of PLATELET LYSIS BUFFER (LYSBUF) in all wells and resuspend the platelet pellet first by gently pipetting up and down. Next, pipette the platelet suspension 10 times vigorously up and down. [This handling induces foaming, which is necessary for efficient lysis of the platelets. Take good care not to cross-contaminate wells.](#)

10.6.2. Incubate for at least 15 minutes at 2 - 8 °C.

10.6.3. Centrifuge at 1050g ± 50g for 15 minutes.

Immune 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.

10.7. ELISA part of the MAIPA procedure

Continue with the ELISA part of the MAIPA procedure as described in sections 9.8 till 9.14.

11. Concise version of the MAIPA procedure

A concise overview of the complete MAIPA procedure is added as annex to this IFU.

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

Interpretation of assay results 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. 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.

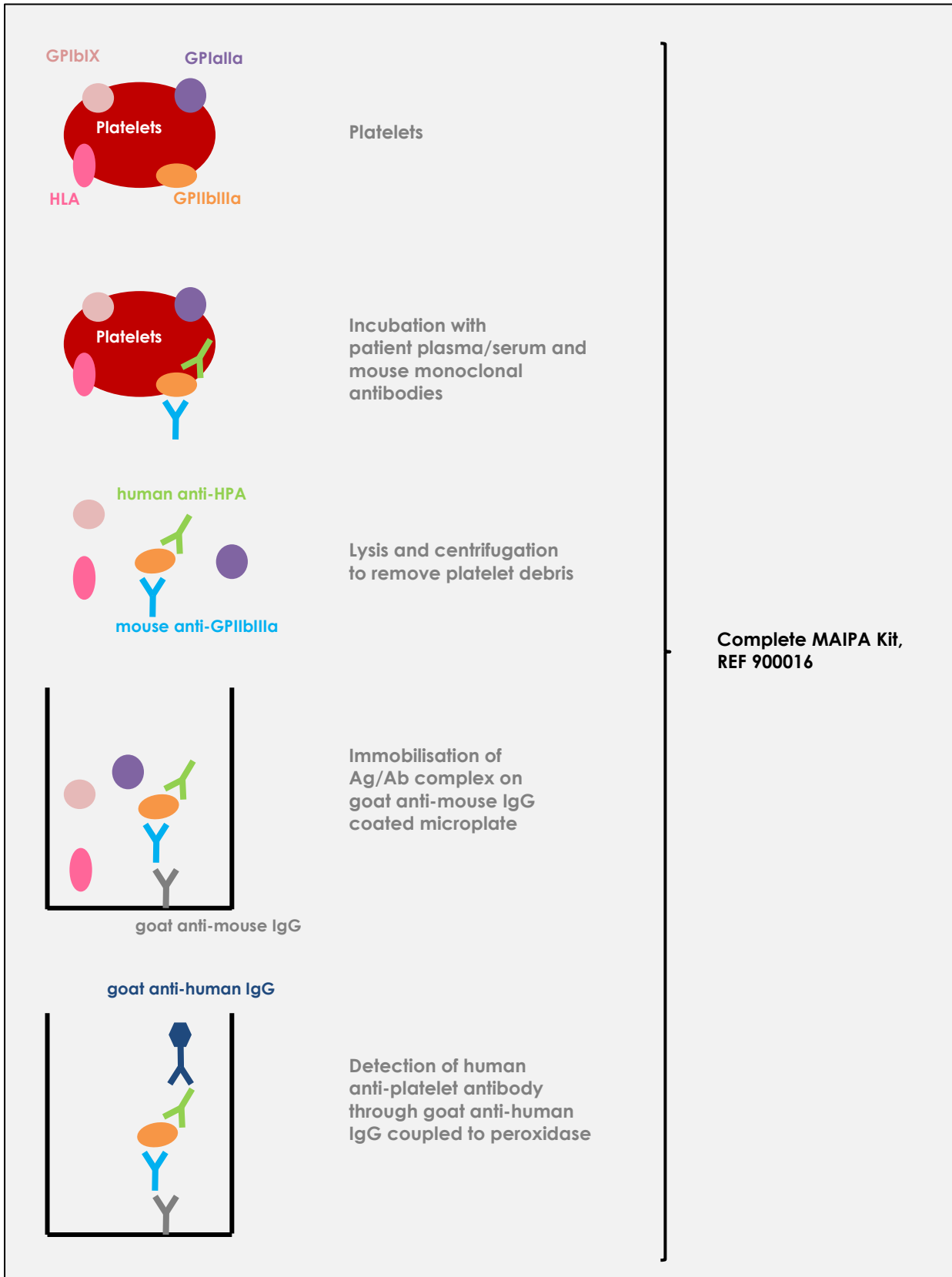
The screening platelets and identification panel cells are not useful for screening and identification of the HPA-15 system. This group is included for information purposes only. For HPA-15a and HPA-15b some specific platelets are required to identify the antibodies. Additionally, a specific monoclonal antibody is required.

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ANNEXES

Test principle of the MAIPA procedure



Concise version of the MAIPA procedure

Indirect screening assay	Direct screening assay	Identification assay
50 µL screening platelets	50 µL patient platelets	50 µL identification platelets
3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm	3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm	3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm
wash 2x: 200 µL CELL WASH BUFFER 1x 3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm	wash 2x: 200 µL CELL WASH BUFFER 1x 3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm	wash 2x: 200 µL CELL WASH BUFFER 1x 3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm
50 µL patient serum/plasma	50 µL CELL WASH BUFFER 1x	50 µL patient serum/plasma
30 min. 36 ± 1 °C	30 min. 36 ± 1 °C	30 min. 36 ± 1 °C
3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm	3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm	3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm
wash 2x: 200 µL CELL WASH BUFFER 1x 3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm	wash 2x: 200 µL CELL WASH BUFFER 1x 3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm	wash 2x: 200 µL CELL WASH BUFFER 1x 3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm
50 µL monoclonal antibody MAB	50 µL monoclonal antibody MAB	50 µL monoclonal antibody MAB
30 min. 36 ± 1 °C	30 min. 36 ± 1 °C	30 min. 36 ± 1 °C
3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm	3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm	3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm
wash 4x: 200 µL CELL WASH BUFFER 1x 3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm	wash 4x: 200 µL CELL WASH BUFFER 1x 3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm	wash 4x: 200 µL CELL WASH BUFFER 1x 3 min. 1050 g (+/- 50 g) decant shake 2 x 10 sec. 700-1000 rpm
130 µL LYSIS BUFFER mix vigorously by pipetting up and down	130 µL LYSIS BUFFER mix vigorously by pipetting up and down	130 µL LYSIS BUFFER mix vigorously by pipetting up and down
minimum 15 min. 2 - 8 °C	minimum 15 min. 2 - 8 °C	minimum 15 min. 2 - 8 °C
15 min. 1050 x g (+/- 50 g)	15 min. 1050 x g (+/- 50 g)	15 min. 1050 x g (+/- 50 g)
100 µL lysate in coated MTP	100 µL lysate in coated MTP	100 µL lysate in coated MTP
30 min. 36 ± 1 °C	30 min. 36 ± 1 °C	30 min. 36 ± 1 °C
decant wash 6x: 200 µL ELISA WASH BUFFER 1x empty wells and tap on absorbent paper	decant wash 6x: 200 µL ELISA WASH BUFFER 1x empty wells and tap on absorbent paper	decant wash 6x: 200 µL ELISA WASH BUFFER 1x empty wells and tap on absorbent paper
100 µL HRP conjugate	100 µL HRP conjugate	100 µL HRP conjugate
30 min. 36 ± 1 °C	30 min. 36 ± 1 °C	30 min. 36 ± 1 °C
decant wash 6x: 200 µL ELISA WASH BUFFER 1x empty wells and tap on absorbent paper	decant wash 6x: 200 µL ELISA WASH BUFFER 1x empty wells and tap on absorbent paper	decant wash 6x: 200 µL ELISA WASH BUFFER 1x empty wells and tap on absorbent paper
100 µL TMB substrate	100 µL TMB substrate	100 µL TMB substrate
15 min. 36 ± 1 °C	15 min. 36 ± 1 °C	15 min. 36 ± 1 °C
100 µL stop solution	100 µL stop solution	100 µL stop solution
read 450/620 nm	read 450/620 nm	read 450/620 nm

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 IaIIa	PLTL PATIENT 2 no serum MAB IaIIa	PLTL PATIENT 3 no serum MAB IaIIa	PLTL PATIENT 4 no serum MAB IaIIa	PLTL PATIENT 5 no serum MAB IaIIa						
C	SCREEN PLTL CONTR Ia 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 IaIIa	PLTL PATIENT 1 no serum MAB IbIX	PLTL PATIENT 2 no serum MAB IbIX	PLTL PATIENT 3 no serum MAB IbIX	PLTL PATIENT 4 no serum MAB IbIX	PLTL PATIENT 5 no serum MAB IbIX						
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 IbIX	SCREEN PLTL SERUM PATIENT 1 MAB IaIIa	SCREEN PLTL SERUM PATIENT 2 MAB IaIIa	SCREEN PLTL SERUM PATIENT 3 MAB IaIIa	SCREEN PLTL SERUM PATIENT 4 MAB IaIIa	SCREEN PLTL SERUM PATIENT 5 MAB IaIIa						
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 IbIX	SCREEN PLTL SERUM PATIENT 2 MAB IbIX	SCREEN PLTL SERUM PATIENT 3 MAB IbIX	SCREEN PLTL SERUM PATIENT 4 MAB IbIX	SCREEN PLTL SERUM PATIENT 5 MAB IbIX						

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 IaIIa						
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 IaIIa						
C	SCREEN PLTL CONTR Ia 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 IaIIa						
D	SCREEN PLTL CONTR 5b MAB IaIIa	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 IaIIa						
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 IaIIa						
F	SCREEN PLTL CONTR NEG MAB IbIX	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 IaIIa						
G	ID PLTL 1 SERUM PATIENT 6 MAB IaIIa	ID PLTL 2 SERUM PATIENT 6 MAB IaIIa	ID PLTL 3 SERUM PATIENT 6 MAB IaIIa	ID PLTL 4 SERUM PATIENT 6 MAB IaIIa	ID PLTL 5 SERUM PATIENT 6 MAB IaIIa	ID PLTL 6 SERUM PATIENT 6 MAB IaIIa						
H	ID PLTL 1 SERUM PATIENT 7 MAB IaIIa	ID PLTL 2 SERUM PATIENT 7 MAB IaIIa	ID PLTL 3 SERUM PATIENT 7 MAB IaIIa	ID PLTL 4 SERUM PATIENT 7 MAB IaIIa	ID PLTL 5 SERUM PATIENT 7 MAB IaIIa	ID PLTL 6 SERUM PATIENT 7 MAB IaIIa						

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 Ialla	PLTL PATIENT 5 no serum MAB Ialla	PLTL PATIENT 6 no serum MAB Ialla						
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 Ialla	ID PLTL 4 SERUM PATIENT 1 MAB IIbIIIa	ID PLTL 4 SERUM PATIENT 2 MAB IIbIIIa	PLTL PATIENT 4 no serum MAB IbIX	PLTL PATIENT 5 no serum MAB IbIX	PLTL PATIENT 6 no serum MAB IbIX						
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 IbIX	ID PLTL 6 SERUM PATIENT 1 MAB IIbIIIa	ID PLTL 6 SERUM PATIENT 2 MAB IIbIIIa	SCREEN PLTL SERUM PATIENT 4 MAB Ialla	SCREEN PLTL SERUM PATIENT 5 MAB Ialla	SCREEN PLTL SERUM PATIENT 6 MAB Ialla						
G	ID PLTL 1 SERUM PATIENT 3 MAB Ialla	ID PLTL 3 SERUM PATIENT 3 MAB Ialla	ID PLTL 5 SERUM PATIENT 3 MAB Ialla	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 Ialla	ID PLTL 4 SERUM PATIENT 3 MAB Ialla	ID PLTL 6 SERUM PATIENT 3 MAB Ialla	SCREEN PLTL SERUM PATIENT 4 MAB IbIX	SCREEN PLTL SERUM PATIENT 5 MAB IbIX	SCREEN PLTL SERUM PATIENT 6 MAB IbIX						

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 IbIIIa											
D	SCREEN PLTL CONTR 5b MAB IaIIa											
E	SCREEN PLTL CONTR HLA MAB HLA											
F	SCREEN PLTL CONTR NEG MAB IbIX											
G												
H												

Revision History

Version number	Description
IFU900016/V03/11.12.2018	Previous version
IFU900016/V04/11-2021	<p>Additions to 'Warnings and precautions' section (clauses 4.2, 4.4, 4.5, 4.6, 4.7, 4.10, 4.11).</p> <p>Additions on required equipment, specifically on centrifuges in section 8.</p> <p>Restructure of MAIPA procedure description:</p> <ul style="list-style-type: none">• Division in separate instructions for Indirect and Direct MAIPA• Inclusion of a concise version of the complete MAIPA procedure in Annex. <p>Application of the legal company name of the manufacturer: 'Advanced Practical Diagnostics BV' in stead of the abbreviation 'apDia'.</p>
IFU900016/V05/02-2023	<p>Addition to sections 9 and 10 «Test Procedure Indirect/Direct MAIPA» (clauses 9.5 and 10.4): for validation of a MAIPA run, the negative control of the kit may not only be tested with detection antibody anti-GPIbIX but antibodies anti-GPIIbIIIa or anti-GPIIa can be used as well.</p>