Platelet Immunofluorescence Test Protocol

This consensus protocol was compiled from the methods used by the participants of the Platelet Serology Exercises organised by NIBSC. More weight was given to the protocols from laboratories with consistently high performance in the exercises, however, it may not perform any better than the original published method (von dem Borne *et al*, Brit. J. Haem. 1978; 39, 195-207).

The protocol has been used successfully at NIBSC but it should be validated locally.

Reagents

FITC-conjugated anti-human Ig (G, M or G+M+A)

PBS/EDTA

PBS	2.5L
$C_{10}H_{14}N_2O_8Na_2.2H_2O$	8.37g

Platelet preparation

1. Take blood from group O donors into EDTA or citrate.
2. Centrifuge at 500g for 10 min in bench-top centrifuge
3. Remove the top 3/4 of the PRP from the top of the tube and transfer to 10mL conical centrifuge tube.
4. Make up to 10mL with PBS/EDTA buffer. Centrifuge at 2,000g for 5 mins and decant supernatant.
5. Re-suspend cells gently in 2mL buffer and repeat step 4. twice more.
6. Re-suspend platelets in PBS/EDTA at 100x 10^9/L approx. Store at 4^{o}C for up to 4 days.

Immunofluorescence test (tube method)

1. For each sample to be tested (no. of tests plus controls x no. of donors) aliquot 50uL platelet suspension into 'FACS tubes'(Falcon 2052)
2. Add 50uL test serum/plasma. Include 2 negative controls and 1 weak positive control which has been calibrated against the National anti-HPA-1a standard (NIBSC code 93/710)
3. Incubate at 37^{o}C for 30 min.
4. Add 4ml PBS/EDTA buffer and spin for 5 min at 1,500g in benchtop centrifuge.
5. Decant supernatant, re-suspend cells, add 4mL PBS/EDTA wash buffer and spin for 5 min at 1,500g.
6. repeat step 5.
7. Decant supernatant, re-suspend cells and add 50uL FITC-conjugated anti-human Ig (G, M or G+M+A, dilution determined by previous experiments). Make sure cells are fully re-suspended.
8. Incubate at 22^{o}C (room temperature) for 30 mins in the dark.
9. Repeat step 4. and decant supernatant.
10. Re-suspend cells in 1mL PBS/EDTA buffer.
11. Store in the dark until FACS analysis.

Immunofluorescence test (microplate method)

1. For each sample to be tested (no. of tests plus controls x no. of donors) aliquot 50uL platelet suspension into U-well microplate and spin at 900g for 3 min.
2. Discard supernatant and re-suspend cells in 50uL test serum/plasma.
3. Include 2 negative controls and 1 weak positive control which has been calibrated against the National anti-HPA-1a standard (NIBSC code 93/710)
4. Incubate at 37^{o}C for 30 min.
5. Add 200ul PBS/EDTA buffer and spin for 3 min at 900g in microplate centrifuge.
6. Decant supernatant, re-suspend cells using microplate shaker at maximum speed for 20 secs, add 200uL PBS/EDTA buffer and spin for 3 min at 900g.
7. repeat step 5.

8. Decant supernatant, re-suspend cells using microplate shaker and add 50uL FITC-conjugated anti-human Ig (G, M or G+M+A, dilution determined by previous experiments). Make sure cells are fully re-suspended using microplate shaker or pipette.
9. Incubate at 22^{o}C (room temperature) for 30 mins in the dark.
10. Repeat step 5. and decant supernatant.
11. Re-suspend cells in 100uL PBS/EDTA wash buffer and transfer to numbered 'FACS tubes' containing 1mL PBS/EDTA wash buffer.
12. Store in the dark until FACS analysis.

N.B If using solid phase technique; use 10uL volumes of platelet suspension, test sera, and FITC-anti-IgG and wash 10 times at steps 6. and 10. If using visual microscopy to read tests carried out in microplates or tubes then cells should be washed twice more after step 10.