NHS
Blood and Transplant

Introduction to Neonatal Alloimmune Thrombocytopenia (NAIT)

Deborah Sage

Histocompatibility and Immunogenetics

Caring Expert Quality

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Overview

- NAIT
- Platelets
  - Glycoproteins on platelets
  - Human Platelet Antigens (HPA)
- Sensitisation/Severity
- Laboratory tests
- Treatment/Management

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NAIT

- Affects 1 in 1,000-2,000 live births
- Can be a cause of miscarriage
- Severe <50x10^9/L
- Most common cause is HPA-1a antibodies
- Can affect first pregnancies (30%)
- 500-600 referrals/year
- 10% detection rate

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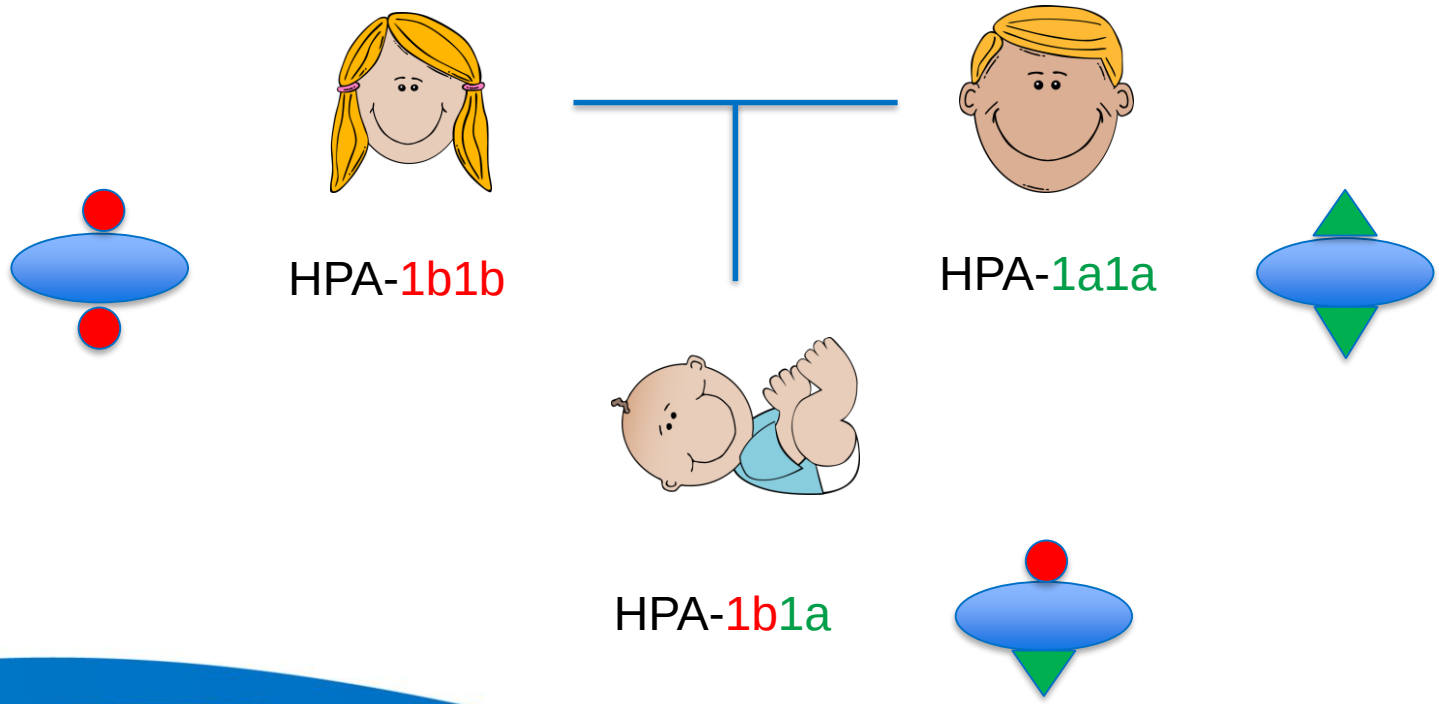
Inheritance of Human Platelet Antigens



How NAIT occurs

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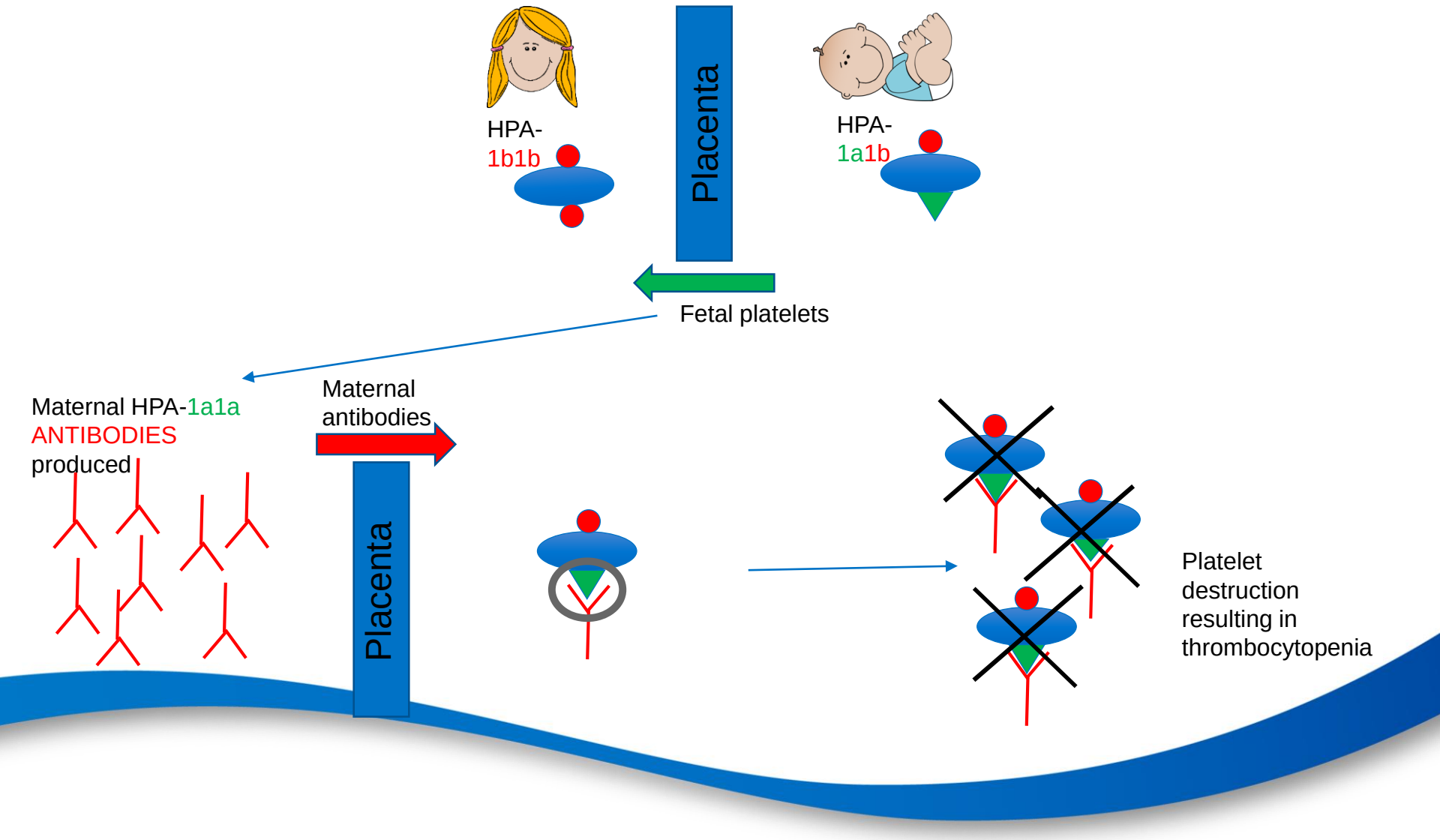

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Clinical Impact and Diagnosis

- Can cause intracranial haemorrhage
  - Death
  - Developmental disabilities
  - Life-long social care
- Blood spots in baby
- Referral to test for maternal antibodies
- Future pregnancies!
- Cannot predict severity from current tests

Antigens on platelets

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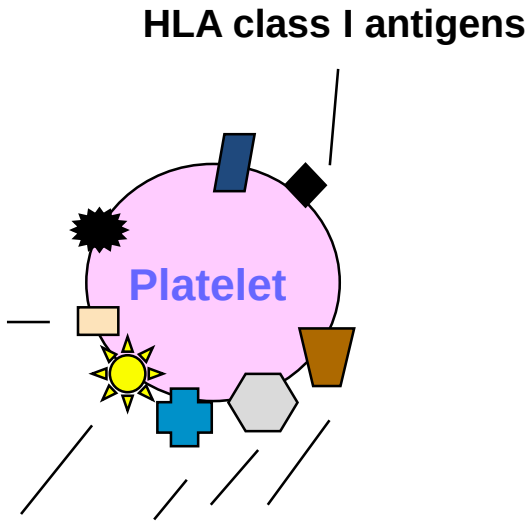
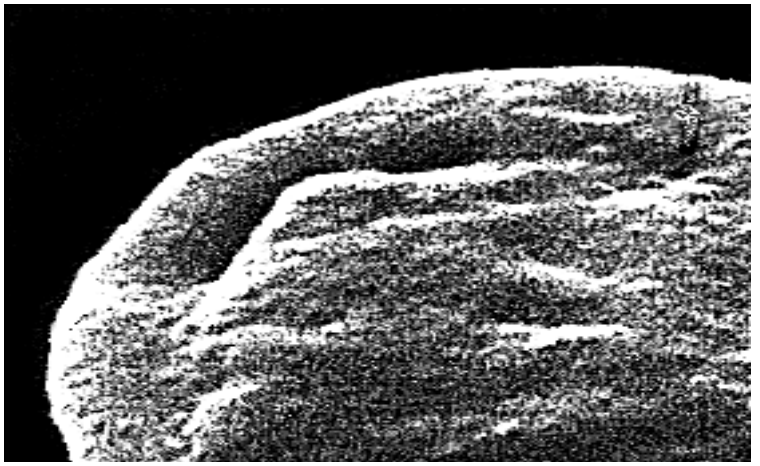
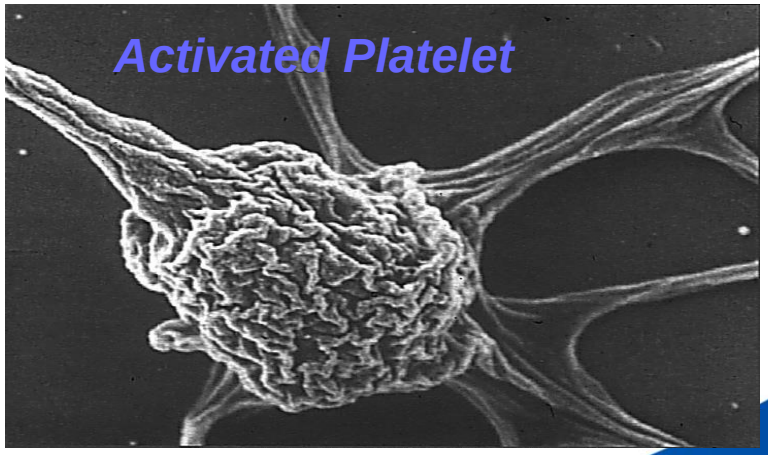


Platelet glycoproteins

GPIIb/IIIa
GPIa/IIa
GPIb/IX/V
CD109

} HPA-1 to 34





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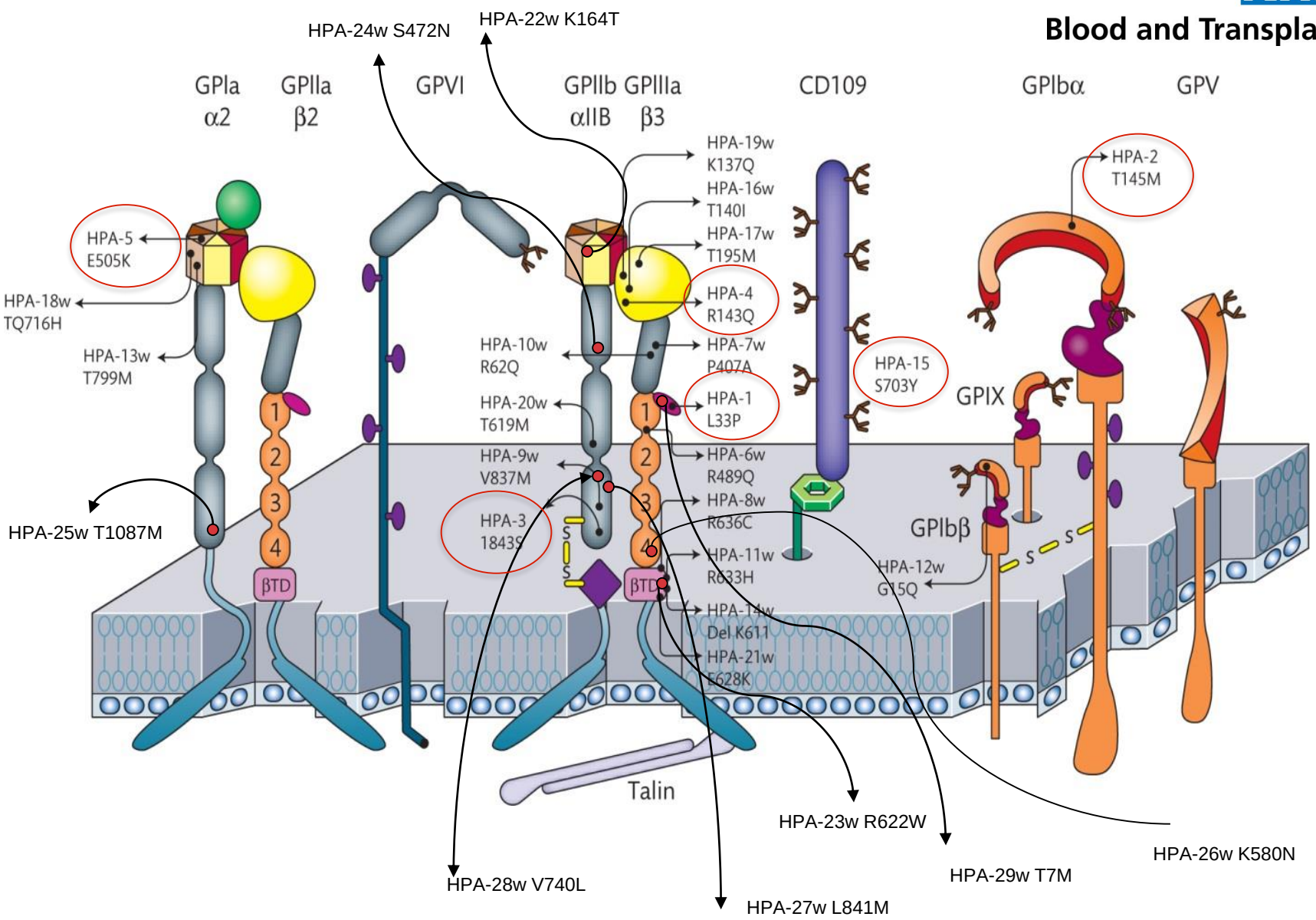

Human Platelet Antigens (HPA)

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There are currently 34 designated HPA systems.

- The majority (21) are associated with the GPIIb/IIIa complex.
- HPA are primarily di-allelic systems, i.e. result in a single amino acid substitution except HPA-14bw, which results from an ‘in frame’ deletion of three nucleotides.
- The ‘a’ allele is always the high frequency form and ‘b’ the low frequency. Three HPA systems have been shown to be tri-allelic; HPA-1c, -5c, -7cw but these mutations are very rare.
- A ‘w’ (workshop) assignment is given to systems where antibodies to only one antigen have been reported – this is the majority of recently identified HPA.

The most clinically significant platelet-specific alloantigens

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Allele	freq. (Cauc)	GP	Copies/cell	GP function
HPA-1a	**84.5 %**	IIIa (CD61)	40K	Fg, vWF, Fn,
HPA-1b	15.5%			Coll, Vn
HPA-2a	89.9%	Ibα (CD42b)	20K	vWF
HPA-2b	10.1%			
HPA-3a	60.3%	IIb (CD41)	40K	Fg, vWF, Fn,
HPA-3b	39.7%			Coll, Vn
HPA-4a	100%	IIIa (CD61)	40K	
HPA-4b	0.0%			
HPA-5a	91.1%	Ia (CD49b)	2-4K	Collagen
HPA-5b	**8.9%**			
HPA-15a	50.0%	CD109	0.5 -2K	Collagen
HPA-15b	50.0%			

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Current Theory of Sensitisation

- Fetal platelets crossing the placenta
- β3 integrin present in saliva/sperm
- αVβ3 on trophoblasts of placenta
- Fetal maternal haemorrhage

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Severity

- HPA-1a antibodies cause ICH
- HPA-5b are said not to be as severe NAIT
- HPA-3a antibodies reported to cause miscarriages
- αVβ3 have been reported to cause ICH
- Cannot predict NAIT severity by lab tests
- Only predictor is subsequent pregnancies are more severe

Laboratory investigations (phase 1)

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Routine investigation

• Screen of maternal serum versus typed HPA donor platelets (PIFT & MAIPA *v* panel of HPA-1, -2, -3, -4, -5, -6, -9,-15 typed platelets)

• Genotype (PCR-SBT) of maternal, paternal & infant sample

• Samples:
  – Maternal = 6ml EDTA & 6ml clot
  – Paternal = 6ml EDTA
  – Neonate = 1ml EDTA

Indirect Immunofluorescence Tests

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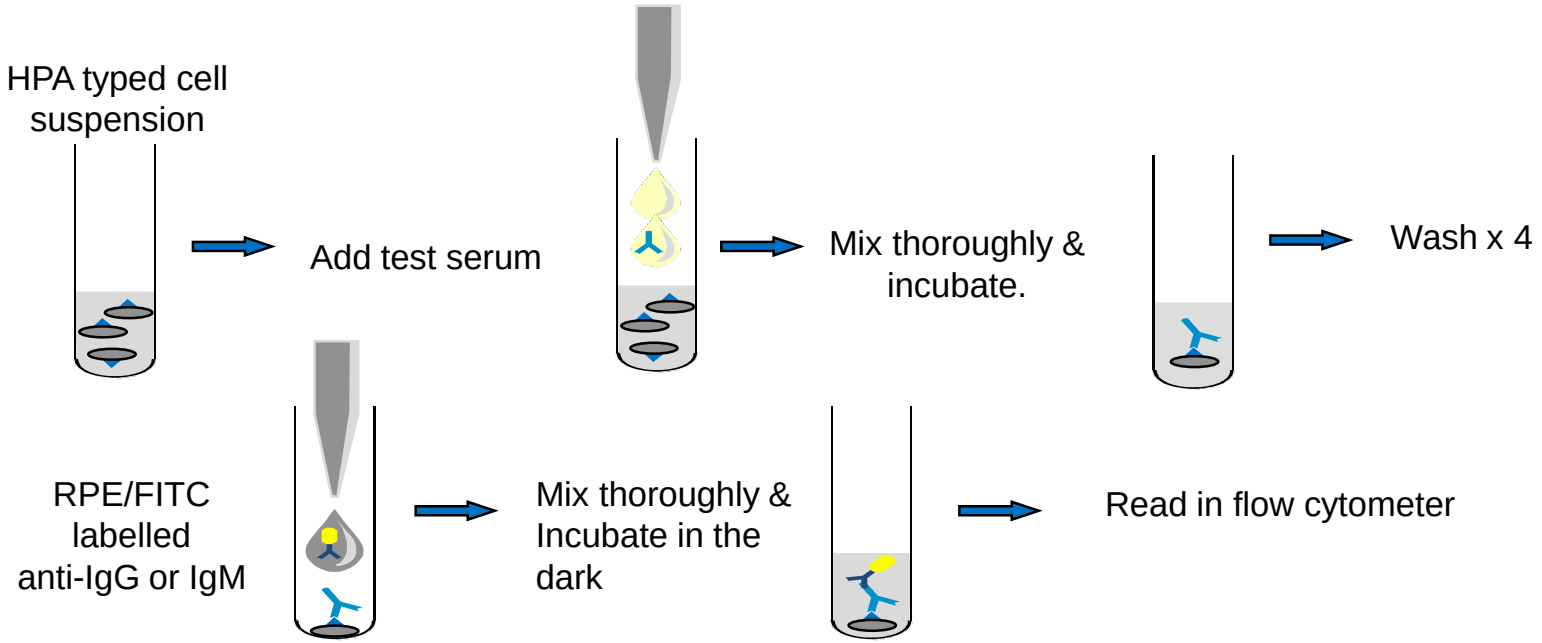

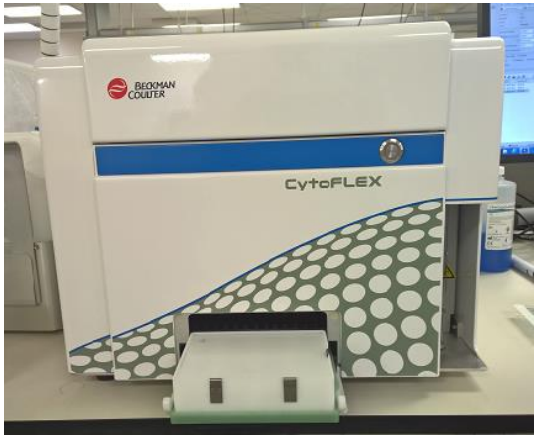

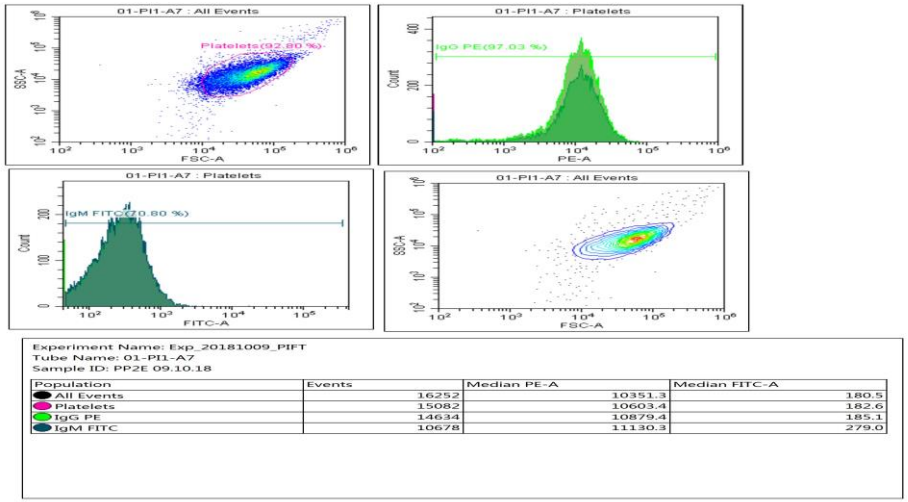

PIFT

Advantages and disadvantages of indirect immunofluorescence tests

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- **Advantages**
  - Sensitive, quick and cheap
  - Whole cell assays with potential to detect all antibodies to the membrane surface – important for some HPA, e.g. HPA-3a
- **Disadvantages**
  - May detect antibodies to HLA class I, ABH; IvIg and immune complexes(?)

MAIPA assay

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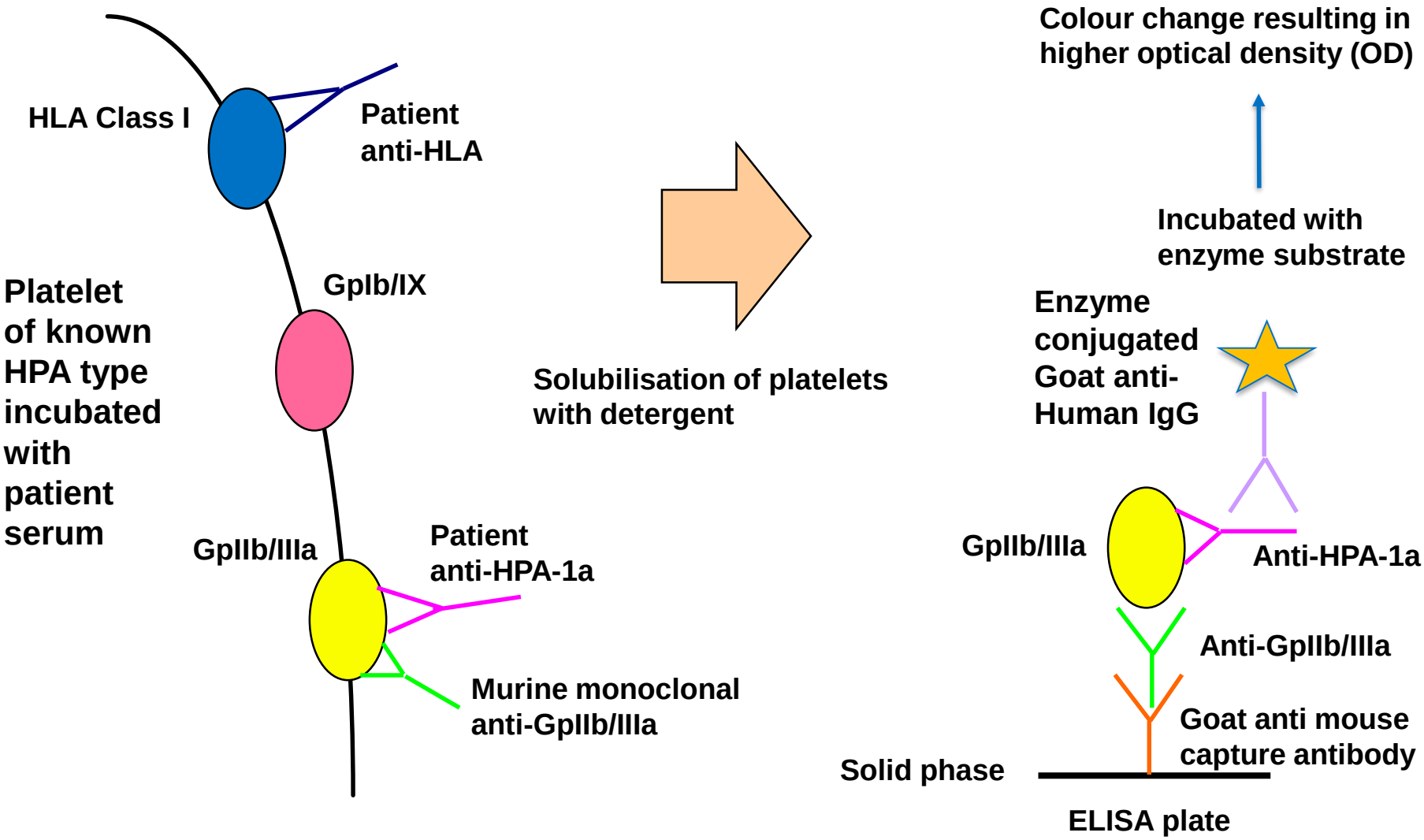

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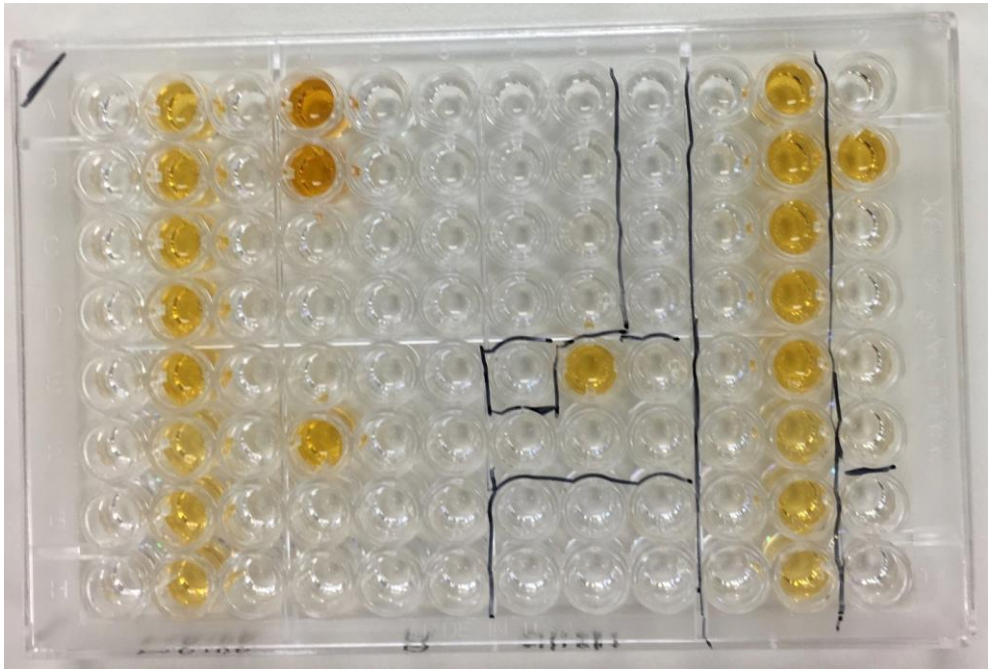

One Day MAIPA DATE: 31/10/2016 Assay number: A HLA Class I

Solubilization buffer batch #729/16 - validation

w6/3+p43

	NEG	2	3	4	5	6	7	8	9	10
PP1A	AB76	PI1	8994/16	8990/16	8991/16	8992/16	8915/16	8974/16		AB76
1a1a3a3a		81.4	1.6	214.8	1.0	1.5	0.9	2.2	0.4	
IIb/IIIa	0.013	1.058	0.021	2.793	0.013	0.020	0.012	0.028	0.005	0.013
PP1B	AB76	PI1	8994/16	8990/16	8991/16	8992/16	8915/16	8974/16	0	AB76
1a1a3b3b		78.3	1.5	183.9	1.0	1.4	1.1	2.5	0.3	
IIb/IIIa	0.015	1.174	0.022	2.758	0.015	0.021	0.016	0.037	0.005	0.016
PP1C	AB76	PI 17	8994/16	8990/16	8991/16	8992/16	8915/16	8974/16	0	AB76
1b1b3a3a		56.2	1.4	1.1	1.0	1.4	1.0	2.9	0.2	
IIb/IIIa	0.021	1.180	0.029	0.024	0.022	0.029	0.020	0.061	0.005	0.024
PP1D	AB76	PI 17	8994/16	8990/16	8991/16	8992/16	8915/16	8974/16	0	AB76
1b1b3b3b		103.4	1.6	1.2	1.1	1.6	1.3	3.2	0.4	
IIb/IIIa	0.012	1.241	0.019	0.014	0.013	0.019	0.015	0.038	0.005	0.013
PP1A	AB76	PI24	8994/16	8990/16	8991/16	8992/16		8931/16	8897/16	AB76
5a5a		91.2	1.5	3.3	0.8	1.4	0.4	50.5	2.5	
Ia/IIa	0.014	1.277	0.021	0.046	0.011	0.020	0.005	1.263	0.062	0.017
PP1C	AB76	PI18	8994/16	8990/16	8991/16	8992/16	8923/16	8927/16	8930/16	AB76
5b5b		23.2	1.5	58.3	1.0	1.4	1.4	1.2	1.7	
Ia/IIa	0.025	0.581	0.037	1.458	0.025	0.036	0.034	0.030	0.043	0.031
PP1B	AB76	PI 15	8994/16	8990/16	8991/16	8992/16			0	AB76
2a2a		52.5	1.6	1.8	1.1	1.7	0.3	0.3	0.3	
Ib/IX	0.015	0.787	0.024	0.027	0.016	0.025	0.005	0.005	0.005	0.017
PP1E	AB76	PI 15	8994/16	8990/16	8991/16	8992/16			0	AB76
2b2b		46.5	1.6	1.7	1.0	1.5	0.3	0.3	0.3	
Ib/IX	0.015	0.697	0.024	0.026	0.015	0.022	0.004	0.005	0.005	0.016

SAMPLE ID	PI 1	AB76
RATIO	101.1	
OD	1.314	0.018
SAMPLE ID	PI 1	HLA
RATIO	80.3	74.8
OD	1.284	1.346
SAMPLE ID	PI 17	8994/16
RATIO	52.6	1.8
OD	1.262	0.033
SAMPLE ID	PI 17	8990/16
RATIO	97.4	1.4
OD	1.266	0.025
SAMPLE ID	P1 24	8991/16
RATIO	72.9	3.8
OD	1.240	0.068
SAMPLE ID	PI 18	8992/16
RATIO	20.8	4.4
OD	0.645	0.079
SAMPLE ID	PI 15	
RATIO	47.2	0.3
OD	0.803	0.005
SAMPLE ID	PI 15	
RATIO	41.6	0.3
OD	0.666	0.005

31.10.16.A.xls

2

Each plate is laid out with glycoproteins from donor platelets in rows whilst test serum for each patient sample is in columns. The results are presented as an optical density, and a ratio of that OD to that of the corresponding negative control serum. An OD >0.150 and a ratio >3 normally constitute a positive.
We only use an anti-IgG conjugated antibody.

The advantages and disadvantages of the MAIPA assay

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- **Advantages**
  - Specific and sensitive
  - Able to identify individual antibody specificities in complex antibody mixtures, differentiation from HLA class I antibodies
- **Disadvantages**
  - Need to know glycoprotein target antigen
  - Choice of monoclonal antibody can be critical
  - Solubilisation may modify the conformation of the native antigen

Laboratory investigations (phase 2)

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Strong clinical evidence of NAIT or HPA-1b1b mother (HPA-1a antibodies not detected)

- Increase serum to cell ratio in PIFT & MAIPA
- Use different capture monoclonal antibodies
- HPA-1b1b, antibody negative women are monitored for antibody production during pregnancy.
- *DRB3*01:01* typing can be useful in cases to provide re-assurance if family is anxious.
- Use PakLx
- Crossmatch of maternal serum versus paternal platelets using PIFT and MAIPA assay.
- Demographics and reaction pattern
- GpIV antibody screening and typing

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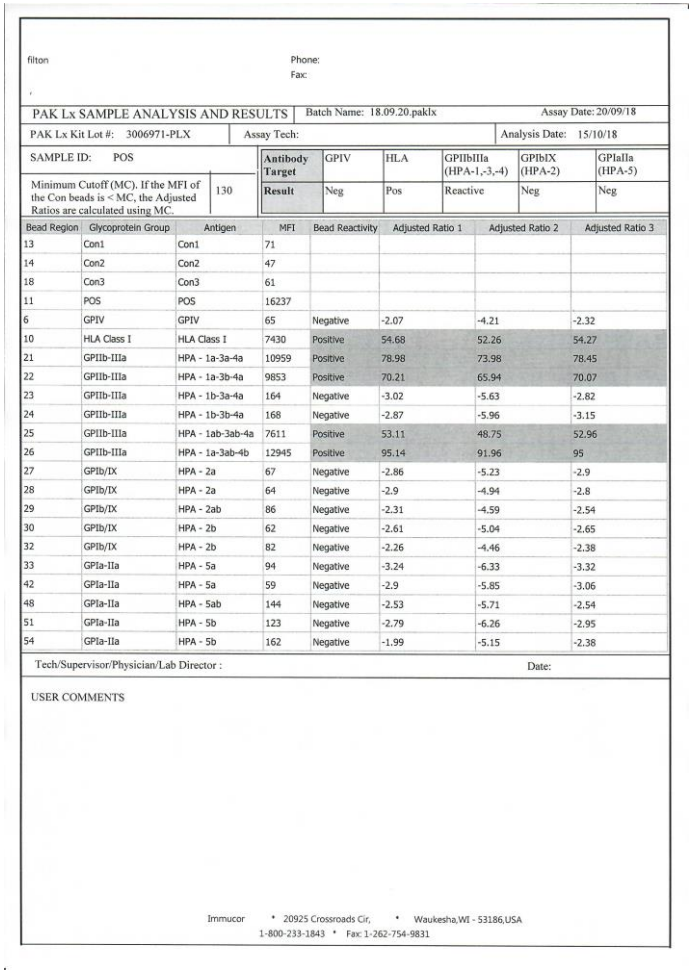

filton Phone:
Fax:

PAK Lx SAMPLE ANALYSIS AND RESULTS | Batch Name: 18.09.20.paklx | Assay Date: 20/09/18

PAK Lx Kit Lot #: 3006971-PLX | Assay Tech: | Analysis Date: 15/10/18

SAMPLE ID: POS

Minimum Cutoff (MC). If the MFI of the Con beads is < MC, the Adjusted Ratios are calculated using MC. 130

Antibody Target	GPIV	HLA	GPIIbIIIa (HPA-1,-3,-4)	GPIbIX (HPA-2)	GPIaIIa (HPA-5)
Result	Neg	Pos	Reactive	Neg	Neg

Bead Region	Glycoprotein Group	Antigen	MFI	Bead Reactivity	Adjusted Ratio 1	Adjusted Ratio 2	Adjusted Ratio 3
13	Con1	Con1	71				
14	Con2	Con2	47				
18	Con3	Con3	61				
11	POS	POS	16237				
6	GPIV	GPIV	65	Negative	-2.07	-4.21	-2.32
10	HLA Class I	HLA Class I	7430	Positive	54.68	52.26	54.27
21	GPIIb-IIIa	HPA - 1a-3a-4a	10959	Positive	78.98	73.98	78.45
22	GPIIb-IIIa	HPA - 1a-3b-4a	9853	Positive	70.21	65.94	70.07
23	GPIIb-IIIa	HPA - 1b-3a-4a	164	Negative	-3.02	-5.63	-2.82
24	GPIIb-IIIa	HPA - 1b-3b-4a	168	Negative	-2.87	-5.96	-3.15
25	GPIIb-IIIa	HPA - 1ab-3ab-4a	7611	Positive	53.11	48.75	52.96
26	GPIIb-IIIa	HPA - 1a-3ab-4b	12945	Positive	95.14	91.96	95
27	GPIb/IX	HPA - 2a	67	Negative	-2.86	-5.23	-2.9
28	GPIb/IX	HPA - 2a	64	Negative	-2.9	-4.94	-2.8
29	GPIb/IX	HPA - 2ab	86	Negative	-2.31	-4.59	-2.54
30	GPIb/IX	HPA - 2b	62	Negative	-2.61	-5.04	-2.65
32	GPIb/IX	HPA - 2b	82	Negative	-2.26	-4.46	-2.38
33	GPIa-IIa	HPA - 5a	94	Negative	-3.24	-6.33	-3.32
42	GPIa-IIa	HPA - 5a	59	Negative	-2.9	-5.85	-3.06
48	GPIa-IIa	HPA - 5ab	144	Negative	-2.53	-5.71	-2.54
51	GPIa-IIa	HPA - 5b	123	Negative	-2.79	-6.26	-2.95
54	GPIa-IIa	HPA - 5b	162	Negative	-1.99	-5.15	-2.38

Tech/Supervisor/Physician/Lab Director : Date:

USER COMMENTS

Immucor * 20925 Crossroads Cir, * Waukesha,WI - 53186,USA
1-800-233-1843 * Fax: 1-262-754-9831

- Recombinant platelet glycoproteins captured on Luminex beads. Better specificity for defining HPA specific antibodies.
- Very sensitive and has picked up antibodies not detected in MAIPA.
- However potential for conformational changes as part of manufacturing process.
- More expensive than an in-house MAIPA.
- No CD109 (HPA-15).
- Labile glycoprotein that dissociates from platelets >24hrs. Requires fresh platelets.

Commercial bead based assay for the detection of HPA antibodies

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- Detects antibodies against HPA-1, -2, -3, -4, -5, GPIV, HLA class I
- **Advantages**
  - Test results available after 3 hours
  - 10uL of serum required
  - Simple assay – beads + serum, wash, add conjugate, wash, test for bead associated fluorescence
  - Sensitive for HPA-1a antibodies
- **Disadvantages**
  - Expensive
  - Limited range of beads with antigen combinations
  - Unable to detect antibodies to HPA-15
  - Relatively insensitive to HPA-3a and HPA-5b antibodies compared to MAIPA
  - Currently, cannot perform crossmatch or test for low frequency HPA

Porcelijn L *et al*. 54; 1486-92 (2014); Cooper N *et al*, Transfusion 56; 115-18 (2016)

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Do we miss antibodies?

Yes

- The proportion of HPA-1b1b mothers in serologically negative NAIT cases is greater than expected

Why?

- Low affinity antibodies
- Isoforms of GPIIb/IIIa
- HPA-1a antibodies are polymorphic

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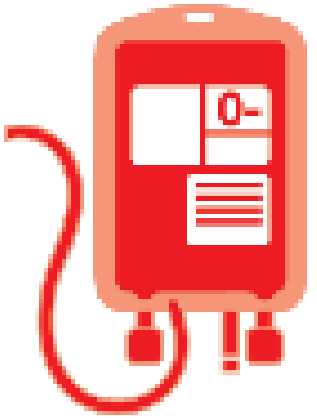
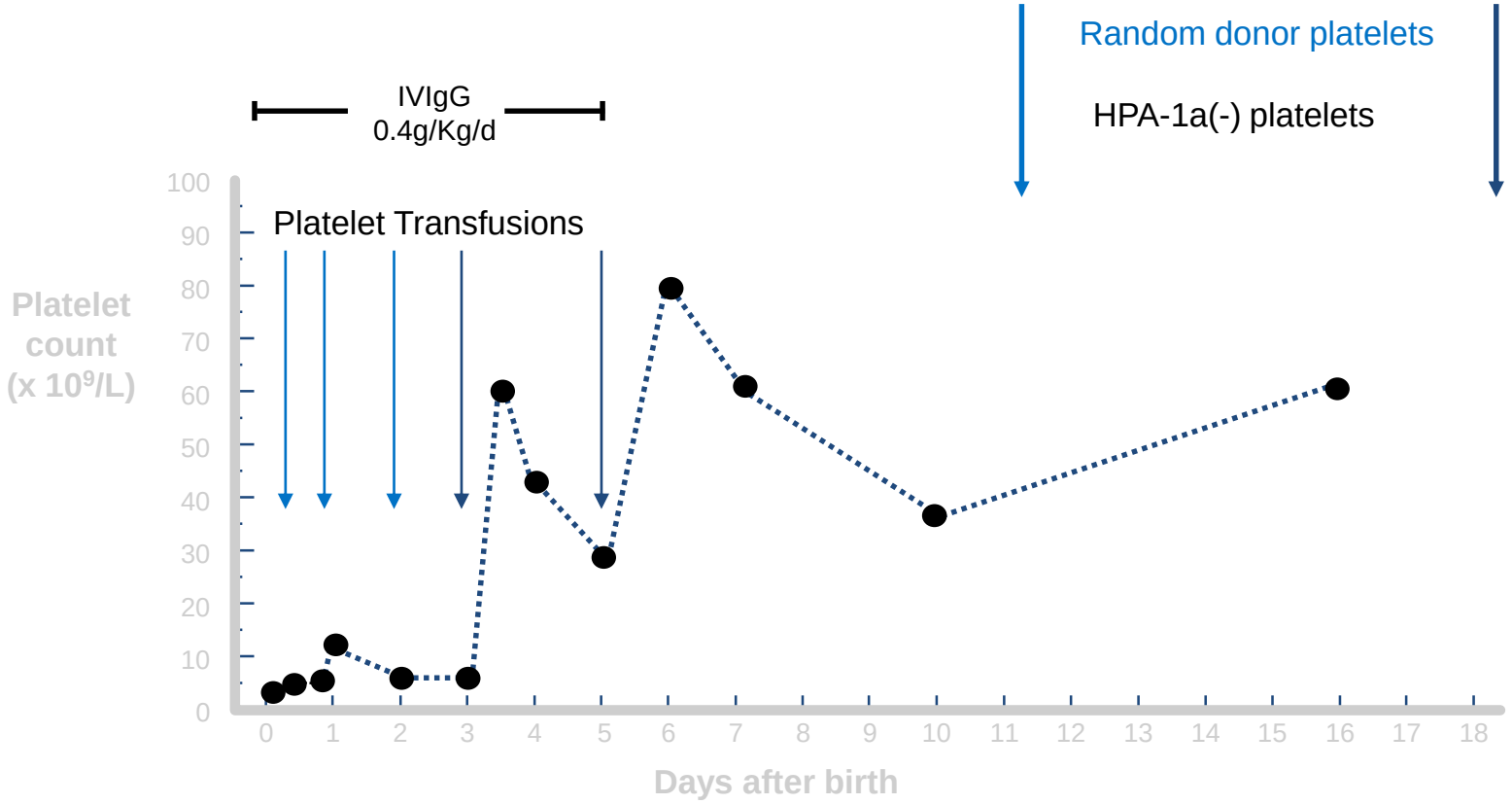
Treatment

- During pregnancy
  - Intravenous immunoglobulin (IVIg)
  - Steroids
  - Platelet transfusions
  - Caesarean

- Following Birth
  - Platelet transfusions



Treatment of NAIT - Neonatal platelet transfusions
Patient ‘C.R.’ - Anti-HPA-1a



Management of HPA alloimmunised women with heterozygous partners

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- **Current**
  - Amniocentesis at ~15 weeks to determine HPA status of fetus
  - Chorionic villus sampling if earlier results required (e.g. if history of early fetal death)
  - HPA determined by PCR-SBT – preliminary result in 48-72 hours, cultured sample result at 14-21 days
  - Invasive procedure – spontaneous abortion & further alloimmunisation leading to increased disease severity
- **Alternatives**
  - Non-invasive, ffDNA typing for HPA-1 from maternal plasma available at some European centres - but earliest typing at 17 weeks and needs repeating later

Samples required for the investigation of NAIT

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Samples required:

- Maternal — 6mL EDTA anticoagulated blood
  6mL clotted
- Paternal — 6mL EDTA anticoagulated blood (18mL for crossmatch)
- Baby — 0.5 to 1mL EDTA anticoagulated blood

Maternal history:

- Ethnic origin
- Medication
- History of thrombocytopenia
- Infant platelet count
- Haemorrhage in infant
- Previous pregnancies - thrombocytopenia in infants?
- Previous transfusions

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Thank you

Any questions?