Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT)

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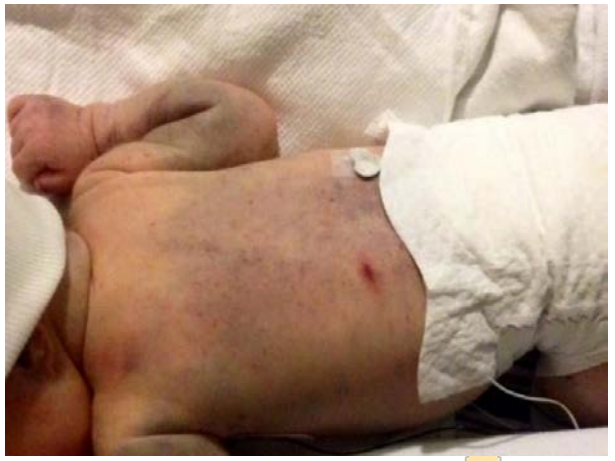
Case Study - Presentation

Pre-term neonate was born with severe thrombocytopenia (<10 x 10^9/L), causing bruising and bleeding.

A cord blood sample is sent for investigation of FNAIT, along with maternal and paternal blood samples.



Images retrieved from: naitbabies.org

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Introduction

Fetal and neonatal alloimmune thrombocytopenia (FNAIT):

- Incompatibility between fetal and maternal platelet antigens.
  - Stimulates production of maternal IgG antibody that crosses the placenta, causing fetal thrombocytopenia
- Occurrence of FNAIT ~1 in 1000 births

Two goals in diagnostic testing for FNAIT:

1. Detect incompatibility between maternal and fetal platelet antigens by HPA genotyping.
2. Detect the presence of maternal platelet alloantibodies.

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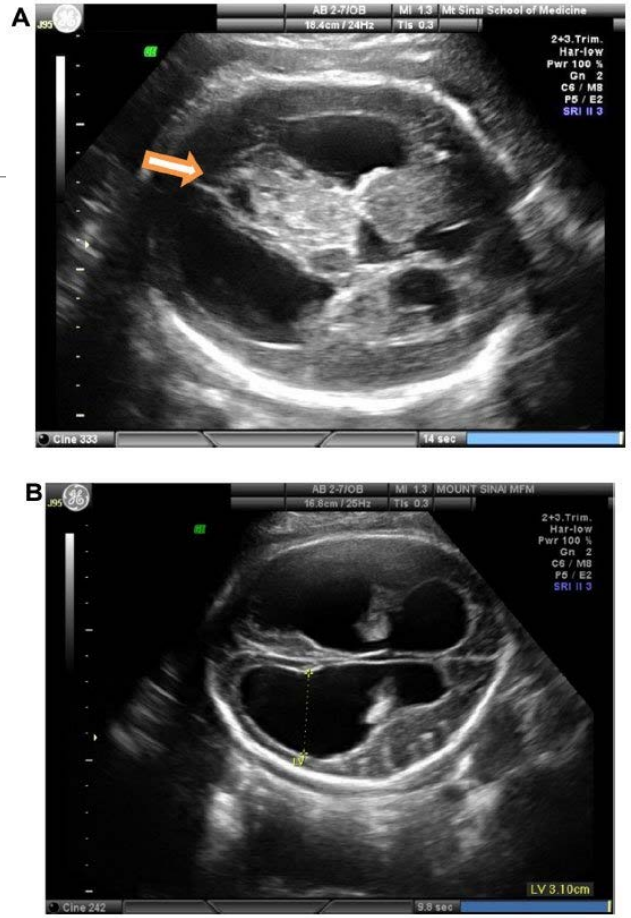
Pathophysiology

- Human Platelet Antigens (HPAs) are expressed by the fetus as early as 16 weeks gestation.
- Fetal platelets can enter maternal circulation and alloimmunization occurs with the production of IgG antibodies against incompatible HPA type.
  - Antibodies cross the placenta and target fetal platelets, and are cleared by reticuloendothelial system.
- Most children affected by FNAIT do not have clinically significant bleeding.
  - However, 20% of cases result in intracranial hemorrhage
    - Fatal and can result in long-term neurological impairment
- Associated incompatible antigen is associated with the severity of the disease.
  - HPA-1a & -3 incompatibility: severe thrombocytopenia & bleeding
  - HPA-5a, -5b, -15a, & -15b: rarely cause severe disease



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Question 1

Which of the following HPA antigen incompatibilities is the most common cause of FNAIT?

a) HPA-1a
b) HPA-3a
c) HPA-5b
d) HPA-15a

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Human Platelet Antigen (HPA)

- Antigens present on platelet surface glycoproteins.
- Polymorphisms due to single nucleotide change (SNP) or in-frame deletion.
- HPA genotyping performed using **PCR techniques**

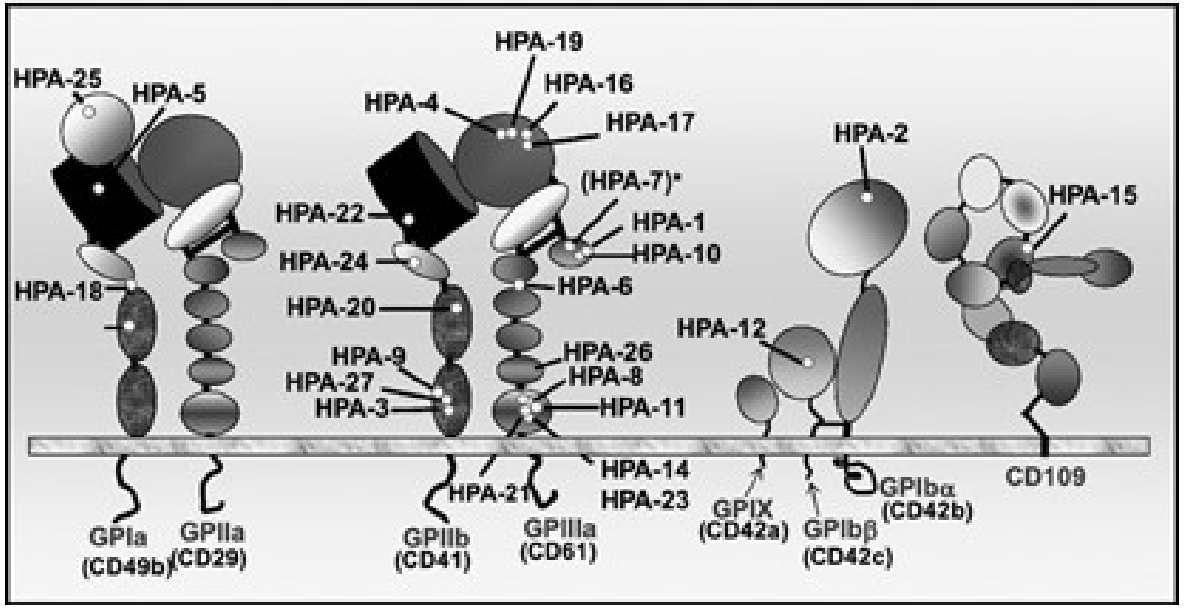

Figure: Curtis & McFarland (2013)

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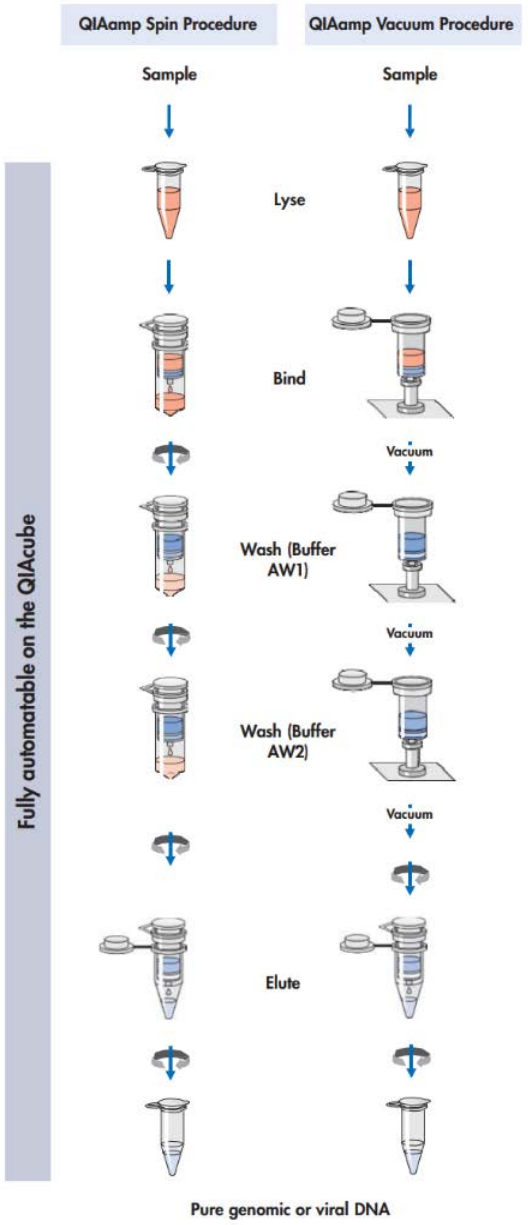

Laboratory Testing – DNA Extraction

- DNA extracted from maternal, paternal, and neonatal/fetal blood samples by solid phase extraction
  - Use spin columns for trapping DNA and removing contaminants
  - Buffers lyse cells in sample and precipitate DNA using ethanol (DNA is trapped in filter in spin column)
  - Wash buffer wash away contaminants
  - DNA is eluted in solution for long-term storage
- DNA concentration & purity:
  - Measure absorbance at 260 nm (for DNA) & absorbance at 280 nm (protein)
  - Ratio of A260/A280 determines purity
  - Optimal DNA purity: 1.8-2.0

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Question 2

What would result in a A260/A280 of 1.6?

a) RNA contamination
b) Protein contamination
c) Incorrect buffers used
d) Incorrect sample type

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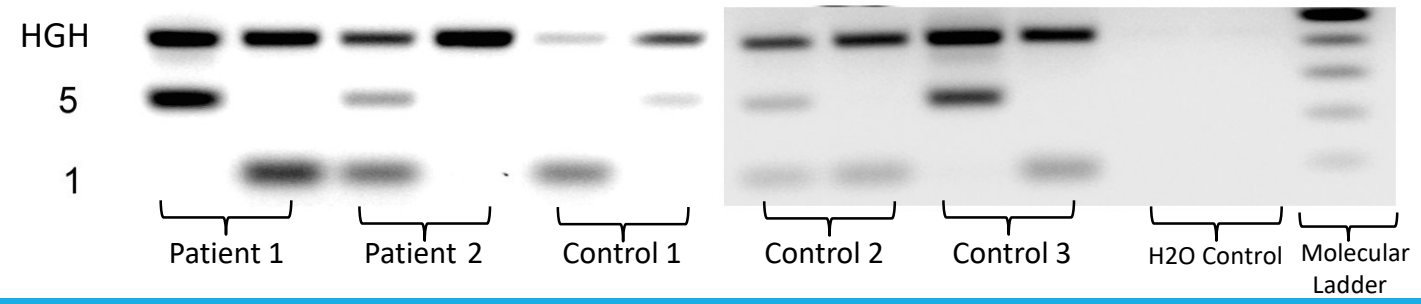
Laboratory Testing – PCR & DNA Gel Electrophoresis

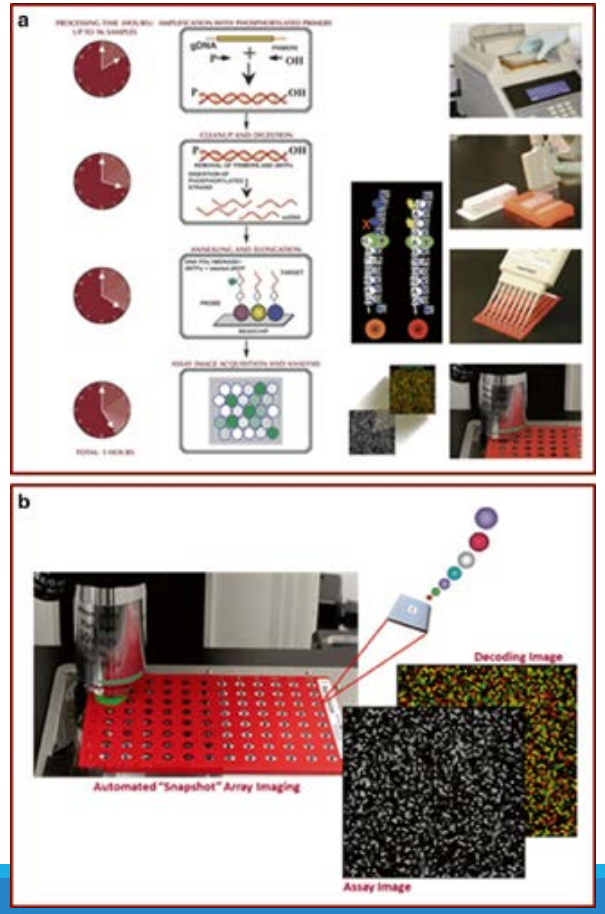
- Once DNA is extracted and the purity is determined, it can be amplified using SSP-PCR & visualized in DNA gel electrophoresis.
- Primers specific to the HPA polymorphisms anneal and amplified the DNA in a thermocycler
- After amplification, DNA is visualized on gel agarose electrophoresis based on length of product

Alleles	a	b	a	b	a	b	a	b	a	b	a	b	
Lanes	1	2	3	4	5	6	7	8	9	10	11	12	13

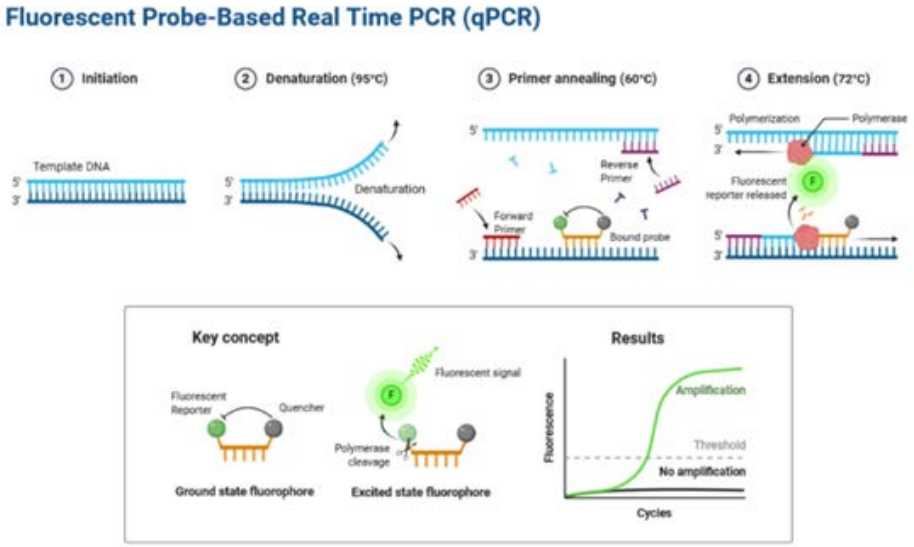


Other Laboratory Techniques

Bead Chip Microarray



qPCR using Fluorescent Probes



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Case Study - HPA Genotype Results

Maternal genotype: 1bb,2aa,3bb,5aa,6aa,9aa,15bb

Paternal genotype: 1aa,2aa,3ab,5aa,6aa,9aa,15bb

Neonatal genotype: 1ab,2aa,3bb,5aa,6aa,9aa,15bb

Question 3: Are there incompatibilities between mother and neonate?

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Case Study - HPA Genotype Results

Maternal genotype: 1bb,2aa,3bb,5aa,6aa,9aa,15bb

Paternal genotype: 1aa,2aa,3ab,5aa,6aa,9aa,15bb

Neonatal genotype: 1ab,2aa,3bb,5aa,6aa,9aa,15bb

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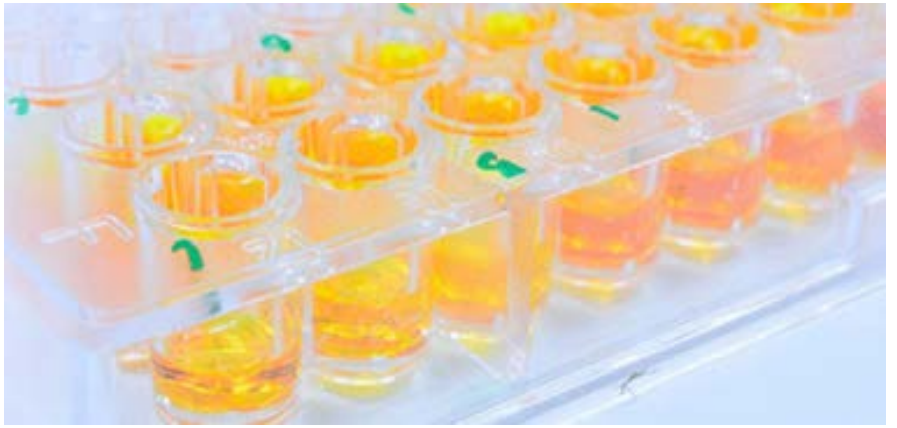
Laboratory Testing for Platelet Antibodies

Platelet glycoprotein-specific assays

- Enzyme Linked Immunosorbent Assay (ELISA):
  - Commonly used
  - Easy to perform
  - Limitations: cannot detect new HPA antibodies, more costly
- Radioimmunoprecipitation (RIP):
  - Higher sensitivity
  - Can detect new antibodies
  - Limitations: cost, technical expertise required, handling radioactive materials



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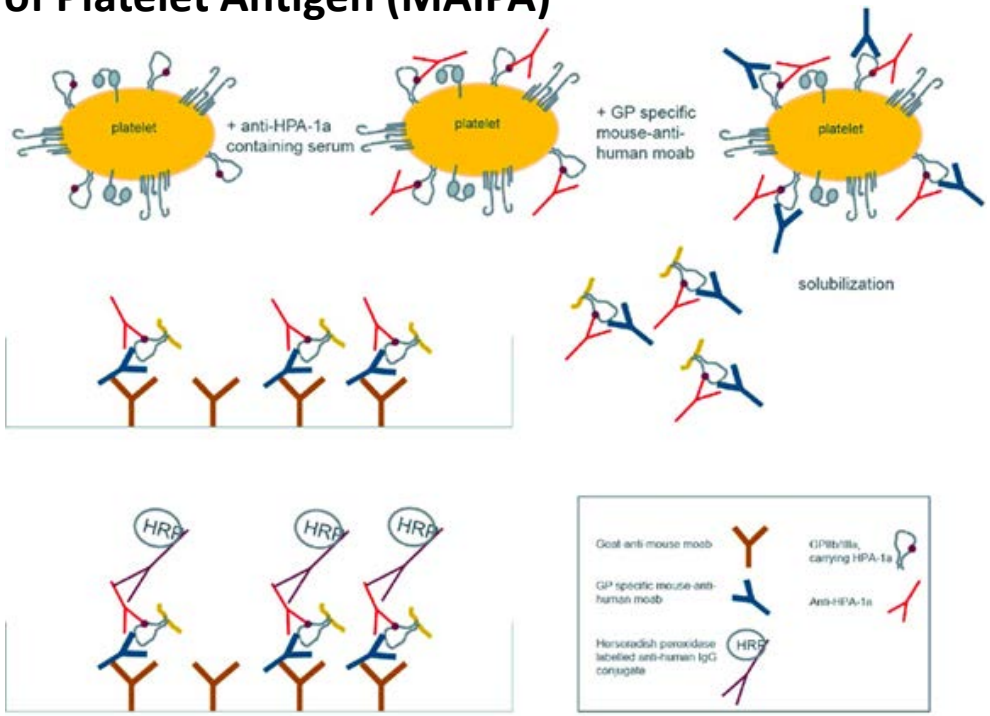
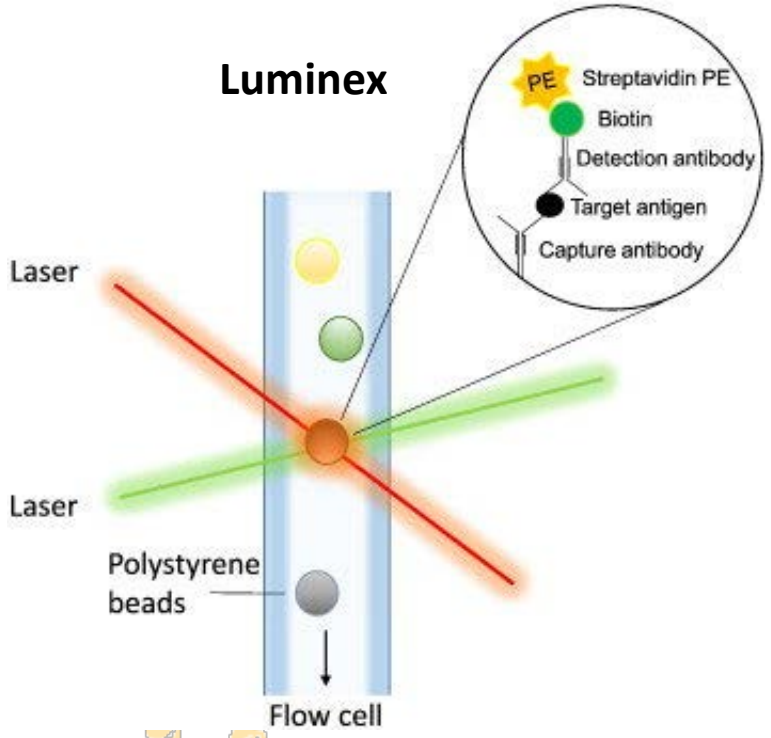
Other Laboratory Techniques

Monoclonal Antibody-Specific Immobilization of Platelet Antigen (MAIPA)



Luminex



Case Study - HPA Antibody Results

Based on testing performed by ELISA kit and in the RIP assay, **anti-HPA-1a** was detected in the **maternal** serum.

Question 4

Which disease is considered the red cell counterpart to FNAIT?

a) Autoimmune hemolytic anemia
b) Drug-induced hemolytic anemia
c) Delayed hemolytic transfusion reaction
d) Hemolytic disease of the fetus and newborn

HDFN vs. FNAIT

	HDFN	FNAIT
Maternal alloimmune sensitization of IgG antibodies	Yes	Yes
Fetal/neonatal symptoms	Anemia, cardiac failure, erythroblastosis fetalis	Thrombocytopenia, bleeding, intracranial hemorrhage
Are first pregnancies affected?	No	Yes
Screening programs available?	Yes	No

Treatment

- IVIG
  - Women with infants previously affected with FNAIT:
    - Previous fetus with ICH: antenatal dose of IVIG 2 g/kg at 12-16 weeks
    - No previous fetus with ICH: antenatal dose of IVIG 1 g/kg at 20-26 weeks
  - Neonates:
    - IVIG 1 g/kg
- Platelets
  - Neonates:
    - To treat severe thrombocytopenia and/or bleeding
    - HPA antigen negative for antibody identified
      - If not readily available, random donor platelets can be given

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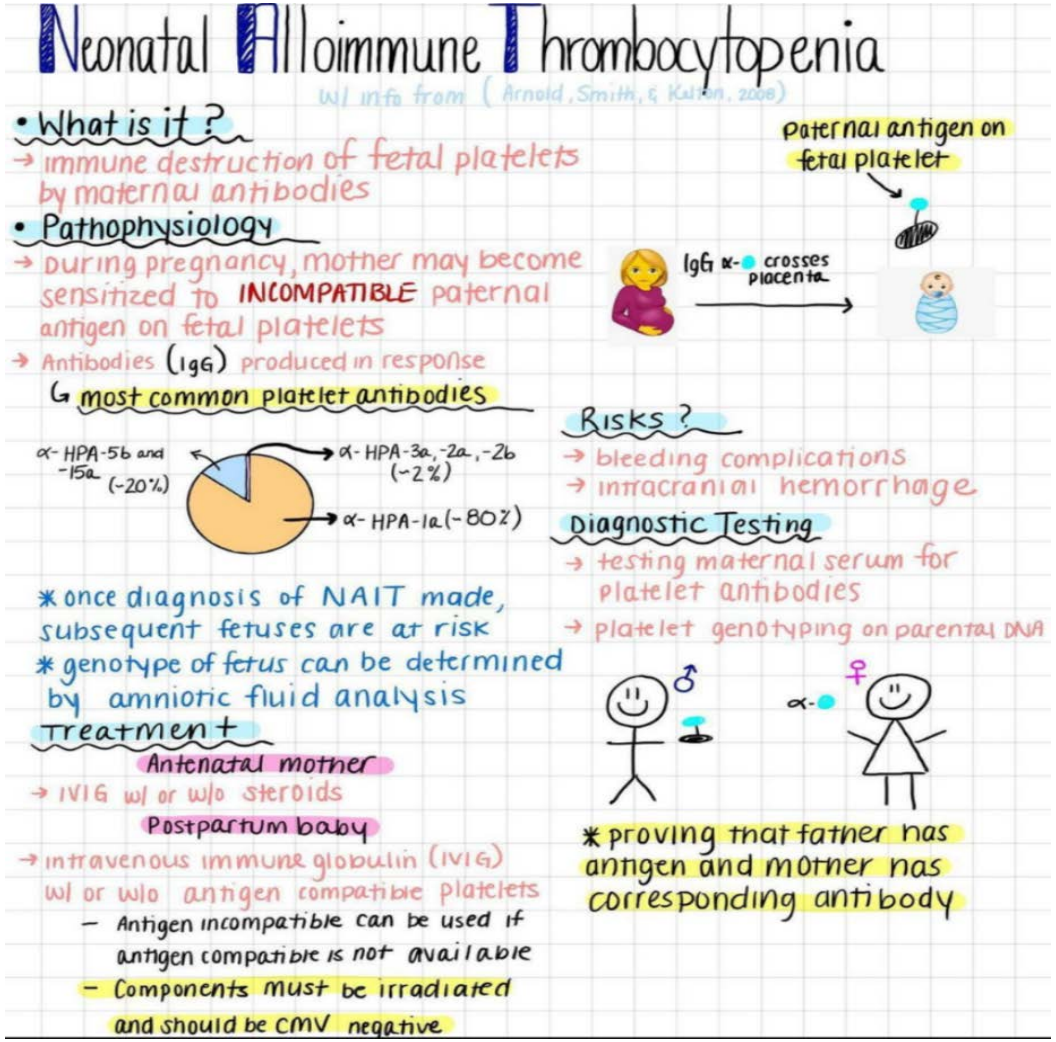
Case Study - Treatment

- In our case study, neonate was given three platelet transfusions
  - Not much improvement in platelet count
- IVIG given & platelet count improved drastically

Future pregnancies:

- Mother closely monitored: IVIG given antenatally
- C-section: HPA-1bb platelets available

Thank You!



By @pathlabandrew & @medlabmaria