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PEARLS OF LABORATORY MEDICINE

# Fetal Maternal Bleed Testing Using Flow Cytometry

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# Hemolytic Disease of the Fetus and Newborn

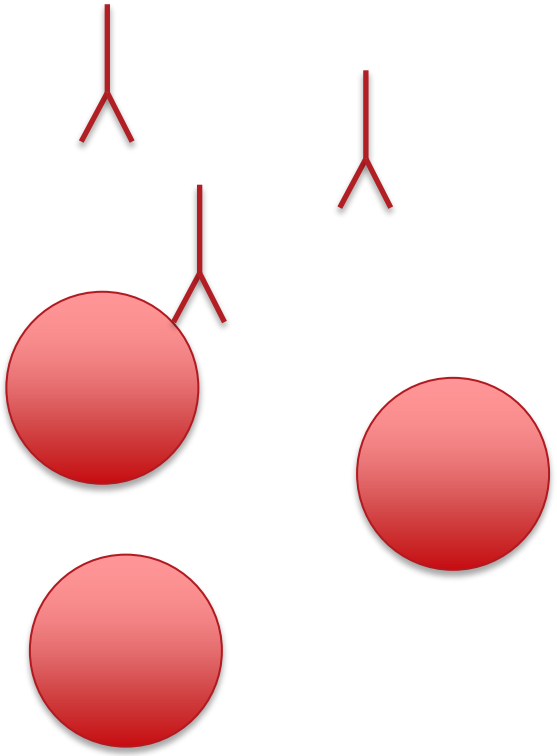
Is a clinical disorder in which fetal red blood cells are destroyed by maternal antibodies targeting paternal antigens expressed on fetal red blood cells

Hemolysis may lead to serious and sometime fatal consequences in utero and after birth

# Pathophysiology

Immunizing event:

- Typically occur during labor and delivery
- Other events
  - Trauma
  - Procedures
  - Abortions





Maitra A. Diseases of Infancy and Childhood. In: Kumar V, Abbas AK, Fausto N, Aster JC, editors. Robbins and Cotran Pathologic Basis of Disease. 8th Ed. Philadelphia (PA): Saunders Elsevier; 2010. p. 447-83. Used with permission.

## Treatment

- Bilirubin lights
- Blood transfusions
  - In utero
  - Exchange
- NICU care

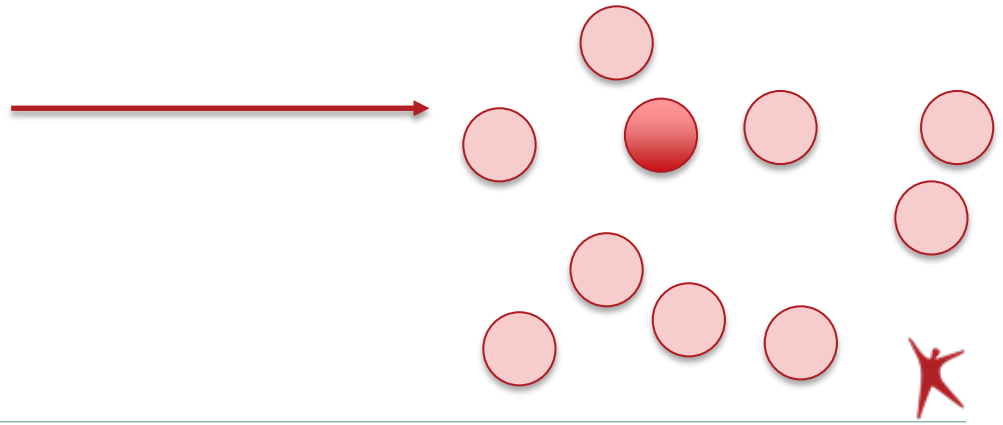
- Morbidity and Mortality is still significant

## Prevention

- 1960's realization that antibody targeting Rh D can prevent most HDNF
- RhIg
  - Anti-D
  - Human sources
- Key to prevention
  - Recognize need
  - Timing
  - Dose

# Qualitative and Quantitative Testing

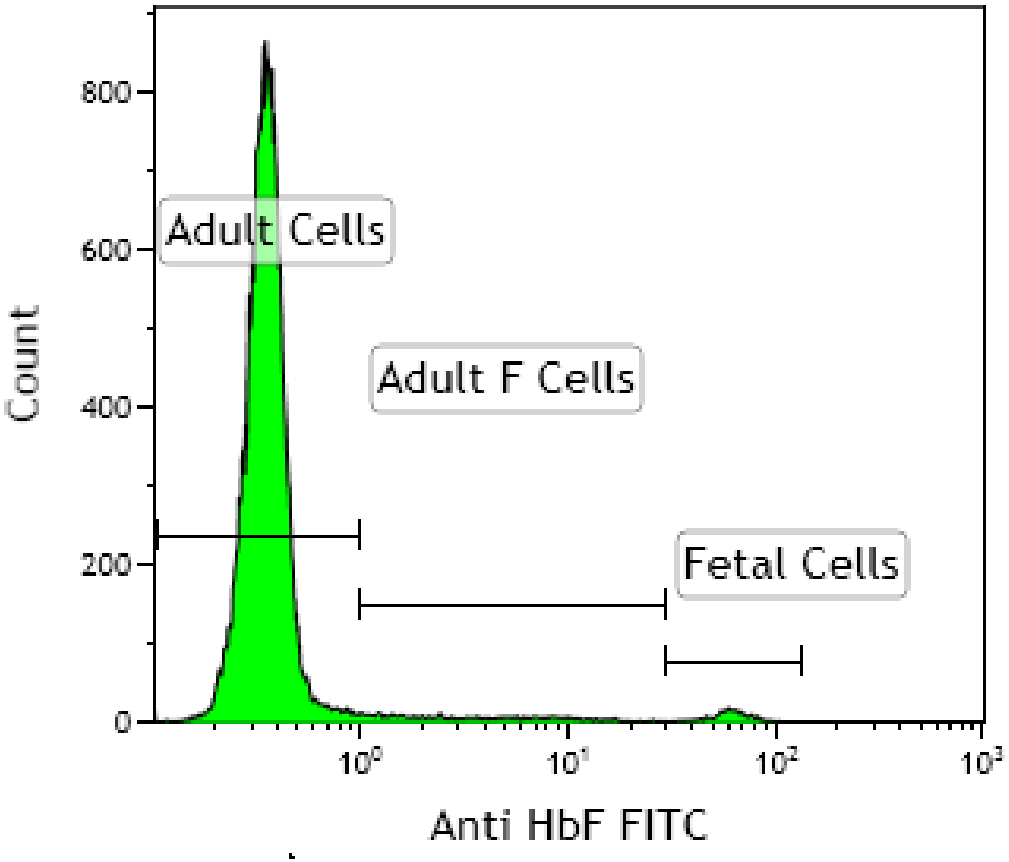
- Typical Coverage
  - 30 ml bleeds (15 ml rbc)= 1 vial RhIg
- Qualitative
  - RosetteTest- to determine if more than 1 vial of RhIg
- Quantitative testing
  - Kleihaur Betke
  - Flow Cytometry



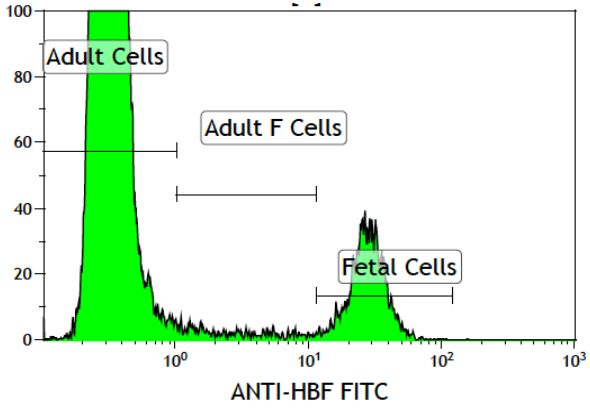


Haemolytic disease of the fetus and the newborn. In: Klein HG, Anstee DJ, editors. Mollison's Blood Transfusion in Clinical Medicine, 12th Ed. Chichester (West Sussex, UK): John Wiley and Sons; 2014. p. 499-548.

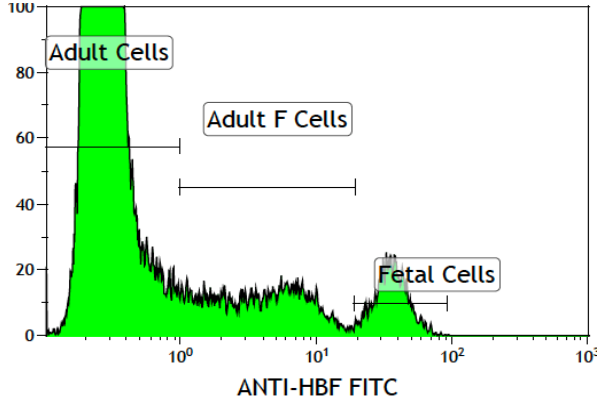
# Flow cytometry



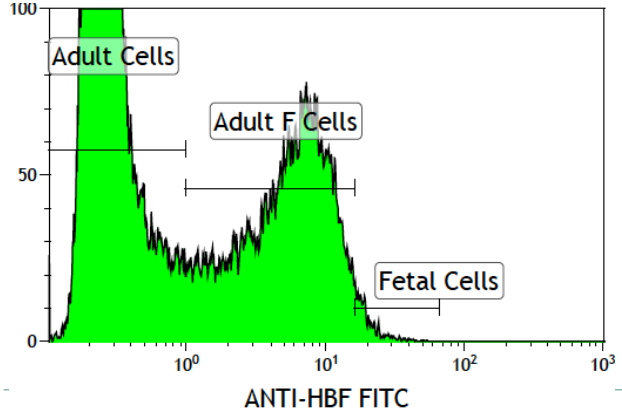

- Distinguish adult vs fetal
  - Intracellular Hgb
    - Fetal Hgb
    - Adult Hgb
      - Fetal
  - Intensity correlated to control cells



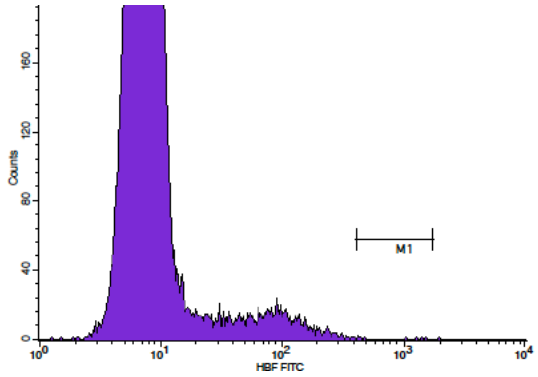

Large true bleed
5.4 %



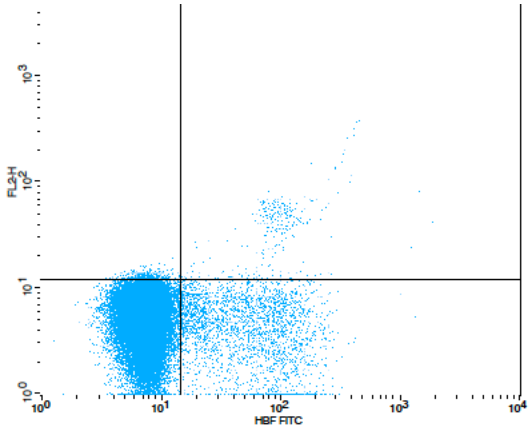

Interfering adult ‘f’ cells
3.3% fetal
7.0% adult



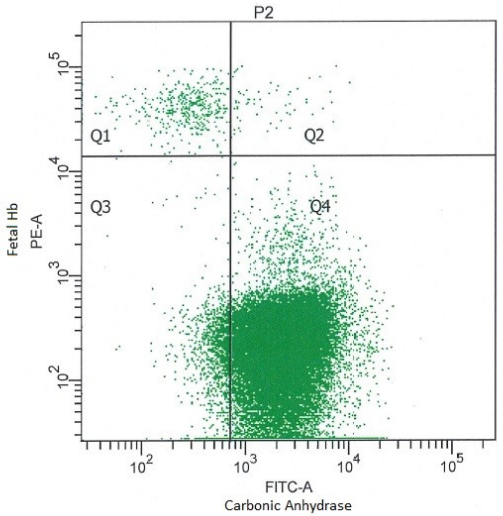

Shoulder due to ‘f’ cells
1.0% fetal
24%  adult




One anti-body:
Gating is key
anti-HgF
? Adult F-cells




2 antibodies
Helps to identify true fetal cells



# Draw backs

- Partial D's
  - Mother
  - Child
- Prior use of anti-D
  - High dose may mask antigen

- Carbonic Anydrase and  Ag with differential age-based expression

# Calculations

Fetal RBC= (Blood Volume)(Percentage Fetal RBC)

RhIg= (Fetal Blood)(1 vial RhIg/30ml whole blood)+1

# Question

Assuming the mother is 70kg and the child is Rh Positive, 1% bleed requires how much RhIg?

1. 1 vial
2. 2 vials
3. 3 vials
4. 4 vials

FMB= (Blood Volume)(% fetal cells)
FMB= (70kg)(70ml/kg)(0.01)
FMB= (4900ml)(0.01)
FMB= 49ml

RhIg=(FMB)(1 vial RhIg/30 ml rbc) +1
RhIg=(49ml fetal cells)(1 vial RhIg/30 ml rbc) +1
=1.6 vials+1 vial
=2+1
= 3 vials

# Which scenario does not require RhIg in an Rh negative mother?

1. Spontaneous abortion at 2nd trimester
2. MVA with FMB detected in 3rd trimester
3. Normal term delivery with an Rh negative fetus and 35ml FMB
4. Normal term delivery with an Rh positive fetus and negative Rosette test
5. Normal term delivery with an Rh positive fetus and a planned hysterectomy

Correct answer: 3.

# References


1. Haemolytic disease of the fetus and the newborn. In: Klein HG, Anstee DJ, editors. Mollison's Blood Transfusion in Clinical Medicine, 12th Ed. Chichester (West Sussex, UK): John Wiley and Sons; 2014. p. 499-548.
2. Maitra A. Diseases of Infancy and Childhood. In: Kumar V, Abbas AK, Fausto N, Aster JC, editors. Robbins and Cotran Pathologic Basis of Disease. 8th Ed. Philadelphia (PA): Saunders Elsevier; 2010. p. 447-83.
3. Delaney M, Svensson AM, Lieberman L. Perinatal issues in transfusion practice. In. Fung MK, Eder AF, Spitalnik SL, Westhoff CM. Technical Manual. 19th Ed. Bethesda (MD); 2017. p. 599-612.

# Disclosures/Potential Conflicts of Interest

*Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:*

- **Employment or Leadership**: employee of the Mayo Clinic which offers Fetal Maternal Bleed testing through its reference laboratory.
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- **Stock Ownership:** No disclosures
- **Honoraria:** No disclosures
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- **Patents:** No disclosures

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