ORBCoN
Ontario Regional Blood Coordinating Network

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Fetal maternal hemorrhage testing: the **why, how & when**

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Faculty Disclosure

In compliance with CPD policy, Temerty Faculty of Medicine requires the following disclosures to the session audience

- This program has received no financial external support
- Heather VanderMeulen has no relevant conflicts of interest to disclose

Objectives

1. **WHY:**
   Understand why to use FMH testing for RhD neg and RhD pos patients

2. **HOW:**
   Appreciate 3 key methods of FMH testing, their value and limitations

3. **WHEN:**
   Consider when to send for flow cytometry

WHY TEST

1) **RhD negative mother**
2) RhD positive (or negative) mother

FMH testing guides RhIg dosing in RhD- patients

> 20 weeks GA **AND** Potentially sensitizing event

↳ **Feto-placental blood volume is 30 mL at 20 wks**

Goal is to identify fetal bleed >30 mL that needs EXTRA RhIg

BCSH Guidelines 2014
AABB Technical Manual
SOGC Guidelines: Prevention of Rh Alloimmunization 2018

Fetal-Placental Volume vs. Gestational Age

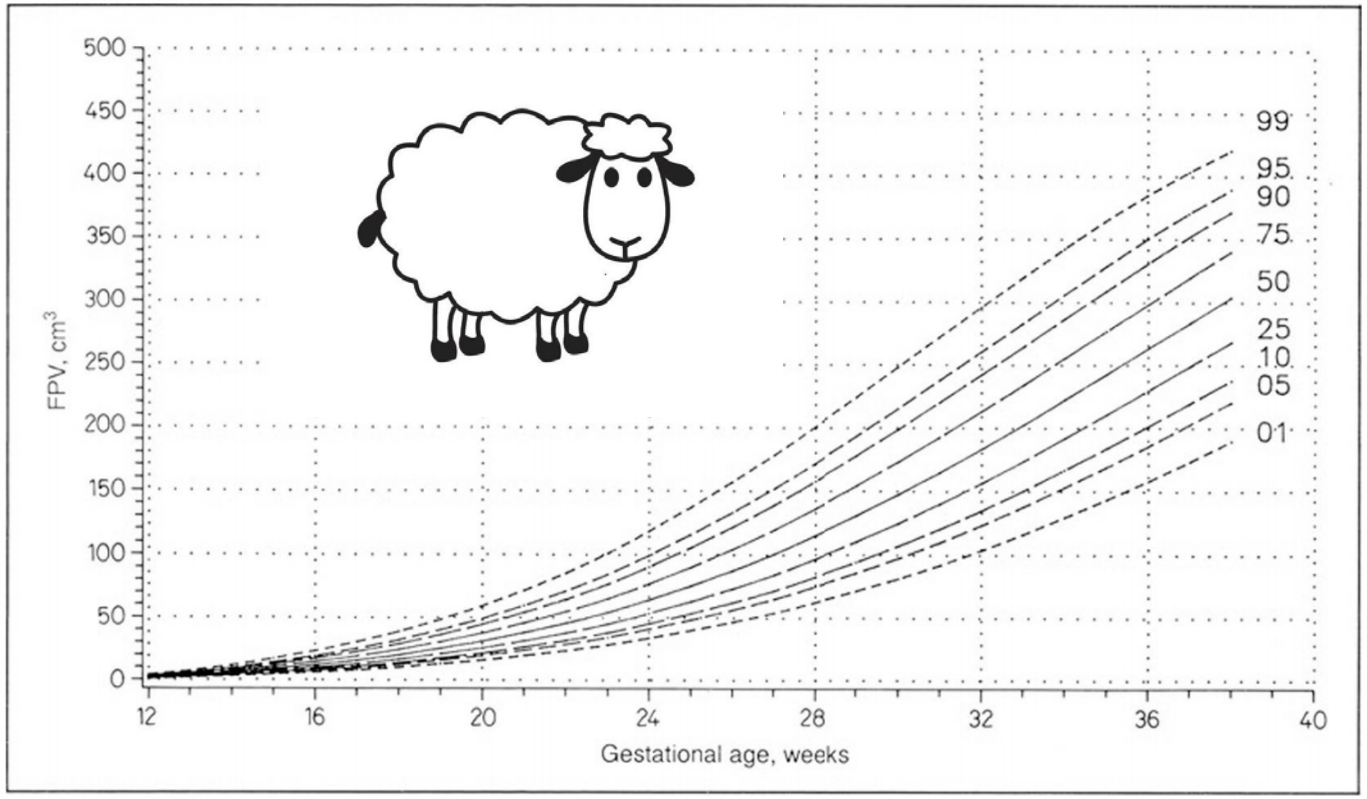


Fig. 3. FPV versus gestational age; 99th to 1st percentiles obtained using exponential model derived from the data of Brenner et al. [11].

Leduc et al., Fetal Diagn Ther, 1990

WHY TEST

1) RhD negative mother
2) **RhD positive (or negative) mother**

Fetal demise

- Prospective cohort study of 1025 fetal deaths ≥20 weeks GA
  - FMH contributed in 10.6%

- SOGC 2020 Guideline for Stillbirth Investigation: consider KB testing

Korteweg, F. J., et al. (2012). Evaluation of 1025 fetal deaths: proposed diagnostic workup. American Journal of Obstetrics and Gynecology, 206(1), 53.e1-53.e12.

Guideline No. 394-Stillbirth Investigation, SOGC Clinical Practice Guideline, January 2020

Maternal trauma

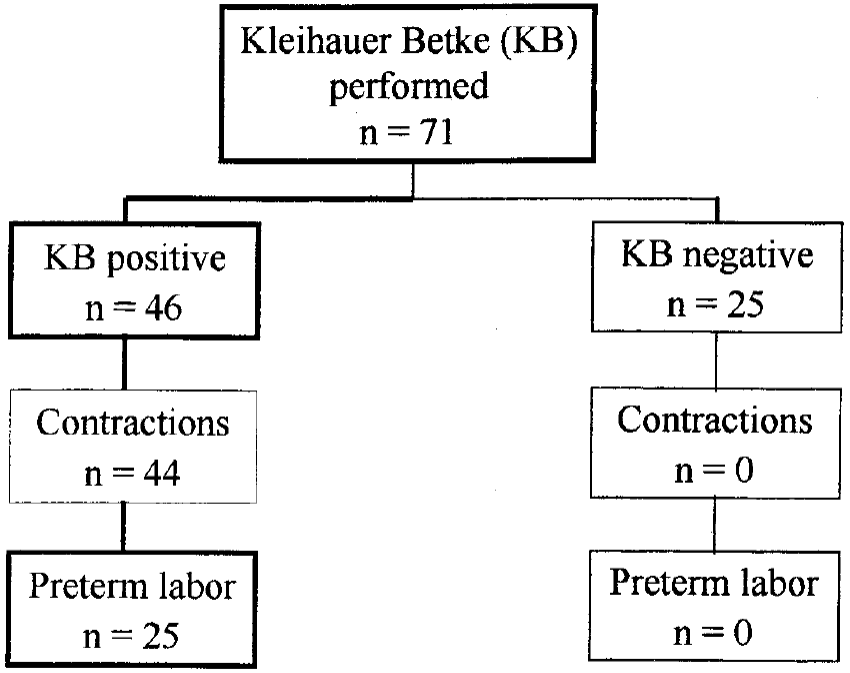


Fig. 1. *Distribution of women.*

+ KB test: LR 20.8 for preterm labor (p < 0.001)

Muench et al., J Trauma, 2004

Maternal trauma

- 98 low-risk 2nd trimester pregnant women
  → 5.8% positive KB

- 151 pregnant trauma patients
  → 2.6% positive KB

- P=0.31

Dhanraj D & Lambers D. Am J Obstet Gynecol. 2004 May;190(5):1461-3.

Fetal outcomes in FMH

Diagnostic accuracy of Kleihauer–Betke (Kb) testing to predict fetal outcomes associated with fetomaternal hemorrhage: a retrospective cohort study

Melanie C. Audette[1,2]✉, Katie Mclean[1,2], Niyati Malkani[2], John Kingdom[1,2] and M[illegible]

- 662 women with fall/abdominal trauma/s[illegible] 641 (97%) KB negative vs. 22 (3%) KB positive
- 1º composite outcome (prete[illegible] GR delivery, fetal anemia requiring transfusion, NICU admission, IUFD, neona[illegible]
- No difference in 1º composite ou[illegible]me between KB negative and KB positive (30% vs. 36%, p=0.54)

No role?

Journal of Perinatology, 2022

SOGC Guidelines for the Management of a Pregnant Trauma Patient (2015)

Though **electronic fetal monitoring is more likely to be clinically useful** in rhesus-positive women, **KB test can be considered** as an optional test to determine the need for prolonged monitoring.

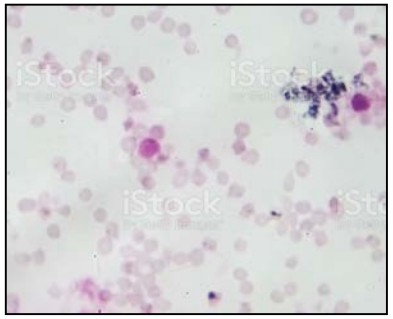
J Obstet Gynaecol Can 2015;37(6):553–571

HOW TO TEST

Selecting the right tool for the job.

How do we detect FMH?

Test	Mechanism	Outcome	Limitations	Turnaround
Rosette	1) Incubate maternal whole blood & anti-D →D+ fetal cells bound 2) Enzyme indicator cells added and bind to anti-D-bound cells → “Rosetting”	Qualitative	• Mother must be D- (false + if weak D) • Fetus must be D+ (false - if weak D) • False + if DAT+	• 1-2 hours



Krywko DM, Yarrarapu SNS, Shunkwiler SM. Kleihauer Betke Test. StatPearls 2020; Cardosa et al., Biomed Research International, 2018

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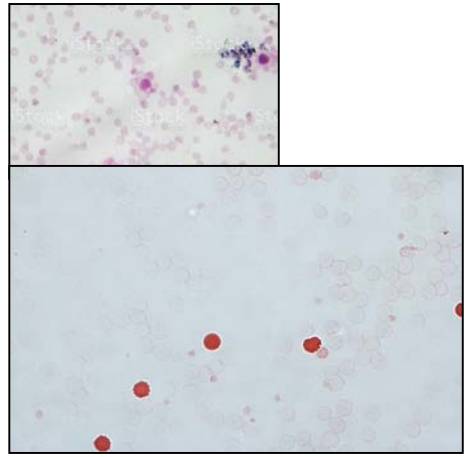
Kleihauer-Betke	• HbF is resistant to acidic denaturation unlike HbA • Fetal RBCs remain intact, adult RBCs turn to "ghosts" • 2000 cells counted	Qualitative vs. Quantitative	• ↑ HbF can give false + • Inter-user variability	• 2 hours



Krywko DM, Yarrarapu SNS, Shunkwiler SM. Kleihauer Betke Test. StatPearls 2020; Cardosa et al., Biomed Research International, 2018

KB test limitations: Inter-user variability

- Imprecision:
  - 2000 cells counted
    - COV 50%
  - 10 000 cells counted
    - COV 18%
  - Anti-HbF flow cytometry (50 000+ cells counted)
    - COV <2%
- CAP Surveys:
  - KB COV 32-80%
  - FC COV <20%
  - 10% of labs using KB reported a 40 mL bleed <30 mL vs. none of labs using FC
  - 50% of labs using KB reported a 20 mL bleed as >30 mL vs. none of labs using FC

AABB Technical Manual: count 2000 cells

Gielezynska et al., 2016; Davis et al. Laboratory Medicine 2007

Variables Contributing to Over- or Underestimation of FMH by the KB Test

Factors leading to overestimation	Factors leading to underestimation
Presence of F cells (1/4 pregnant patients in 2nd tri, HPFH, SCD, thalassemia)	Failure to adjust for ↑ maternal circulating volume (if >70 kg)
Ghost cells may be difficult to identify	Incomplete staining of fetal cells (only 90% stain dark pink)

Kim & Makar, AJH 2012;

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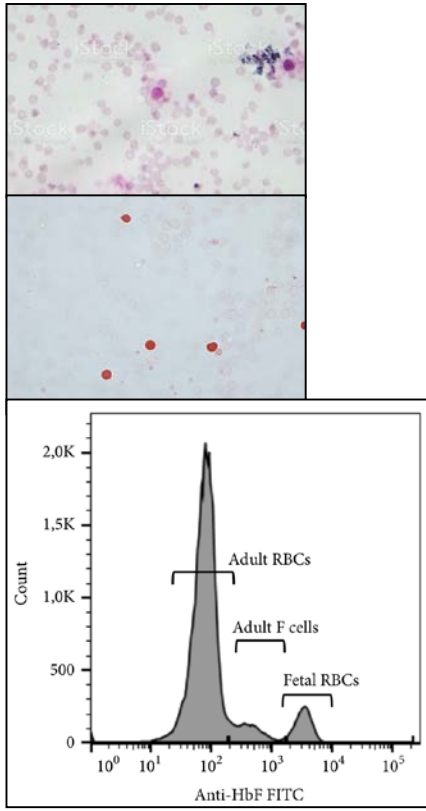
Flow cytometry	• Monoclonal antibodies targeted vs. HbF	Quantitative (most accurate, precise, sensitive & specific)	• Expensive • Availability • Validation for FMH	• Centre-specific



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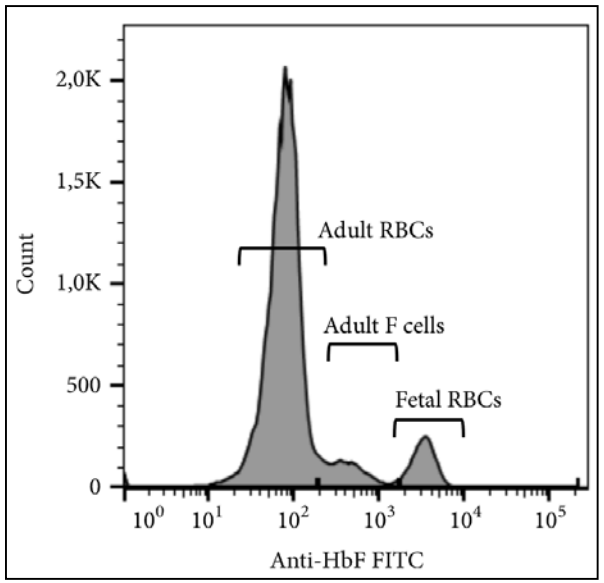
WHEN TO GO WITH THE FLOW

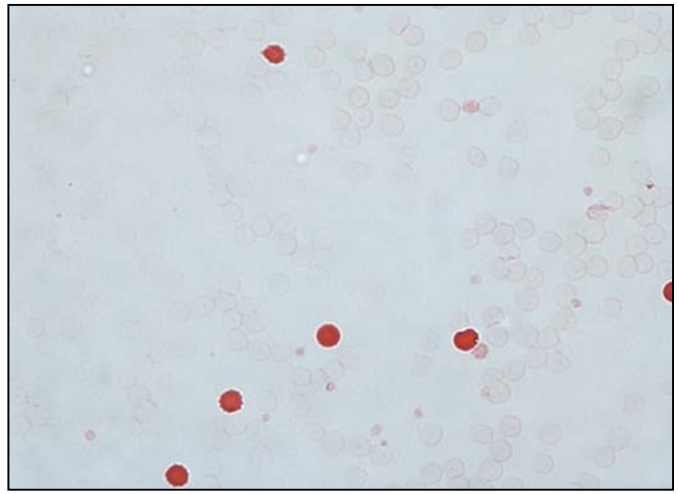
When to “go with the flow”

1.↑ maternal hemoglobin F:

persistence of fetal hemoglobin, sickle cell, β thalassemia



Flow distinguishes maternal F cells vs. fetal cells



KB does <u>not</u> distinguish maternal F cells vs. fetal cells

Kim & Makar, AJH 2012

When to “go with the flow”

2. Assessment of +KB:

- KB can both overestimate (more commonly) or underestimate FMH
- 19% of large bleeds (>10 mL) received inadequate RhIg due to KB readings
- Sensitivity of KB for FMH >30 mL by flow cytometry:

KB result cut-off	Sensitivity	Specificity	Accuracy
10 mL	92% (22/24)*	81% (271/334)	82%
20 mL	92% (22/24)*	91% (304/334)	91%
30 mL	87% (21/24)	93% (331/334)	93%

* Sensitivity excluding 2 adult HbF cases: 100%

- Most important to send for flow if KB shows large bleed (>10 mL)

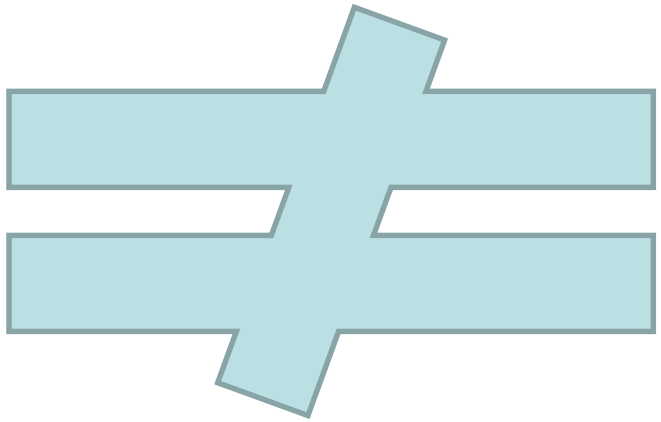
Lafferty et al., 2003 ; Nance et al., 1988; Kim & Makar, AJH 2012 ; Abstract: Dube et al., AABB 2020

When to “go with the flow”

3. Discrepancy between KB and fetal status

- Distinguish F cells (↑ in ¼ pregnant women in 2nd tri) vs. fetal cells

- A fetus with large FMH will not look “well”



Kim & Makar, AJH 2012

Conclusions

- **WHY:**
  - RhD negative mother: identify the need for extra RhIg
  - RhD negative or positive mother: fetal demise ?maternal trauma

- **HOW:**
  - Rosette: Fast, available, inexpensive, attention to maternal/fetal D expression
  - KB: High inter-user variability, large bleeds may be underdosed with RhIg, caution with hemoglobinopathies
  - Flow cytometry: Most sensitive, precise, sens & spec. but limited availability/slow turnaround time /$$$

- **WHEN to use flow:**
  - High maternal HbF
  - +KB → especially if large bleed suspected
  - Discrepancy between KB and baby

Questions?
Comments?

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