

**IMMUNOCYTOMETRIC QUANTITATION  
OF RHD POSITIVE FETAL RBC  
IN BLOOD OF RHD NEGATIVE MOTHERS  
USING ABBOTT CELL DYN SAPPHIRE**

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***COMPARATIVE STUDY USING  
KLEIHAUER TEST AS REFERENCE***

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# RHD HAEMOLYTIC DISEASE OF THE NEWBORN (1)

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## THE DISEASE

Hemolytic Anemia (risk of fetal death)

Neonatal hyperbilirubinemia (risk of Kernicterus)

## MECHANISM

Hemolysis of RHD positive RBC coated with maternal IgG anti-D antibodies transferred via the placenta.

## ORIGIN OF MATERNAL ANTI-D PRODUCTION:

Fetal RHD positive RBC entering the circulation of RHD negative mother during pregnancy or at delivery :

foeto-maternal haemorrhage or FMH



# RHD HAEMOLYTIC DISEASE OF THE NEWBORN (2)

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## CURATIVE TREATMENT

**Antenatally** : Fetal (cord) transfusion of RHD negative RBC  
Premature delivery

**Postnatally** : Transfusion, Phototherapy

## PREVENTIVE TREATMENT: RH Immunoprophylaxis

(Immune neutralisation of RHD positive fetal RBC)

Ante and postnatal administration of RHD Immunoglobulin in RHD negative pregnant women

# RHD HAEMOLYTIC DISEASE OF THE NEWBORN (3)

FRANCE	INCIDENCE (for 1000 births)	MORTALITY & SEVERE MORBIDITY
<u>1966</u>	<p>5 to 6 ‰</p> <p>↓</p> <p>RHD immunoprophylaxis (RH immune globulin)</p> <p>↘ of fecondity index</p> <p>↓</p>	<p>≥ 12% of cases</p> <p>↓</p> <p>Biological antenatal screening</p> <p>Improvements in neonatal and fetal medecine</p> <p>IV fetal transfusion</p> <p>↓</p>
<u>2006</u>	<p>≈ 0,8 ‰ (700 cases per year)</p>	<p>≈ 4% of cases</p>



# IMMUNOSUPPRESSIVE EFFICACY of RHD Immunoglobulin RELIES ON :

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- **The delay of RHD Ig administration** after passage of RHD antigen in maternal circulation: optimal administration time  $\leq 72$  hours
- **The ratio of anti-D IgG/volume of fetal RHD+ RBC:**
  - **Anti-D IgG  $\geq$  at 20  $\mu\text{g}/\text{mL}$  RBC: 100% efficacy**

Optimal ratio derived from 9 studies of dose efficacy in RHD negative male volunteers:

    - Anti-D IgG  $\leq 5$   $\mu\text{g}/\text{mL}$  RBC : no or minimal efficacy
    - Anti-D IgG = 10 to 17.4  $\mu\text{g}/\text{mL}$  RBC : sub-optimal efficacy



# RH IMMUNOPROPHYLAXIS RECOMMENDATION

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- **GUIDELINES** exist throughout Europe for the use of RHD Ig
- The French guidelines (updated in 2005) require:
  - For 100 % efficacy, the patient must received at least 20  $\mu\text{g}$  anti-D IgG/ml of fetal RHD+ RBC  
*(example : For an hemorrhage of 50 ml fetal RBC :  
1000  $\mu\text{g}$  anti-D IgG (i.e 50 x 20  $\mu\text{g}$ )*
  - Quantification of FMH to adapt the dose in case of :
    - Delivery
    - Fetal death
    - Abdominal trauma



# QUANTITATION OF FMH

- **KLEIHAUER TEST** is performed **routinely** for quantitation of FMH in our laboratory

*September 2005 to November 2006 : 6490 Kleihauer tests*

	<i>NUMBER</i>	<i>FREQUENCY</i>
O Fetal RBC/ 10 000 Maternal RBC	4899	76 %
< 1 to 273 Fetal RBC/10000 maternal RBC	1369	21 %
Uninterpretables cells*	222	3 %

- **PROBLEMES WITH KLEIHAUER TEST:**

- .Manual technique, without standardization, requiring trained technicians.

- .Difficulties to differentiate fetal cells from maternal cells with High content of HbF (\*false positive or uninterpretable smears)



# AIM OF THE STUDY

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## TO VALIDATE AN INTERESTING ALTERNATIVE TO QUANTITATE FMH USING AN IMMUNOCYTOMETRIC METHOD

- Technique derived from the Abbott Cell-Dyn CD4000 (*Little, 2005*) :  
Using a Fluorescein Isothiocyanate-labelled monoclonal anti-RHD (BRAD3)
- Correlation of this technique adapted to the *Cell Dyn Sapphire* with the Kleihauer test in selected clinical specimen with or without FMH
- Determination of Low Limit of quantification (LLQ)
- Testing the impact of the number of aspirations with *Cell Dyn Sapphire*





# MATERIAL AND METHOD

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## MATERIAL :

- Comparative analysis of 86 selected clinical blood specimens of RHD negative mothers at delivery based on Kleihauer test (63 pos, 23 neg)

## METHOD :

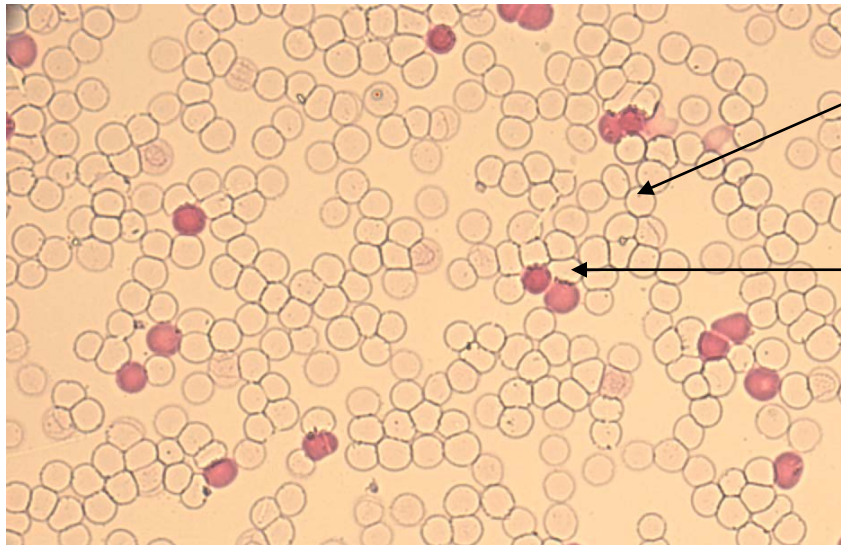
### KLEIHAUER TEST

- . Blood smear : fixed in alcohol (10')
- . Acidic elution at 41 °C (5')
- . Staining with Phloxin (10')
- . Microscopic examination (x25)

### *CELL-DYN SAPPHIRE*

- . Whole Blood : 50  $\mu$ l
- . Anti-RHD FITC (BRAD3) : 5  $\mu$ l
- . Incubation (30' at 37 °C)
- . Addition of 100  $\mu$ l of sapphire diluent
- . 3 aspirations
- . Data collection
- . Analysis of data : FCS Express Software

# RESULTS : KLEIHAUER TEST



**ADULT RBC : de-haemoglobinized  
appear as « ghosts »**

**FETAL RBC : still haemoglobinized  
are staining by phloxin**

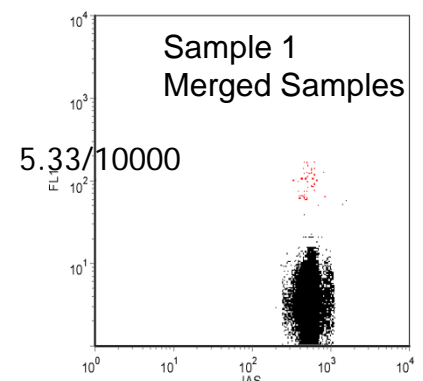
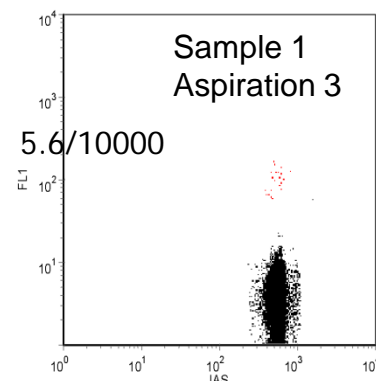
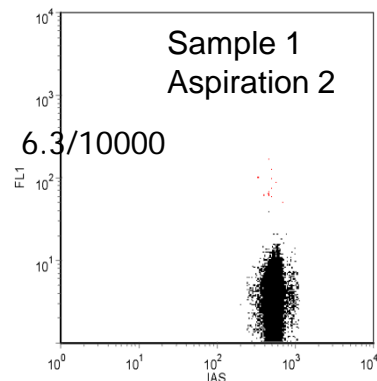
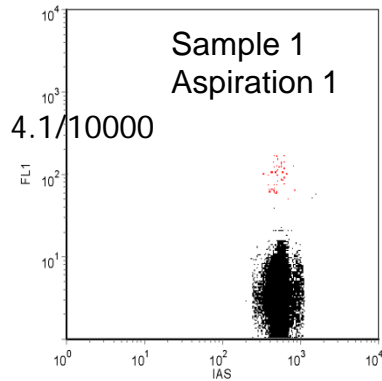
**Result of a Kleihauer test at 500 fetal RBC /10 000 Maternal RBC : 250 ml of fetal blood**

**Duration of testing : 1 hour**

# RESULTS : CELL DYN SAPPHIRE

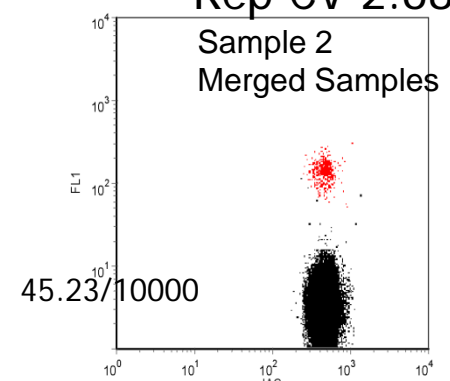
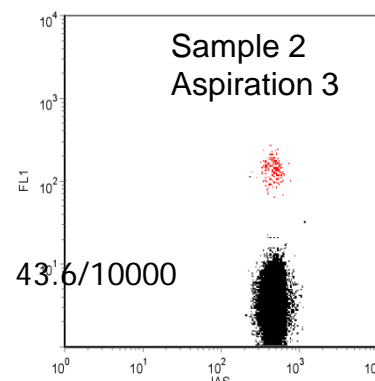
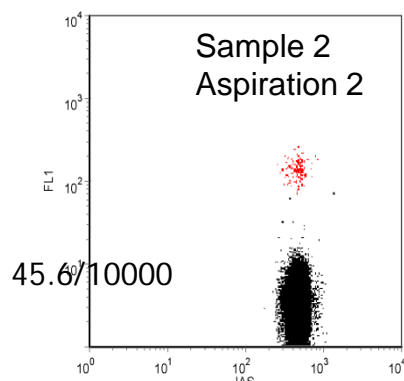
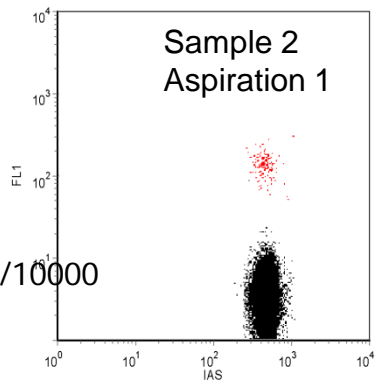
**Sample 1: KT 3/10,000 RBC**

Rep CV 17 %



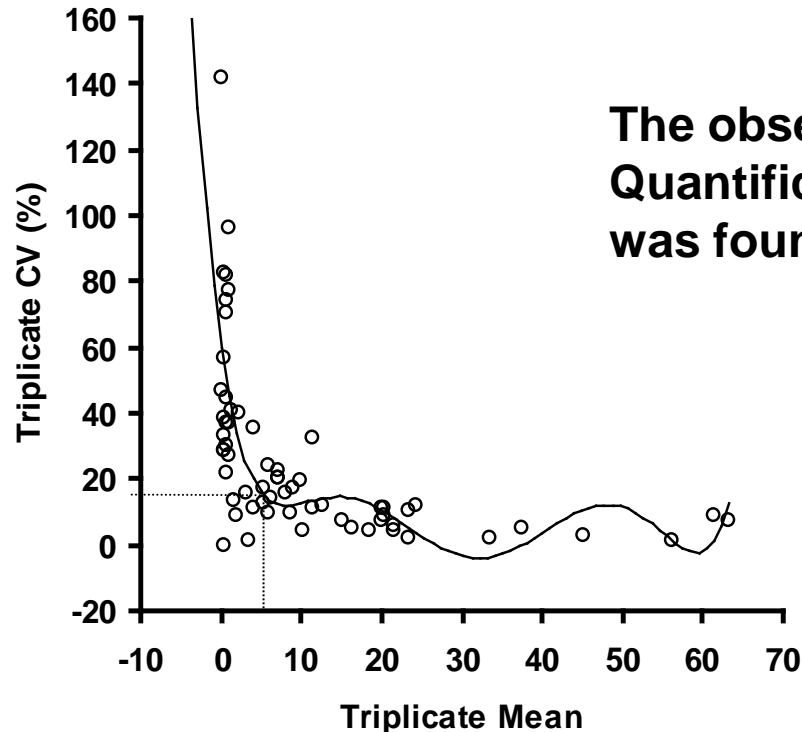
**Sample 2: KT 36/10,000 RBC**

Rep CV 2.68%



**Graphical displays of two representative samples comparing single and merged samples analyses.**

# RESULTS : DETERMINATION OF LLQ



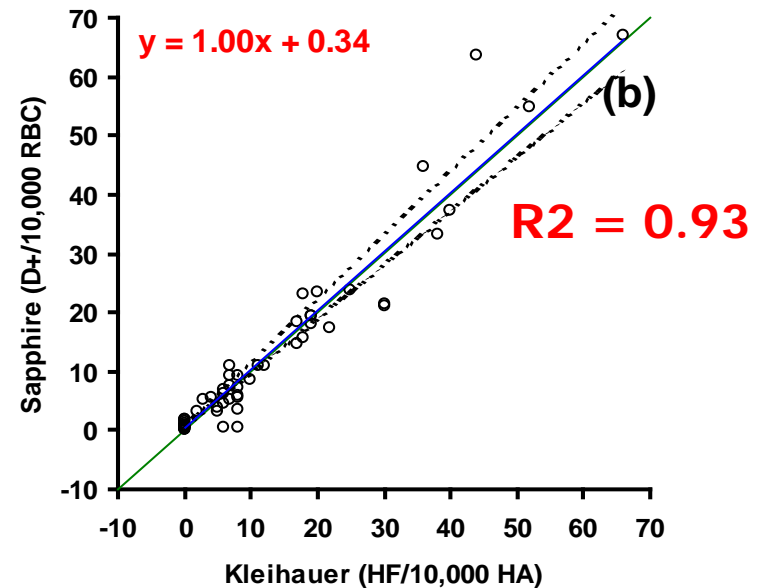
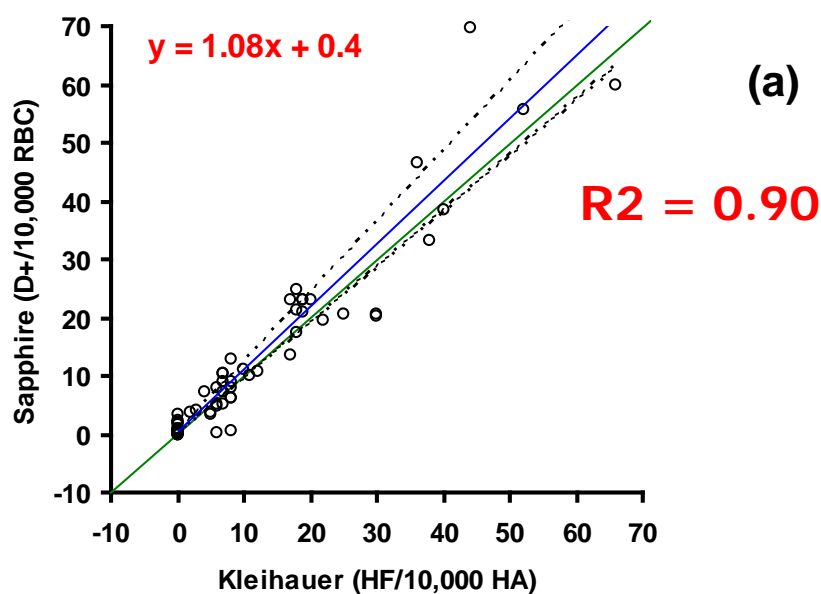
The observed LLQ (Lower Limit of Quantification) with a CV of less than 15% was found at a value of **5 HF/10,000 HA**

Polynomial regression analysis determining the relationship between triplicate mean and CV%.

Analysis of 58 samples; Kleihauer test ranging between 0 and 66 HF/10,000 HA.

# RESULTS : CORRELATION

Analysis of 58 samples with Kleihauer test ranging between 0 and 66 HF/10,000 HA



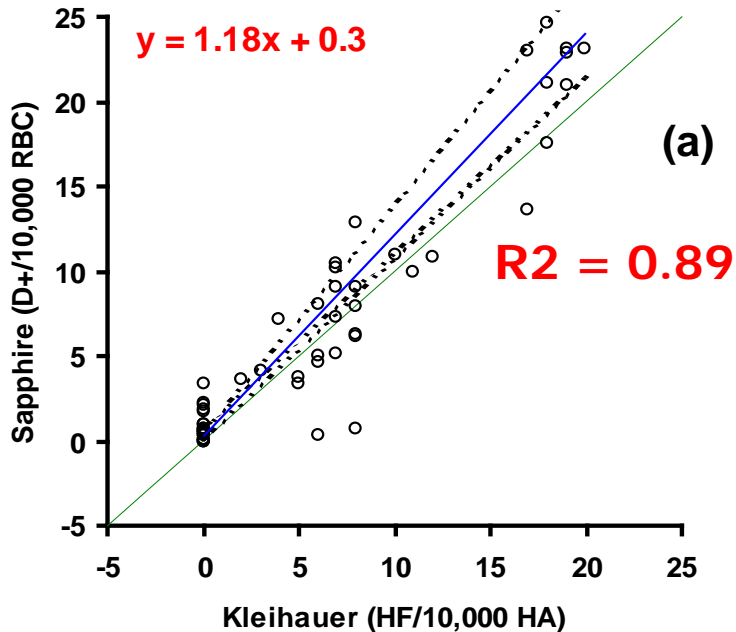
Plot (a): single aspiration with the *Cell Dyn Sapphires*

Plot (b): merged triplicate aspirations with *Cell Dyn Sapphires*

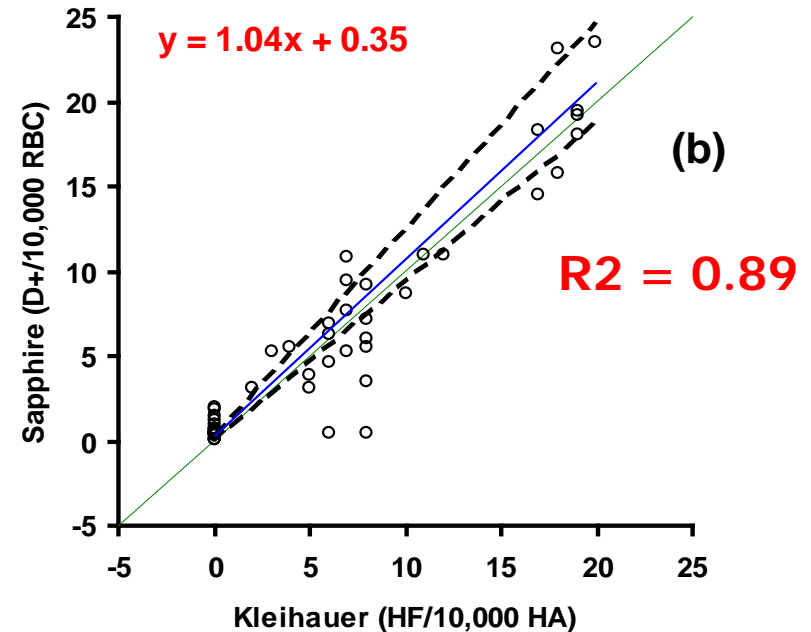
Passing & Bablok analysis comparing Kleihauer test and cell-Dyn Sapphires

# RESULTS : CORRELATION

Analysis of 48 samples with a Kleihauer test ranging between 0 and 20 HF/10,000 HA.



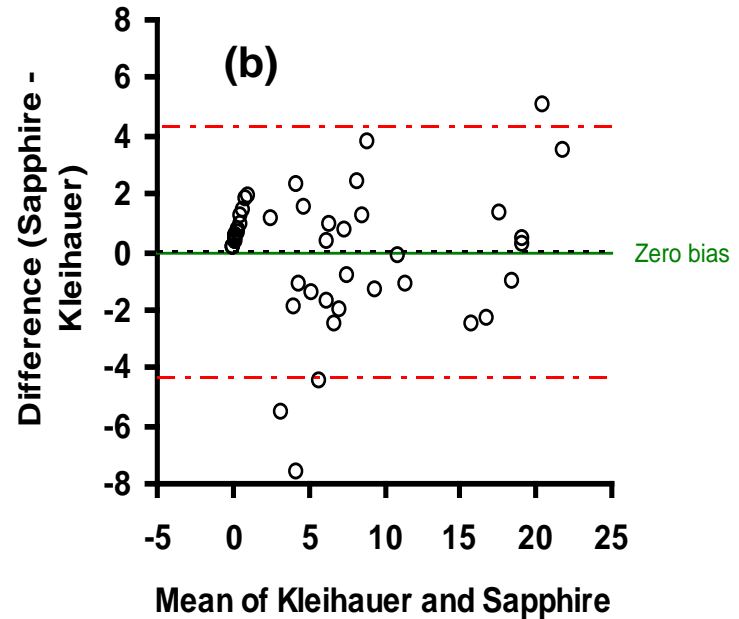
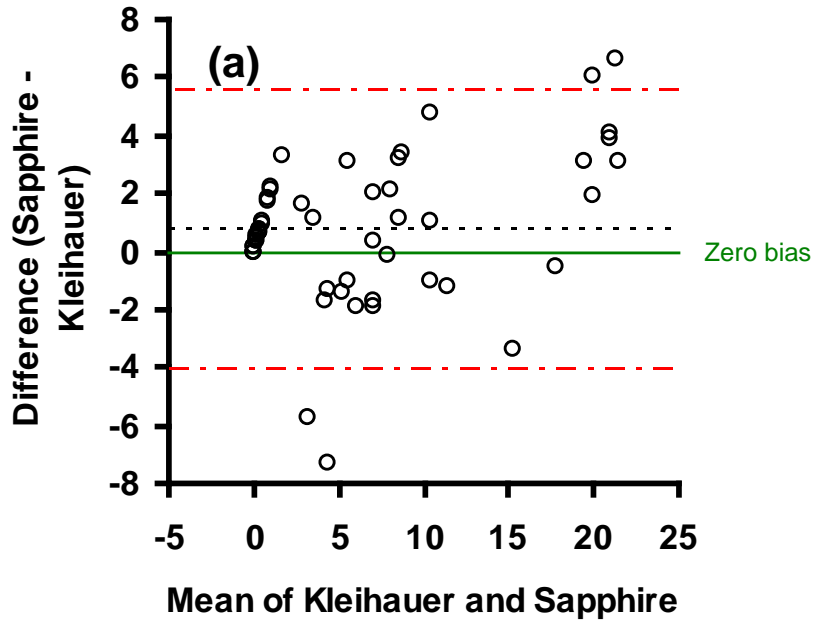
Plot (a): single aspirations  
with **Cell Dyn Sapphire**



Plot (b): merged triplicate aspirations  
with **Cell Dyn Sapphire**

Passing & Bablok analysis comparing Kleihauer and **Cell Dyn Sapphire**

# RESULTS



Altman-Bland bias analysis of agreement between the Kleihauer and *Cell Dyn Sapphire* estimates of FMH for analysis of 48 samples with Kleihauer test ranging between 0 and 20 HF/10,000 HA.

Plot (a): analysis for single aspirations with Cell-Dyn Sapphire. Mean bias for *Cell Dyn Sapphire* is **+0.76**.

Plot (b): analysis for merged triplicate aspirations with the Sapphire. The mean bias for *Cell Dyn Sapphire* is **+0.00**.



# DISCUSSION

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- **RESULTS are EXCELLENT :**
  - Good correlation of FMH quantification with *Cell Dyn Sapphire* compared with Kleihauer test.
  - Acceptable sensitivity: LLQ : 5/10 000
- **ONE PRECAUTION:** *Cell Dyn Sapphire* analysis should include data from 3 aspirations
- **ADVANTAGES OF CELL-DYN SAPPHIRE :**
  - Easier and faster than KLEIHAUER for laboratories not using routinely this manual test. Well adapted to emergency
  - Solution for uninterpretable results
  - BEST REPEATABILITY, REPRODUCIBILITY than Kleihauer
- **DISADVANTAGES :**
  - Limits of indication to RHD immunoprophylaxis. Not usable for FMH in all pregnant women
  - SOFTWARE not integrated in Sapphire: necessity of data transfer
  - ABSENCE of quality controls





# CONCLUSION

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## FMH QUANTITATION ON *CELL DYN SAPPHIRE*

- A simpler technique for laboratories not trained to perform Kleihauer test to quantitate FMH for RHD immunoprophylaxis
- Specificity, sensibility and precision of this technique makes it a reliable alternative
- Quantitation of FMH possible in few minutes at every time for all laboratory equipped with a *Cell Dyn Sapphire* running 24/7