

## Marsh Scores in Automated Anti-D (RH1) and Anti-c (RH4) Titration Assay Using Glass Microbeads Columns correlate with Continuous Flow Analysis quantification results and precises the risk for Hemolytic Disease of the Fetus and Newborn

Cécile TOLY-NDOUR<sup>1</sup>, Stéphanie HUGUET-JACQUOT<sup>1</sup>, Jenny BEAUD<sup>1</sup>, Jérôme BABINET<sup>1</sup>, Elodie MAENULEIN<sup>1</sup>, Christelle MARION<sup>1</sup>, Mathieu REGOUBY<sup>1</sup>, Agnès MAILLOUX<sup>1</sup>

<sup>1</sup>Fetal and Perinatal Hematology Department, National Reference Center for Perinatal Hematology, Saint-Antoine Hospital, Paris

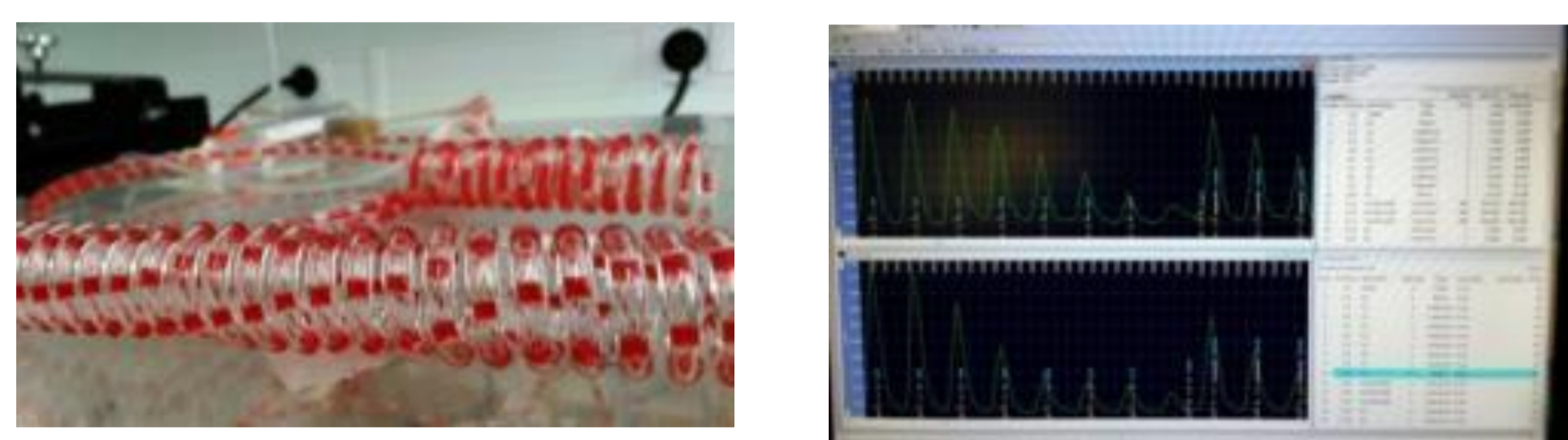
We wanted to correlate the results of the anti-D (RH1) and anti-c (RH4) Marsh scores calculated manually based on the reaction intensities of the different wells in the automated glass microbeads column titration technique (QuidelOrtho® VISION MAX) with quantitative assay values obtained by continuous-flow hemagglutination on Astoria (WHS) and Proxima (AMS Alliance) systems (two-step technique).

### Anti-D (RH1) and anti-c (RH4) quantification assays by continuous-flow hemagglutination on Astoria or Proxima autoanalyzer

Method described in the European Pharmacopoeia.

Indirect measurement of the intensity of D+C+E+c+e+ (RH:1,2,3,4,5) bromelain-treated red blood cells agglutination, induced in the continuous flow of the autoanalyzer in the presence of anti-D (RH1) or anti-c (RH4) antibodies present in the patients' plasma/serum (two-step method using methylcellulose as a "roll-inducing" agent)

Using a WHO international standard (16/332), the anti-D concentration can be determined in arbitrary local units (UHP/ml) or in IU/ml (250 UHP/ml = 5 IU/ml), and the anti-c concentration in UHP/ml (conversion to IU/ml : 500 UHP/ml equivalent to ≈ 7.5 IU/ml)



### Glass microbeads column titration method on the QuidelORTHO VISION MAX automated system

Automated serial 2-fold dilutions of patient plasma/serum (from undiluted (1:1) to a 1:1024 dilution).

Reagents: RH:1,2,3,4,5 (D+C+E+c+e+) KEL:1,2 (K+k+) Red blood cells marketed by the UPR (EFS) (ref MPO02) adjusted to a concentration of 0.9% in Biovue Red Cell Diluent).

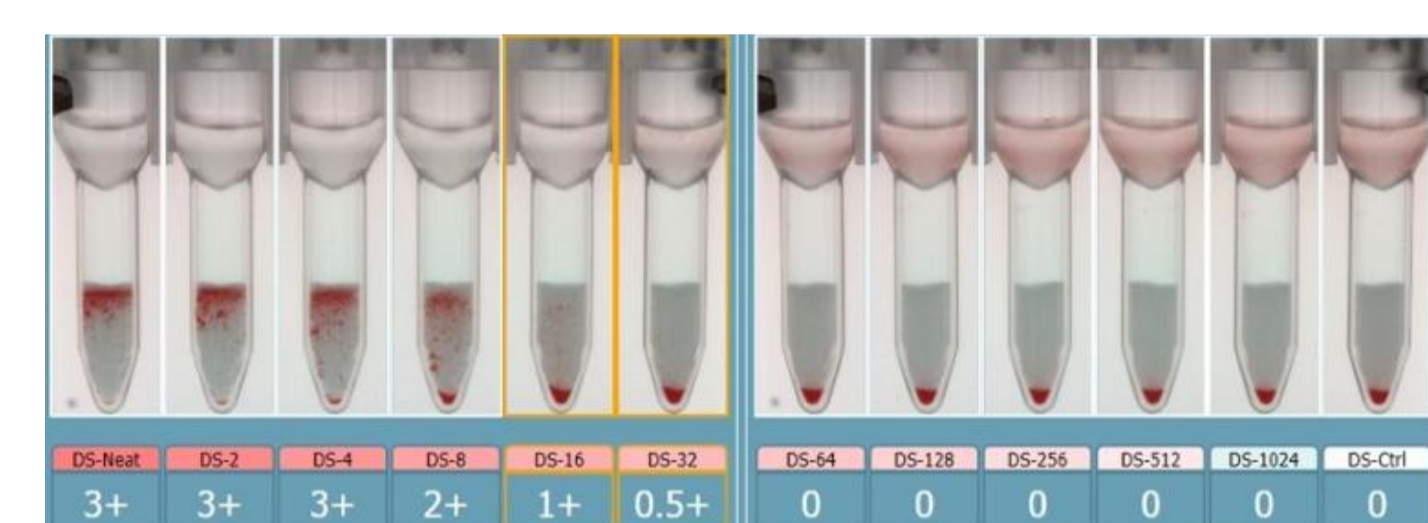
Incubation of the serum/plasma dilutions with the red blood cell suspension for 15 minutes at 37° C in the reaction chamber of the Biovue anti-human globulin anti-IgG cassettes.

Centrifugation and reading of the reaction by the instrument's camera. Interpretation with an endpoint of 1+.



Manual calculation of scores (Marsh):

4+ = 12  
3+ = 10  
2+ = 8  
1+ = 5



Example :  
Titre 16  
score 43

### Biological thresholds for triggering specific fetal ultrasound monitoring to detect indirect signs of severe fetal anemia

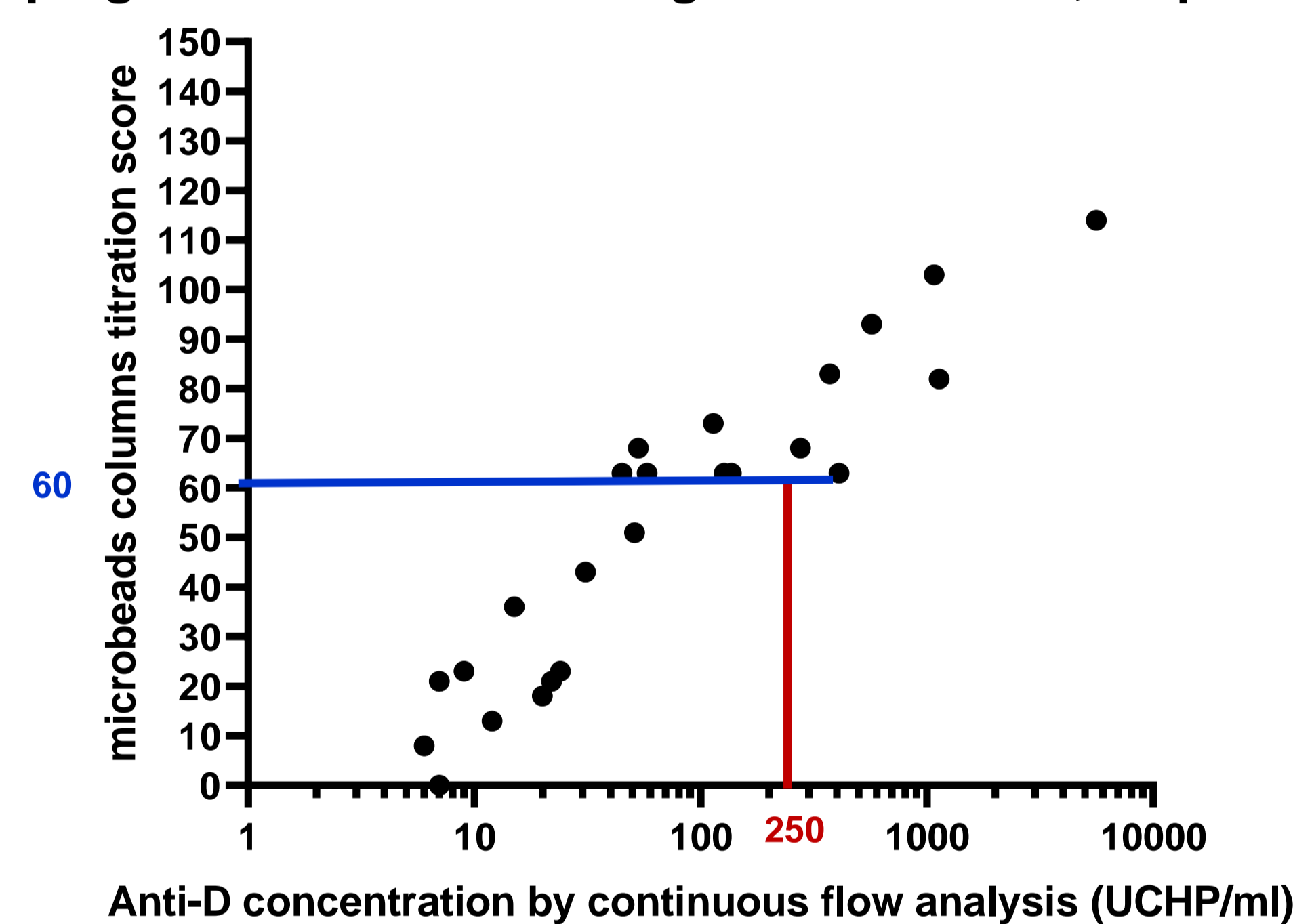
Hemolysis of fetal red blood cells caused by maternal anti-D (RH1) and anti-c (RH4) antibodies that have crossed the placenta, can lead to severe fetal anemia, hydrops, and fetal death in utero. In cases of severe alloimmunization, defined by an antibody concentration greater than 250 UHP/ml (5 IU/ml) for anti-D and 500 UHP/ml (equivalent to 7.5 IU/ml) for anti-c, fetal ultrasound monitoring with measurement of the systolic peak velocity in the middle cerebral artery—which indirectly reflects anemia—is initiated weekly starting at 18 weeks of gestation.



**Results:** A total of 24 and 25 samples from pregnant women containing anti-D or anti-c, respectively, were tested.

**Figure 1:** Correlation between scores from the glass microbeads column titration technique and anti-D (RH1) concentrations obtained by continuous flow hemagglutination on autoanalyzer

Samples with titers > 256 (tube) or > 1024 (microbeads) were not included



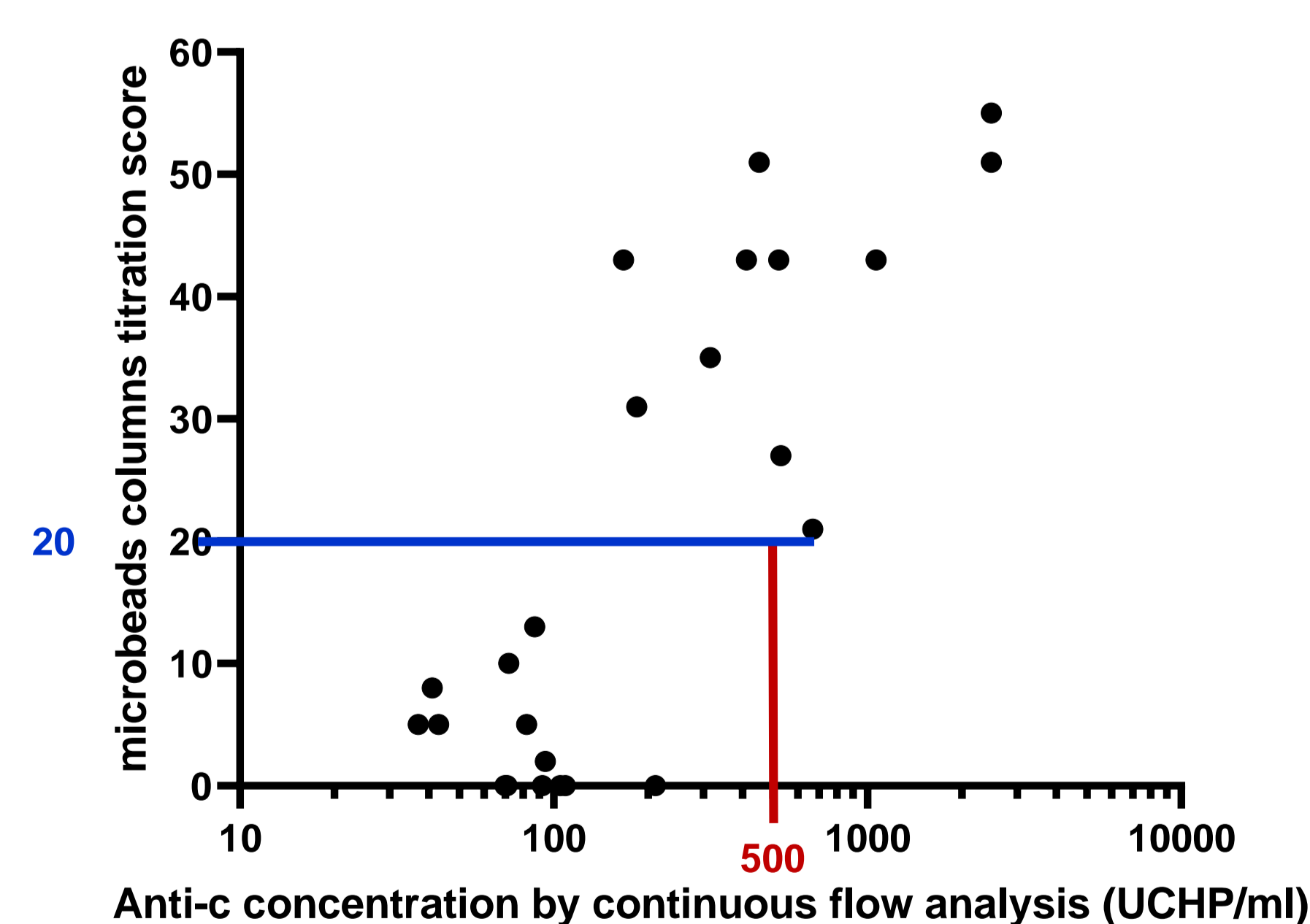
\*\*Spearman correlation between Marsh glass microbeads column scores and antibody concentrations using continuous flow hemagglutination assay (two-step) for anti-D

n = 24, p = 0.0031,  
r=0.5774 [0.2268–0.7955]

Marsh scores obtained using the glass microbeads titration technique correlate significantly with anti-D concentration (p = 0.0031, r = 0.58).

The **threshold score for anti-D** corresponding to the threshold concentration of 250 UHP/ml (= 5 IU/ml) appears to be at least **60 for the glass microbeads technique**

**Figure 2:** Correlation between scores from the glass microbeads column titration technique and anti-c (RH4) concentrations obtained by continuous flow hemagglutination on autoanalyzer



\*\*\* Spearman correlation between Marsh glass microbeads column scores and antibody concentrations using continuous flow hemagglutination assay (two-step) for anti-c

n = 25, p = 0.0001,  
r=0.6868 [0.3899–0.8544]

Titer scores using the glass microbeads technique are significantly correlated with the anti-c concentrations obtained by continuous flow hemagglutination assay (p = 0.0001, r = 0.69)

The **cut-off score for anti-c** corresponding to a threshold concentration of 500 UHP/mL (equivalent to 7.5 IU/mL) appears to be at least **20 for the glass microbeads assay**.

### Conclusion:

Calculation of Marsh scores in automated anti-D (RH1) and anti-c (RH4) titration using glass microbeads columns shows a significant correlation with quantitative assay values, providing added value compared with titer reading alone. Further studies involving a larger number of samples, supported by clinical data, should be considered to consolidate and validate these results.