AP-HP. Sorbonne Université

ISBT BARCELONA 2024

CNRHP Centre National de Référence en Hémobiologie Périnatale

# Automated gel card anti-D (RH1) titration: determination of risk thresholds for severe hemolytic disease of the fetus and newborn from clinical outcomes


C. Toly-Ndour[1], L. Augier[1], S. Huguet-Jacquot[1], J. Beaud[1], J. Babinet, H. Delaby[1] , P. Maurice[2], M.G. Guillemin[2], A. Mailloux[1]

[1] Fetal and Perinatal Hemobiology Laboratory Department - National Reference Center for Perinatal Hemobiology (CNRHP), Saint-Antoine Hospital , AP-HP, 75012 Paris, France

[2] Fetal and Perinatal Hemobiology Clinical Unit - National Reference Center for Perinatal Hemobiology (CNRHP), Trousseau Hospital AP-HP, 75012 Paris, France


**Background:**

In case of anti-D (RH1) alloimmunization, during pregnancy, maternal antibodies can cross the placenta and cause Hemolytic Disease of the Fetus and Newborn (HDFN). In severe cases, fetal anemia can develop and results, if untreated, to hydrops and intrauterine fetal death. In the event of significant alloimmunization defined in France by an antibody titer greater than 16 (indirect antiglobulin test tube method) or an anti-D quantification greater than 5 UI/ml (continuous flow analysis (CFA)), fetal ultrasound monitoring with weekly measurement of the middle cerebral artery peak systolic velocity (MCA PSV) is undertaken from the 18$^{th}$ week of gestation. If ultrasound signs of severe fetal anemia are observed, Intrauterine Transfusion (IUT) can be realized.

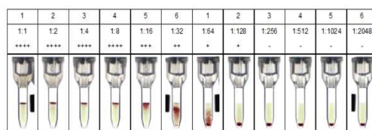
Anti-D titrations and quantifications also make it possible to assess the risk for severe Hemolytic Disease of the Newborn (neonatal anemia and hyperbilirubinemia) as well as to anticipate care for the newborn (phototherapy, transfusion, transfusion exchange…).

But titration by tube method is known to have high intra- and interassay imprecisions and CFA is only performed in specialized laboratories. Recently, automated titration methods on different supports has been developed by several manufacturers.

## Gel Titration method on IH-500 system

Low Ionic Strenght Solution (LISS) antiglobulin procedure:
Automated dilutions of plasma/serum and distribution of the dilutions and the Red Blood Cells suspension (Bio-Rad diluent (LISS)) in the gel containing anti-IgG.
Incubation (15 min – 37°C) /
Centrifugation
Reading End-point : 1+, macroscopically

Scoring (Marsh):
4+ = 12
3+ = 10
2+ = 8
1+ = 5

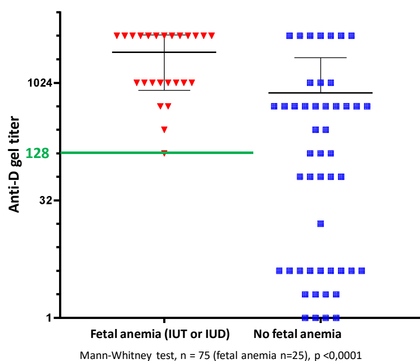



**Aims:** Our laboratory has evaluated an automated titration method using LISS Coombs gel cards (IH-500 Bio-Rad) in 2018 and the anti-D titers were found on average 3 times greater than the reference tube method. However, for some tube titers, the respective gel titers were 2 to 6 times higher making direct extrapolation of the tube threshold of 16 to a new gel card threshold difficult. We also demonstrated a correlation between the titer score in gel card and the anti-D quantification. The cutoff value of 5 IU/ml corresponded to a titer score of approximately 75 in gel card.
We wanted to determine new cutoffs for anti-D titer and titer score in gel card based on clinical data.

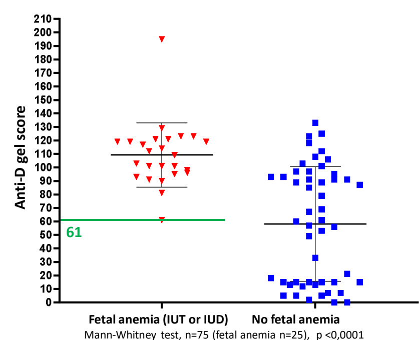
**Methods:** From 01/01/2021 to 30/05/2022, data (gel card titers and titer scores) were extracted from the Laboratory Information System used at the National Reference Center in Perinatal Hemobiology (CNRHP) for anti-D alloimmunized pregnant women followed at Trousseau hospital in Paris. The number of fetal and/or neonatal transfusions and hemoglobin and bilirubin values at birth were collected.

**Results:** 75 patients were included. 25 fetuses developed severe fetal anemia. The minimum gel anti-D titer associated with a severe fetal anemia was 128 with a titer score of 61. Among the newborns who did not develop fetal anemia (n= 50), 19 presented anemia at birth (cord hemoglobin < 14g/dl) and 15 had hyperbilirubinemia (cord bilirubin > 80 µmol/l). The minimum gel titers and titer scores associated with neonatal anemia and severe jaundiced were 128, 69 and 256, 87 respectively.

### Fetal anemia

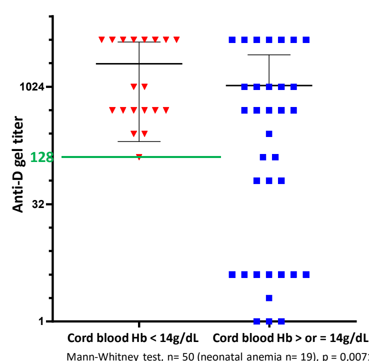


Mann-Whitney test, n = 75 (fetal anemia n=25), p <0,0001

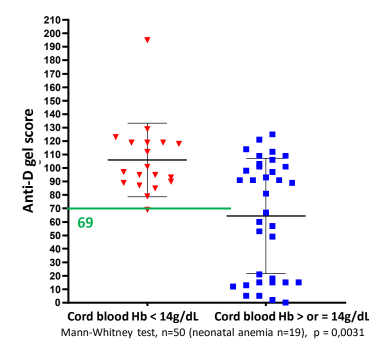


Mann-Whitney test, n=75 (fetal anemia n=25), p <0,0001

### Neonatal anemia

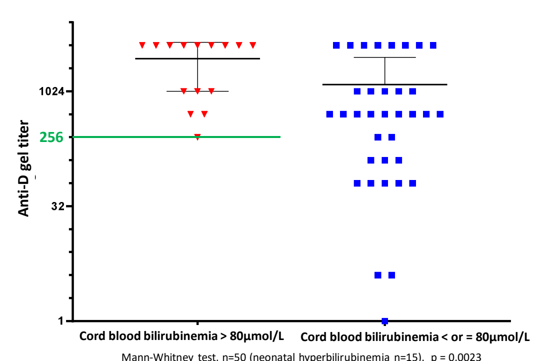


Mann-Whitney test, n= 50 (neonatal anemia n= 19), p = 0,0071

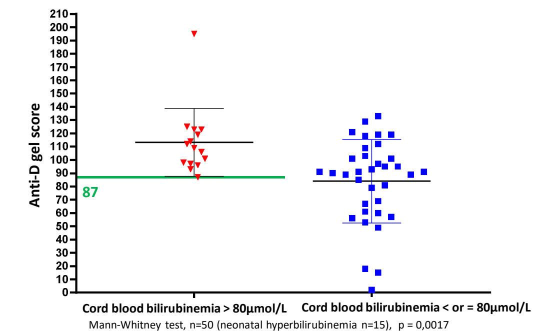


Mann-Whitney test, n=50 (neonatal anemia n=19), p = 0,0031

### Neonatal hyperbilirubinemia



Mann-Whitney test, n=50 (neonatal hyperbilirubinemia n=15), p = 0,0023



Mann-Whitney test, n=50 (neonatal hyperbilirubinemia n=15), p = 0,0017

**Conclusion: For high-risk pregnancies due to anti-D alloimmunization, based on clinical data, automated gel titer and titer score thresholds with IH-500 triggering ultrasound monitoring and anticipating severe hemolytic disease of the newborn have been set at 128 and 61, respectively.**
**Titer results correlate with the average of 3 dilutions difference with the tube technique and for the titer score, results are slightly lower than the previous estimate based on biological correlation.**