

INPUT OF TITER SCORES DETERMINED BY AUTOMATED COLUMN AGGLUTINATION TECHNOLOGY IN THE MANAGEMENT OF PREGNANCIES COMPLICATED BY ANTI-D (RH1) AND ANTI-c (RH4) IMMUNIZATION

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Background : In France, for pregnancies complicated by anti-D (RH1) and anti-c (RH4) allo-immunization, the tests currently used to quantitate maternal antibodies are tube method titration and continuous flow analysis determination of the antibodies concentration. Recently, an automated assay was developed using the column agglutination technology on the IH-500 system (Bio-Rad®).

Aims: We wanted to evaluate the score, calculated from the agglutination profile of the antibodies on the IH-500 system, as a quantitative data to appreciate the level of maternal antibodies.

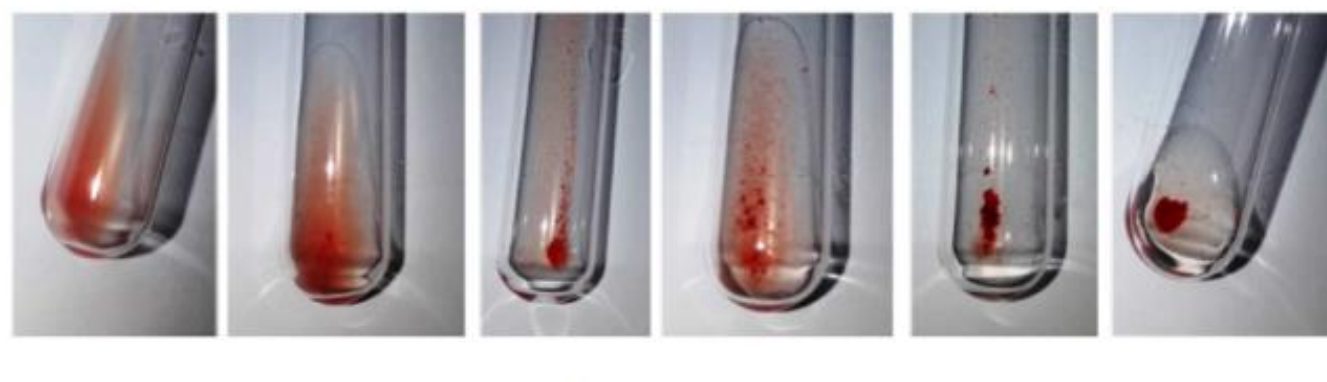
Methods: Titers from 29 samples containing anti-D and 20 containing anti-c have been established using the semi-automated tube method performed since decades in our lab and the fully automated gel method on the IH-500 system. Scores were calculated manually in both cases. Antibodies concentrations were also determined for all samples by continuous flow analysis (CFA) on our auto-analyzer device (Evolution III AMS Alliance). We looked for a possible correlation between anti-D and anti-c scores and the corresponding concentrations using the Spearman correlation test.

Tube titration method used in the CNRHP laboratory on Tecan Evo Clinical Base

Saline (NaCl 0,9%) antiglobulin procedure:
Automated dilutions of plasma/serum
Red Blood Cells suspension (NaCl 0,9%) addition
Incubation (60 min at 37° C) / Wash x 3 / Anti-IgG addition / Centrifugation and resuspension
The antibody titer is defined as the greatest dilution of the plasma/serum at which a detectable hemagglutination is still visible.
Reading: End point : (+), macroscopically

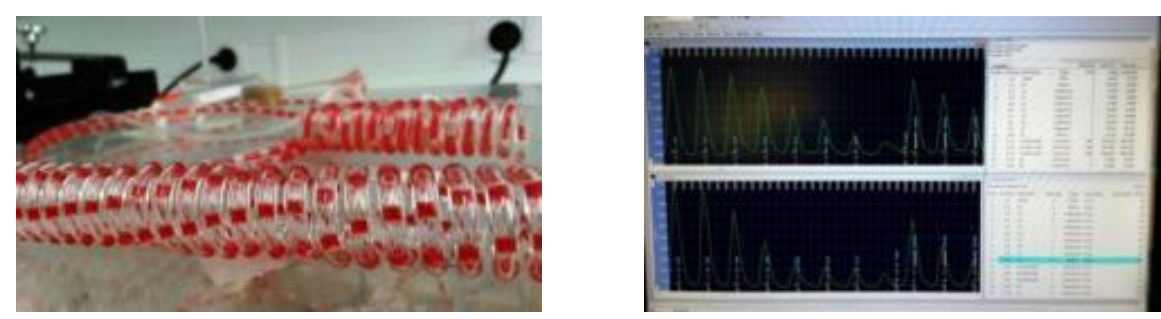


Scoring (Marsh)
4+ = 12
3+ = 10
2+ = 8
1+ = 5
(+) = 2



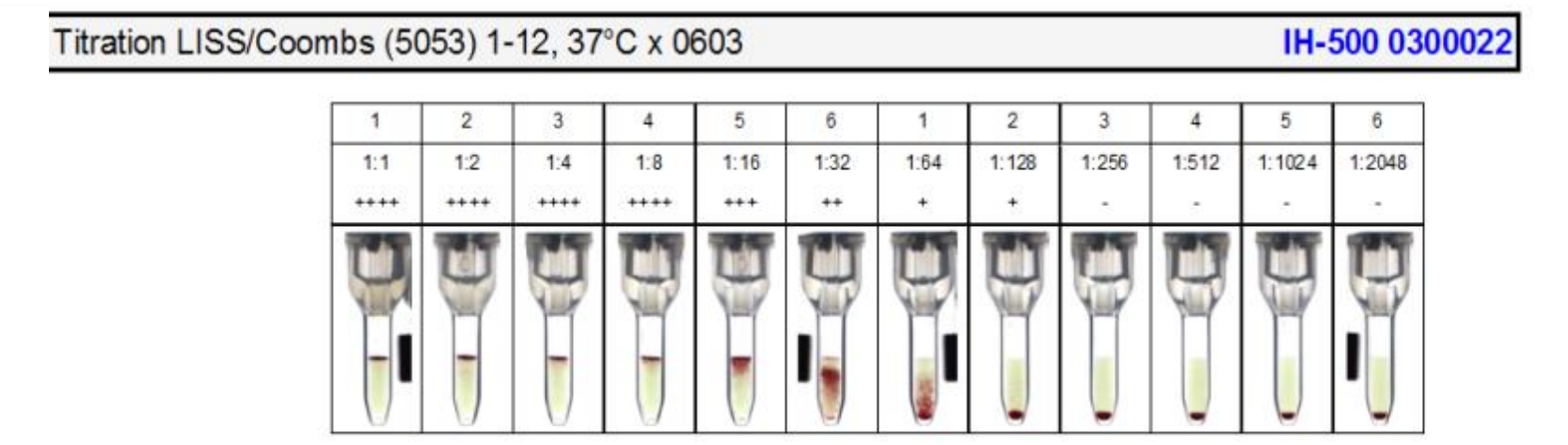
Anti-D quantitation by continuous flow analysis (CFA) on Alliance Evolution III

Method described in the European Pharmacopeia
Measure of the agglutination of prebromelized RH:1,2,3,4,5 red blood cells induced in the continuous flow of the Autoanalyzer by the anti-D (RH1) or the anti-c (RH4) antibodies present in the serum of the patients (2-stages method with polyvinyl pyrrolidone)
The WHO International Standard anti-D typing serum was used to determine the anti-D concentration in arbitrary local units (UHP/ml) and in IU/ml (250 UHP/ml = 5 IU/ml) and the anti-c concentration in arbitrary local unit (UHP/ml) converted in equivalent IU/ml (500 UHP/ml equivalent to 7,5 IU/ml)



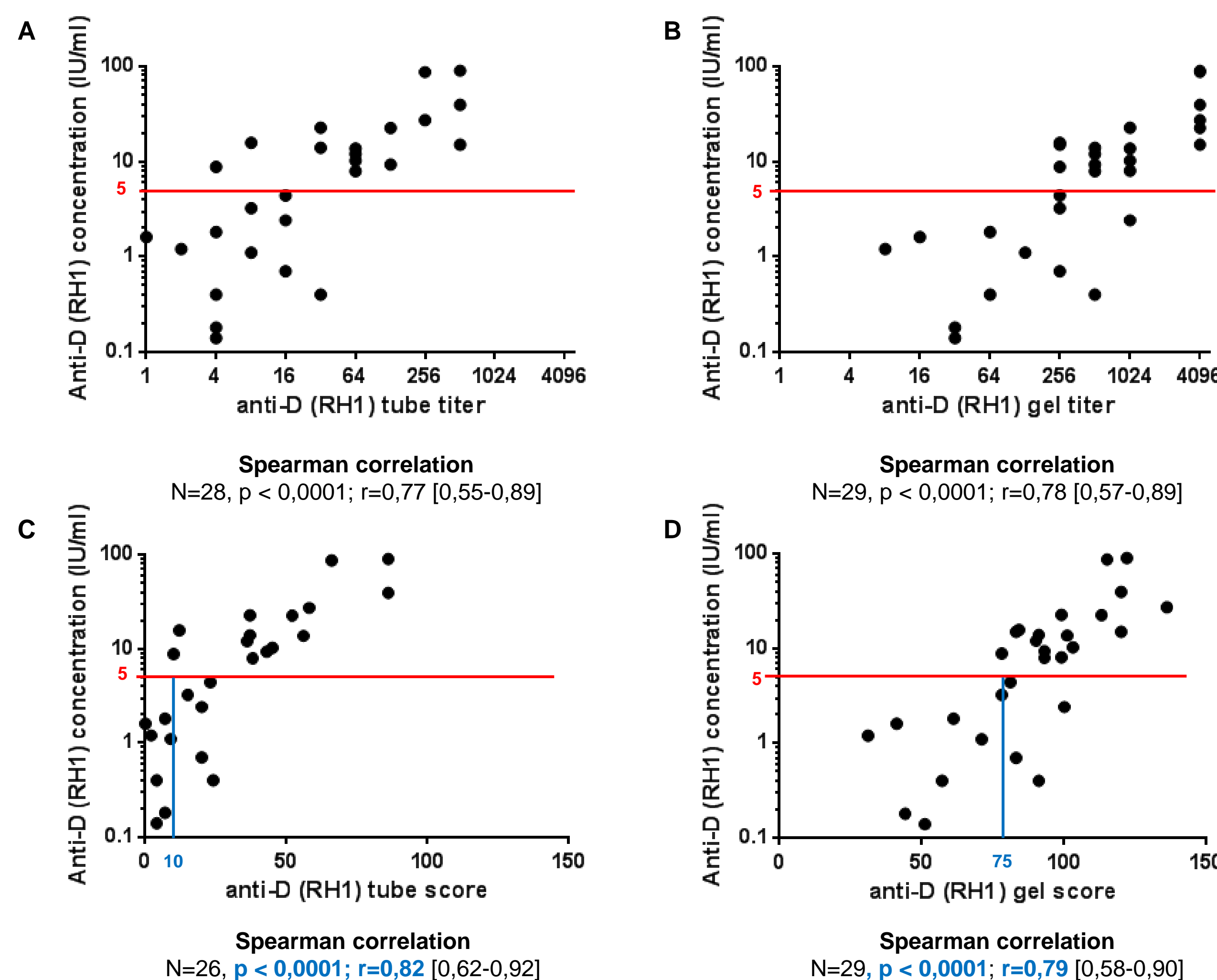
Gel Titration method on IH-500 system

Low Ionic Strength 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



Results:

Figure 1: Correlation between anti-D (RH1) titers and CFA concentrations by tube [A] and gel methods [B] and between anti-D (RH1) scores and CFA concentrations by tube [C] and gel [D] methods



Anti-D tube and gel scores were significantly correlated with the anti-D concentration values (p < 0.0001, r = 0.79 and p < 0.0001, r = 0.82 respectively).

Spearman correlations between titers and concentrations show lower "r" coefficients. It seems more difficult to extrapolate anti-D titer thresholds from CFA concentrations than score thresholds.

The determined **score thresholds**, corresponding to 5 IU/ml (250 UHP/ml) of anti-D, were respectively **10 for tube and 75 for gel methods**

Biological thresholds to trigger ultrasonographical fetal surveillance:

Fetal red blood cells hemolysis due to maternal anti-D and anti-c antibodies could lead to severe fetal anemia, hydrops fetalis and fetal death. In cases of **severe maternal allo-immunization**, defined in France by **anti-D (RH1) or an anti-c (RH4) CFA concentrations respectively above 250 UHP/ml (5 IU/ml) and 500 UHP/ml (equivalent 7,5 IU/ml)**, fetus are monitored every week since 16WG by ultrasonography. Fetal anemia could be diagnosed in a non invasive way by measurement of the peak systolic velocity in the middle cerebral artery (MCA-PSV)

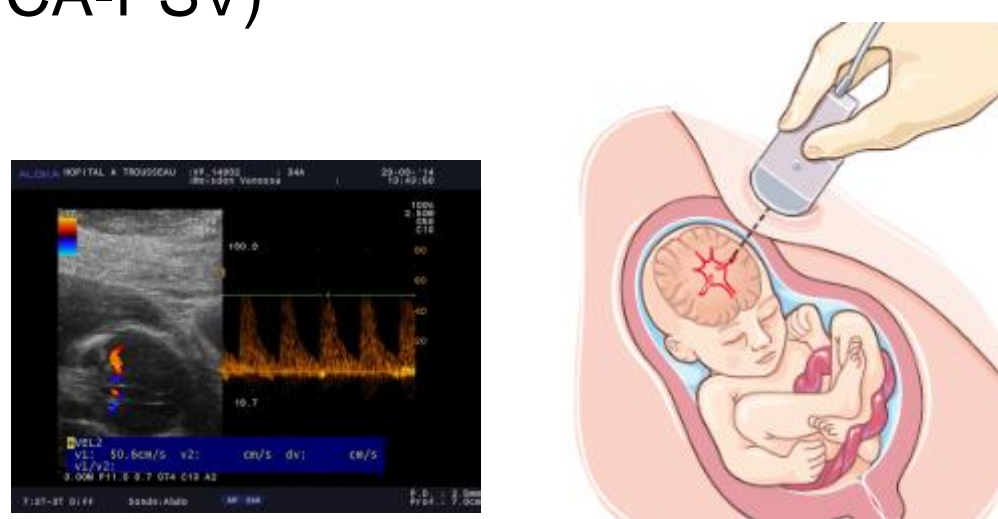
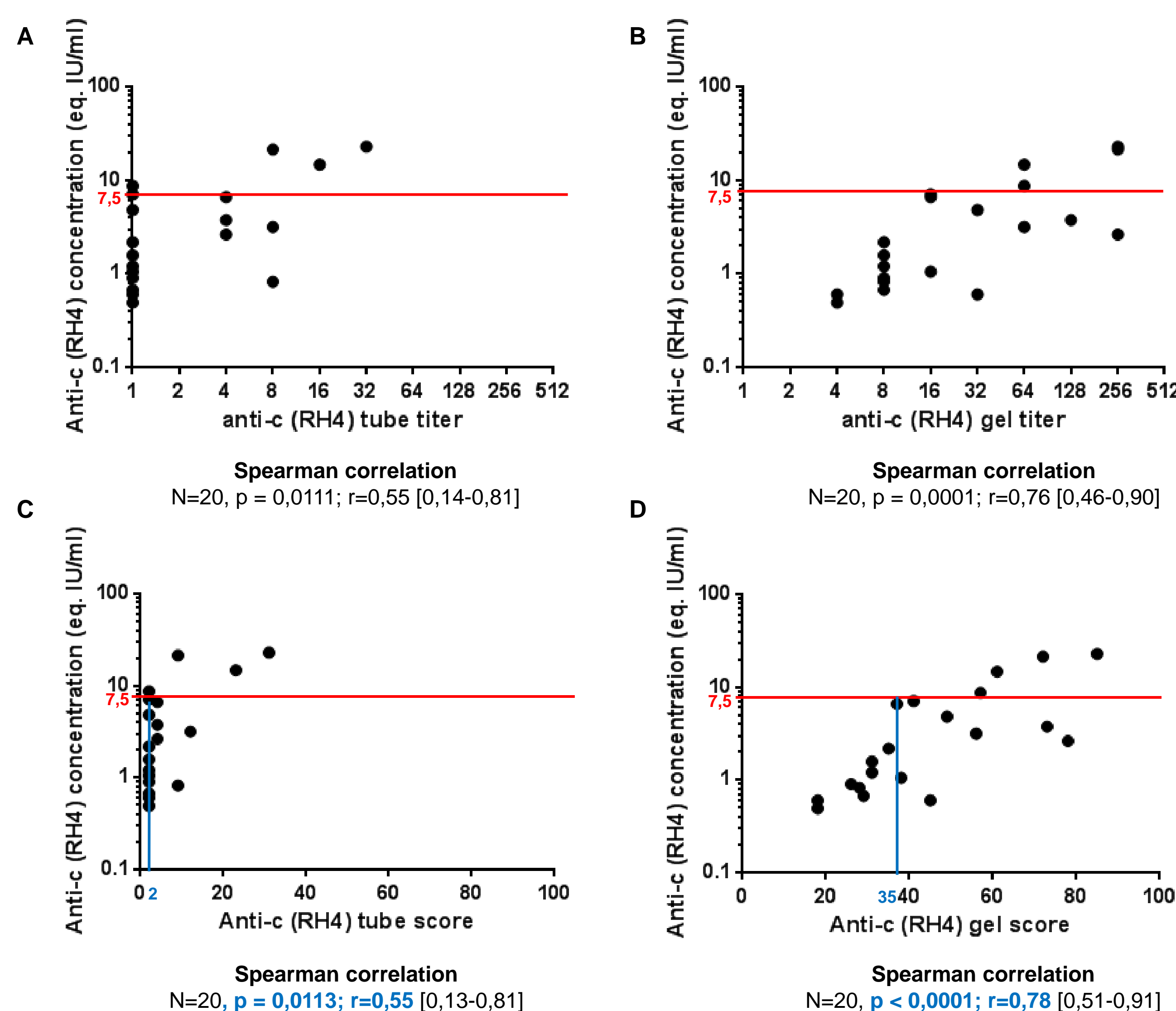


Figure 2: Correlation between anti-c (RH4) titers and CFA concentrations by tube [A] and gel methods [B] and between anti-c (RH4) scores and CFA concentrations by tube [C] and gel [D] methods



Anti-c scores were also significantly correlated with anti-c concentration values (p<0.0001) but gel scores have a better correlation coefficient than tube scores (r = 0.78 versus 0.55).

Spearman correlations between titers and concentrations show also a higher "r" coefficient for anti-c gel titers. Like anti-D, it seems more difficult to extrapolate anti-c titer thresholds from CFA concentrations than score thresholds.

The determined **score thresholds**, corresponding to equivalent 7,5 IU/ml (500 UHP/ml) of anti-c, were respectively **2 for tube and 35 for gel methods**

It was easier to extrapolate gel score thresholds than tube score thresholds from the autoanalyzer values, especially for anti-c alloimmunization, with the aim of triggering fetal monitoring by ultrasounds and measurements of the peak systolic velocity in the middle cerebral artery only for risk pregnancies. The determined gel score thresholds were 75 and 35, corresponding respectively to 5 IU/ml (250 UHP/ml) of anti-D and 7.5 equivalent IU/ml (500 UHP/ml) of anti-c.

Conclusions :

Calculating the score from the hemagglutination profile displayed by the IH-500 system provides added values compared to the sole reading of the titer. For anti-c immunization, gel scores are more discriminant than tube ones and better correlated to the concentration values established by continuous flow analysis. The proposed score thresholds to trigger fetal antenatal monitoring need, however, to be confirmed on more samples and to be clinically documented.