ANTI-RH1 QUANTIFICATION ASSAY USING IH-500(BIO-RAD®): PROMISING RESULTS FOR MONITORING RH:- 1 PREGNANT WOMEN

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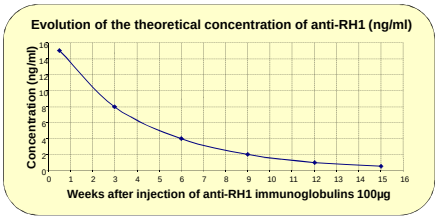
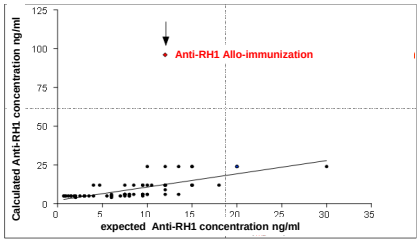
BACKGROUND

The generalization of immunoprophylaxis by anti-RH1 immunoglobulins since 1970 complicates the interpretation of the anti-red blood cell antibodies screening during pregnancy. To distinguish an alloantibody from a passive one, many laboratories in France use anti-RH1 microtitration. It is a column agglutination technology using red blood cells RH:1,-2,-3,4,5 (R0r). It permits to quantify low levels of anti-RH1 in comparison to a range of an anti-RH1 standard. Then the value is compared to anti-RH1 concentration expected after injection which allows to conclude on the passive or immune nature of the anti-RH1. Performed since 1999 at the CNRHP and automated on Evo clinical Base Tecan in 2008 (dilutions and distribution), anti-RH1 microtitration is well adapted to Rh prophylaxis.



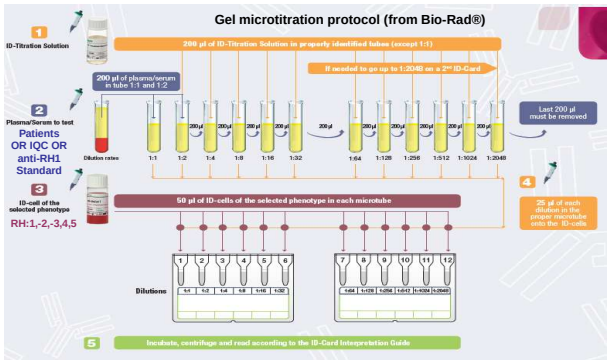


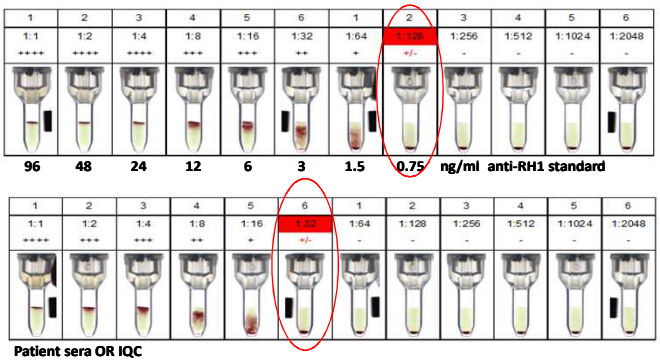
AIMS

The aim of this study was to evaluate the anti-RH1 microtitration on the IH-500 system from Bio-Rad®

METHODS

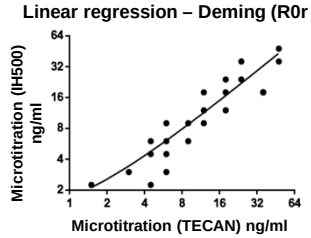
On IH-500, the reactivity of the Bio-Rad® reagents was compared with the CNRHP reagents (red blood cells R0r, anti- RH1 standard). The performances of the method were evaluated using three internal quality control (IQC) (2 CNRHP home-made at 2 and 12 ng/ml and 1 Bio-Rad® at 12 ng/ml) on papainized Liss coombs R0r (PLC) and native Liss coombs R0r (NLC). A comparison of results from patient sera ranging from 1.5 to 48 ng/ml was done between IH-500 and Evo clinical Base Tecan.



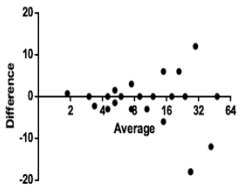


Calculation of the anti-RH1 concentration = 32 X 0.75 = 24 ng/ml

Linear regression – Deming (R0r PLC)



Difference vs average – Bland-Altman
Microtitration anti-RH1
CNRHP vs IH500 with R0r PLC

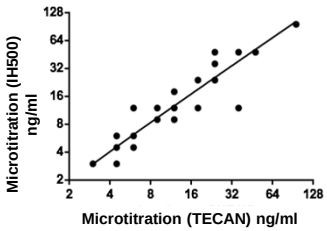


RESULTS

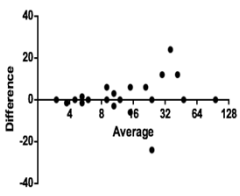
The results of the 3 IQC are similar between the different reagents used.
There is no significant difference between the 2 types of red blood cells except for the limit of detection: 1.5 ng/ml in PLC - 6ng/ml in NLC.
For the 3 IQC, the intra and interassay imprecision based on the dilution degree show coefficients of variation between 0 and 15%, similar to those found with the Evo Clinical Base.
The correlation with the CNRHP technique performed on 50 samples in PLC and 44 samples in NLC was satisfactory (Deming)

PLC: r2 = 0.80 Y = 0.89X + 0.78
NLC: r2 = 0.86 Y = 1.08X-0.25

Linear regression – Deming (R0r NLC)



Difference vs average – Bland-Altman
Microtitration anti-RH1
CNRHP vs IH500 with R0r NLC



CONCLUSIONS

The anti-RH1 microtitration on the IH-500 offers similar performances to the method conducted at the CNRHP. The IH-500 allows automated reading of gel cards.

However, it does not have calculation or interpretation algorithm and does not directly give the concentration of anti-RH1. This final part remains manual and requires trained staff.