COMPARISON OF THE INTENSITY OF ANTI-RH1 DETECTION USING GALILEO®(IMMUCOR) AND GEL MICROTITRATION: A SEMI QUANTITATIVE AUTOMATED APPROACH TO DETERMINE THE ORIGIN OF ANTI- RH1.

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Background

In 2005, the French National College of Gynecologists and Obstetricians published guidelines recommending systematic injections of anti-D immunoglobulins at 28 weeks of pregnancy for all RH:-1 women. Since, the rate of positive antibody screening has increased. It therefore becomes essential to differentiate passive anti-RH1 from allo-anti RH1. In order to resolve this issue the CNRHP developed in 2000, the anti-RH1 microtitration method, a semi-quantitative assay used to determine if an anti-RH1 results from administration of IgRH or from alloimmunization.
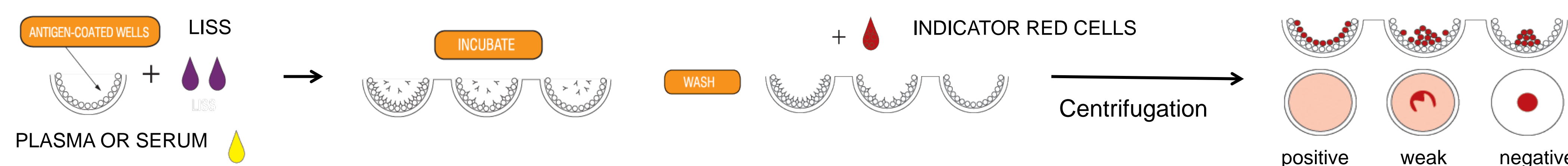
Aim

The intensity of agglutination of antibody screening using Capture® technology on Galileo® (Immucor) was compared to a microtitration method in pregnant women serum with anti-RH1.

Materials and methods

Blood samples of pregnant women were screened for antibodies using Capture-R (4 cell) technology on Galileo®. Samples with anti-RH1 were then analyzed by microtitration using gel column technology. For each range of concentration, the mean of the Capture-R agglutination intensities was calculated and then compared to the result of the microtitration.

Capture test procedure on Galileo®

PLASMA OR SERUM
ANTIGEN-COATED WELLS
LISS
INCUBATE
WASH
INDICATOR RED CELLS
Centrifugation
positive   weak   negative

Intensity of reaction with Capture-R technology : Galileo Signal UA
From 0 to 20 = Negative
From 20 to 30 = Indeterminate
From 30 to 45 = 1+
From 45 to 65 = 2+
From 65 to 90 = 3+
From 90 to 99 = 4+

Technology of gel microtitration

Reagents
Red blood cells : R_0r treated with papaïne
Gel column : LISS Coombs Diamed
Anti-RH1 standard : performed in CNRHP from NISBT standard (24ng/ml)

Analyzer
Freedom Evo Clinical from TECAN

Protocol
1- Preparation of serial dilutions of standard and samples of patients from 1/2 to 1/32
2- Distribution 50 µl of red blood cells R_0r treated by papaïne on LISS Coombs gel
3- Distribution 25 µl of dilutions of samples or anti-RH1 standard on LISS Coombs gel
4- Incubation 15 min 37°C
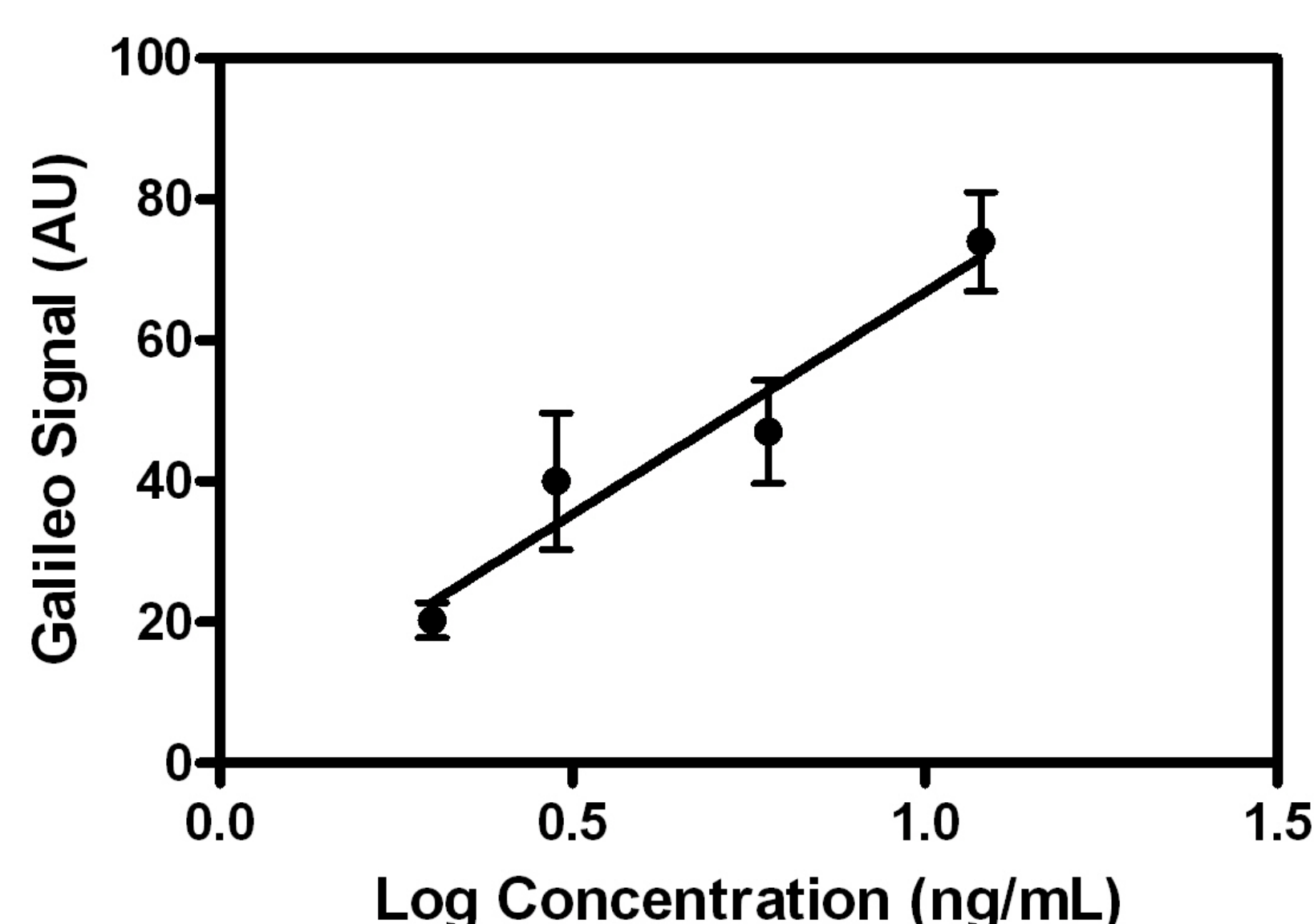5- Centrifugation and visual reading of the intensity of agglutination

Interpretation

Concentration of anti-RH1 = Inverse of last reactive dilution of patient $\times$ Concentration of dilution of standard with same intensity of reaction

6 ng/ml   3   1.5   0.75   Pur   1/2   1/4   1/8   1/16   1/32
STANDARD   SAMPLE OF PATIENT
Concentration = 8 x 1.5= 12 ng/ml
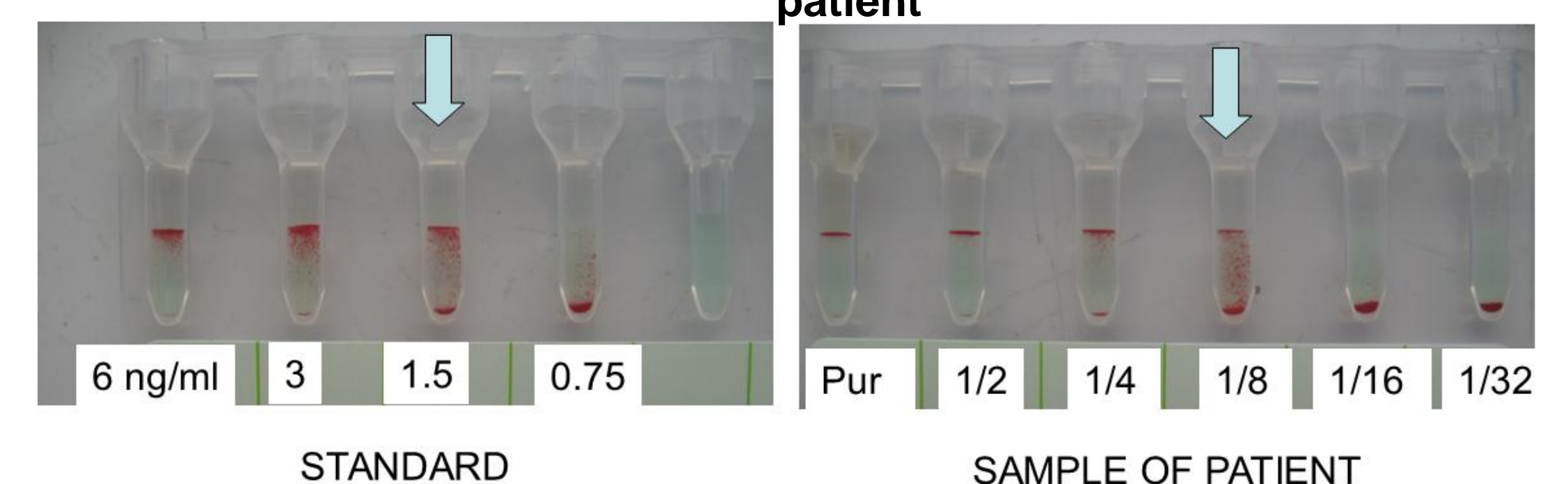
Results

37 anti-RH1 positive samples were analysed with both methodologies: 6 samples at 1.5 ng/ml, 9 at 3 ng/ml, 15 at 6 ng/ml, 7 at 12 ng/ml. A good correlation was found between the 2 methods $R^2 = 0.948$ with the logarithm function: Slope 63.00 ± 10.83, Y-intercept 3.815 ± 7.827, X-intercept -0,06055, 1/slope 0,01587

CONCENTRATION ng/ml	CEL1*	CEL2*	MEAN	STD ERROR
1,5	14	28		
1,5	12	13		
1,5	26	18	20	6
1,5	28	28		
1,5	23	19		
1,5	15	20		
3	10	12		
3	12	8		
3	19	21		
3	29	49	40	29
3	60	82		
3	14	20		
3	71	72		
3	87	85		
3	24	53		
6	74	80		
6	79	80		
6	92	86		
6	17	17		
6	9	10		
6	27	33		
6	25	27		
6	22	24	46,5	28
6	19	21		
6	58	69		
6	34	32		
6	62	76		
6	59	78		
6	75	82		
6	7	21		
12	37	33		
12	71	72		
12	82	75		
12	94	98	74	19
12	83	81		
12	69	70		
12	89	81		

* CEL 1, CEL 2 : 2 different wells with RH1 antigen coated

Conclusion

The work presented here is a first step before a complete validation of the method. These preliminary results indicate that the fully automated Galileo® technology can be used to design a semi quantitative approach of anti RH1 determination. The concentration found by the Galileo can be compared with the expected concentration of anti RH1 following IgRHD injection knowing both the date and the dose of the injection. If the result of the Galileo measurement exceeds the expected concentration, an alloimmunization can be suspected. This method will be very useful in the monitoring of RH:-1 women when IgRH have been previously injected.