ASSESSING THE PERFORMANCE OF AUTOMATED ANTI-RED BLOOD CELL ANTIBODIES TITRATION BY COLUMN AGGLUTINATION TECHNOLOGY ON THE IH-500 SYSTEM (Bio-Rad®)

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Background :In France, since May 2018, the legislation does not promote anymore the use of the reference tube method for titration of anti-red blood cells antibodies. This opened the way to the use of newly developed automated anti-red blood cells antibodies quantitation by column agglutination technology.

Aims: We wanted to assess the performance of titration and scoring by the ID-gel test on the IH-500 system (Bio-Rad®) and to compare it with the performance established for the reference tube method, used in our lab since decades. Another objective of the study was to determine titer thresholds for the gel method, to trigger fetal monitoring by ultrasounds and measurements of the peak systolic velocity in the middle cerebral artery.

Methods: The antibody titer is defined as the greatest dilution of the plasma/serum at which a detectable hemagglutination is still visible.

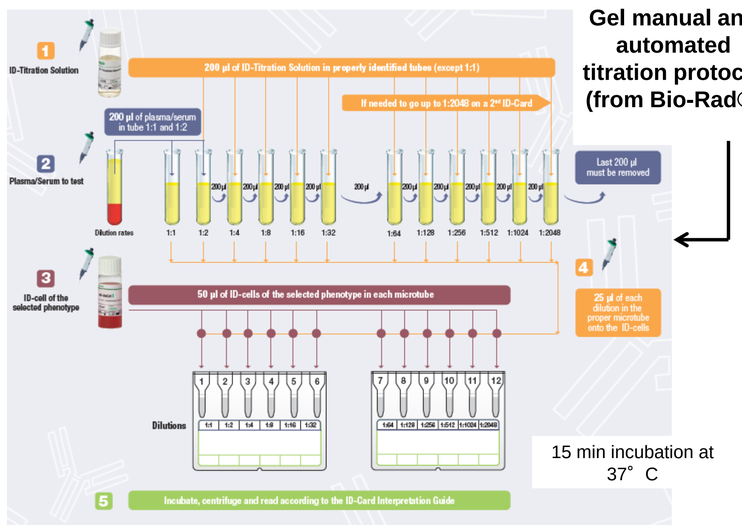
Gel Titration method

IH-500 system

Gel titration results

Reading End-point : 1+, macroscopically
Scoring (Marsh):
4+ = 12
3+ = 10
2+ = 8
1+ = 5

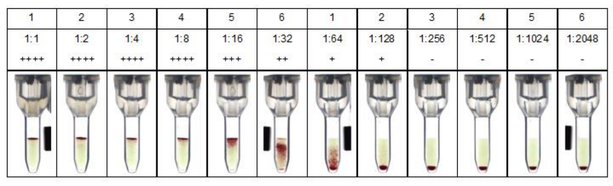
Gel manual and automated titration protocol (from Bio-Rad®)



15 min incubation at 37° C

Test Réalisé: Titration LISS/Coombs (5053) 1-12, 37°C x 0603 IH-500 0300022

Analysé:
support / 20/07/2018 12:38
Vérifié:
support / 25/07/2018 17:48

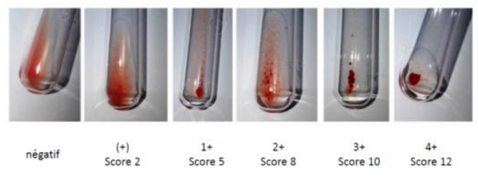


Tube titration method used in the CNRHP laboratory

Automated dilution of fresh samples (starting dilution :100 µl of samples + 100 µl NaCl 0,9%) (Tecan Freedom Clinical Base)
Reagent = fresh red blood cells (RBC) (< 15 days)
Red blood cell concentration used :4% in 0,9% NaCl
Incubation of 50µl of red blood cells + 100 µl of diluted samples at 37° C +/- 2 ° C for 60 +/- 15 min
Washes: 3 times
Testing phase: Antiglobulin (Diagast AGH Maestria IgG) diluted to 1/3, added at room temperature (22 +/- 3° C)
Centrifugation and read:
End point : (+), macroscopically

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

Tecan system



Results:

An home-made internal quality control (IQC) prepared and calibrated using the international anti-D Standard (01/572) was used to determine the intraassay and interassay imprecisions, regarding the score and the titer results.

	Intraassay imprecision			Interassay imprecision		
Titration method	**N=**	**Titer results (CV %)**	**Score results (CV %)**	**N=**	**Titer results (CV %)**	**Score results (CV %)**
Tube manual dilution	19	**9,2**	**11,5**	29	**10,3**	**20,9**
Tube automated dilution	26	**0**	**6,1**	30	**3,7**	**6,4**
Gel manual dilution	19	**6,3**	**6,4**	8	**10,1**	**7,9**
Gel automated dilution (IH-500)	20	**0**	**1,25**	9	**0**	**1,9**

Figure 1: Intra and interassay imprecisions of automated and manual tube and gel titration methods determined with an home-made anti-D (RH1) internal quality control

(value of the IQC titer = 32 (tube method) / 128 (gel method))
The CV (coefficient of variation) for titer results were calculated based on the number of the last positive dilution with the respecting equivalence titer 2 = dilution 1, titer 4 = dilution 2, titer 8 = dilution 3, titer 16 = dilution 4, titer 32 = dilutions 5, titer 64 = dilution 6, titer 128 = dilution 7, titer 256 = dilution 8, titer 512 = dilution 9, titer 1024 = dilution 10, titer 2048 = dilution 11

The intra and interassay imprecisions of the titer/score determined by automated gel method show a low coefficient of variation (CV) (<2%) compared to non-automated gel method (6 to 8 %) or tube methods (6 to 20 %).

Patients samples for testing were chosen during the 2-months assay period, regarding the specificity of the antibodies and the tube titer in order to cover a wide range of situations. Comparison of the results obtained from the same clinical samples with both methods was carried out.

	N	N with identical titer in both methods	N with a higher titer with the tube method	N with a higher titer with the gel method	mean of the difference (gel-tube) (number of dilutions)
anti-D (RH1)	26	0	0	26	**3,96**
Anti-C (RH2)	3	1	0	2	**1,33**
Anti-E (RH3)	11	1	0	10	**2,27**
Anti-c (RH4)	21	2	0	19	**3,29**
Anti-e (RH5)	2	0	0	2	**3,5**
Anti-Cw (RH8)	3	0	0	3	**1,67**
Anti-Kell (KEL1)	16	2	1	13	**1,81**
Anti-Kpa (KEL3)	1	0	0	1	**2**
Anti-Jka (JK1)	8	1	0	7	**1,38**
Anti-Jkb (JK2)	3	1	0	2	**1**
Anti-Fya (FY1)	2	0	0	2	**2,5**
Anti-Fyb (FY2)	2	1	0	1	**1**
Anti-M (MNS1)	15	5	1	9	**1,13**
anti-S (MNS3)	4	1	0	3	**1,75**
anti-s (MNS4)	2	0	0	2	**2**
anti-U (MNS5)	3	0	0	3	**3,33**
TOTAL	**122**	**15**	**2**	**105**	

Figure 2: Comparison of the titer results obtained with automated tube method and with the IH-500 gel method from the same clinical samples
(N = 122)

On the 122 samples tested: 105 have higher titer values with the column agglutination technology, 15 have equal values and 2 have lower values. The **highest differences** (more than 2 to 3 dilutions higher; highlighted in orange) were seen for **antibodies directed against RH system antigens** . Among the other specificities, **Anti-K (KEL1) and anti-M (MNS1) antibodies** show the most samples with **equal or lower titers compared to the tube method** (highlighted in green)

A

Anti-D (RH1) n= 26	Gel titer												
tube titer	1	2	4	8	16	32	64	128	256	512	1024	2048	> 2048
<2					1								
2				1									
4						1	1		1				
8								1	2				
16									2		1		
32										2	2		
64										2	2		
128										1			1
256													2
> 256													3

B

Anti-K (KEL1) n= 16	Gel titer												
tube titer	1	2	4	8	16	32	64	128	256	512	1024	2048	> 2048
<2	1		2										
2		1											
4													
8													
16						1							
32					1				1				
64									2	2			
128													
256										1		1	1
> 256											1		1

Figure 3:
Determination of gel thresholds for triggering ultrasonographical fetal surveillance. Comparison of the titer results obtained with automated tube and IH-500 gel methods from each sample used containing anti-D (RH1) [A] and anti-K (KEL1) [B] antibodies

For all **anti-D (RH1) antibodies** samples, the gel titer was higher compared to the corresponding tube titer. **The tube threshold of 16** used since decades in our lab (see yellow line) **may be extrapolated to at least 3 dilutions higher for the gel method** - i.e. 128 if we take into account the measurement incertainty.
For **anti-K (KEL1) antibodies**, gel titers were equal, slightly higher or even lower depending on the sample tested. **The tube threshold of 16** used in our lab **may be also relevant for gel titers.**
Further testing on more samples are required.

Conclusions :

Automated anti-red blood cell antibodies titration by column agglutination technology on IH-500 system shows better intra and interassay CVs compared to the tube method. It is explained by the fully automated process that includes the reading step. Titer results are almost always higher with the gel technology.
Thus, it seems possible to safely extrapolate the titer thresholds defined for anti-red blood cells antibodies by the tube method to the gel method. However, based on future clinical studies and fetal/neonatal outcomes, it would probably be necessary to increase these thresholds, at least for anti-RH antibodies, in order to avoid heavy, expensive, stressful and useless monitoring of some pregnancies.