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Performance of an Anti-Red Blood Cells Antibody Titration Method Using an Automated Glass Microbeads Column Technique

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We wanted to assess the performance of the titration method using glass microbeads columns on the QuidelOrtho® VISION MAX analyzer and to compare it with the performance established for the reference tube method, used in our lab since decades.

Titration method on VISION MAX

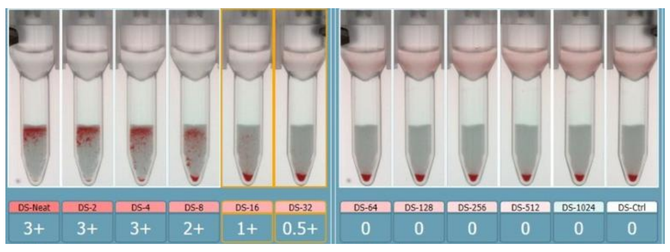
Automated serial 2-fold dilutions of patient plasma/serum (from undiluted (1:1) to a 1:1024 dilution).

Reagents: RH:1,2,3,4,5 (D+C+E+c+e+) KEL:1,2 (K+k+) red blood cells marketed by the UPR (EFS) (ref MPO02) prepared to a concentration of 0.9% in Biovue Red Cell Diluent.

Incubation of serum/plasma dilutions with the red blood cell suspension for 15 minutes at 37° C in the reaction chamber of the Biovue anti-human globulin anti-IgG cassettes.

Centrifugation and reading of the reaction by the instrument's camera

Example :



Titer 16
Score 43

Minimum sample volume: 320 µl

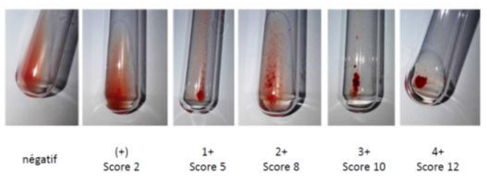
Rendering time for the 1-er -test: 28 min
Then a result approximately every 10 minutes.

Estimated daily throughput: about 50 tests

For each sample: automated reading of reaction intensities for each well
Manual determination of the titer by end-point reading (1+), and manual calculation of the Marsh score (4+=12, 3+=10, 2+=8, 1+=5)

Tube-based titration method used at the CNRHP

Automated 2-fold dilutions of samples (Tecan Freedom Clinical Base)
(starting dilution: 100 µl of sample + 100 µl of 0.9% NaCl)
Anti-D, anti-c, and anti-E titrations: use of RH:1,2,3,4,5 (D+C+E+c+e+) red blood cells from a donor (blood collection within the last 15 days).
Anti-K titers: use of KEL:1,2 (K+k+) red blood cell from a donor (UPR (CNRGS) panel). Concentration of the red blood cell suspension: 4% in 0.9% NaCl
Incubation of 50 µl of test red blood cells + 100 µl of the sample dilution at 37 ± 2 ° C for 60 ± 15 min
3 washes
Addition at room temperature (22 ± 3° C) of antiglobulin (anti-IgG) (Diagast AGH Maestria IgG) diluted 1:3.
Centrifugation and macroscopic observation of agglutinates: end-point reading (+) by the operator (titration performed in duplicate) and manual calculation of the overall Marsh score by summing the scores for each positive dilution according to the chart below.



Results: **Intra-assay (n=10) and inter-assay (n=7) imprecision tests, performed using the anti-D internal quality control (IQC) prepared at the CNRHP, show coefficient-of-variation (CV) values for titers on the automated system of approximately 5% and 6% (end-point reading 1+) and CV values for scores of 2% and 3%, respectively. These results are better than the CVs established for manual titration techniques.**

Figure 1: Intra-assay and inter-assay imprecisions of different titration methods

IQC target values in tube: titer 32 ± 1 dilution, score 30 ± 5
In gel: titer 512 ± 1 dilution, score 90 ± 10
Immunocapture: titer > 256
On glass microbeads column : 512 ± 1 dilution, score 85 ± 10

The coefficients of variation (CV) for the titers were calculated based on the dilution ranks according to the following correspondence: titer 2 = dilution rank 1, titer 4 = dilution rank 2, titer 8 = dilution rank 3, titer 16 = dilution rank 4, titer 32 = dilution rank 5, titer 64 = dilution rank 6, titer 128 = dilution series 7, titer 256 = dilution series 8, titer 512 = dilution series 9, titer 1024 = dilution series 10, titer 2048 = dilution series 11

* Calculation based on dilution ranks
** Due to a maximum value of 256 in the immuno-capture technique, the CV value for the titer is only approximate

	Intra-assay imprecision (IQC titration)				Inter-assay imprecision (IQC titration)		
Titration method	N=	Average Titer	Titer* (CV %)	Score* (CV %)	N=	Titer* (CV %)	Score* (CV %)
Tube manual dilution	19	32	9.2	11.5	29	10.3	20.9
Tube Automated dilution	26	32	0	6.1	30	3.7	6.4
Gel Manual dilution	19	512	6.3	6.4	8	10.1	7.9
Gel Automated dilution	20	512	0	1.25	9	0	1.9
Immunocapture: automated technique	7	> 256	9**	4	5	9**	5
Automated glass microbeads	10	512	5	2	7	6	3

Note: The number of values is not comparable across different methods. This number is low for immunocapture and glass bead techniques, which may result in less precise results.
Confirmation using a larger number of values and with other IQC levels is expected.

Titers obtained using the tube method (end point (+)) and the microbead column method (end point 1+) were compared for 116 samples from pregnant women containing clinically significant antibodies: 27 anti-D, 28 anti-c, 29 anti-E, and 32 anti-K.

For anti-D (RH1) and anti-K (KEL1) titers, the differences were statistically significant, with results on average 1.8 and 0.7 dilutions higher, respectively, using the automated microbeads column technique (Wilcoxon, p<0.0001). For anti-c (RH4) and anti-E (RH3) titers, the differences were smaller and not statistically significant.

Antibody specificity	n (total)	n with lower titer in ORTHO cassette	n with equal title in ORTHO cassette	n with higher titer in ORTHO cassette	mean of titer differences (cassette – tube) (number of dilutions)	standard deviations of titer differences (cassette – tube) (number of dilutions)
anti-D (RH1)	27	0	5	22	**1.8***	1.1
anti-c (RH4)	28	1	17	10	**0.5**	0.8
anti-E (RH3)	28	2	21	5	**0.2**	1.2
anti-K (KEL1)	31	2	11	18	**0.7***	1

* Statistically significant

Figure 2: Comparison of titer results obtained from samples tested using the semi-automated tube method at the CNRHP and the automated method using glass microbeads columns

A Anti-D n=27

tube titer \ Microbeads column titer	0	1	2	4	8	16	32	64	128	256	512	1024	2048
<2		3	1	7									
2													
4					1	1	1	1					
8								2					
16								2	1				
32								1		2			
64											1		
128									1				
256												1	
> 256												1	

B Anti-K n=31

tube titer \ Microbeads column titer	0	1	2	4	8	16	32	64	128	256	512	1024	2048
<2	4	1	2	2									
2					1								
4							1						
8			1										
16						1	2						
32						1	1	2	1				
64								1	2				
128									1	1	2		
256										1	1	3	
> 256												1	

C Anti-c n=28

tube titer \ Microbeads column titer	0	1	2	4	8	16	32	64	128	256	512	1024	2048
<2	9	5	3		1								
2					1								
4				1	1	1							
8						2							
16							1						
32							1						
64							1	1					
128													
256													
> 256													

Figure 3: Comparison of titer results obtained using the tube method and the glass microbeads method for each sample containing anti-D (RH1) [A], anti-K (KEL1) [B], or anti-c (RH4) [C] and determination of threshold titers for initiating fetal ultrasound monitoring to detect indirect signs of severe fetal anemia

For samples containing **anti-D,** the **tube-based cutoff titer of 16** defined in our laboratory appears to correspond to a titer **of approximately 64 using the automated microbead column technique**.
For samples containing **anti-K, the threshold titer of 16 in the tube** used in our laboratory appears to be applicable as the threshold titer **in the automated microbead column technique as well.**
For samples containing **anti-c**, **the threshold titer of 4 in the tube** used in our laboratory appears to be applicable as the threshold titer **in the automated microbead column technique as well.**
For anti-E, due to excessive variability in results, we were unable to establish a corresponding threshold titer between the two techniques.

Conclusion: The automated titration method using glass microbeads columns demonstrates good performance. The titers obtained differed significantly from those obtained using the tube-based technique, with variations observed depending on the samples and the antibody specificity. To establish threshold titers using this technique that correlate with the risk of severe fetal or neonatal hemolytic disease, further studies involving a larger number of samples and incorporating clinical data on pregnancy outcomes should be considered.