EVALUATION OF THE GALILEO® AUTOMATED INSTRUMENT (IMMUCOR®) FOR SCREENING IRREGULAR RED BLOOD CELL ANTIBODIES IN PREGNANT WOMEN: EXPERIENCE OF FRENCH NATIONAL REFERENCE CENTER FOR PERINATAL HEMOBIOLOGY

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Background

In France, all pregnant women, regardless of their RhD type, should be tested for clinically significant antibodies. The RhD positive women must be screened at the first and third trimester while RhD negative are screened at the first trimester, at 6 months (before the IgRH injection) and 8 months. The screening for antibodies followed by their identification is used to determine the need for additional analysis to monitor the risk of the haemolytic disease of the fetus and newborn (HDFN). The detection of antibodies, even at very low concentration, is considered critical for our laboratory, reference centre in France, because the monitoring of the pregnancy as well as the care of the newborn both depend on this result. Although 150 new RH1 immunizations are diagnosed in France every year, the attention should not only be on anti-RH1 antibodies but also on others such as anti-RH4 or anti-KEL1.

The aim of this study is to evaluate antibody screening and the sensitivity of detection of irregular red blood cell antibodies in pregnant women on the Galileo® automated instrument in our center.

Materials and methods

85 samples of pregnant women were analysed. The Capture-R (4 cell) technology used on the Galileo® for screening was compared with the gel test method performed routinely and manually in our lab (LISS Coombs gel, DIAMED®).
To evaluate the sensitivity of detection of anti-RH1 we used 6 dilutions of the NISBC (National Institute for Biological Standards and Control) anti-RH1 standard: from 0.75 ng/ml to 24 ng/ml.

Results

1- For the **50 negative samples tested** we found **50 negative results with Galileo®.**

2- 14 specificities of allo-antibodies were tested : anti-RH3, anti-RH4, anti-RH5, anti-RH8, anti-KEL1, anti-KEL3, anti-FY1, anti-JK1, anti-MNS1, anti-MNS3, anti-MNS4, anti-MNS5, anti-LU1, anti-LU2.
Galileo® detected **30 of the 35 positive** samples and the 5 antibodies not detected were: 2 anti-RH8, 3 anti-MNS1.

3- Another assay was performed to test positive results samples with serial dilution of sera (in albumin medium). Dilutions were chosen in function of initial antibody titer.

POSITIVE SAMPLES			GALILEO	LISS COOMBS GEL DIAMED
ANTIBODIES	TITERS*	SERIAL DILUTION TESTED	CAPTURE-R INTENSITY**	AGGLUTINATION INTENSITY***
5 Anti-RH3	< 1/2	Pur	87	1,5+
	< 1/2	Pur	88	1+
	<1/2	Pur	83	1+
	<1/2	Pur	56	(1+)
	1/8	Pur	88	2,5+
3 Anti-RH4	1/2	Pur	99 99 95	2+
	1/4	Pur	99 99 99	2,5+
	1/256	1/50	99 99 86	3+
		1/100	99 99 90	2,5+
		1/200	99 87 83	2,5+
1 Anti-RH5	<1/2	Pur	99 99 99	2+
5 Anti-KEL1	1/8	Pur	64	2+
	1/32	1/12	85	1+
		1/25	67	-
		1/50	neg	-
	1/64	1/25	90	1+
		1/50	41	-
		1/100	14	-
	1/128	1/50	96	2+
		1/100	89	1+
		1/200	74	0,5+
	1/256	1/50	93	2,5+
		1/100	99	2+
		1/200	94	1+
1 AntiKEL3	1/32	1/40	62	2+
2 Anti-RH8	1/2	Pur	25	2+
	1/4	Pur	86	3+

***Titration** performed by a saline antiglobulin procedure (60 minutes incubation at 37°C and using of anti-IgG)

****Intensity of reaction with Capture-R technology** : correspondence
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+

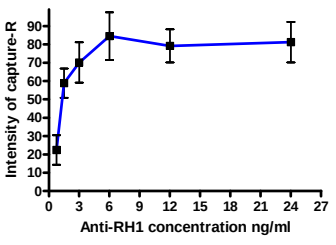
***** Intensity of agglutination with LISS Coombs gel**
From – to 4+

POSITIVE SAMPLES			GALILEO	LISS COOMBS GEL DIAMED
ANTIBODIES	TITERS*	SERIAL DILUTION TESTED	CAPTURE-R INTENSITY**	AGGLUTINATION INTENSITY***
3 Anti-FY1	1/8	Pur	44 71 74	2,5+
	1/32	1/12	90 94 96	2,5+
		1/25	88 89 99	2+
		1/50	83 90 95	1+
	1/128	1/50	91 87 99	2+
		1/100	86 90 98	1,5+
		1/200	54 61 67	0,5+
2 Anti-JK1	1/4	Pur	17 20 34	2+
	1/32	Pur	87 99 87	2+
3 Anti-MNS3	1/8	Pur	29 42	1,5+
	1/64	1/25	99 80	2,5+
		1/50	89 83	2+
		1/100	53 78	1,5+
	1/128	1/50	85 80	2+
		1/100	86 82	1+
		1/200	55 64	-
1 Anti-MNS4	1/128	1/50	87 81 86	2,5+
		1/100	81 97 99	2,5+
		1/200	99 99 99	2,5+
1 Anti-LU1	1/4	Pur	32 51	2,5+
1 Anti-LU2	1/256	1/50	77	2+
		1/100	neg	-
		1/200	neg	-
1 Anti-MNS5	1/256	1/50	97 94 91 99	3+
		1/100	99 93 94 92	2,5+
		1/200	86 99 99 99	2+

We observed a better sensitivity with the Capture-R Immucor® Technology than with the gel test method for all antibodies detected by Galileo®, except for 2 antibodies (1 Anti-RH8, 1 Anti-LU1).

4- The sensitivity of detection of anti-RH1 was found between 0.75 and 1.5 ng/ml for Galileo (versus 6 ng/ml for gel test method).

		Anti-RH1 concentration					
		24 ng/ml	12 ng/ml	6 ng/ml	3 ng/ml	1,5 ng/ml	0,75 ng/ml
Intensity of capture-R	Number of values	10	10	10	10	10	10
	Minimum	66	59	60	55	46	13
	Maximum	99	89	99	87	77	36
	Mean	81,2	79,2	84,5	70,1	58,9	22,4
	Std. Deviation	11,12	9,886	13,76	11,53	8,962	8,027
	Std. Error	3,518	3,126	4,352	3,647	2,834	2,538
	Coefficient of variation	13.70%	12.48%	16.29%	16.45%	15.22%	35.83%



Conclusion

Galileo® offers a high sensitivity for the 14 specificities of antibodies tested during this evaluation to monitor clinically significant antibodies in pregnant women. The Capture-R (4 cell) screening method is a satisfactory technology with a better sensitivity for anti-RH1 detection than the gel method. Furthermore, the 5 antibodies not detected (2 anti-RH8 and 3 anti-MNS1) were IgM antibodies which cannot cross the placenta and therefore would not be the cause of HDFN.