#COD#

IMPLEMENTING ANTI-RH1 MICROTITRATION EXTERNAL QUALITY CONTROL PROGRAM (EQC)

S. Huguet-Jacquot[3], C. Toly-Ndour[3], H. Delaby[3], J. Beaud[3], J. Babinet[3], A. Vassault[1], M. Vaubourdolle[2], A. Mailloux[3]

1. ASQUALAB - Bâtiment Leriche – 8 rue Maria Helena Vieira Da Silva - 75014-Paris, FRANCE
2. DMU BioGeM – AP-HP.SorbonneUniversité - Site Saint-Antoine – 184 rue du Faubourg St Antoine – 75012 – PARIS, France
3. Service d'Hémobiologie fœtale et périnatale - Centre National de Référence en Hémobiologie Périnatale (CNRHP), Hôpital Saint-Antoine, AP-HP, 72012 Paris, FRANCE

Microtitration of anti-RH1, an indirect haemagglutination gel diffusion test, allows quantification of low concentrations of anti-RH1. It is suitable for passive anti-RH1 following the injection of IgRh in anti-RH1 prophylaxis. With the prospect of wider use of this technique thanks to automation and with the legal obligation for French laboratories to be accredited came the need for an EQC. The CNRHP and ASQUALAB has set up one and send twice a year, a plasma sample with a clinical case study.

First exercice : 14 laboratories

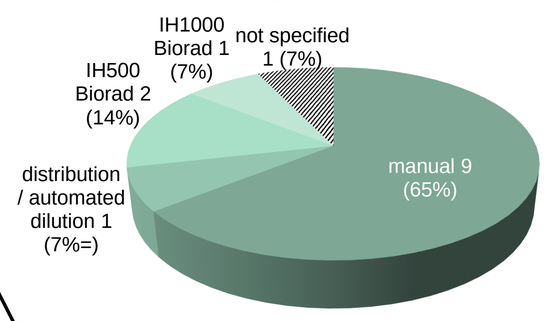
Sample MT1901 = Sample taken on 18/12/2019 at 27 SA from Mrs. M. with a positive anti-RH1 .
Patient RH:-1, positive fetal RHD genotyping, having received an injection of IgRh 200 µg on 09/18/19 following metrorrhagia. There was no other injection of IgRh .

Evaluation of microtiter

Target value: 24 µg/l (ng / ml)
Acceptable values: 12 to 48 µg/l (ng /ml)
Raw average: 23.5 µg/l, raw CV: 23.3%.
Robust Average: 24.0 µg/L, Robust CV: 11.0%.
13 laboratories out of 14 returned a result within the acceptable values set .

Results ng /ml	12	13.92	12 to 24	24	28	30	not returned
Number of laboratories	1	1	1	7	1	2	1

Techniques used



standard	Test cells used		total
	natives	papainized	
House	2	4	6
commercial	1	6	7
total	3	10	13

Advice assessment

Advice in the form of choices to be ticked off among several proposals.

interpretative clinico -biological advice and % of correct answers:

- ☐ Concentration of anti-RH1 compatible with the expected concentration. 0/14 – 100%
- ☑ **Concentration of anti-RH1 higher than the expected concentration . 14/14 – 100%**
- ☑ **Patient probably immune to anti-RH1 . 10/14 – 71%**
- ☐Patient to be treated with IgRh within 72 hours . 0/14 – 100%
- ☑ **Systematic injection of IgRh from the 28th week useless. 8/14 – 57%**
- ☐ Systematic injection of IgRh from the 28th week recommended . 0/14 – 100 %
- ☑ **Antibody screening test + dosage to be checked in 2 weeks. 14/14 – 100 %**
- ☑ **Quantification of anti-RH1 recommended . (proposal not checked acceptable because depending on the practices of each laboratory – quantification requested from a titre of 8 in indirect Coombs , tube technique) 9/14 – 64%**

Second exercice : 17 laboratories

Sample MT2001 = Sample taken on 03/06/2020 from Mrs M RH:-1 for an "antibody screening test" prescribed for her systematic injection in the 28th week. We find a positive result with an anti-RH1. Fetal RHD genotyping is positive and this patient received an injection of IgRh 200µg on 06/05/2020 for metrorrhagia. There was no other injection of IgRh .

Evaluation of microtiter

Target value: 12 µg/l (ng / ml)
Acceptable values: 6 to 24 µg/l (ng /ml)
Raw average: 13.9 µg/l, raw CV: 28.8%.
All the laboratories returned a result within the acceptable values set.

Results ng /ml	6.96	10	12	13	14	15	23
Number of laboratories	1	1	6	1	1	5	2

Advice assessment

Advice in the form of choices to be ticked off among several proposals.

interpretative clinico -biological advice and % of correct answers:

- ☑ **Concentration of anti-RH1 compatible with the expected concentration. 17/17 – 100%**
- ☐ Concentration of anti-RH1 higher than the expected concentration . 1/17 – 94%
- ☐ Patient probably immunized to anti-RH1 . 0/17 – 100%
- ☐ Systematic injection of IgRh from the 28th week useless. 1/17 – 94%
- ☑ **Systematic injection of IgRh from the 28th week recommended . 12/17 – 71%**
- ☐ antibody screening test + dosage to be checked in 2 to 3 weeks . 4/17 – 76%
- ☐quantification of anti-RH1 recommended . 0/17 – 100%

Fird exercice : 17 laboratories

Sample MT2002 = Sample taken on 20/10/2020 from Mrs M RH:-1 for an "antibody screening test" prescribed for her scheduled caesarean section the following week. We find a positive result with an anti-RH1. The fetal RHD genotyping is positive and this patient received her routine prophylaxis for the 28th week, i.e. 300 µg on 08/20/2020.

Evaluation of microtiter

Target value: 6 µg/l (ng /ml)
Acceptable values: 3 to 12 µg/l (ng /ml)
Raw average: 5.5 µg/l, raw CV: 21.9%.
16 laboratories out of 17 returned a result within the acceptable values set.

Results ng /ml	3	3.48	4.5	4.6	4.7	5	6	6.5	7	Neg
Number of laboratories	1	1	1	1	1	1	6	1	3	1

The result returned negative is a misinterpretation due to the use of native red blood cells whose detection threshold for anti-RH1 (approximately 6 ng/ml) is higher than with the use of papain red blood cells ,

Advice assessment

Advice in the form of choices to be ticked off among several proposals.

interpretative clinico -biological advice and % of correct answers:

- ☑ **Concentration of anti-RH1 compatible with the expected concentration. 17/17 – 100%**
- ☐ Concentration of anti-RH1 higher than the expected concentration . 0/17 – 100%
- ☐ Concentration of anti-RH1 lower than the expected concentration. 2/17 – 88%

(proposal checked acceptable because the 2 laboratories also checked compatible concentration)

- ☐ Patient probably immunized to anti-RH1 . 0/17 – 100%
- ☐Rhesus prophylaxis at childbirth unnecessary . 0/17 – 100%
- ☑ **Rhesus prophylaxis during childbirth recommended . 16/17 – 74%**
- ☑ antibody screening test **to remotely control childbirth (6 months) 10/17 – 76%**

(proposal not checked acceptable linked to the variability of practices and without impact for the current pregnancy)

- ☐quantification of anti-RH1 recommended . 0/17 – 100%

Since 2019, 3 exchanges were proposed with about fifteen participants. The majority of laboratories use a manual technique on papainized red blood cells. More than 90% of them had a correct result. The 2 erroneous results correspond to a not returned result and a negative result, an inappropriate term for an anti-RH1 whose concentration was below the detection threshold of the method. The interpretation on the passive or immune nature of anti-RH1 was correct for all the laboratories. Nevertheless, differences in practice can be observed for anti-RH1 prophylaxis advice, which is not carried out by all.

Microtitration technique is simple, generally well mastered by all the participants and a valuable aid in the monitoring and management of RH1 negative pregnant women.