P0050

# EXTERNAL QUALITY ASSESSMENT PROGRAM FOR ANTI-RED BLOOD CELL ANTIBODIES TITRATION : HETEROGENEOUS RESULTS FOR ANTI-M TITERS

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**Anti-red blood cells (RBC) titration with indirect antiglobulin test allows to quantitate maternal antibodies during pregnancy and to assess the risk for hemolytic disease of the fetus and newborn. The diversification of titration techniques made possible by the French decree of May 2018 and the requirement for all laboratory tests to have external quality control led the The French National Reference Center in Perinatal Hemobiology , in accordance with its missions (Ministerial Circular DGOS 2004), to collaborate with ASQUALAB to implement an external quality assessment program for anti-RBC antibodies titration, distributing 2 samples per year per participant and proposing clinical cases and questions to answer.**

| Exercise | Antibody specificity | Expected titer | Titer results | Expected advices (boxes to tick to adapt the biological follow-up and the pregnancy monitoring) | Adapted advices |
|---|---|---|---|---|---|
| 19-01 | Anti-D (RH1) | Saline Tube method 32 (16-64)<br>LISS Gel method 128 (64-256) | 6/6 (tube)<br>1/1 (gel) | Risk of fetal anemia and fetal ultrasound and MCA-PSV monitoring/ Risk of severe HDN with IPT and ET or top-up transfusion. Father phenotyping / non invasive *RHD* fetal genotyping. Regular antibody quantification during all the pregnancy. Pregnancy management by a Multidisciplinary Center for Prenatal Diagnosis / Delivery to occur in an hospital with technical facilities and experienced neonatologists | 6/6 (tube)<br>1/1 (gel) |
| 20-01 | Anti-c (RH4) | Saline Tube method 8 (4-16)<br>LISS Gel method 64 (32-128) | 6/6 (tube)<br>1/1 (gel) | Risk of fetal anemia and fetal ultrasound and MCA-PSV monitoring/ Risk of severe HDN with IPT and ET or top-up transfusion. Father phenotyping / non invasive *RHc* fetal genotyping. Regular anibody quantification during all the pregnancy. Pregnancy management by a Multidisciplinary Center for Prenatal Diagnosis / Delivery to occur in an hospital with technical facilities and experienced neonatologists/ | 6/6 (tube)<br>1/1 (gel) |
| 20-02 | Anti-M (MNS1) | Saline Tube method 2-4 (<1-16)<br>LISS Gel method 32 (16-64) | 6/6 (tube) but wide range of answers<br>1/1 (gel) | No risk of fetal anemia or severe hemolytic disease of the newborn<br>Father phenotyping<br>Antibody quantification at the 3rd trimester | 3/6 (tube)<br>No answer (gel) |
| 21-01 | Anti-D (RH1) | Saline Tube method 32 (16-64)<br>LISS Gel method 128 (64-256) | 6/6 (tube)<br>1/1 (gel) | Risk of fetal anemia and fetal ultrasound and MCA-PSV monitoring/ Risk of severe HDN with IPT and ET or top-up transfusion. Father phenotyping / non invasive *RHD* fetal genotyping. Regular antibody quantification during all the pregnancy. Pregnancy management by a Multidisciplinary Center for Prenatal Diagnosis / Delivery to occur in an hospital with technical facilities and experienced neonatologists. | 6/6 (tube)<br>1/1 (gel) |
| 21-02 | Anti-K (KEL1) | Saline Tube method 128 (64-256)<br>LISS Gel method 128 (64-256) | 7/7 (tube)<br>1/1 (gel) | Risk of fetal anemia and fetal ultrasound and MCA-PSV monitoring/ Risk of severe HDN with IPT and ET or top-up transfusion. Father phenotyping / non invasive *KEL1* fetal genotyping. Regular antibody quantification during all the pregnancy. Pregnancy management by a Multidisciplinary Center for Prenatal Diagnosis / Delivery to occur in an hospital with technical facilities and experienced neonatologists | 7/7 (tube)<br>1/1 (gel) |
| 22-01 | Anti-D (RH1) | Saline Tube method <2 (0-2)<br>LISS Gel method 8 (4-16) | 7/7 (tube)<br>1/1 (gel) | No Risk of fetal anemia or severe HDN. Father phenotyping / non invasive *RHD* fetal genotyping. Regular antibody quantification during all the pregnancy. Microtitration test and/or anti-D concentration determination by continuous flow analysis recommended. | 7/7 (tube)<br>1/1 (gel) |
| 22-02 | Anti-E (RH3) | Saline Tube method 4 (2-8)<br>LISS Gel method 16 (8-32) | 7/7 (tube)<br>1/1 (gel) | No Risk of fetal anemia. Risk of mild HDN. Father phenotyping +/- non invasive *RHE* fetal genotyping. Regular antibody quantification during all the pregnancy. Anti-E concentration determination by continuous flow analysis recommended. Delivery to occur in an hospital with technical facilities and experienced neonatologists. | 7/7 (tube)<br>1/1 (gel) |

MCA-PSV: peak systolic velocity in the middle cerebral artery / HDN: hemolytic disease of the newborn / IPT: intensive phototherapy / ET: exchange transfusion

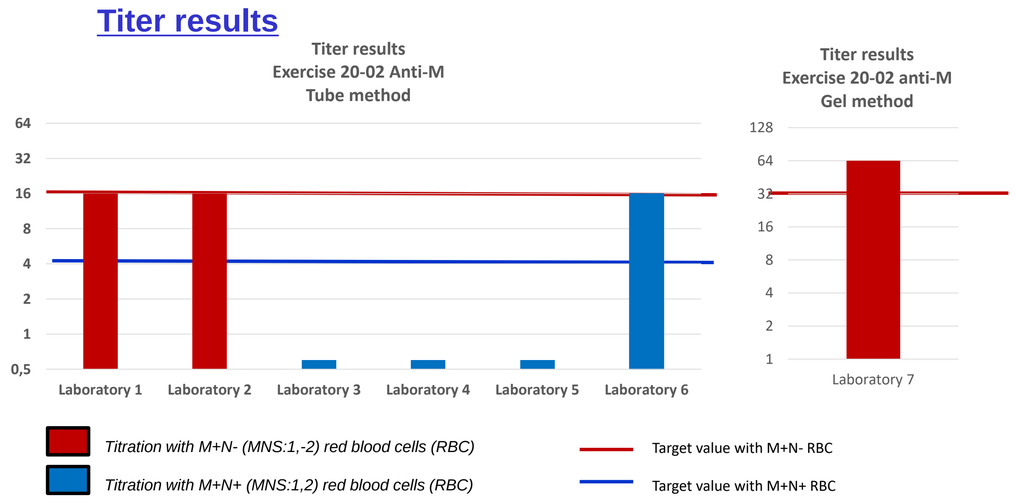
Since 2019, 7 exercises have been proposed. For exercises with anti-D (RH1) (n=3), with anti-c (RH4) (n=1), with anti-E (RH3) (n=1) and with anti-K (KEL1) (n=1), 7 laboratories/7 have found a result inside the expected titer range, with adapted advices associated for the biological follow-up and for the clinical (ultrasound) monitoring of the pregnancy.

For the exercise with anti-M (MNS1), the found titer values varied between <1 and 16 with the saline tube method. The advice given with the results were different, depending not only on the titer value but also on the global consideration for the hemolytic risk related to anti-M, due to different concern based on literature and their own experimental data.

## Exercise 20-02 anti-M (MNS1)

**TIT 20 02 clinical case =** sample of Mme G. parity 2. Positive screening at the 1st trimester : identification of **anti-M (MNS1) IgG.**
Patient with a M negative phenotype

### Titer results




**Possible causes to explain the different titers observed**

- M antigen on Glycophorin A, consisting of 5 amino acids, 3* of which are O-glycosylated (Ser-Ser*-Thr*-Thr*-Gly).
- Sugars = disialotetrasaccharides (mainly) +/- monosialotrisaccharides +/- trisialopentassacharides ➢ Impact of the type of sugars and the interactions sugars/ sialic acids/ amino acids on the conformation of the antigen. Type of glycosylation and variable amount of antigen expressed from subject to subject (high dose effect)

Most anti-M antibodies recognize a conformational epitope composed of a combination of amino acids/sugars and sialic acid.
There are probably several types of antibodies according to the exact pattern recognized, with an IgG component and a variable thermal optimum, which explains why the levels of antibodies found can fluctuate strongly depending on the titration techniques and the type of red blood cells used.

**How to better standardize the results of anti-M titrations and how to better evaluate the hemolytic risk?**

- Serological titration of the previous sample in parallel required
- In case of high titer, titrate antibodies after serum treatment with DTT (to destroy the IgM component)
- Have a "standardized" M positive red blood cells pool (test cells coming from several donors, large "pool" to freeze if necessary?)
- If clinical signs with seemingly low titer: titrate the maternal anti-M antibodies with the father's red blood cells (after adsorption of anti-A/B if applicable)

### Expected advices with results

| | Laboratory 1 | Laboratory 2 | Laboratory 3 | Laboratory 4 | Laboratory 5 | Laboratory 6 | Laboratory 7 |
|---|---|---|---|---|---|---|---|
| **Compliance of the given advices with the expected one** | 100% | 100% | 75%* | 75%* | 75%* | 50* → 75%$ | N/A** |

* *Recommendation of more frequent antibody quantifications ((from the 2nd trimester)*
** *No recommendation given*
$ *More cautionary approach : Risk of severe HDN / Pregnancy management by a Multidisciplinary Centre for Prenatal Diagnosis / Delivery to occur in an hospital with technical facilities and experienced neonatologists*
→ *advices finally accepted as correct (see literature review below)*

**Experience of our Center and review of the literature on the obstetric dangerousness of anti-MNS1 (M):**

Antibody reported as responsible for fetal anemia and/or severe hemolytic disease of the newborn (HDN) in rare cases.

- Since 2000 at the CNRHP : 3 cases of fetal anemia at the 3rd trimester of the pregnancy with an anti-MNS1 titer at least equal to 64 with the tube method (majority IgG).

- Since 1959 in the literature *(revue Stetson B et al Am J Perinatol Rep 2017):*

  - 13 cases of patients with one or more pregnancies with severe fetal anemia, sometimes from the end of the 2nd trimester : anti-MNS1 titer at least equal to 64 (tube method) except for 1 patient with a titer of 1 *(Wikman A et al. Transfusion 2007).*
  - About 40 cases of severe HDN (aregenerative anemia +/- jaundice) having required a transfusion or exchange transfusion (ET) with titers generally greater than 16 (tube) but may be weaker (from 1) and direct antiglobulin tests (DAT) often negative in newborns.

➢ Severe HDN not so frequent given the number of pregnancies with anti-M (10% of pregnancies with positive screening test). Exclusive imputability of the anti-M antibodies to HDN in cases observed with low titers?

Role of the mechanism of action of the antibody (destruction of erythroid precursors) on these observations?
Impact of difficulties in standardizing titration on these results?

**Summary/conclusion : These results underline the good interlaboratory standardization of the titration methods used in France, concerning the RH and the KEL antibody specificities, as well as homogeneous results interpretations and advices for pregnancy management. But they show that important differences could exist with other antibody specificities like anti-M, for which there is variable individual antigenic expression and for which the risk for fetus and newborn hemolytic disease could be difficult to evaluate.**