Transfusion clinique et biologique 33 (2026) 235–240

Contents lists available at ScienceDirect

Transfusion clinique et biologique

journal homepage: www.elsevier.com/locate/tracli

Original article

Clinical input of anti-D (anti-RH1) microtitration in the management of D negative (RH:-1) pregnant women. Experience of the French National Reference Center For Perinatal Hemobiology (CNRHP)

Marion Coux[a], Jenny Beaud[a], Cecile Toly-Ndour[a], Vincent Thonier[a], Stephanie Huguet-Jacquot[a], Elodie Maenulein[a], Michel Vaubourdolle[b], Agnès Mailloux[a,*]

[a] Fetal and Perinatal Hemobiology Department–National Reference Center for Perinatal Hemobiology (CNRHP), DMU of Medical Biology and Genomics (BioGeMH), Hospital Saint-Antoine, AP–HP, Sorbonne University, 184, rue du Faubourg Saint-Antoine, 75012 Paris, France
[b] Department of Biochemistry, Hormonology and Therapeutic Monitoring, Saint-Antoine Hospital, DMU BioGeMH, AP–HP, Sorbonne University, Paris, France

ARTICLE INFO

Keywords:
Anti-D
Titration
Fetomaternal hemorrhage
Pregnancy
Immunoprophylaxis

ABSTRACT

Background and objectives: The administration of anti-D immunoglobulin (RhIg) has significantly reduced the incidence of anti-D alloimmunization. However, it complicates the interpretation of antibody screening, as it is necessary to distinguish passive anti-D from early alloimmunization. Microtitration is the reference method for making this distinction. The aim of this study was to evaluate the impact of anti-D microtitration on the immuno-hematological and clinical management of RH:-1 (D-) pregnant women in the CNRHP in 2024.
Materials and methods: This retrospective observational study included all anti-D microtitrations performed between January 1st and December 31st, 2024 in the CNRHP. The anti-D microtitration impact was analyzed in three settings: antenatal care, delivery, and fetomaternal hemorrhage (FMH).
Results: A total of 3281 microtitrations were performed. Antenatally, 84.6% of results matched the expected anti-D levels, while 10.5% were higher, leading to 11 diagnoses of alloimmunization. At delivery, 603 microtitrations identified two new alloimmunizations and prevented RhIg injections in 30 patients. In FMH setting 11.3% of the cases required multiple doses, with microtitration being essential for assessing neutralization. This was especially observed in massive FMH.
Conclusion: The CNRHP's experience highlights that anti-D microtitration is an essential tool for distinguishing passive from immune anti-D, enabling early detection of alloimmunization, avoiding unnecessary monitoring and RhIg injections, and optimizing dose adjustment in cases of FMH.

1. Introduction

1.1. Background

The Haute Autorité de santé (HAS) recommends immune-hematological monitoring during pregnancy based on the screening and identification of irregular red blood cell alloantibodies [1]. The identification of these antibodies enables the diagnosis of anti-erythrocyte alloimmunization [2]. If the fetus carries the corresponding antigen, fetomaternal erythrocyte incompatibility (FMEI) occurs [3]. Once detected, these antibodies, which can cause hemolytic disease of the fetus and/or newborn (HDFN), should be regularly quantified throughout pregnancy [4]. D (RH1)-related FMEI is the leading cause of non-ABO FMEI and is responsible for 50% of symptomatic cases and 25% of severe forms [5].

In this context, detection of anti-D alloimmunization as well as effective Rh prophylaxis represent major public health priorities.

In France, for D- (RH:-1) patients, screening tests are performed before 10GW, then at 6 and 8 months of pregnancy. Prevention of anti-D alloimmunization relies on a combined strategy systematic injections of anti-D immunoglobulin (RhIg) at 28 weeks of gestation (WG) and within 72 hours after delivery, as well as targeted prophylaxis following any obstetric event associated with a risk of fetomaternal hemorrhage (FMH) [6,7].

1.2. Biological tools for prevention

Immunoprophylaxis optimization relies on several tests. The Kleihauer Test (KT) quantifies fetomaternal hemorrhage from 15 WG

* Corresponding author.
E-mail address: agnes.mailloux@aphp.fr (A. Mailloux).

https://doi.org/10.1016/j.tracli.2026.07.002

Available online 18 July 2026
1246-7820/© 2026 Published by Elsevier Masson SAS on behalf of Société francophone de transfusion sanguine (SFTS).

and defines the RhIg dosage during pregnancy and at delivery [8]. A RBC antibody screen must also be performed at least 7 days before any injection (whether for targeted or systematic prophylactic injections). A negative result excludes alloimmunization, while a positive one may reflect either passive antibodies from prior injections or early alloimmunization [7,9]. Anti-D microtitration is the gold standard test to distinguish between passive and immune antibodies [4,10].

This study assessed the impact of anti-D microtitration on immune-hematological and clinical management of RhD negative (RH:-1) pregnancies in the French national reference center for perinatal hemobiology (CNRHP) in 2024. Impacts were analyzed in 3 different settings: antenatal care, delivery, and FMH management.

2. Materials and methods

2.1. Study population

This retrospective observational study included all RH:-1 pregnant women who underwent anti-D microtitration in our laboratory between January 1st and December 31st, 2024. The women's referral contexts were divided into 3 clinical settings: antenatal routine screening, at delivery and suspected fetomaternal hemorrhage. Data were retrospectively extracted from the laboratory information management system (Clinisys™ GLIMS).

2.2. Techniques

Microtitration is a gel-based hemagglutination technique consisting of an indirect antiglobulin titration test (IAT). We used the column filtration LISS/Coombs (Bio-Rad®). This technique relies on twofold serial dilutions of the patient's plasma or serum incubated with RH:1 papain-treated RBCs. These O R0r (RH:1,-2,-3,4,5) RBCs are supplied by the French Blood Establishment. They were subsequently papain-treated in our laboratory and then resuspended at 0.8% in ID-Diluent 2 (Bio-Rad®) [11].

For each batch of patients, a thawed in-house anti-D standard is tested under the same conditions. It is a polyclonal anti-D solution which is prepared from a 1500 IU vial of Rhophylac® (RhIg) in order to have a final concentration of 0.12 IU/mL (24 ng/mL) and subsequently frozen [4,11].

This test is routinely performed in our laboratory whenever the antibody screening test is positive for anti-D in D-negative or variant D patients.

2.3. Technical steps

Twofold serial dilutions are performed with an isotonic sodium chloride solution (0.9% NaCl) (Versylene®): 6 dilutions of plasma or serum (from pure to 1/32 dilution) and 4 dilutions of the standard (from 1/4 dilution to 1/32 dilution). A volume of 50 µL of R0r papain-treated RBC is distributed into each microcolumn of the Bio-Rad® LISS/Coombs gel card. Then 25 µL of each sample dilution or standard dilution are added. After a 15 to 20 minutes incubation at 37 ± 2 °C and a 10 minutes centrifugation, using the ID Centrifuge 24S (Bio-Rad® program), hemagglutination reactions in the gel cards are rated [4,11].

2.4. Reading and calculation of anti-D concentration

The intensities of hemagglutination reactions are read macroscopically. The hemagglutination intensities of the different dilutions of the patient's sample are compared to those of the anti-D standard. They are rated from “negative”, “(+)”, “1+”, “2+”, “3+” to “4+”. The concentration of anti-D present in the patient's sample equals the inverse of the last reactive dilution of the sample multiplied by the concentration of the anti-D standard dilution with the same reaction intensity (Fig. 1). It is expressed in IU/mL or ng/mL. According to this technique, the anti-D concentration is therefore a discrete value: < 0.0075, 0.0075, 0.01125, 0.015, 0.0225, 0.03, 0.045, 0.06, 0.09, 0.12, 0.18, 0.24, 0.36, 0.48, > 0.48 IU/mL (< 1.5, 1.5, 2.25, 3, 4.5, 6, 9, 12, 18, 24, 36, 48, 72, 96, > 96 ng/mL) [11].

2.5. Interpretation

The anti-D concentration estimated by anti-D microtitration is compared with the anti-D concentration expected after an anti-D immunoglobulin injection. Determining this theoretical concentration requires knowledge of the dose injected and the time between injection and blood sampling. It is calculated using the following formula: C (ng/mL) = $n \times 15/e^{0.03t}$ (n: number of 500 IU (100 µg) doses injected, 15: concentration extrapolated to time 0 after an IV injection of 500 IU (100 µg) of anti-D immunoglobulin (after intra and extravascular equilibrium), 0.03: daily fraction of IgG catabolized and t the time in days since injection) (Table 1) [11].

In practice, if the measured concentration is very close (within ± 1 dilution) or slightly below the expected concentration (up to one dilution lower), the detected anti-D is considered to be of passive origin. No specific monitoring is required. For microtitrations found to

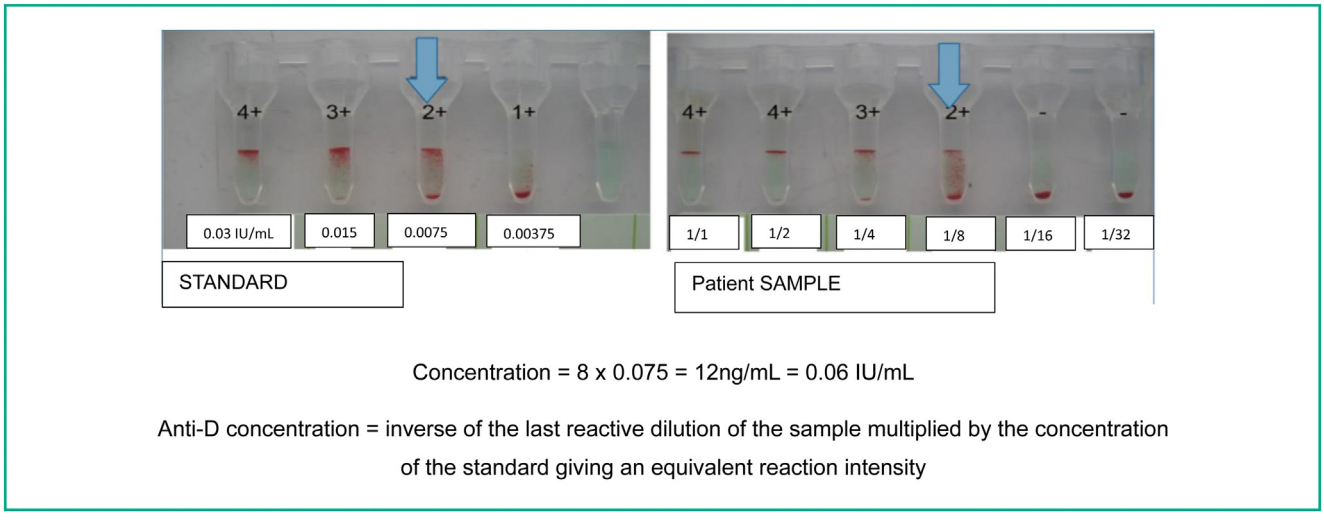


Fig. 1. Example of anti-D concentration calculation after microtitration (based on Huguet-Jacquot et al., 2015).

Table 1
Theoretical expected serum anti-D concentrations IU/mL (ng/mL) after Rhophylac® injection over time [11].

Time after injection	Serum anti-D concentrations IU/mL (ng/mL) after Rhophylac® injection	
	1000 IU (200 µg)	1500 IU (300 µg)
48 hours	0,15 IU/mL (30 ng/mL)	0,225 IU/mL (45 ng/mL)
1 week	0,12 IU/mL (24 ng/mL)	0,18 IU/mL (36 ng/mL)
3 weeks	0,075 IU/mL (15 ng/mL)	0,1125 IU/mL (22.5 ng/mL)
6 weeks	0,038 IU/mL (7.6 ng/mL)	0,057 IU/mL (11.4 ng/mL)
9 weeks	0,019 IU/mL (3.8 ng/mL)	0,0285 IU/mL (5.7 ng/mL)
12 weeks	0,009 IU/mL (1.8 ng/mL)	0,0135 IU/mL (2.7 ng/mL)
15 weeks	0,0045 IU/mL (0.9 ng/mL)	0,00675 IU/mL (1.35 ng/mL)

be much lower (> 2 dilutions below) or higher than the expected concentration (> 1 dilution above), the CNRHP recommends to perform a new test 3 weeks later, which corresponds to the half-life of the molecule, in order to verify the kinetics (gradual decrease) of antibody concentration and thus confirm its passive nature [12].

However, this kinetic monitoring should not prevent or delay the administration of RhIg when prophylaxis is indicated. Indeed, monitoring can be continued after RhIg administration, provided that the expected post-injection antibody concentrations are taken into account.

If the anti-D level has not decreased significantly and even more if it has increased, anti-D alloimmunization should be considered and appropriate biological monitoring should be implemented. In the case of microtitrations above 0.12 IU/mL (24 ng/mL) the continuous flow hemagglutination assay (CFHA) is performed. If its value is above 0.5 IU/mL (25 UCHP/mL or 100 ng/mL), the probability of passive anti-D is low but still possible. This is the case when a sample is immediately drawn after an IV injection or the infusion of several doses of anti-D immunoglobulin [4,12]. As a result, only monitoring of the kinetics of the anti-D level can distinguish passive anti-D from anti-D alloimmunization.

When low anti-D is detected at concentrations consistent with passive anti-D (microtitration between 0.0075 and 0.48 IU/mL (1.5–96 ng/mL) and/or CFHA < 0.5 IU/mL (25 UCHP/mL) but without any record of RhIg prophylaxis, recent injection should be considered. If the patient is confirmed not to have received any anti-D immunoglobulin, the possibility of low-level anti-D alloimmunization must be evaluated [12]. In this case, the strategy consists of performing kinetic monitoring of antibody levels. If antibody levels increase, the presence of anti-D alloimmunization can be confirmed, and Rh immunoprophylaxis is no longer indicated for these patients. If antibody levels remain stable and low (< 0.06 IU/mL [12 ng/mL]), the presence of “mimicking antibodies” cannot be excluded, and Rh immunoprophylaxis remains recommended by the CNRHP.

2.6. Situations in which a microtitration test is performed in the CNRHP laboratory

2.6.1. Antenatal context

During pregnancy, microtitration is performed when the indirect antiglobulin screening test on a gel card shows a weak reaction which is defined either by < 2+ on untreated RBC or only reactive on enzyme-sensitized (papain-treated). It is also performed when the indirect antiglobulin titer by the reference tube method is ≤ 4 [12] [4,10].

2.6.2. Postpartum Rh disease prevention tests

At delivery, Rh disease prevention assessment is performed. It includes RBC alloantibody screening. When an anti-D is detected and its raw data is higher than the ones of a 0.03 IU/mL or 6 ng/mL standard, microtitration is carried out [4,12].

2.6.3. Fetomaternal hemorrhage (FMH)

In the context of FMH, effective neutralization of fetal RH:1 RBC requires anti-D immunoglobulin levels exceeding 100 IU/mL (20 µg/mL) of fetal RBC [8]. Anti-D microtitration is performed to confirm that a sufficient level of anti-D remains in the maternal circulation following prophylaxis. This can be evaluated using the formula: MT × 3 > 20 × KT × 0.25, where MT represents the microtitration (ng/mL), 3 corresponds to the maternal plasma volume at term (in liters), and KT refers to the result of the KT, expressed as fetal RBC per 10,000 maternal RBC. In this context, one fetal RBC per 10,000 maternal RBC is equivalent to approximately 0.25 mL of fetal blood, assuming a hematocrit of 50%.

2.7. Statistical analysis

All analyses were performed using GraphPad Prism. Anti-RhD concentrations measured by microtitration were analyzed as grouped mean values for each expected concentration level. The relationship between measured and expected mean concentrations was assessed using Deming regression and Pearson's correlation coefficient. Agreement between measured and expected values was further evaluated using a Bland-Altman plot, reporting mean bias, standard deviation, and 95% confidence intervals. A significance threshold of $P < 0.05$ was applied for all analyses.

3. Results

Over the course of 2024, a total of 3281 anti-D microtitration tests were performed in the CNRHP laboratory.

3.1. Antenatal care

3.1.1. Measured vs. expected anti-D concentrations

In 2024, 1290 anti-D microtitration tests were carried out in a post-anti-D immunoglobulin injection context (cases for which the injection date was known).

For 1092 samples, the anti-D concentrations measured were consistent with the expected concentrations; for 62 samples, the anti-D concentrations were significantly lower, and for 136 samples, the anti-D concentrations were higher.

Among these 136 samples, there were:

- 25 anti-D alloimmunizations (11 newly diagnosed and 14 already known);
- 72 samples requested a 3-week follow-up. Twenty-five patients were finally found to have a passive anti-D as their anti-D concentration halves every 3 weeks as expected from a prophylactic anti-D. The initial high levels of measured versus expected antibody concentration were likely due to a low volume of distribution in these patients (small-gabled patients) compared to the mean volume of distribution of patients used to establish RhIg pharmacokinetic curves. For the remaining 47 patients, there was no follow-up;
- 39 samples for which the final conclusion was that the measured concentration was compatible with the expected concentration:
  - 17 samples with microtitrations > 0.12 IU/mL (24 ng/mL) and for which the CFHA dosage was more accurate to conclude a non-immunization of these patients,
  - 22 samples showed low microtitration values ≤ 0.06 IU/mL (12 ng/mL). However, the deviation from the expected value for these low concentrations is not considered significant, given the measurement uncertainty.

Fig. 2 illustrates the relationship between anti-D concentrations measured by the microtitration assay and the corresponding expected anti-D concentrations throughout 2024. The relationship between

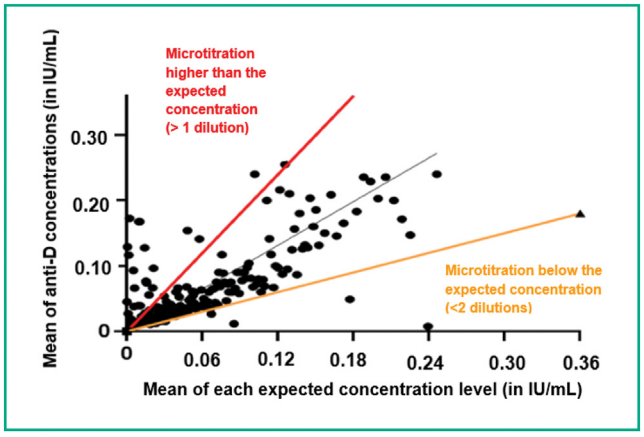


Fig. 2. Comparison of measured and expected anti-D concentrations by microtitration over the year 2024. The 1290 individual results were grouped into 182 expected concentration classes ranging from 0 to 0.246 IU/mL. At low concentrations, classes were defined using 0.0005 IU/mL intervals, with progressively wider intervals at higher concentrations according to the distribution of the data. Each point represents the expected anti-D concentration (x-axis) and the corresponding mean anti-D concentration measured by microtitration (y-axis) for one concentration class. The solid line represents the Deming regression and the dashed line the line of identity (y = x).

measured and expected concentrations was assessed using Deming regression. The regression equation was Y = 0.8975X + 0.3828 (95% CI for slope: 0.7568–1.038; $P < 0.0001$). The slope, which is close to 1, indicates good overall proportional agreement between measured and expected concentrations, whereas the small positive intercept suggests only a limited constant bias. Pearson's correlation analysis also demonstrated a significant positive correlation between the two variables ($r = 0.7006$, $P < 0.0001$), indicating that measured anti-D concentrations generally increased with the expected concentrations, although substantial variability remained between concentration classes.

Fig. 3 presents the Bland-Altman analysis of agreement between measured and expected anti-D concentrations. The mean bias was 1.074 (SD = 21; 95% limits of agreement [–37.08, 45.23]), indicating good overall agreement but substantial variability between concentration classes.

Over the year 2024, among the 1290 anti-D microtitration tests performed after anti-D immunoglobulin injections, these tests allowed the identification of 11 new alloimmunization during pregnancy (Table 2).

Table 2
2024 post partum Rh disease prevention assessment data from the CNRHP.

Women to be treated	*n* (%)
D positive (RH:1) newborn	2052 (58%)
Newborn/fetus RH indeterminate (IUFD)	92 (3%)
Positive fetal RHD genotyping	1368 (39%)
Total	3512
Women not to be treated	***n*** **(%)**
D negative (RH:-1)	1631 (97,2%)
Anti-D immunized women	15 + 2 (1%)
D positive (RH:1) newborn but MT > 0.06 IU/mL (12 ng/mL) + injection < 3 weeks + KT = 0	21 (1.2%)
Positive fetal RHD genotyping but MT > 0.06 IU/mL (12 ng/mL) + injection < 3 weeks + KT = 0	5 (0.3%)
Undetermined fetal/newborn RH1 phenotype but MT > 0.06 IU/mL (12 ng/mL) + injection < 3 weeks + KT = 0	4 (0.3%)
Total	1678

3.2. Impact of anti-D microtitration at delivery

3.2.1. Rh disease prevention

During 2024, the CNRHP received 5216 samples for determining the need for anti-D immunoprophylaxis (called prevention assessments), 26 of which were excluded because they were sent by error for D positive (RH:1) patients. These 5190 samples were from several maternity wards in the Greater Paris region which have agreements with our laboratory. Among these patients, 3512 required prophylaxis, while 1678 did not ultimately need it.

In this context, 603 microtitrations were performed: 86% for patients requiring prophylaxis, 12% for patients who were ultimately not treated (Table 2), and 2% for patients for whom true anti-D immunizations were suspected due to high anti-D concentrations of the microtitration test.

Patients assigned “to be treated” are those whose child is RH:1 or with fetal RHD genotyping determined positive. In cases of intrauterine fetal death (IUFD), when fetal RHD genotyping is inconclusive or not performed, patients are treated as a precaution. Nevertheless, treatment may be withheld if three criterias are simultaneously met: a previous injection within the last 3 weeks, an anti-D microtitration greater than 0.06 IU/mL (12 ng/mL), and a negative KT (0 fetal RBC/10,000 maternal RBC) [3]. In 2024, 30 patients met these criteria and new injections were avoided because the persistence of passive anti-D immunoglobulins is

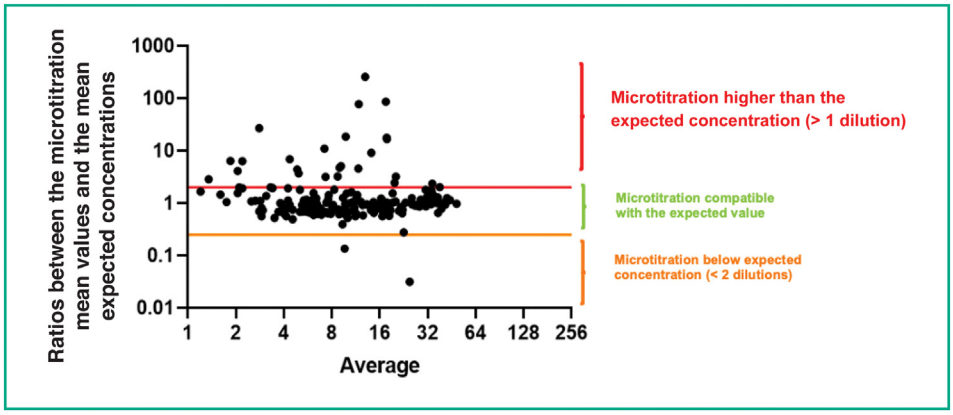


Fig. 3. Bland-Altman plot of the ratios between the microtiter mean values and the mean expected concentrations. Bland-Altman plot comparing measured and expected anti-D concentrations. The analysis was performed using the 182 expected concentration classes derived from the 1290 individual results obtained in 2024. Each point represents one concentration class and corresponds to the difference between the mean measured and mean expected anti-D concentrations plotted against their mean concentration. The solid line represents the mean bias, and the dashed lines represent the 95% limits of agreement.

considered sufficient to ensure maternal protection. Some patients do not require anti-D prophylaxis. This is the case for women whose child is RH:-1, women harboring D variants specifically Weak D variants type 1, 2, or 3 and women who are already anti-RH1 immunized in whom RhIg is ineffective.

3.2.2. Diagnosis of anti-D alloimmunization

Among the 2024 prevention assessments performed in our laboratory, microtitration detected two previously unknown anti-D alloimmunizations. Fifteen other immunizations had already been identified during pregnancy, making unnecessary to provide prevention counselling.

The two newly diagnosed immunizations had high microtitrations (> 0.48 IU/mL [96 ng/mL]) and were both confirmed by CFHA dosage which showed concentrations incompatible with prophylaxis.

3.3. Impact of microtitration in FMH

In 2024, 722 FMH (KT ≥ 1 fetal RBC/10,000 maternal RBC) were diagnosed in both RH:-1 and RH:1 women. Of these, 159 cases (RH:-1 or D variant women) resulted in Rh disease prevention counselling. Among the 159 cases analyzed in 2024, 18 patients (11.3%) required multiple injections of RhIg.

3.3.1. Massive FMH (n = 17)

In 2024, 17 patients had a KT result ≥ 24 fetal RBC/10,000 maternal RBC and a negative RBC alloantibody screen, indicating the injection of RhIg. Among those, one patient had a KT result of 672 fetal RBC/10,000 maternal RBC. The infusion of 18 doses of 1000 IU (200 μg) was recommended. The 72-hour follow-up showed a KT result of 5 fetal RBC/10,000 maternal RBC and a microtitration > 0.48 IU/mL (96 ng/mL), ensuring sufficient coverage using the formula $96 \times 3 > 20 \times 5 \times 0.25$ ($288 > > 25$). Twenty-four hours later, the KT was negative, requiring no additional injections.

3.3.2. FMH in one patient harboring a D variant

A patient with a D variant had a positive KT of 72 fetal RBC/10,000 maternal RBC and a negative RBC alloantibody screen at delivery. Three doses of 1000 IU (200 μg) were recommended. After 48 hours, the KT showed 49 fetal RBC/10,000 maternal RBC and the microtitration reached 0.36 IU/mL (72 ng/mL), leading to the administration of two additional doses. Forty-eight hours later, the KT results remained high (51 fetal RBC/10,000 maternal RBC) while the microtitration decreased to 0.12 IU/mL (24 ng/mL). Flow cytometry analyses of two latter samples confirmed the absence of a strong D expression on fetal RBC. The newborn also had a weakened D phenotype suggesting that he is carrying a D variant, probably identical to the one carried by the mother. This was eventually confirmed by molecular biology.

4. Discussion

This study highlights the key role of anti-D microtitration in managing RH:-1 pregnancies. The analysis of 3281 tests in 2024 shows its importance for both managing immunoprophylaxis and guiding therapy. Microtitration remains the only reliable method to distinguish passive from immune anti-D alloantibodies, particularly when reactivity intensities are inconsistent with injection timing or when clinical history is incomplete [10].

During pregnancy's follow-up in 2024, the majority of cases (84.6%) showed anti-D concentrations consistent with expected levels following immunoglobulin injection, confirming their passive origin and avoiding unnecessary monitoring. However, 10.5% of microtitrations revealed higher-than-expected concentrations, resulting either in a diagnosis of anti-D alloimmunization (25/1290, i.e., 1.9%) or in the initiation of kinetic monitoring to resolve uncertainty about the nature of the antibody. These findings highlight the risk of overlooking active alloimmunization without a quantitative approach, as illustrated by cases where microtitration enabled timely detection of alloimmunization, whereas its absence contributed to severe neonatal outcomes [13]. Overall, these observations focus on the critical role of the clinical pathologist in interpreting results and referring patients to a reference laboratory when needed.

The significant correlation observed between the measured and expected mean concentrations (Deming regression, $r = 0.70$) confirms the overall robustness of the method, while highlighting significant individual variability. This variability fully justifies the dynamic approach recommended by the CNRHP, based on the analysis of the kinetics of microtitration three weeks apart, corresponding to the half-life of anti-D immunoglobulin elimination.

At the time of delivery, analysis of 5190 Rh disease prevention assessments confirmed the value of microtitration in the anti-D prophylaxis strategy. The diagnosis of anti-D alloimmunization at the time of delivery requires immediate management of the newborn. A complete assessment of FMEI (ABO grouping, RH-KEL phenotyping, direct antiglobulin test, elution, bilirubin) allows for the rapid identification and treatment of anemia or hemolytic jaundice [14]. The child must then be followed up the months following birth to screen for possible delayed anemia [5].

In 2024, 603 microtitrations were performed in this context, identifying two previously unknown anti-D alloimmunizations and avoiding unnecessary anti-D immunoglobulin injections in 30 patients. These results are highly important given that RhIg is a blood-derived medicine. Their use must be carefully considered in view of its potential adverse effects, ethical aspects and supply issues.

Finally, in the context of FMH, the 2024 data confirm that microtitration is an indispensable tool for adjusting anti-D immunoglobulin dosage. Of the 159 cases requiring advice for immunoprophylaxy, 11.3% required multiple injections. In addition to the KT, microtitration made possible the assessment of the effective neutralizing capacity of circulating anti-D immunoglobulin. This is especially true in complex situations like massive FMH or persistent positivity of the KT in D variant patients [15]. Microtitration provides information that RBC alloantibody screening alone cannot offer and allows the decision to administer an additional dose.

In the absence of harmonized practices or national guidelines, these results strongly support the routine use of anti-D microtitration alongside RBC alloantibody screening. Moreover, automation can facilitate its integration into daily clinical practice, including antenatal care, post-delivery prophylaxis assessment, and management of FMH [16].

Our study and conclusions have however some limitations: the generalization of the use of the microtitration technique would imply that laboratories have an anti-D standard with a known concentration and linked to the international anti-D standard (NIBSC 16/332). There are commercially available standards, but their cost can be a barrier to the implementation of the systematic technique in laboratories. The same goes for the papain-treated red blood cells used in the test. An alternative would be to send only certain samples to a reference laboratory so that the microtitration technique can be carried out.

5. Conclusion

The CNRHP's experience shows that anti-D microtitration is an indispensable tool in the management of RH:-1 pregnant women. It is the gold standard test to make the difference between passive and immune anti-D. Consequently, it limits diagnostic errors and optimizes the fetomaternal management.

Data from 2024 show that microtitration enables early detection of alloimmunization with a high clinical impact, avoids unnecessary

monitoring and anti-D immunoglobulin injections, and guides dosage adjustment in cases of FMH.

Human and animal rights

The authors declare that the work described has not involved experimentation on humans or animals.

Informed consent and patient details

The authors declare that the work described does not involve patients or volunteers.

Funding

This work did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

All authors attest that they meet the current International Committee of Medical Journal Editors (ICMJE) criteria for Authorship.

Disclosure of interest

The authors declare that they have no competing interest.

References

[1] PDF. Accessed February 27, 2026. https://www.has-sante.fr/upload/docs/application/pdf/suivi_des_femmes_enceintes_-_recommandations_23-04-2008.pdf.
[2] HAS. Suivi et orientation des femmes enceintes en fonction des situations à risque identifiées. Recommandation de bonne pratique - Mis en ligne le 26 mai 2016
[3] Cortey A, Mailloux A, Huguet-Jacquot S, et al. Incompatibilités fœtomaternelles érythrocytaires. EMC Pediatr Mal Infect. 2012;7:1–22, http://doi.org/10.1016/S1637-5017(12)40368-4
[4] Toly-Ndour C, Huguet-Jacquot S, Delaby H, Maisonneuve E, Cortey A, Mailloux A. Quantification des anticorps anti-érythrocytaires chez la femme enceinte. Rev Biol Med. 2019;347:16–34, http://doi.org/10.3917/rbm.347.0016
[5] Franchinard L, Maisonneuve E, Friszer S, et al. Perinatal risk factors associated with severity of haemolytic disease of the foetus and newborn due to Rhc maternal-foetal incompatibility: a retrospective cohort study. Vox Sanguinis. 2022;117:570–9, http://doi.org/10.1111/vox.13215
[6] Masson E. Diagnostic et suivi biologiques des allo-immunisations anti-érythrocytaires chez la femme enceinte. EM-Consulte. Accessed February 27, 2026. https://www.em-consulte.com/article/964831/diagnostic-et-suivi-biologiques-des-allo-immunisat.
[7] Fung-Kee-Fung K, Wong K, Walsh J, Hamel C, Clarke G. Directive clinique n° 448 : prévention de l'allo-immunisation Rhésus D. J Obstet Gynaecol Can. 2024;46:102448, http://doi.org/10.1016/j.jogc.2024.102448
[8] Huguet-Jacquot S, Toly-Ndour C, Delaby H, Beaud J, Mailloux A. Test de Kleihauer : recommandations du centre national de référence en hémobiologie périnatale. Rev Biol Med. 2022;365:29–39, http://doi.org/10.3917/rbm.365.0029
[9] Arrêté du 15 mai 2018 fixant les conditions de réalisation des examens de biologie médicale d'immuno-hématologie érythrocytaire.
[10] Dupont M, Gouvitsos J, Dettori I, Chiaroni J, Ferrera V. Intérêt de la technique de microtitrage des anticorps anti-RH1 dans le suivi immunohématologique des femmes enceintes. Transfus Clin Biol. 2007;14:381–5, http://doi.org/10.1016/j.tracli.2007.10.001
[11] Mailloux A. Technique de microtitrage des anticorps anti-D – étude comparative inter-laboratoires. Congrès Société Francophone de Transfusion Sanguine; 2007 [Accessed February 27, 2026. https://cnrhp.fr/docs/com2007SFTSmicrotitrage.pdf]
[12] Adiogo A. Titrage des anticorps anti-érythrocytaires : place dans le suivi des allo-immunisations fœto-maternelles, comparaison de méthodes, et étude de robustesse de la technique de référence.
[13] Watchko JF, Tiribelli C. Bilirubin-induced neurologic damage-mechanisms and management approaches. N Engl J Med. 2013;369:2021–30, http://doi.org/10.1056/NEJMra1308124
[14] Toly-Ndour C. Utilité de la prévention Rh systématique chez les patientes présentant un antigène D partiel. Congrès Société Francophone de Transfusion Sanguine; 2023.
[15] Beaud J, Delaby H, Toly-Ndour C, Mailloux A, Huguet-Jacquot S. Evaluation au CNRHP de l'automate IH-500 (Bio-Rad®) pour le microtitrage des anti-D (anti-RH1). Transfus Clin Biol. 2019;26:S58–9, http://doi.org/10.1016/j.tracli.2019.06.084
[16] Beaud J, Augier L, Huguet-Jacquot S, Toly-Ndour C, Delaby H, Mailloux A. Test d'un algorithme de calcul du microtitrage anti-RH1 automatisé sur IH-500 (bio-rad®). Congrès Société Francophone de Transfusion Sanguine.