Clin. Lab. 2020;66:XXX-XXX

ORIGINAL ARTICLE

Red Blood Cell Alloantibody Titration - Does the Titration Method Matter?

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SUMMARY

Background: **Red blood cell (RBC) alloantibody titration is a quasi-quantitative method to assess antibody concentration and is considered a useful means of estimating maternal alloimmunization during pregnancy. Traditionally, titration is performed using conventional tube test (CTT). The gel microcolumn agglutination-based method (GMA) has been proven reliable for many immunohematology tests. Our study compared CTT with GMA of two different, commercially available GMA systems for RBC alloantibody titration.**

Methods: **Serum samples with significant RBC-alloantibodies were evaluated in our study. Each sample was titrated concurrently with CTT, with ID-DiaMed-GmbH, Cressier, Switzerland (GMA1), and with DG Gel Coombs Diagnostic Grifols, Passeig Fluvial, Spain (GMA2).**

Results: **One hundred thirty-seven titration tests including 50 anti-D, 25 anti-Kell, 10 anti-E, 9 anti-Jka, 8 anti-c, 5 anti-Cw, 5 anti-Fya, 7 anti-M, 6 anti-Kpa, 3 anti-Lua, 1 anti-e, 3 anti-G, and 2 anti-Cha were performed and evaluated. Samples tested by CTT versus GMA1 and GMA2 generated mostly equal or higher titers by GMAs. The results of both comparisons were in good agreement ($W = 0.91$, $p < 0.0001$, and $W = 0.92$, $p < 0.0001$, respectively). For all antibody specificities, the mean absolute difference in titers ranged from 1 - 3 for both GMA1 and GMA2 versus CTT. Samples tested by GMA1 vs. GMA2 were in almost perfect agreement ($W = 0.95$, $p < 0.0001$).**

Conclusions: **Although both GMAs were found slightly more sensitive than CTT for alloantibody titration, the differences were not significant and the agreement between all methods was very good, possibly indicating GMA as a suitable alternative to CTT in RBC antibody titration.**

(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2019.191021)

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Manuscript accepted December 1, 2019

KEY WORDS

red blood cell antibody titration, alloimmunization, titration method

INTRODUCTION

RBC-antibody titration represents a quasi-quantitative method to assess antibody plasma concentration. ABO-antibody titration has been a useful tool in setting therapeutic strategies and evaluating patient outcomes in ABO-incompatible solid organ and bone marrow transplants. Rhesus-D and other non-ABO antibodies' titration is often applied to evaluate maternal alloimmunization during pregnancy. A precise titer when compared to a previous one performed by the same technique, can detect an increased production of maternal antibody and weigh the need to monitor pregnancy with additional methods such as Doppler ultrasonography [1-4].

Titration performed by the conventional tube test (CTT) is the method that most laboratories use and the one recommended for determining alloantibody titers in pregnancy. CTT is a reliable cost-effective method. Nevertheless CTT has several limitations including reproducibility as well as difficulties in automation and standardization [5,6]. Gel microcolumn agglutination technique (GMA) has already been used for a variety of immunohematology tests such as indirect antiglobulin test, RBC alloantibody identification and crossmatch tests. GMA-method is compatible with automation, ameliorates reproducibility and objectivity, and permits storage of immunohematology tests' results [7,8]. Although GMA has already been used to determine ABO antibodies' titers, limited data exists regarding titration of RhD and other non-ABO antibodies.

We performed a study to compare conventional tube test with gel microcolumn agglutination assay of two different, commercially available GMA systems for RBC antibody titration in pregnant women and patients.

MATERIALS AND METHODS

Samples and RBC reagents

Serum samples with a positive indirect antiglobulin test (IAT) detected and identified during routine pretransfusion testing (Autovue & Biovue Ortho Clinical Diagnostics Bridgend, United Kingdom) containing clinically significant IgG alloantibodies were evaluated in our study. Blood samples were centrifuged (1,500 g, 10 minutes) and immediately stored at -25°C. Titration was performed within 15 days after collection. All testing was performed by the same laboratory technician scientist. The study was conducted from March 2012 until July 2014 and was approved by the Scientific and Ethics Committee, Aretaieion Hospital and Nikaia General Hospital, Athens, Greece.

Serum samples were thawed immediately before testing. Eleven serum sample serial two-fold dilutions (i.e., 1, 2, 4, …1,024) were made with normal saline. Each master dilution was tested with CTT and by two different, commercially available GMA systems concurrently.

The test-erythrocytes used for the CTT procedure were the commercially available 3 ± 1% Reagent RBCs (Ortho Clinical Diagnostics, Bridgend, United Kingdom), which were also used to prepare the 0.8% Reagent RBCs for the titration with the GMA technique as previously described [9,10]. The selection of RBCs for the titration of each alloantibody was made in such a way that the RBCs expressed, if feasible, a double dose of the corresponding antigen.

Titration methods

For the titration in CTT, 100 µL of each serum dilution sample was transferred into 11 test tubes along with 50 µL of 3 - 5% RBC-suspension. Each tube was incubated for 30 minutes at 37°C and then washed three times in an automated cell washing centrifuge. After adding 100 µL of polyspecific-AHG (Millipore, Livingston, UK & Immundiagnostika GmbH, Dietzenbach, Germany), the tubes were centrifuged at 1,000 g for 15 seconds, and resuspended with gentle agitation. The agglutination was graded from 0 to 4+ according to the AABB Technical Manual [11].

Titration in GMA was performed using gel-cards: ID-DiaMed-GmbH, Cressier, Switzerland for GMA1 and DG Gel Coombs Diagnostic Grifols, Passeig Fluvial, Spain for GMA2 following a procedure used for antibody detection and identification, according to manufacturers' instructions.

The reported titer by both methods was the reciprocal of the highest serum dilution showing agglutination grade (1+). In this study at least two negative serum samples of subsequent higher dilution confirmed the final positive result.

Statistical analysis

The statistical analysis was performed by SAS 9.4 for Windows (SAS Institute Inc., NC, USA) [12]. Two methods were applied in order to assess agreement among the different tests for the obtained results. The first method is the mean absolute difference between two tests. Initially we calculated the mean difference between the base2 logarithm (log2) of the dilution showing that the test was positive. Since a negative result is assigned the number zero (0) which does not have a logarithm, we added 1 on every dilution number, so for dilution 4 the log2 (4) + 1 was 2 + 1 = 3, etc. Moreover, the differences of the logarithms for the result of two tests can be a positive or negative number, and therefore the final mean value may not reflect the real discordance between the tests; thus, the mean value was calculated on the absolute difference. For example, if for three results the difference is -1, 0, and 1 then the mean value is 0, while the mean value of the absolute differences is 2/3 = 0.67, a result indicative that the two

tests differ (which is not reflected if the absolute value is not used).

The second comparison technique was performed using Kendall's test that takes into account information related to the proximity of the test results [13] and introduces a metric (W) ranging from no agreement ($W \leq 0$) in cases that the results are considered as random, to complete agreement ($W = 1$). In the authors' opinion, Kendall's test is more representative of the techniques' agreement as it takes into account the "distance" between the results.

RESULTS

A total of 137 serum samples from 107 alloimmunized patients were tested in our study. Alloimmunization was due to transfusion (67/107, 62.62%), to pregnancy (20/107, 18.69%), to both transfusion and pregnancy (10/107, 9.35%), and to passive alloimmunization (10/107, 9.35%).

Out of 107 patients, 21/107 (19.62%) were pregnant women or had newborn children; 41/137 (29.93%) titration tests concerned this group of pregnant women.

In 91 patients, a single serum sample was tested (anti-D (28), anti-Kell (23), anti-E (9), anti-c (6), anti-Jka (9), anti-Kpa (2), anti-Fya (4), anti-M (3), anti-Cw (3), anti-Lua (3), anti-e (1). Multiple samples in the same patient were tested at different time points mainly during pregnancy or titration was performed for each one of more than two alloantibodies in the same patient; more specifically in 11 patients 2 samples were tested, (anti-D (1), anti-c (1), anti-Cha (1), anti-Cw (1), anti-D & C (3), anti-Kpa (1), anti-Kell & Kpa (2), anti-E & Fya (1)), in 2 patients 3 samples were tested (anti-D, anti-G), in 2 patients 4 samples (anti-D, anti-M), and in 1 patient 10 samples (anti-D).

The antibody specificities were distributed as follows anti-D: 32/107 (29.91%) patients, anti-Kell: 23/107 (21.50%), anti-E: 9/107 (8.41%), anti-Jka: 9/107 (8.41%), anti-c: 7/107 (6.54%), anti-Cw: 4/107 (3.74%), anti-Fya: 4/107 (3.74%), anti-M: 4/107 (3.74%), anti-Kpa: 3/107 (2.80%), anti-Lua: 3/107 (2.80%), anti-e: 1/107 (0.93%), anti-G: 1/107 (0.93%), anti-Cha: 1/107 (0.93%), while 6/107 (5.61%) patients had double alloimmunization, anti-C & anti-D: 3/107 (2.80%), anti-Kell & anti-Kpa: 2/107 (1.87%), anti-E & anti-Fya: 1(0.93%).

In regards to the comparison of CTT vs. GMA1 (by Diamed) titration method, it was found that titers were identical in 18 cases (13.14%) by both methods, in 118 cases (86.13%) were higher by GMA1, (48 by one serial dilution, 45 by 2, 17 by 3, 6 by 4, and 2 by 5) and in 1 case (0.73%) was lower (one serial dilution) with GMA1 as depicted in Table 1a. Correlation of titrations with CTT versus GMA1 by antibody specificity is shown in Table 1b. The CTT technique compared to GMA1 using the Kendal's method showed good/satisfactory agreement $W = 0.91$, $p < 0.0001$.

Regarding CTT versus GMA2 (by Griffols), titers were identical in 10 cases (7.3%), in 127 cases (92.7%) were higher by GMA2 (45 by one serial dilution, 44 by 2, 27 by 3, 8 by 4, and 2 by 5, and 1 by 6) while there was no titer case higher by the CTT method as shown in Table 2a. CTT versus GMA2 according to antibody specificity is shown in Table 2b. Comparing CTT and GMA2 techniques using the Kendal's method again revealed good/satisfactory agreement $W = 0.92$, ($p < 0.0001$). When comparing the two gel microcolumn titration methods (GMA1 vs. GMA2), it was found that titers were identical in 73 cases (53.28%), in 49 cases (35.77%) were higher by GMA2 (40 by one position, 8 by 2, and 1 by 3) and in 15 cases (10.95%) higher by GMA1, (12 by one position, 2 by 2, and 1 by 3), Table 3b. The results obtained by the two different microcolumn techniques (GMA1 vs. GMA2) were in almost perfect agreement with $W = 0.95$, $p < 0.0001$.

In total, the comparison of the three methods simultaneously revealed good agreement ($W = 0.90$ $p < 0.0001$) according to Kendal's test.

DISCUSSION

Red blood cell ABO-antibody titration is an established method in the course of ABO incompatible solid organ and bone marrow transplant. Titration of RhD and other clinically significant antibodies has been proven to be a useful tool to evaluate maternal alloimmunization during pregnancy and in guiding the decision to assess fetal anemia by non-serologic means. Traditionally, the manual tube method has been largely used in RBC cell agglutination testing due to its simplicity. During the last decades new automated or semi automated gel microcolumn methods have been implemented in red cell serology testing. They have been proven to be flexible, reliable, and highly accurate with a sensitivity > 97.58% and a specificity > 99.93% in antibody identification [14-16]. Similarly, they provide highly reproducible results and enhance antibody identification as they have been found to be superior to tube LISS-IAT in detecting clinically significant red blood cell alloantibodies [16, 17]. However, although ABO-antibody titration reported data suggests that antibody titers by GMA would clinically benefit the management of ABO incompatible solid organ transplant patients [18], data regarding gel methodology in the evaluation of RBC-antibody titration is limited. Considering that titration levels are often evaluated according to antibody critical levels and that critical antibody levels were originally established by the CTT, it is currently difficult to determine whether these critical levels can be applied to titers assed by microcolumn gel methodology.

Our study compared CTT with two different, commercially available GMA systems (GMA1 - DiaMed and GMA2 - Grifols) for RBC-antibody titration.

When comparing CTT and GMA1 (by DiaMed), we found that most cases (86.13%) were higher by GMA1;

Table 1. Conventional Tube Test (CTT) versus Gel microcolumn agglutination based method 1 (GMA 1) by DiaMed GmbH, Cressier, Switzerland.

Table 1a. Antibody titer by CTT compared to GMA1 (by Diamed).

	GMA1												
CTT	**0**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**Total**
0	**10**	**31**	**18**	**6**	**3**	**1**							**69**
1		**2**	**5**	**10**	**3**	**2**							**22**
2			**1**	**7**	**3**	**1**	**1**						**13**
4			**1**	**3**	**2**	**3**							**9**
8						**1**	**3**	**3**		**1**			**8**
16						**1**	**1**	**3**	**3**				**8**
32							**1**		**4**				**5**
64											**1**		**1**
128													**0**
256											**1**	**1**	**2**
Total	**10**	**33**	**25**	**26**	**11**	**9**	**6**	**6**	**7**	**1**	**2**	**1**	**137**

Table 1b. Correlation of samples in CTT versus GMA1 (by Diamed) by antibody specificity.

Antibody	Number (% of total titrations)	Identical (% for antibody)	Higher by CTT (% for antibody)	Higher by GMA1 (% for antibody)	Mean difference in titers (GMA1-CTT) *	Mean absolute difference in titers (GMA1-CTT) **
Anti-D	**50 (36.5)**	**4 (8)**	**0 (0)**	**46 (92)**	**1.80**	**1.80**
Anti-KELL	**25 (18.25)**	**6 (24)**	**0 (0)**	**19 (76)**	**1.16**	**1.16**
Anti-E	**10 (7.3)**	**1 (10)**	**0 (0)**	**9 (90)**	**1.30**	**1.30**
Anti-Jka	**9 (6.57)**	**1 (11.11)**	**1 (11.11)**	**7 (77.78)**	**1.67**	**1.89**
Anti-c	**8 (5.84)**	**1 (12.5)**	**0 (0)**	**7 (87.5)**	**2.00**	**2.00**
Anti-M	**7 (5.11)**	**2 (28.57)**	**0 (0)**	**5 (71.43)**	**1.14**	**1.14**
Anti-Kpa	**6 (4.38)**	**0 (0)**	**0 (0)**	**6 (100)**	**2.00**	**2.00**
Anti-Cw	**5 (3.65)**	**2 (40)**	**0 (0)**	**3 (60)**	**0.80**	**0.80**
Anti-Fya	**5 (3.65)**	**0 (0)**	**0 (0)**	**5 (100)**	**2.00**	**2.00**
Anti-C	**3 (2.19)**	**0 (0)**	**0 (0)**	**3 (100)**	**2.67**	**2.67**
Anti-G	**3 (2.19)**	**0 (0)**	**0 (0)**	**3 (100)**	**2.00**	**2.00**
Anti-Lua	**3 (2.19)**	**1 (33.33)**	**0 (0)**	**2 (66.67)**	**0.67**	**0.67**
Anti-Cha	**2 (1.46)**	**0 (0)**	**0 (0)**	**2 (100)**	**3.00**	**3.00**
Anti-e	**1 (0.73)**	**0 (0)**	**0 (0)**	**1 (100)**	**3.00**	**3.00**
Total	**137**	**18 (13.14)**	**1 (0.73)**	**118 (86.13)**	**1.62**	**1.64**

*** Expressed in logarithmic scale to the Base 2 + 1. Negative titrations were assigned the number 0.**
**** Expressed by the absolute value of logarithmic scale to the Base 2 + 1. Negative titrations were assigned the number 0.**

however, the majority of differences observed were by one 41.18% or by two dilutions 37.82%, showing good agreement $W = 0.91$, $p < 0.0001$ between the two methodologies. Our results are consistent with the results of Bromilow et al. [19] (test for proportions, $p > 0.05$) who studied the titer in cases of clinically significant alloantibodies by CTT and GMA1 (by Diamed) and reported higher titer with the gel method in 31/34 (91.18%) cases. The three cases with a higher CTT titer were one anti-Kell, one anti-S, and one anti-Fya while in our study the only case with a higher titer by CTT was an anti-Jka.

Regarding the comparison of CTT vs. GMA2 (by Grifols) our study revealed that although in most cases samples' titers (92.7%) were higher by GMA2, the majority of differences were by only one (35.43%) or two

Table 2. Conventional Tube Test (CTT) versus Gel microcolumn agglutination-based method 2 (GMA 2) by DG Gel Coombs Diagnostic Grifols, Passeig Fluvial, Spain.

Table 2a. Antibody titer by CTT compared to GMA2 (by Griffols).

	GMA2												
CTT	**0**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1,024**	**Total**
0	**5**	**32**	**18**	**11**	**1**	**1**	**1**						**69**
1			**3**	**9**	**5**	**4**	**1**						**22**
2				**3**	**6**	**2**	**2**						**13**
4				**3**	**4**	**1**	**1**						**9**
8						**1**	**4**	**2**	**1**				**8**
16						**2**	**1**	**2**	**3**				**8**
32									**3**	**2**			**5**
64											**1**		**1**
128													**0**
256											**1**	**1**	**2**
Total	**5**	**32**	**21**	**26**	**16**	**11**	**10**	**4**	**7**	**2**	**2**	**1**	**137**

Table 2b. Correlation of samples in CTT versus GMA2 (by Griffols) by antibody specificity.

Antibody	Number (% of total titrations)	Identical (% for antibody)	Higher by CTT (% for antibody)	Higher by GMA2 (% for antibody)	Mean difference in titers (GMA2-CTT) *	Mean absolute difference in titers (GMA2-CTT) **
Anti-D	**50 (36.5)**	**5 (10)**	**0 (0)**	**45 (90)**	**1.92**	**1.92**
Anti-KELL	**25 (18.25)**	**4 (16)**	**0 (0)**	**21 (84)**	**1.48**	**1.48**
Anti-E	**10 (7.3)**	**0 (0)**	**0 (0)**	**10 (100)**	**1.60**	**1.60**
Anti-Jka	**9 (6.57)**	**1 (11.11)**	**0 (0)**	**8 (88.89)**	**2.67**	**2.67**
Anti-c	**8 (5.84)**	**0 (0)**	**0 (0)**	**8 (100)**	**2.38**	**2.38**
Anti-M	**7 (5.11)**	**0 (0)**	**0 (0)**	**7 (100)**	**1.43**	**1.43**
Anti-Kpa	**6 (4.38)**	**0 (0)**	**0 (0)**	**6 (100)**	**2.50**	**2.50**
Anti-Cw	**5 (3.65)**	**0 (0)**	**0 (0)**	**5 (100)**	**1.40**	**1.40**
Anti-Fya	**5 (3.65)**	**0 (0)**	**0 (0)**	**5 (100)**	**2.40**	**2.40**
Anti-C	**3 (2.19)**	**0 (0)**	**0 (0)**	**3 (100)**	**2.33**	**2.33**
Anti-G	**3 (2.19)**	**0 (0)**	**0 (0)**	**3 (100)**	**2.33**	**2.33**
Anti-Lua	**3 (2.19)**	**0 (0)**	**0 (0)**	**3 (100)**	**1.00**	**1.00**
Anti-Cha	**2 (1.46)**	**0 (0)**	**0 (0)**	**2 (100)**	**3.00**	**3.00**
Anti-e	**1 (0.73)**	**0 (0)**	**0 (0)**	**1 (100)**	**3.00**	**3.00**
Total	**137**	**10 (7.3)**	**0 (0)**	**127 (92.7)**	**1.91**	**1.91**

*** Expressed in logarithmic scale to the Base 2 + 1. Negative titrations were assigned the number 0.**
**** Expressed by the absolute value of logarithmic scale to the Base 2 + 1. Negative titrations were assigned the number 0.**

dilutions (34.64%). The mean absolute difference for the non-matching titrations was 2.06 times higher by GMA2. Existing data in the literature comparing CTT vs. GMA2 (by Grifols) is scarce and regards the titers of IgG component of anti-A and anti-B in the ABO-titration setting, which were found 2.17 and 2.61 times higher, respectively, with GMA2 than CTT (both $p < 0.001$) [20]. Our results also suggest that higher titers by GMA2 do not differ significantly from those mentioned above (test for proportions $p > 0.05$). However, it is worth noticing that in our study the differences between CTT and GMA2 were not so great and thus the agreement between the two methods was good $W = 0.92$, ($p < 0.0001$).

When comparing the two gel methods GMA1 (by Diamed) vs. GMA2 (by Grifols), identical titers were

Table 3. Gel microcolumn agglutination-based method 1 (GMA 1) by DiaMed GmbH, Cressier, Switzerland versus Gel microcolumn agglutination-based method 2 (GMA 2) by DG Gel Coombs Diagnostic Grifols, Passeig Fluvial, Spain.

Table 3a. Antibody titer by GMA1 (by Diamed) compared to GMA2 (by Griffols).

	GMA 2												
GMA1	**0**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1,024**	**Total**
0		**8**	**1**	**1**									**10**
1	**3**	**20**	**6**	**4**									**33**
2	**1**	**4**	**13**	**7**									**25**
4	**1**		**1**	**13**	**9**	**2**							**26**
8				**1**	**6**	**4**							**11**
16					**1**	**4**	**4**						**9**
32							**5**		**1**				**6**
64						**1**	**1**	**4**					**6**
128									**5**	**2**			**7**
256									**1**				**1**
512											**2**		**2**
1,024												**1**	**1**
Total	**5**	**32**	**21**	**26**	**16**	**11**	**10**	**4**	**7**	**2**	**2**	**1**	**137**

Table 3b. Correlation of samples in GMA1 (by Diamed) versus GMA2 (by Grifols) by antibody specificity.

Antibody	Number (% of total titrations)	Identical (% for antibody)	Higher by GMA1 (% for antibody)	Higher by GMA2 (% for antibody)	Mean difference in titers (GMA1-GMA2) *	Mean absolute difference in titers (GMA1-GMA2) **
Anti-D	**50 (36.5)**	**22 (44)**	**11 (22)**	**17 (34)**	**-0.12**	**0.72**
Anti-KELL	**25 (18.25)**	**13 (52)**	**2 (8)**	**10 (40)**	**-0.32**	**0.48**
Anti-E	**10 (7.3)**	**9 (90)**	**0 (0)**	**1 (10)**	**-0.30**	**0.30**
Anti-Jka	**9 (6.57)**	**2 (22.22)**	**0 (0)**	**7 (77.78)**	**-1.00**	**1.00**
Anti-c	**8 (5.84)**	**4 (50)**	**1 (12.5)**	**3 (37.5)**	**-0.38**	**0.63**
Anti-M	**7 (5.11)**	**5 (71.43)**	**0 (0)**	**2 (28.57)**	**-0.29**	**0.29**
Anti-Kpa	**6 (4.38)**	**3 (50)**	**0 (0)**	**3 (50)**	**-0.50**	**0.50**
Anti-Cw	**5 (3.65)**	**3 (60)**	**0 (0)**	**2 (40)**	**-0.60**	**0.60**
Anti-Fya	**5 (3.65)**	**3 (60)**	**0 (0)**	**2 (40)**	**-0.40**	**0.40**
Anti-C	**3 (2.19)**	**2 (66.67)**	**1 (33.33)**	**0 (0)**	**0.33**	**0.33**
Anti-G	**3 (2.19)**	**2 (66.67)**	**0 (0)**	**1 (33.33)**	**-0.33**	**0.33**
Anti-Lua	**3 (2.19)**	**2 (66.67)**	**0 (0)**	**1 (33.33)**	**-0.33**	**0.33**
Anti-Cha	**2 (1.46)**	**2 (100)**	**0 (0)**	**0 (0)**	**0.00**	**0.00**
Anti-e	**1 (0.73)**	**1 (100)**	**0 (0)**	**0 (0)**	**0.00**	**0.00**
Total	**137**	**73 (53.28)**	**15 (10.95)**	**49 (35.77)**	**-0.29**	**0.57**

*** Expressed in logarithmic scale to the Base 2 + 1. Negative titrations were assigned the number 0.**
**** Expressed by the absolute value of logarithmic scale to the Base 2 + 1. Negative titrations were assigned the number 0.**

found in most samples tested (73/137, 53.28%), while among discrepant titers most cases were higher by GMA2 by Grifols (49/64, 76.57%). However, the majority of differences (81.25%) between the two methods were by only one dilution and thus, the two gel methodologies tested revealed an almost perfect agreement ($W = 0.95$, $p < 0.0001$) (Table 3a). Accordingly, Cid et al. [15] compared three microtube column agglutination

systems for screening and titration in 100 samples and reported that antibody titration with GMA1 by Diamed, GMA2 by Grifols, and Ortho Biovue showed similar results (mean score (range) 30.3 (3 - 112), 34.31 (5 - 119), and 37.38 (3 - 112), respectively). On the other hand a study, that evaluated the GMA1 and GMA2, for antibody screening and identification in the pretransfusion setting, concludes that for antibody identification, the accuracy was not different ($p > 0.05$) for Grifols DG Gel system and for Bio-Rad (96.03% and 94.44%, respectively) [21].

Regarding antibody specificity and when comparing CTT with GMAs (GMA1 and GMA2), both GMAs generated titer values higher than the values by CTT with a mean absolute difference in titers greater than one (1.62 for GMA1 vs. CTT and 1.91 for GMA2 vs. CTT). That was the case for all alloantibodies titrated (anti-D, anti-Kell, anti-E, anti-Jka, anti-c, anti-Cw, anti-Fya, anti-M, anti-Kpa, anti-Lua, anti-e, anti-G, anti-Cha), with the exception of a single anti-Jka case that produced a higher titre with CTT vs. GMA1 by one dilution (Table 1b). Finck et al. compared CTT with a different microtube system by Ortho-BioVue systems (that contains a glass bead matrix) also found that for most alloantibodies titrated with Ortho-BioVue (anti-E, anti-e, and anti-c), on average, titers generated were higher than the titers by CTT. However, titer values differed by less than one dilution, a finding which was significantly different from our results (test for proportions $p < 0.05$) [22].

When focusing on anti-D only, which concerned most of the samples in our study ($n = 50$, frequency 36.50%), it was observed that most cases 46/50, (92%) were higher by GMA1 vs. CTT while there was identical titration in the remaining 4/50 (8%) (Table 1b). The mean absolute difference for the non-matching titrations was 1.96 times higher by GMA1. Similar data has been published by Thakur et al. in which they tested GMA1 by Diamed and CTT titers for anti-D. They found that 90.4% of the cases were higher by the GMA1 or identical in the remaining 9.6%, anti-D titers were a one to five-fold higher by GMA1 [10] (test for proportions, $p = 0.9996$). Another study by Novaretti et al. [23] found that anti-D titers by GMA1 were consistently higher (100%) than those by CTT, which does not differ significantly from our results ($p = 0.0748$). However, with respect to the difference in the titration values they reported that the GMA1 method produced 3.4-fold higher titers than CTT which is higher than our findings (test for proportions $p < 0.05$).

Regarding anti-K, we found similarly higher titer values by both gels (GMA1 by 1.16 and GMA2 by 1.48) in all 25 cases tested, as opposed to Frink et al., where anti-K samples ($n = 15$) tended to generate lower titer values with an Ortho Biovue System by almost one dilution (0.9) versus CTT. Regarding Jka alloantibodies, it is worth mentioning that anti-Jka samples revealed one of the greatest differences in titration with both GMAs vs. CTT in most cases tested (Tables 1b, 2b). Especially for GMA2 by Grifols vs. CTT, the mean absolute difference in titers was 2.67. This seems to be consistent with previous observations according to which the Grifols DG Gel system is associated with increased sensitivity to the detection of anti-Jka [21].

Greater differences between CTT and both GMAs were found in samples with anti-e and anti-Cha with a mean difference in titers of 3.00 for both comparisons. Anti-Cha cases' titers were 16 by CTT and 128 by both GMA1 and GMA2, as expected by a high titer low avidity HTLA antibody [24]. However, the restricting small number of both anti-Cha (two cases) and anti-e (one case) can be considered as a limiting factor.

It is worthy of note regarding limitations, that several parameters in our study may be misconstrued as limitations but are actually method specific. Although RBC suspensions for all methods (CTT, GMA1, GMA2) were prepared using the same commercially available test RBCs to prevent differences between RBC antigen profiles as a variable. A RBC-suspension was prepared from test RBCs of $3 \pm 1\%$ by adding normal saline to a final concentration of 0.8% for gel methods, as previously utilized by Cid et al. [15] and Thakur et al. [10]. However, this along with the use of both GMAs in titration assays are not included in either of the official manufacturers' recommendations.

It is worth mentioning that differences in commercial gel methodologies, different inter-laboratory practices, (i.e., different test RBC antigen profile, different dilution means) along with different sample sizes may be a reasonable explanation for some of the detected discrepancies among different studies.

To the best of our knowledge our study comprises the largest number of titration samples, a total of 137 samples tested each at the same time with conventional tube test and with gel microcolumn agglutination assay of two different, commercially available GMA systems (GMA1 by Diamed and GMA2 by Grifols) for RBC antibody titrations in pregnant women and patients. Titration samples' dilution was made with saline and titrations for both tube and gel methods were performed from the same dilution tube, rendering the primary difference in precision, if any, method specific.

Taking into account our results showing higher titration values with both GMAs when compared with tube test, our data suggest that the gel microcolumn assay seems to be more sensitive than the tube technique for alloantibody titration.

This is in accordance with the finding that the gel seems to be more sensitive in the detection of RBC alloantibodies [10,25]. However, the differences were not significant in our study and the agreement between all methods was very good, possibly indicating GMA as a suitable alternative to CTT also for non-ABO RBC-antibody titration.

Acknowledgment:
We would like to thank Mrs. Marianna Preveziotis for English Editing of the manuscript.

Financial Disclosure:
None.

Declaration of Interest:
The authors have disclosed no conflicts of interests.

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