Precision of Antibody Titration in Gel versus Tube

Abstract

BACKGROUND: The titration of an alloantibody to a red cell antigen is a useful semi-quantitative screening tool that, when compared to a previous titer performed by the same technique, can detect an increased production of maternal antibody during pregnancy. This study compared gel titration and tube titration for precision as an indicator of which method can more accurately indicate that a true rise in titer has occurred.

STUDY DESIGN AND METHODS: A total of 42 samples containing IgG red cell antibodies were evaluated for both antibody titer and score. Each sample was tested a total of 3 times, on different days, by each method. Samples were stored frozen between test days.

RESULTS: The precision of repeat titer results performed in gel was slightly more consistent ($p = 0.0095$) when compared to tube titration. There was no difference in titer in any given sample when testing was repeated by gel methodology, while 7 of 42 (17%) showed a difference in titer of 1 dilution when repeat testing was performed using tubes. Although 7% of titrations in gel demonstrated slight changes in score, 28% of the tube titers demonstrated changes.

CONCLUSION: Results of this study demonstrated that more variation in antibody titer result and titration score were noted upon repeat testing of the same sample when testing was performed in tubes as compared to repeat testing in gel. Although the titers and scores in gel are higher than tubes, performing antibody titration using gel technology is worth serious consideration and validation in a laboratory using gel technology.

INTRODUCTION

The titration of an alloantibody to a red cell antigen is a useful semi-quantitative screening tool that, when compared to a previous titer performed by the same technique, may detect an increased production of maternal antibody during pregnancy. A four-fold or greater increase in titer (2 dilution increase) is considered to be an indicator to monitor the pregnancy by additional methods such as color Doppler ultrasonography.[4] Judd et al suggested that gel column technology should not be used for prenatal antibody titration until there are substantial data showing correlation between gel and tube titers.[1] Proficiency survey results published by the College of American Pathologists indicate that, based on published correlation of variations, titrations in tube produce a large disparity of values amongst participants.

The AABB *Technical Manual* states that titration is a semi-quantitative method and is quite technique dependent.[2] Because many variables can affect testing results, the procedure is relatively imprecise. Parallel titrations are performed to compare titer results of a previous sample with a current sample in order to mitigate technique-dependent variables. Additionally, enhancement techniques or gel testing is not recommended because elevated titer values may be obtained, even though these same enhancement techniques are used for antibody detection and identification by laboratories using gel technology. A study by Steiner et al concluded that antibody titers in gel produce higher titers than those performed in tubes.[3]

Deciding which semi-quantitative method is more "accurate" than the other is a matter of opinion. The goal of this study is not to establish whether titrations in gel correlate with tubes, but rather, to compare the precision of the two methods. Precision

would serve as a more useful indicator of which method could reproducibly predict a rise in titer vs. a variation seen due to technique.

MATERIALS AND METHODS

Samples

A total of 42 plasma samples containing IgG antibodies commonly associated with HDFN were evaluated.

Master Dilutions

Serial two-fold dilutions were made in normal saline. The first dilution was made by mixing 200 μL of plasma with 200 μL of saline, which represents a 1:2 dilution. Subsequent serial dilutions were made by adding 200 μL of diluted plasma to a tube containing 200 μL of saline. The contents of the tube were mixed, and 200 μL was transferred to the next tube containing 200 μL of saline. Pipette tips were changed after the transfer of each dilution. From each master dilution, 100 μL was used for tube testing and 25 μL for gel testing. Using the same master dilution tube for both gel and tube testing reduces the likelihood of variation in titration value and titration score relevant to the preparation of serial two-fold dilutions.

Gel Testing: Preparation of Red Cells

Cells carrying a presumed double-dose expression of the antigen corresponding to the antibody in the sample were selected for the titration. For example, R_2R_2 red cells were selected for titration testing of samples containing anti-D. Cells used for the titration were 0.8% cell suspension (Ortho Clinical Diagnostics, Raritan, NJ) obtained from panel cells intended for gel testing. Cells were used without further modification.

Gel Titration Procedure

Titration in gel was performed using gel cards (Ortho Clinical Diagnostics, Raritan, NJ) following the same procedure used for antibody detection and identification. Briefly, 50 μL of well-mixed cells was added to the gel column, followed by 25 μL of diluted plasma sample. Following standard 15-minute incubation at 37 °C, the gel cards were centrifuged and reactions were immediately graded.

Tube Testing: Preparation of Red Cells

Cells carrying a presumed double-dose of the antigen corresponding to the antibody in the sample were selected for the titration. For example, R_2R_2 red cells were selected for titration testing of samples containing anti-D. Two percent cells (Immucor-Gamma, Norcross, Ga.) were prepared by centrifuging 5 mL of 5% cells, removing the diluent, and replacing it with 2 mL of saline.

Tube Titration Procedure

Titration was performed using 12-mm test tubes following the accepted procedure in the AABB *Technical Manual*.[2] Briefly, 100 μL diluted plasma was placed into a test tube, and 50 μL of 2% cells were added. The tube was incubated at 37° C for 60 minutes. After washing four times with saline, two drops of anti-IgG (Immucor, Norcross, GA) were added. The tubes were centrifuged and read for macroscopic agglutination.

Testing, Grading and Scoring

All titration testing was performed by the same individual. The antibody specificity of each plasma sample was known so appropriate cells could be selected for titration. However, the sample identity was blinded. Titration and scoring for each plasma sample was repeated a total of three times. On each test day, one plasma sample was tested by

both methods, and then frozen for future testing. At least 2 days elapsed between test dates, and the same panel cell lot number and specific cell was used for each repeat titration. A new master dilution was prepared each time a sample was used for testing. Expired panel cells were never used for testing. The titer is the reciprocal the highest dilution producing a 1+ reaction, and the strength of reactions were scored as described in the AABB *Technical Manual*.[2]

RESULTS

The gel titration method showed no difference in antibody titer in any of the samples tested (Table 1), although there was some difference in score (Table 2). Of the titers performed in tubes, 7 of 42 (17%) had a difference of 1 dilution. There was no difference above one titration dilution. With regards to titration score, 3 out of 42 titrations in gel (7%) produced slight changes in titration score while 28% (12/42) of the tube titers produced slight changes in scores. None of the changes in scores was above 10 (Fig.1).

The score results in gel were equal to or slightly more consistent ($p = 0.0095$) compared to tube titration. These testing results suggest that gel titration is more precise, and thus more reproducible, than titrations in tubes. The precision of titers in tubes, based on differences in titration value and titration scores on the same samples, was low to moderate. When the antibody titer was higher, there was a more discernible difference between the precision of the two methods, which is another indication that the use of gel technology is worth consideration for use in alloantibody titration.

DISCUSSION

Hemolytic Disease of the Fetus and Newborn (HDFN) is the destruction of fetal and newborn red cells by maternal alloantibody directed against a red cell antigen expressed on fetal cells. Maternal IgG antibodies enter fetal circulation by crossing the placenta and bind to the corresponding fetal red cell antigen. The resultant IgG-coated cells interact with fetal macrophages in a manner that facilitates the removal of these red cells in the fetal spleen. Subsequent complications to the fetus may include compensatory erythropoiesis in the fetal liver and spleen, resulting in organ enlargement and portal hypertension. If left unmanaged, severe complications to fetal hepatic and cardiovascular systems may follow, potentially leading to fetal demise.

Titration of an alloantibody to a red cell antigen is a useful semi-quantitative screening tool that can detect an increased production of maternal antibody during pregnancy. A significant increase in antibody titer or score may be an indication that the mother is mounting an anamnestic response to a previously encountered antigen. Clinically significant alloantibodies commonly encountered in prenatal testing include anti-D, -K, -C, -c, and -Fy^a.

A rise in antibody titer is not necessarily an indication of the degree of the severity of HDFN, but merely an indication that closer monitoring of the pregnancy may be appropriate. Severe fetal disease can begin as early as 18-20 weeks gestation, so titrations should begin as soon as both pregnancy and antibody identification have been established.[2] Kell system antigens are present on early red cell precursors, so monitoring a patient with anti-K should begin immediately. For the fetus at risk for HDFN, once the 18-20 weeks gestation mark has been reached, specific monitoring techniques can be

considered. One such technique is the non-invasive color Doppler ultrasonography to assess cerebral artery blood flow. Increased cerebral artery blood flow is an indicator that fetal anemia is a distinct possibility. Invasive procedures such as amniocentesis or cordocentesis, with subsequent analysis of amniotic fluid and fetal blood, respectively, can also be considered.[5] Molecular testing of maternal plasma for fetal DNA can be performed during the second trimester.[6] DNA probes for the most common antigens associated with HDFN are available.

The accepted titration methodology is the tube method.[2] A difference of 2 dilutions or a score of 10 is an indication of significant change in antibody production, normally due to re-exposure of the maternal immune system to a red cell antigen expressed on fetal red cells. This rise in titer can be used as a guide in deciding when to employ various fetal monitoring techniques. These titer criteria apply to clinically significant antibodies, especially anti-D.

Titration using gel column technology may result in titers several dilutions higher than the tube method. Steiner et al reported antibody titers and scores in gel to be consistently higher than titers and scores in tubes.[3]

Antibody titration in gel has been shown to be a more precise method with less variation due to technique than antibody titration in tubes when performing ABO titers. A European study concluded that the use of the gel technique significantly decreases inter-center variation when compared with the tube technique for performing ABO titers in renal transplant patients.[6] Titration using gel technology has also been used in determining the ABO titers in single donor platelets.[7]

There are, however, indications that a change from tube antibody titration to gel titration for the purpose of monitoring maternal alloantibody production will take time to be implemented. A recent report[8] states "*Large differences in titer can be seen in the same patient between different laboratories, and a newer gel technique produces higher titer results than the older tube method. Therefore, standard tube methodology should be used to determine critical titer, and a change of more than 1 dilution represents a true increase in maternal antibody titer*." Titration methodology at an institution should be validated to clinical data to ensure appropriate interpretation of the data by obstetricians.

This study compared gel titration and tube titration for precision as an indicator of which method can more accurately predict a true rise in titer as compared to a variation due to technique. Results of this study showed subtle imprecision in tube testing compared to gel titration. A subtle difference in titer and score based solely on the precision of the methodology in combination with a slight rise in antibody titer in a subsequent sample could lead to inconclusive results when the antibody titer has truly risen significantly. Since the titrations for both tube and gel methods were performed from the same dilution tube, the primary difference in precision, if any, was method specific.

In conclusion, although the titers and scores in gel are higher than in tubes, performing antibody titration using gel technology is worth serious consideration and validation in a laboratory using gel technology. Additionally, use of automated processes in conjunction with gel technology to perform critical steps, such as automated serial two-fold dilutions, could also be investigated.

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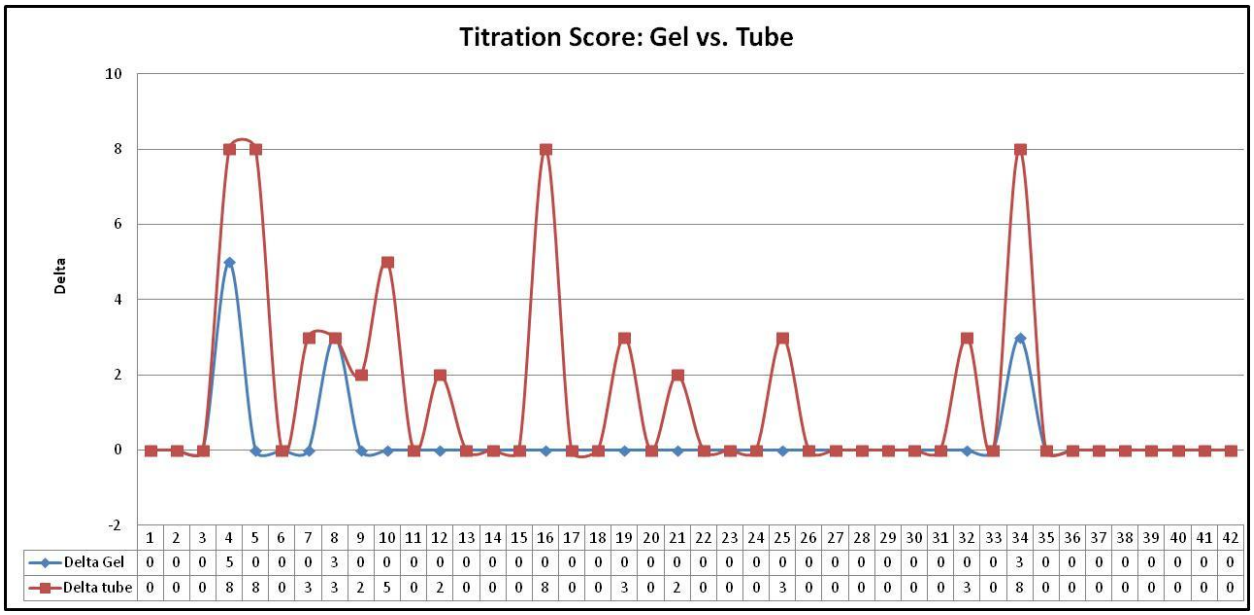
Table 1. Titration Results of Gel and Test Tube: Delta Titer

	Titration - Gel			Titration - Tube				
ID	#1	#2	#3	#1	#2	#3	Delta Gel	Delta tube
1617	8	8	8	4	4	4	0	0
4198	32	32	32	16	16	16	0	0
9829	4	4	4	2	2	2	0	0
1714	256	256	256	128	64	64	0	1
7093	256	256	256	128	128	64	0	1
B0714	64	64	64	16	16	16	0	0
591	128	128	128	64	64	32	0	1
9892	1024	1024	1024	512	256	512	0	1
4049	32	32	32	8	8	8	0	0
50205	256	256	256	64	64	128	0	1
8101	32	32	32	32	32	32	0	0
2840	32	32	32	16	16	16	0	0
3519	16	16	16	8	8	8	0	0
2608	16	16	16	8	8	8	0	0
7787	64	64	64	32	32	32	0	0
5632	64	64	64	16	16	32	0	1
7673	32	32	32	16	16	16	0	0
5323	64	64	64	32	32	32	0	0
9180	64	64	64	32	32	32	0	0
4223	64	64	64	32	32	32	0	0
3749	64	64	64	16	16	16	0	0
B1018	8	8	8	4	4	4	0	0
B0928	8	8	8	2	2	2	0	0
8960	8	8	8	4	4	4	0	0
6743	16	16	16	8	8	8	0	0
B9012	32	32	32	16	16	16	0	0
8452	4	4	4	2	2	2	0	0
1551	8	8	8	4	4	4	0	0
327	4	4	4	4	4	4	0	0
7343	2	2	2	1	1	1	0	0
B0824	16	16	16	8	8	8	0	0
1269	32	32	32	16	16	16	0	0
2373	16	16	16	8	8	8	0	0
1825	32	32	32	16	8	8	0	1
7506	16	16	16	8	8	8	0	0
4610	8	8	8	4	4	4	0	0
4269	8	8	8	4	4	4	0	0
4663	64	64	64	16	16	16	0	0
4239	8	8	8	4	4	4	0	0
4663	64	64	64	32	32	32	0	0
240	8	8	8	4	4	4	0	0
5873	16	16	16	8	8	8	0	0

Table 2. Titration Results of Gel and Test Tube: Delta Score

	Titration Score - Gel			Titration Score - Tube				
ID	#1	#2	#3	#1	#2	#3	Delta Gel	Delta tube
1617	28	28	28	21	21	21	0	0
4198	53	53	53	31	31	31	0	0
9829	18	18	18	13	13	13	0	0
1714	69	74	69	62	54	54	5	8
7093	73	73	73	64	64	56	0	8
B0714	63	63	63	38	38	38	0	0
591	64	64	64	56	56	53	0	3
9892	512	256	512	72	69	72	3	3
4049	49	49	49	28	28	26	0	2
50205	93	93	93	72	72	77	0	5
8101	46	46	46	42	42	42	0	0
2840	46	46	46	38	36	38	0	2
3519	33	33	33	28	28	28	0	0
2608	33	33	33	28	28	28	0	0
7787	58	58	58	46	46	46	0	0
5632	52	52	52	36	36	44	0	8
7673	42	42	42	31	31	31	0	0
5323	58	58	58	42	42	42	0	0
9180	58	58	58	42	42	39	0	3
4223	53	53	53	42	42	42	0	0
3749	49	49	49	36	36	34	0	2
B1018	37	37	37	16	16	16	0	0
B0928	29	29	29	13	13	13	0	0
8960	28	28	28	18	18	18	0	0
6743	39	39	39	26	23	23	0	3
B9012	41	41	41	33	33	33	0	0
8452	21	21	21	13	13	13	0	0
1551	29	29	29	18	18	18	0	0
327	21	21	21	21	21	21	0	0
7343	10	10	10	5	5	5	0	0
B0824	34	34	34	26	26	26	0	0
1269	42	42	42	34	31	34	0	3
2373	36	36	36	28	28	28	0	0
1825	47	47	44	36	28	28	3	8
7506	36	36	36	28	28	28	0	0
4610	26	26	26	18	18	18	0	0
4269	23	23	23	18	18	18	0	0
4663	52	52	52	39	39	39	0	0
4239	26	26	26	18	18	18	0	0
4663	54	54	54	42	42	42	0	0
240	28	28	28	18	18	18	0	0
5873	34	34	34	23	23	23	0	0

Fig. 1. Titration Results of Gel and Test Tube: Delta Score



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Delta Gel	0	0	0	5	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
Delta tube	0	0	0	8	8	0	3	3	2	5	0	2	0	0	0	8	0	0	3	0	2	0	0	0	3	0	0	0	0	0	0	3	0	8	0	0	0	0	0	0	0	0