Comparison of gel test and conventional tube test for antibody detection and titration in D-negative pregnant women: study from a tertiary-care hospital in North India

M.K. Thakur, N. Marwaha, P. Kumar, S.C. Saha, B. Thakral, R.R. Sharma, K. Saluja, H.K. Dhawan, and A. Jain

Conventional tube testing was used for antibody screening and titration in D– pregnant women in our hospital until the recent introduction of the gel test. In this study we assessed the sensitivity of the gel test in our setup and tried to establish a correlation between these tests for determining antibody titer. We collected 652 blood samples from 223 antenatal D– women during a span of 1 year. The samples were tested separately by the conventional tube technique and the gel test for antibody detection and titration. The tube test detected 84 (12.8%) positive samples as compared with 93 (14.2%) by gel test, indicating the latter to be more sensitive ($p < 0.01$). The gel test picked up weakly reactive anti-D that the tube test missed. We did not use any enhancing media such as LISS in titration studies performed by either method in an effort to establish a correlation. However, much higher titers (one- to fivefold) were obtained by the gel test with no clear correlation with the corresponding tube values. When comparing the titer values to the finding of hydrops on ultrasound and Liley's chart OD reading on amniocentesis, a value of less than 128 (i.e., 64) by gel test corresponded to normal results. Through this study, we thus conclude that the gel test is more sensitive for antibody detection, although a linear correlation could not be established for titers. Clinical correlation may point toward a critical titer of 64 for the gel test, but further studies need to be done to support this finding. ***Immunohematology* 2010;26:174–77.**

Key Words: alloantibodies, antibody screening, titration, gel test, D-negative women

Hemolytic disease of the fetus and newborn (HDFN) was first reported in 1609,[1] although the discovery of the Rh system was made in 1939 and the implication of D antigen in its pathogenesis in 1940.[2] However, to the present day, researchers and clinicians alike have worked to unravel its pathogenesis, effects, prevention, and treatment.

Anti-D is by far the most common cause of HDFN although its incidence has drastically fallen with antenatal D immunoglobulin use as prophylaxis. Other antibodies are also implicated, and their prevalence has been studied in the Western world but such data are lacking in the Indian population. The antibodies can be identified as well as semiquantitated from the mother's serum. Titer determination of anti-D by tube test helps in the clinical decision to proceed with an invasive procedure such as amniocentesis.

The conventional tube test has stood the test of time in both antibody detection and titration. The gel test introduced by Lapierre et al.[3] in 1990 has gained popularity as a result of its standardized performance, technical ease, stable end point, and versatility of methodology. There have been many studies to show its superiority versus the tube test for antibody detection. The blood transfusion services in our country are gradually introducing gel technology for grouping, compatibility testing, and alloantibody detection owing to the many advantages. However, in some situations such as HDFN not only the specificity of alloantibody but also its titer has a direct impact on the fetus. Therefore, we evaluated the gel test and the conventional tube test for antibody screening and titration to determine if there is any correlation between the results obtained by using both technologies and to find out whether critical levels of alloantibody could be determined. We further studied obstetric management such as ultrasonography and intrauterine transfusion. We tried to correlate the titers with these clinical interventions.

Material and Methods

The duration of study was from July 2005 to June 2006, during which a total of 652 clotted samples were collected from 223 antenatal D– women. The study was conducted after approval from the Institute Ethics Committee. A written informed consent was taken from the patients for the study.

Sample Collection

A 3-mL clotted sample was collected, which was then centrifuged and the serum separated. Serum was stored in a frozen state at –80°C in two aliquots each until further testing. The first sample was taken in the first trimester or at the time of the first visit. Subsequent samples were taken in every trimester and at 28 weeks before the administration of anti-D immunoglobulin. Immunized women were followed up every 3 to 4 weeks.

Antibody Detection

The serum was thawed to room temperature before being tested. Antibody detection was done in parallel by the conventional tube technique and the gel test. A commercially available three-cell screening panel (DiaScreen; DiaMed, Cressier sur Morat, Switzerland) was used. For tube testing,

a 3% suspension in normal saline was used, and for gel testing, a 0.8% suspension in LISS was used.

Tube IAT

One drop of RBC suspension was added to two drops of serum to be tested into labeled test tubes. The tubes were incubated in a water bath at 37°C for 60 minutes. After three washes with normal saline solution, polyspecific anti-human globulin was added to the RBC button and the tube was centrifuged at 3000 *x g* for 15 seconds in an appropriate centrifuge. The tubes were examined for agglutination. The reactions were graded and recorded as per the *AABB Technical Manual*.[4]

Gel Test

We dispensed 50 µL of RBCs into labeled gel cards and added 25 µL of the serum to be tested. The cards were incubated for 15 minutes at 37°C in specially designed incubators. They were then centrifuged at 1050 rpm for 10 minutes. The reactions were graded and recorded. Antibody identification was done using commercially available panels (DiaMed) according to the manufacturer's instructions.

Antibody Titration

Titration was performed on those samples that were positive on antibody screening. Serial twofold dilutions were made in normal saline solution in clean test tubes. The dilutions were tested in parallel for both tests. In-house prepared R_1R_1 RBCs from a single donor were used for D antibodies, whereas RBCs with heterozygous expression were used for Le^a and Le^b antibodies. The RBCs were washed three times in normal saline solution and resuspended to a final concentration of 3% and 0.8% in normal saline solution for tube testing and gel testing, respectively. Critical titer was taken as 16 by the tube technique followed in our institute.

Tube Test

The method used was the same as that for antibody detection.

Gel Test

The RBCs used were suspended in normal saline solution to a final concentration of 0.8%, and the cards incubated at 37°C for 60 minutes. The remainder of the procedure was the same as for antibody detection. Titers were taken as the highest dilution that gave 1+ agglutination. The gel test had been modified from the manufacturer's guidelines for titration. LISS was not used in the process, and the time of incubation was increased from 15 minutes to 60 minutes. We tested several samples in parallel with the standard method and found that deviating from the original method did not miss any antibodies.

The women who were positive for alloantibodies were investigated with ultrasonography for features of hydrops. The findings were compared with the titer values obtained by both methods. All women with titer values of 16 or above by the conventional tube method were further evaluated by amniocentesis. The OD values were plotted on Liley's chart[5] and compared with the titer results by both methods.

Statistical analysis was performed using SPSS software version 13.0 (SPSS, Inc., Chicago, IL).

Results

On antibody screening, the tube technique detected 84 (12.8%) positive results, whereas the gel test detected 93 (14.2%) positive results individually. On comparison, 83 of the 84 positive by tube were also positive by gel, whereas 1 of the 84 was missed by gel. On the other hand 10 samples that were positive by gel had been missed by the tube technique. On identification the sample missed by gel was anti-Le^a in specificity, and all samples missed by tube were anti-D in specificity. Both the tests showed a positive correlation in antibody detection that was significant with a Spearman's correlation of +0.842 ($p < 0.01$). However, the gel technique proved to be more sensitive than the tube technique ($p < 0.01$). Table 1 shows the profile of antibodies detected.

Table 1. Comparison of antibodies detected by both methods

Sample tested	Tube	Gel
Total	652	652
Tests positive	84	93
Anti-D (all)	73	83
Anti–D (passive)	11	21
Anti-C*	2	2
Anti Le^a	6	5
Anti Le^b	5	5

*Anti-C was found in association with anti-D.

Table 2 compares the titer values obtained by both methods for D antibodies. The Lewis antibody titer values are compared in Table 3. The titers on gel were generally higher than those obtained by the tube technique, and as the tube titers increased, the gel titers also increased. The titer increase with the gel test was higher when compared with the tube test. The values varied from onefold to fivefold (mean, 1.6-fold). The observed differences were onefold in 21 sera, twofold in 32, threefold in 18, fourfold in 3, and fivefold in 1.

Obstetric Studies

Ultrasonography

The titers were compared with the fetal findings on ultrasonography, i.e., normal vs. hydropic. All the women with alloantibodies were investigated. The mean titer value for those who had normal ultrasonographic findings was 62.39 by tube technique with a standard deviation of 114.5 and titers ranging from 1 to 512. By the gel technique, the

Table 2. Comparison of antibody titers on tube with those on gel test for anti-D

Tube titers	Gel titers												
	Neg	1	2	4	8	16	32	64	128	256	512	1024	2048
Neg	—	—	6	4	—	—	—	—	—	—	—	—	—
1	—	2	2	5	2	1	—	—	—	—	—	—	—
2	—	—	3	3	3	—	—	—	—	—	—	—	—
4	—	—	—	—	2	2	2	—	—	—	—	—	—
8	—	—	—	—	2	2	—	1	1	—	—	—	—
16	—	—	—	—	—	—	1	2	1	—	—	—	—
32	—	—	—	—	—	—	—	1	4	3	1	1	—
64	—	—	—	—	—	—	—	—	1	3	2	—	—
128	—	—	—	—	—	—	—	—	1	7	4	3	—
256	—	—	—	—	—	—	—	—	—	—	2	1	—
512	—	—	—	—	—	—	—	—	—	—	—	—	2
	Total samples—83												

Table 3. Titer values for Lewis antibodies by both methods

Lewis antibody type	Tube titer value	Corresponding Gel titer	Number of samples per titer value
Le[a]	1	Neg	1
	2	2	5
Le[b]	4	4	4
	8	4	1
Total			11

mean value was 271.04 with a standard deviation of 460.27 and a range of 2 to 2048. The mean titer value for those who had hydropic findings on ultrasound was 182.86 by tube technique with a standard deviation of 145.138 and titers ranging from 128 to 512. By the gel technique, the mean value was 841.04 with a standard deviation of 604.259 and a range of 256 to 2048.

When compared using nonparametric tests, the difference between the two groups was significant by the tube method ($p = 0.02$) and also by gel test ($p = 0.03$). Table 4 shows the median values for normal and hydropic findings using both techniques.

Table 4. Median titer values by both methods for antenatal ultrasound (USG) showing normal vs. hydropic changes

Median titer	Normal on USG	Features of hydrops
Tube	32	128
Gel	32	256

Amniocentesis

The titer values by both methods were compared with the need for amniocentesis, which was performed on all women with a tube titer greater than 16. The values for the corresponding zones on Liley's chart were compared by both methods. The comparison is shown in Table 5.

Table 5. Median titer values by both methods for different zones of amniocentesis

Zone as per Liley's chart (n)	Tube titer range (median)	Gel titer range (median)
Low zone (4)	16–64 (32)	64–512 (128)
Mid zone (6)	32–512 (64)	128–2048 (256)
High zone (8)	128–512 (128)	256–2048 (718)

n = Number of women falling in each group.

Discussion

Rh antigens are by far the most common cause of alloimmunization in pregnant women. The D antigen is the most immunogenic; yet, alloimmunization caused by it has been virtually eliminated in the Western world. Use of antibody screening and titration studies aid the obstetrician in deciding among early preventive or treatment modalities to manage HDFN.

Risk of HDFN can be diagnosed early in pregnancy with noninvasive serologic methods such as the indirect antiglobulin test to detect the presence of irregular antibodies in the maternal serum. Usually the firstborn is the initiator of sensitization to antigens present on fetal RBCs. This occurs mostly at delivery. During subsequent pregnancy reexposure to the same antigen initiates a secondary immune response and with the potential for the pathogenesis of HDFN. Antibody that has been detected in maternal serum must be identified. The antibody can then be quantitated using laboratory methods such as titration studies. The titration studies are useful in guiding the timing of clinical intervention required in utero. The noninvasive serologic methods precede the more invasive methods such as amniocentesis, which is associated with fetal morbidity and mortality.

The higher sensitivity and versatility of the gel test compared with the tube test have been reported in various studies.[6,7] A recent study comparing the conventional tube test with the gel technique for crossmatch also showed the latter to be more sensitive.[8] Other positive points of the gel test include smaller volume of sample required, elimination of washing steps, stable results, and easy readability. The tube test in our study missed 10 examples of anti-D that the gel test detected. However, the anti-D in these samples were all attributable to antenatal anti-D immunoprophylaxis and they became undetectable after 4 to 6 weeks. Thus, the tube test did not miss any of the significant antibodies. Data are also presented that indicate the propensity of the gel test to miss clinically insignificant antibodies like Le^a.[7] The gel test in our study missed one sample of anti-Le^a. Nine samples of anti-Le^a and anti-Le^b were, however, detected by both methods. The titration of antibodies by both methods showed variable results. Similar data have been published by Novaretti et al.[9] in which they tested gel and tube titers for anti-D. They found the titers to be threefold to eightfold higher. Our study showed the titers to be one- to fivefold higher. The gel titers tended to be higher when compared with the tube titers. However, no correlation between the two methods could be found.

The recommended titration study by Judd[10] is a saline antiglobulin procedure with 60-minute incubation at 37°C. In our study, we did not use LISS for gel titration, which is a known enhancing medium, in an attempt to establish a correlation, if any exists, between the conventional tube technique and the gel technique. Judd also stated that until substantial data are available that show correlation between gel microcolumn assay and saline tube antiglobulin titers IgG gel column technology should not be used for prenatal antibody titration. The critical tube titers of 16 corresponded to gel titers ranging from 32 to 128. Hence, it is difficult to arrive at a definitive conclusion of critical gel titers when a direct correlation is attempted. When the titers were compared with ultrasonography for hydrops and amniocentesis OD values as per Liley's chart, a gel value of less than 128 (i.e., 64) corresponded with normal ultrasonography and low zone OD values. However, this value should be interpreted with caution until more studies support it.

Based on the above observations, we conclude that the gel test is a better method than the conventional tube test for antibody detection because of its higher sensitivity and technical safety. Titration by gel, however, should not be considered for antenatal HDFN management as gel titers do not show linear correlation with tube titers, which predict fetal outcome in RhD sensitized women. Developing countries work under resource constraints, and such studies would optimize cost-effective use of this technology.

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Manish K. Thakur, MD (corresponding author), Senior Resident, and Neelam Marwaha, MD, Professor and Head, Department of Transfusion Medicine, Praveen Kumar, MD, DM(Neonatology), Additional Professor, Department of Pediatrics, Subhash C. Saha, MD, Associate Professor, Department of Obstetrics and Gynecology, and Beenu Thakral, MD, Assistant Professor, Ratti Ram Sharma, MD, Assistant Professor, Karan Saluja, MD, Assistant Professor, Hari Krishan Dhawan, MD, Senior Resident, Department of Transfusion Medicine, and Ashish Jain, MD, Senior Resident, Department of Transfusion Medicine, Post Graduate Institute of Medical Education and Research, Sector 12, Chandigarh, India 160012.