

Evaluation of particle gel immunoassays for the detection of severe immunoglobulin A deficiency and anti-human immunoglobulin A antibodies

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BACKGROUND: Immunoglobulin A (IgA)-deficient patients with anti-IgA (Ab) require transfusions using blood components with less than 0.05 mg IgA/dL as they are known to be safe for these patients. Identification of severely IgA-deficient (IgA SD) donors involved preliminary screening by the Ouchterlony double immunodiffusion assay followed by confirmatory testing at the required level of sensitivity for IgA and Ab at an external reference laboratory. Two in vitro particle gel immunoassays (ID-PaGIA IgA deficiency test and anti-IgA test) were also evaluated for their suitability in identifying IgA SD individuals and determining their Ab status.

STUDY DESIGN AND METHODS: Samples from 198 donors and 36 patients, subjected to confirmatory testing for IgA SD and Ab over a 2-year period, were also evaluated using the ID-PaGIA kits.

RESULTS: DiaMed test sensitivity and specificity for detection of IgA SD in donors was 98% whereas for Ab, test sensitivity was 91% at a specificity of 94%. In patients, sensitivity was 94% for IgA SD and 67% for Ab, both tests at a specificity of 100%.

CONCLUSIONS: The ID-PaGIA IgA deficiency test was a sensitive and specific tool for identifying IgA SD donors or patients. Sensitivity of the Ab test was high for donors but reduced for patients and of high specificity in both groups. Further studies with patients are needed to confirm this latter observation. Implementation of these tests would make it possible to supply appropriate products from IgA SD donors to prevent anaphylactic transfusion reactions in patients.

Identification of patients as IgA deficient with anti-IgA (Ab) is critical in preventing adverse transfusion reactions since they must either receive blood products from donors known to be IgA deficient or receive products derived from normal, healthy individuals that have been processed in a manner shown to substantially reduce IgA levels.¹⁻⁷ While passive transfusion of Ab to IgA in products to a recipient who is not IgA deficient has not been shown to elicit transfusion reactions,⁸⁻¹⁰ an IgA-deficient patient's antibody status still remains of concern in the event they receive products containing IgA. For this reason, Canadian Blood Services (CBS) has been using a screening assay to identify possibly severely IgA-deficient (IgA SD) donors.¹¹ Subsequently, these samples were sent to an external reference laboratory for confirmatory testing of IgA levels and determination of Ab status. Samples from patients were also sent for confirmatory testing without prior screening at CBS.

The current testing algorithm involves the use of a screening test (Ouchterlony double immunodiffusion assay) at CBS, which requires an 18- to 24-hour incubation before IgA test results are known. Any sample found to be possibly deficient is forwarded for confirmatory IgA and Ab testing at an external reference laboratory with results being available on average within 14 days from the date

ABBREVIATIONS: Ab = anti-IgA; CBS = Canadian Blood Services; IgA NSD = IgA not severely deficient; IgA SD = IgA severely deficient; NPV = negative predictive value; PaGIA = particle gel immunoassay; PPV = positive predictive value.

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of sample receipt. We evaluated two commercial kits currently licensed in Europe for the determination of IgA deficiency and the presence of human antibodies to IgA. There are a number of potential benefits in using particle gel immunoassays (DiaMed ID-PaGIA IgA deficiency test and the ID-PaGIA anti-IgA test, DiaMed GmbH, Cressier, Switzerland). Results would be available within 30 minutes and use of the kits is relatively easy. Other particle gel immunoassays are already in use at CBS for RBC antibody screen and identification. Use of these kits offers additional advantages with respect to regulatory issues by provision of standardized kits and controls and their applicability for testing both donors and patients.

A limited number of reports are available concerning use of the gel card assays for detection of IgA SD and anti-IgA. A previous report by Salama and colleagues¹² reported that the DiaMed ID-PaGIA anti-IgA was unreactive to antibodies to IgM and IgG and unreactive to normal serum samples from healthy blood donors. However, it was reactive to Ab in six patients with IgA deficiency and to four patients with common variable immunodeficiency. A later report by Brown and colleagues¹³ with a limited number of patients indicated a favorable outcome using the DiaMed gel card system for IgA compared to IgA quantitation by nephelometry and for Ab determination at titers of at least 1024 compared to the results obtained by a hemagglutination assay. In this study, we assessed the sensitivity and specificity of the DiaMed gel card assays compared with the results obtained from 198 donor and 36 patient samples determined at an external reference laboratory that used previously published methods for determination of severe IgA deficiency by an enzyme-linked immunosorbent assay (ELISA) method and detection of Ab to IgA by a passive hemagglutination assay. This report presents the results of this evaluation.

MATERIALS AND METHODS

Sample screening and confirmatory testing

During the period November 2007 to December 2009, CBS screened 99,772 donor samples for possible IgA deficiency by a double immunodiffusion assay as previously described.¹¹ Donor samples found to be possibly deficient (IgA < 2-4 mg/dL) were sent to the American Red Cross National Reference Laboratory, Penn-Jersey Region, Philadelphia, PA (hereafter referred to as the external reference laboratory) for confirmatory testing of IgA levels as either less than 0.05 or at least 0.05 mg/dL and of Ab to IgA1 or IgA2 (sensitivity 0.5 mg/dL) using previously published methods.^{14,15} Donors were considered to be confirmed as IgA SD if their IgA levels were found to be less than 0.05 mg/dL on two consecutive donations. If

their IgA levels were found to be at least 0.05 mg/dL then they were considered to be not severely deficient (IgA NSD). Additional details of sample selection, the IgA screening test method, and confirmatory testing for IgA SD and Ab have been previously published.¹¹ Except where otherwise indicated, all Ab test results presented in this report refer to anti-IgA1.

Additionally, samples from hospital patients referred to CBS from November 2007 to September 2010 because of low IgA levels, transfusion reactions, primary immune deficiency, or variable immunodeficiency were not screened for IgA levels but forwarded to the external reference laboratory for determination of IgA levels and Ab status. Where sufficient sample volume was available, donor and patient samples were also subjected to testing using the DiaMed gel card kits described below. Ethylenediaminetetraacetate plasma samples from donors and plasma or serum samples from patients were stored frozen at -20°C or colder before testing.

DiaMed PaGIA test method

Sample IgA levels and anti-human IgA were determined using the DiaMed-ID microtyping system (six microtubes per gel card with six gel cards per kit) following the package inserts provided by the manufacturer (DiaMed GmbH). For the ID-PaGIA IgA deficiency test, 10 µL of sample was incubated with 50 µL of a suspension of red, high-density synthetic polymer particles coated with anti-IgA in the reaction chamber on top of each microtube. For the ID-PaGIA anti-IgA test, 10 µL of sample was incubated with 50 µL of suspended polymer particles coated with polyclonal human IgA. After 5 minutes of incubation at 18 to 25°C, each gel card was centrifuged in an ID-MTS centrifuge (Model 5150-60, Sparton Electronics, DeLeon Springs, FL, for Micro Typing Systems, Inc., an Ortho-Clinical Diagnostics Co., Pompano Beach, FL). Centrifugation at 895 ± 25 rpm (80-90 RCF) for 10 minutes separates agglutinated from nonagglutinated particles in the gel filtration matrix of each microtube. Positive and negative IgA or anti-IgA controls were also run on each gel card as appropriate. The gel cards were read within 15 minutes after recovery from the centrifuge.

A positive reaction for IgA (IgA > 0.05 mg/dL) corresponds to an agglutination reaction seen as a red line on top of the gel, a strong red coloration dispersed within the upper part of the gel or dispersion throughout the gel. A negative reaction (IgA < 0.05 mg/dL) corresponds to complete sedimentation of the particles as a pellet at the bottom of a microtube with no agglutinated particles visible within the gel. For the ID-PaGIA anti-IgA test (sensitivity, 0.02-0.05 mg/dL), agglutination of particles is caused in the presence of IgA antibodies of any immunoglobulin class. A strong positive anti-IgA reaction results in a red line at the top of the microtube gel matrix

or as agglutinates partially distributed only within the upper part of the gel. For a weak reaction, some particles may reach the bottom of the gel but with agglutinates still visible in the upper part of the gel or dispersed throughout it. For a negative anti-IgA reaction, complete sedimentation of the particles as a pellet must occur with no agglutinated particles visible within the gel matrix. All samples were tested singly for IgA and Ab concurrently. For the relatively few instances when results were obtained that were different than confirmatory test outcomes, samples were retested for both IgA and Ab to obtain a final determination. To maintain objectivity in evaluating the gel card results, one technologist prepared the samples for testing and carried out the gel card assays and a second technologist blinded to the previously documented confirmatory test results recorded the results.

Statistical analysis

The data were analyzed by standard methods for the determination of DiaMed test accuracy¹⁶ compared with the external reference laboratory test results that were considered to be the "gold standard." Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with their 95% confidence intervals (CIs) were calculated using computer software (GraphPad PRISM, Version 4.03, GraphPad Software, Inc., San Diego, CA). Sample sizes required to accurately measure true sensitivity and specificity were evaluated based on the CI approach using the exact method for a binomial distribution. The calculations were based on the assumed true rates of agreement between the DiaMed and external reference laboratory results (90%-100%) and an allowed margin of error (precision of 2.5%-10.0%) for a one-sided 95% CI. Statistical software (SAS, Version 9.1, 2002-2003, SAS Institute, Inc., Cary, NC) was used for the calculation.

RESULTS

Comparison of DiaMed and American Red Cross test result outcomes

Donors

A comparison of IgA and Ab results obtained using the DiaMed gel card assays and those obtained at the external reference laboratory is shown in Table 1. Results are categorized by both IgA and Ab outcomes when both IgA and Ab results are in agreement with one another, and when differences were observed they are listed by IgA and/or Ab outcomes. There were 139 donor samples that were confirmed as having IgA of less than 0.05 mg/dL (IgA SD) and another 59 with IgA of at least 0.05 mg/dL (IgA NSD). Of 139 donors who were IgA SD, 57 were positive for Ab. None of the IgA NSD donors had detectable Ab. Using the DiaMed gel card assays, IgA SD was detected in 136 of a possible 139 samples and Ab in 52 of a possible 57 samples. Test sensitivity, specificity, PPV, and NPV for detection of IgA SD or Ab are shown in Table 2 as are the associated 95% CIs. We noted that test sensitivity and specificity for IgA SD were 5% to 7% higher than observed for Ab. Of the 59 IgA NSD donors, none of whom had detectable Ab, the DiaMed gel card assay detected 58 donors as IgA NSD.

Patients

For patients, as no initial screening at the National Testing Laboratory had occurred, only confirmatory results were available for IgA levels. There were 17 patients confirmed as IgA SD and 19 as IgA NSD (Table 3). Ab was detected in 10 IgA SD patients and in two IgA NSD patients. By DiaMed assays, IgA SD was detected in 16 of a possible 17 samples whereas IgA NSD was found in 17 of 17 samples. Ab was detected in 7 of 10 possible samples from IgA SD patients and from one of two IgA NSD patients. Measures of DiaMed test accuracy for the detection of IgA SD or Ab are shown in Table 4. Test sensitivity was much lower for Ab than for IgA SD although specificity remained at 100%.

TABLE 1. Comparison of DiaMed and reference laboratory results for both donor IgA and anti-IgA status*

IgA/Ab outcomes	Total number of reference laboratory results	Total number of DiaMed test results	Differences observed by DiaMed assay		
			IgA	Anti-IgA	IgA and anti-IgA
IgA SD/Ab (-)	82	75	1 IgA NSD	5 Ab (+)	1 IgA NSD/Ab (+)
IgA SD/Ab (+)	57†	51	1 IgA NSD	5 Ab (-)	0
Σ	139	126	2	10	1
IgA NSD/Ab (-)	59	56	0	2 Ab (+)	1 IgA SD/Ab (+)
IgA NSD/(Ab +)	0	0	0	0	0
Σ	59	56	0	2	1
All data	198	182	2	12	2

* Donor test outcomes were assessed as either IgA < 0.05 mg/dL (IgA SD) or as IgA ≥ 0.05 mg/dL (IgA NSD) without (-) or with (+) Ab to IgA. The number of outcomes in agreement for both IgA and anti-IgA are indicated for the DiaMed test results. Details concerning any differences between test methods are also provided.

† Includes four donors who were confirmed as IgA SD/anti-IgA2 (+), of which three were assessed as IgA SD/Ab (+) and one as IgA SD/Ab (-) by the DiaMed gel card tests.

TABLE 2. Assessment of DiaMed IgA SD and anti-IgA test accuracy for donors

DiaMed result	Gold standard result (reference laboratory)		DiaMed test accuracy (95% CIs)			
	True positive (IgA SD)	True negative (IgA NSD)	Sensitivity	Specificity	PPV	NPV
IgA SD	136	1	97.8%	98.3%	99.3%	95.1%
IgA NSD	3	58	(93.8%-99.6%)	(90.9%-100%)	(96.0%-100%)	(86.3%-99.0%)
Total	139	59				
	True Ab positive	True Ab negative				
Ab (+)	52	9	91.2%	93.6%	85.3%	96.4%
Ab (-)	5	132	(80.7%-97.1%)	(88.2%-97.0%)	(73.9%-93.0%)	(91.7%-98.8%)
Total	57	141				

TABLE 3. Comparison of DiaMed and reference laboratory results for both patient IgA and anti-IgA status*

IgA/Ab outcomes	Total number of reference laboratory results	Total number of DiaMed test results	Differences observed by DiaMed assay		
			IgA	Anti-IgA	IgA and anti-IgA
IgA SD/Ab (-)	7	7	0	0	0
IgA SD/Ab (+)	10	6	1 IgA NSD	3 Ab (-)	0
Σ	17	13	1	3	0
IgA NSD/Ab (-)	17	17	0	0	0
IgA NSD/Ab (+)	2†	1	0	1 Ab (-)	0
Σ	19	18	0	1	0
All data	36	31	1	4	0

* Patient test outcomes were assessed as either IgA < 0.05 mg/dL (IgA SD) or as IgA ≥ 0.05 mg/dL (IgA NSD) without (-) or with (+) Ab to IgA. The number of outcomes in agreement for both IgA and anti-IgA are indicated for the DiaMed test results. Details concerning any differences between test methods are also provided.

† Includes one patient confirmed as IgA NSD/anti-IgA2 (+) which tested as IgA NSD/Ab (-) by the DiaMed gel card tests.

TABLE 4. Assessment of DiaMed IgA SD and anti-IgA test accuracy for patients

DiaMed result	Gold standard result (reference laboratory)		DiaMed test accuracy (95% CIs)			
	True positive (IgA SD)	True negative (IgA NSD)	Sensitivity	Specificity	PPV	NPV
IgA SD	16	0	94.1%	100%	100%	95.0%
IgA NSD	1	19	(71.3%-99.9%)	(82.4%-100%)	(79.4%-100%)	(75.1%-99.9%)
Total	17	19				
	True Ab positive	True Ab negative				
Ab (+)	8	0	66.7%	100%	100%	85.7%
Ab (-)	4	24	(34.9%-90.1%)	(85.8%-100%)	(63.1%-100%)	(67.3%-96.0%)
Total	12	24				

Detection of anti-IgA2 (Ab2)

There were six unrelated individuals found to have Ab to IgA2 (Ab2) at the reference laboratory. Of these, there were four IgA SD donors of which Ab2 in one donor was not detected when tested by the gel card method. In addition, there were two patients that were IgA NSD of which Ab2 in one patient was not detected by the gel card method.

Comparison of DiaMed test accuracy for donor and patient samples

DiaMed test sensitivity was slightly reduced comparing IgA SD results from patients (Table 4) to those of donors

(Table 2) although test specificity was 100% for patients compared to 98% for donors. Sample size was much smaller for the IgA SD patients (n = 17) compared to IgA SD donors, however (n = 139). Similarly for detection of Ab, a reduced sensitivity was noted comparing patients to donors although test specificity was 100%. Based on the posttest analysis, sample size was sufficient to determine sensitivity or specificity at a precision of 5% for the IgA SD test or at 10% precision for the IgA Ab test when considering the data from donors. However, sample size was not sufficient for patient data at either level of precision. If a higher precision is needed (<5%), then a larger cohort must be tested for both the donor and the patient groups.

DISCUSSION

This study showed that the DiaMed gel card tests gave a high level of sensitivity and specificity for the detection of IgA SD in either donors or patients when compared to confirmatory test results. Both the DiaMed gel card and the external reference laboratory ELISA methods have identical limits of detection for IgA (<0.05 mg/dL). The DiaMed gel card assay for Ab was also of high sensitivity in donors but reduced for patient samples. This reduced sensitivity may reflect lower antibody titers in patients than were present in donors included in this study. However, test specificity was high for both donors and patients. As sample size was much smaller for the patient group compared to donors, the 95% CIs around each measure of DiaMed test accuracy reflect a much wider margin of error for patient samples.

While antibodies to IgA would be expected in some individuals who are IgA SD,¹¹ we noted that some patients who were IgA NSD (≥ 0.05 mg/dL) also had detectable Ab. Unlike the donor samples tested, we had no estimate of the upper limit of their IgA levels. We suspect that they were low, however, and fell in the same range as has been previously reported in other studies for individuals who were not severely IgA deficient but had detectable antibody nonetheless.¹⁷⁻¹⁹

Based on data from this study, the PaGIA test for IgA SD would be considered acceptable for use in testing of donor and patient samples. The PaGIA gel card test for Ab detection would also be considered acceptable for donor testing since the passive transfusion of a blood product from a donor who was IgA SD with Ab has not been shown to be detrimental to any recipients.⁸⁻¹⁰ So a false-negative Ab result in a donor would not result in an inappropriate recommendation for transfusion therapy of a patient.

Where DiaMed and confirmatory test results, determined at the external reference laboratory, for matched samples did not agree it is possible that antigen or antibody levels were close to the sensitivity limits of the different methods. Slight variations in sampling accuracy and test sensitivity may give different results even if these samples were repeatedly tested. Apparent changes in donor or patient IgA levels or Ab status may also be due to actual changes in IgA or Ab levels over time. Laschinger and coworkers¹ noted that donors with serum IgA of less than 0.05 mg/dL had a coefficient of variation of 143% over time that could certainly account for a change in apparent IgA status from IgA SD to IgA NSD or vice versa. However, in this study, testing was only performed on matched samples for which the reference laboratory results were known.

While this study compared DiaMed test results to an external reference laboratory method of similar sensitivity for IgA, this was not the case in the study by Brown

and coworkers.¹³ In that study although 9 of 24 patients were identified as IgA deficient at the limit of detection by nephelometry (<6.7 mg/dL), only eight were found to be IgA SD by the more sensitive DiaMed assay method. Of the remaining 15 patients whose IgA levels ranged from 56 to 681 mg/dL by nephelometry, all were correctly assessed as IgA NSD by the DiaMed test. Our study presents a more rigorous challenge of the sensitivity and specificity of the DiaMed gel card test for IgA levels as it included a large cohort of IgA SD ($n = 139$) and IgA NSD donors ($n = 59$) all of whom had a maximum IgA level of less than 2 to 4 mg/dL as assessed by the screening assay at CBS. This study also included a larger number of IgA SD patients than were reported by Brown and colleagues.¹³

Concerning DiaMed Ab results, the study by Brown and colleagues¹³ indicated that of 18 patients with Ab titers in the range 2 to 128 determined by a hemagglutination assay, only two also had detectable Ab by the DiaMed test. They suggested that the PaGIA gel card method either had a relative insensitivity to IgA Ab in normal or IgA-deficient patients at low antibody titers (range, 2 to <128) previously determined by hemagglutination or that the hemagglutination assay itself might be detecting a nonspecific (false-positive) reaction in 16 of 16 patients with normal IgA levels and in two of two individuals that were IgA deficient. Of the remaining six patients with Ab titers in the range 1024 to 524,288, all were also Ab positive by the DiaMed test.¹³ In our study, antibody titers are not determined for samples sent to the external reference laboratory for confirmatory testing. This study and that of Brown and colleagues¹³ both differ from that of Salama and colleagues,¹² which did not compare DiaMed results to those obtained using a reference method for Ab to IgA.

Investigation of the consistency of donor IgA SD or Ab status over time was not within the scope of this comparative study. However, our previous study¹¹ showed that the majority of donors confirmed at the external reference laboratory as IgA SD/Ab (–) on their first donation consistently showed the same outcome on their second or, where repeat testing occurred at the reference laboratory, in any subsequent donations (94.3%). Similarly, donors confirmed at the external reference laboratory as IgA SD/Ab (+) on their first donation typically had the same outcome on their second and any subsequent donations (92.3%). In that study, consistency of confirmatory test results for IgA NSD with or without Ab was not determined because those individuals were not part of the target group we were trying to identify. Consistency of positive Ab status over time was also not routinely monitored after a second confirmatory test in that study or this one because once Ab was detected, no further testing would normally occur.

In summary, the DiaMed ID PaGIA IgA deficiency test can be used to detect IgA deficiency in patients who may be at risk of anaphylactic reactions if transfused with blood products containing IgA. This test may also be used to test donors with severe IgA deficiency whose blood can be used for the clinical treatment of patients with anti-IgA. The DiaMed ID PaGIA anti-IgA test may be used to detect specific IgA antibodies from any immunoglobulin class in pretransfusion screening. Both of these tests offer significant benefits in terms of turnaround times for results reporting. Running four samples with two controls per gel card provides test results in less than 30 minutes once the gel cards are loaded. Each kit includes enough gel cards for 24 samples all of which can be loaded onto the ID centrifuge at the same time. Staff reported no difficulties in determining whether or not a sample was positive or negative for IgA or Ab. Total assay time from start to finish was less than 1 hour per 6 × 6 gel card kit including pipetting, incubation, centrifugation, and results assessment.

This study extends the findings of earlier reports in the literature of the sensitivity and specificity of the DiaMed particle gel immunoassays, with a larger cohort of IgA SD donors and patients whose IgA and Ab status have been previously determined by confirmatory tests at an external reference laboratory. Based on our results, the particle gel IgA deficiency immunoassay would be extremely useful to identify IgA-deficient donors and patients. The particle gel anti-IgA test appeared to be less sensitive for the detection of antibodies in donors and to a greater degree for patients than the passive hemagglutination assay currently used by the external reference laboratory. However, only a relatively small number of patient samples were available for testing, and more data are necessary to confirm this observation. Furthermore, in the absence of Ab titer information, we can speculate that Ab titers may have been lower in some patients than in donors and this was reflected in a reduction of test sensitivity for Ab in patients. Actual use of the test in Canada will still depend on licensure and the manufacturer's intended use specifications.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest relevant to the manuscript submitted to **TRANSFUSION**.

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