

## **Title Page**

### **Impact of Uniform Methods on Inter-laboratory Antibody Titration Variability**

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## **Abstract**

**Background-** Substantial variability between different antibody titration methods prompted development and subsequent introduction of uniform methods in 2008. We sought to determine whether uniform methods consistently decrease inter-laboratory variation in proficiency testing.

**Study Design and Methods-** Proficiency testing data for antibody titration between 2009 and 2013 were obtained from College of American Pathologists. Each laboratory was supplied plasma and red cells to determine anti-A and anti-D titers by their standard method: gel or tube by uniform or other methods at different testing phases (immediate spin and/or room temperature (anti-A), and/or anti-human globulin (AHG: anti-A and anti-D)) with different additives. Inter-laboratory variations were compared by analyzing the distribution of titer results by method and phase.

**Results-** A median of 574 and 1100 responses were reported for anti-A and anti-D titers, respectively over five years. The three most frequent methods performed for anti-A: uniform tube room temperature (25.3%), uniform tube AHG (24.9%), other tube AHG (16.7%); and for anti-D: other tube (41.0%), uniform tube (36.4%) and uniform gel (12.4%) methods. Of the larger reported methods, uniform gel AHG phase for anti-A and anti-D had the most participants with the same result (mode). Significant reduction in titer variability for anti-A: 1/8 (uniform vs other tube room temperature), 0/8 (uniform vs other tube AHG); and anti-D: 0/8 (uniform vs other tube), 0/8 (uniform vs other gel) tests noted.

**Conclusion-** Uniform methods harmonize laboratory techniques but do not consistently reduce inter-laboratory titer variance in comparison with other methods.

**Key Words:** Antibody titration; uniform methods.

## Introduction

Antibody titration (ABT) is a semi-quantitative method used to detect the reactivity of antibodies present in the patient's plasma<sup>1</sup>. ABT is used prenatally to screen for risk of hemolytic disease of the fetus and newborn (HDFN), hemolytic reactions or in transplantation field. If the mother has a clinically significant alloantibody, ABT is performed. Once the antibody and the titer strength is identified, they are periodically performed throughout pregnancy, where plasma samples are compared in parallel with the previously frozen samples to determine increase in titer strength. With the advent of middle cerebral artery doppler there is decreased reliance on titers alone to predict the wellbeing of baby<sup>2</sup>. However each laboratory must still define a critical titer where further fetal assessment, with doppler, ultrasound or amniocentesis needs to be initiated. ABT is also commonly used for screening blood products, particularly platelets and plasma. In order to decrease the risk of hemolytic transfusion reactions due to passive anti-A/anti-B, group O products are tittered and those with high titers (typically >1:100) are labeled and used for group O individuals only<sup>3</sup>. In addition, ABT has a role in preventing graft rejection for ABO incompatible solid organ transplants of heart, liver, and lung as well as hematopoietic progenitor cell transplants<sup>1,4,5</sup>.

Laboratories have multiple variables to determine in their ABT procedures, including technology, diluent, incubation time, strength of reading cut-off, and testing phase<sup>6</sup>.

Traditionally, ABT is carried out in a test tube, however, gel methods appear to be more sensitive and less dependent on test performer so many laboratories are switching to the gel method using saline as the diluent<sup>7</sup>. The AABB Technical Manual's method<sup>7</sup>, which is well

adopted for non-ABO antibodies, is with saline, 60 minutes incubation at 37° C and anti-IgG using the tube technology<sup>8</sup>. Variation is also seen in the cut-off strength of the reaction to determine the titer, such as w+ versus 1+. With the increased sensitivity of gel versus tube the strength of titer reading is usually 1+ reactivity in gel versus w+ reactivity in tube<sup>9</sup>. Since patients often seek care at different institutions with varying standard operating procedure (SOP) for ABT, titer strength reported could easily be in a wide range and inadvertently subject patients to high risk procedures<sup>10,11</sup>. Hence it is critical to not just identify the appropriate antibody and their exact strength, but also to make sure test methodology is well defined to get reproducible results in different settings. Hence College of American Pathologists (CAP) and Biomedical Excellence for Safer Transfusion/(BEST) committee in 2008 introduced a well-defined standardized procedure (uniform method), based on the findings of a pivotal study that showed significant reduction in inter-laboratory titer variability<sup>12</sup>. To assess the impact of introduction of uniform methods for ABT, we analyzed data from consecutive CAP proficiency testing (PT) conducted post uniform method introduction.

## Methods

CAP PT survey was conducted bi-annually post uniform procedure announcement (Period: 2009-2013, 10 surveys) for anti-A and anti-D titration in 2008<sup>9</sup>. Laboratories reported their results based on their primary testing method, as per proficiency testing standards. Each survey contained two separate sera (anti-A and anti-D) and a Group A1 RhD-positive (D+) red cells for labs to perform antibody titration. For anti-A, titers were performed both at immediate spin (IS) and/or room temperature (RT) and the anti-human globulin (AHG) phase, and for anti-D at AHG phase either by using uniform or other methods as per laboratories' primary SOP. A detailed description of uniform methods is available in Tables 1&2. Table 3 illustrates commonly used abbreviations in the manuscript. Results of PT were submitted using a standardized form, which collected information on the laboratory's methodology and titer results. Considering the number of participants varied throughout the five year study period, median percentage for individual methods are used for comparison in the manuscript (Table 4). To simplify interpretation of wide range of distribution of titers, results of both the tube and gel methods were assessed by determining the proportions of titers that were identified within 1 dilution range from mode (mode defined as the titer with highest frequency of responses). As a general trend a small percentage of results were determined outside of mode  $\pm 2$  range and hence not reported in results section, but summarized in Figure 1. Finally, all reported titers were converted to log<sub>2</sub> and the standard deviation and variance was calculated for each of the methods (log<sub>2</sub> default value for a titer of 1 was 0.1). Since titration results with different methods had varying response rates (number of titer results reported/method), for final titer variance reduction analysis data from the top three or four methods reported for anti-A and

anti-D determination were included for statistical comparisons (Tables 4 and 5). Also in determining reduction in titer variance, only data from 2010-2013 were included in analysis, since the 2009 surveys permitted labs to report results with multiple attempts of testing and hence deemed not suitable to check for variance for that particular year.

## Statistical analysis

Results were grouped according to antibody (anti-A vs anti-D) and platform (tube or gel) and for anti-A separately in the IS/RT and the AHG phases. While analyzing for ABT variance between uniform and other methods for anti-A, uniform tube (UT) vs other tube (OT) methods was compared at both the RT and AHG phases. For anti-D, since all the methods were performed at AHG phase, statistical significance for titer variance were calculated separately for different platforms, uniform tube (UT) vs other tube (OT) and uniform tube (UT) vs uniform gel (UG). After analyzing all titer values, visual outlying titers were removed to prevent bias to the variability estimates. Therefore in the years 2011 (survey A), 2012 (survey A) and 2013 (Survey B), 3, 1 and 2 outlier values from the respective PT were removed for analysis. The response rate with OG, UG methods for anti-A and OG methods for anti-D was small, hence statistics could not be represented accurately for these methods. Thus, a direct comparison to check for titer reduction between UG vs UT or UG vs OG for anti-A, and OG vs UG for anti-D were not evaluated in our study. The primary statistical test performed was the variance ratio test (F-test) to compare the significance of the ratio of the variance estimates of the two methods.

## Results

### Anti-A Titer

**Technique** - There were a median of 574 (range 509-682) anti-A titers over five years study period (Table 4). Uniform methods were reported most frequently over other methods both at RT (25.3% vs 16.4%) and AHG (25% vs 16.7%) phase with the tube platform. Also with the gel platform, uniform methods (UG RT- 3.3% and UG AHG -4.1%) were reported more frequently than other methods (OG- 2.1%). Only a minority of responders chose to report anti-A separately at IS phase (5.6%) and with polyspecific AHG reagent (1.9%) in other tube methods.

**Distribution of Titers**- With the exception of UG RT method more than 90% of titers were detected within 1 dilution from mode (i.e. mode + mode  $\pm$  1dilution) by utilizing gel platform (OG AHG- 93.6%, UG AHG-91.6%). In contrast, tube platform methods could detect > 80% but < 90% of anti-A titers within 1 dilution from mode (OT IS- 88.8%, OT RT- 88.0%, UT AHG-86.0%, and OT AHG- 84.4 %), barring the rare exception of UT RT (79.8%) method. When analyzing the range of distribution of titers at different phases individually, OT IS phase captured 88.9% of titers within 1 dilution from mode. At RT phase also majority of titers were detected within 1 dilution from mode, but had a wide range of results based on platform and method applied (UT RT 79.8%, OT RT- 88% and UG RT- 67.9% )( (Figure 1). At the AHG phase, irrespective of the method used (uniform or other) > 80% of titers was reported with in 1 dilution from mode (UT AHG- 86.1%, OT AHG- 84.5%, UG AHG- 91.7% and OG AHG-93.6%).

**Variance** - In the cumulative analysis, at RT none (0/8) of the PT surveys comparing UT vs OT methods showed a statistically significant reduction in titer variance, in fact, 6/8 (75.0%) had

statistically significant higher variance. However in AHG phase, 1/8 (12.5%) surveys comparing UT vs OT showed statistically significant reduction in anti-A titer variance and none had statistically significant higher variance. (Table 5 and Figure 2).

### **Anti-D Titer**

**Technique-** There were a median of 1100 (range 1059- 1211) anti-D titers reported over five year period (Table 4). With tube platform, other methods with IgG AHG (OT -41.0%) were preferred more often than uniform method (UT AHG- 36.5%). Gel platform was utilized less often with majority of laboratories opting for uniform method over other methods (UG- 12.5%, OG- 1.4%). With the tube platform apart from using IgG AHG (41.0%) method, titers were also reported with 6% albumin diluent (5.2%), 22% albumin diluent (1.7%) and with polyspecific AHG (2.1%) reagents by different laboratories as per laboratories' SOP during the study period.

**Distribution of Titers-** All titers were reported at AHG phase. Gel platform methods had > 90% of titers detected within 1 dilution from mode (OG-94.0%, UG-91.5%) (Figure 1). Tube platform methods with both uniform and other methods detected > 80% but < 90% of anti-D titers within 1 dilution from mode (OT IgG AHG- 88.2%, UT- 88.2%, OT 22% albumin- 86.5%, OT 6% albumin- 86.4% and OT polyspecific AHG- 82.6%).

**Variance** – In the cumulative analysis, none (0/8) of the PT surveys comparing UT vs OT and UT vs UG methods showed statistically significant reduction in titer variance (Table 5 and Figure 2).

However, for 5/8 (62.5%) PT surveys, UT had statistically higher variance than UG and for 1/8 (12.5%) surveys, UT had statistically higher variance than OT.

## Discussion

Significant variation in titer reporting between different laboratories has been reported previously<sup>13</sup>. BEST/CAP Transfusion Medicine Resource Committee (TMRC) in 2008 published the uniform method to be incorporated in clinical practice to decrease inter-laboratory titer variation<sup>12</sup>. Evaluating clinical practice via PT surveys for ABT our study demonstrates several key current trends. 1) Uniform methods (tube + gel platform) is practiced by about half of the laboratories around the country (50.3% for anti-A and 49.0% for anti- D). 2) Tube platform is more commonly used in comparison with the gel platform (91% vs 9% for anti-A and 86% vs 14% for anti-D). 3) Assessing the phase at which anti-A is frequently reported- our findings suggest almost equal number of participants report at RT (UT- 25.3%, UG- 3.3%, OT- 16.4%) or AHG (UT- 25.0%, UG- 4.1%, OT- 16.7%) phase irrespective of the method (uniform or other) or platform (tube or gel) chosen. 4) Gel platform appears to be more sensitive than tube platform to detect antibodies within one dilution around mode (> 90% of titers vs > 80% but < 90%). 5) Comparing the four or three most commonly performed techniques for anti-A and anti-D respectively, application of uniform methods did not show statistically significant inter-laboratory reduction in titer variance consistently, except on rare occasion (Table 4).

In the pivotal study reported by Aubuchon et al<sup>12</sup>, reduced titer variability was noted with a w+ endpoint and not with 1+ endpoint using uniform tube method. The same endpoints were used in our study but with different outcomes (Tables 1&2). In comparison to Aubuchon et al study which had 19 laboratories (14 from USA) participating, our study had a higher number of

participants. It is highly likely that with numerically higher participants as noted in our study, consistently reproducing the success of uniform method could be a difficult task due to several reasons. First, although uniform method provides clear technical notes for performing ABT procedure, grading the strength of agglutination (i.e. distinguishing between w+ and 1+) is done manually. Based on the expertise of individuals from a diverse pool of laboratories participating in PT surveys, interpreting titration end points and thereby antibody strength could be subject to variations (operator bias). Prior studies have shown even in a single laboratory, titer reports can substantially vary and give credence to operator dependent bias hypothesis as one of the factors contributing to variance<sup>14</sup>. Second, the equipment (centrifuge machines, speed/duration of centrifuge, test tube size, diluents, etc) used for the ABT process between different institutions participating in PT surveys could be different and thereby introducing additional bias (laboratory or circumstantial bias). Finally, for both anti-A and anti-D the use of uniform methods in several PT's showed an increase in titer variance, rather than reduction in variance. Although our study did not specifically explore reasons for this discrepancy, the real problem with standardizing ABT could be truly multifactorial. Hence, further research to identify additional factors influencing ABT needs to be prioritized.

Another key observation in our study is the platform (tube > gel) used to do ABT. This preference could be because despite many advantages like increased automation, retrospective supervisory review and decreased reliance on man power, utilizing gel technique is not without disadvantages<sup>15</sup>. Persistent concerns with increased sensitivity for antibody detection (including

non-specific) and reporting higher titers of antibody with the gel in comparison with tube techniques in the absence of robust evidence linking gel titer levels with clinical outcomes could be limiting more widespread gel technique incorporation<sup>1,16</sup>. Also high rates of antibody detection with gel technology can impede the process of cross match and theoretically increase phenotyping procedures performed in labs to provide antigen negative units, thereby adding additional cost<sup>17</sup>. Hence in our current budget conscious health care system, incorporation of the gel micro agglutination techniques could be a slow and gradual process. Within its limitations (not adequately powered), ours and Aubuchon et al studies have shown the use of gel method is associated with high proportion of labs detecting the same antibody strength (mode). Thus this platform holds promise to replace tube platform as an effective alternative with improved precision. To facilitate this transition, these findings needs to be re-confirmed in well-designed future clinical studies adequately powered for statistical and clinical relevance<sup>18</sup>. Also future studies should address reasons for lower adaptation of gel technique in clinical practice for ABT and understand more pros and cons with this platform.

Currently there is no consensus on temperature/phase at which anti-A titers should be reported (IS, RT or AHG). Many laboratories for ABO typing pre-transfusion, perform either IS or RT testing to determine IgM component and perform AHG phase to determine IgG component when there is suspicion for hemolytic events or determine titers for transplantation. We note with interest that there are multiple methods with different phases at which laboratories are currently reporting anti-A titers. There appears to be almost equal number of labs reporting

either at RT or AHG phase irrespective of tube/gel platform, based on whether uniform or other tube methods are preferred (Table 4). Prior report from the ABO incompatible kidney transplant program at Johns Hopkins Hospital, the conduct of IS/RT ABT is considered redundant due to its inability to offer additional clinically meaningful outcomes in comparison with AHG phase. Authors of this study also opined the use of AHG phase alone either with tube or gel platform instead of RT phase can provide rapid turn-around time for reporting titers<sup>19,20</sup>. Considering several of our respondents in PT used RT or IS phase for anti-A reporting, more focused work to address the role of reporting anti-A titers (either at RT or IS, or AHG phase) in different ABO incompatible clinical scenarios needs to be pursued in future. Until further credible evidence emerges laboratories might have to continue to report anti-A titers based on their current SOP or physician preference. For anti-D, all titers were reported at AHG phase. Since HDFN and other hemolytic events commonly associated with anti-D are predominantly IgG, identifying the phase of anti-D was not the focus of our study. Rather, the focus was to evaluate if the application of uniform methods either by using tube or gel platform could decrease titer variance, which it failed to demonstrate consistently (Table 5). Thus, titrating the old frozen plasma concurrently with the most recent plasma sample for anti-D using the same platform should continue to be a common clinical practice.

## **Limitations**

First, there were fewer anti-A titer results reported compared with anti-D titers and even amongst them, far fewer responses with gel platform was noted thereby reducing the power of the study to determine statistical significance. Second, this study did not analyze the reactivity of other clinically significant allo-antibodies (Rh, Kell, Kid etc) implicated in hemolytic transfusion reactions and HDFN as these are not included in this PT survey. Therefore, our data cannot be extrapolated to other alloantibodies.

## **Future Perspective**

Since discrepancies and inconsistencies with the current methods for ABT persists despite implementation of uniform methods, exploring alternative novel techniques using enzyme linked immunosorbent assay, flow cytometry, surface plasmon resonance and KODE technology in the near future could be prudent<sup>21-27</sup>.

## **Conclusions**

Our study reflects current practice for ABT post uniform method introduction in different laboratories. Standardization of antibody titration techniques aimed at improving precision of results continue to remain an elusive and complex task despite implementing the uniform methods. Our understanding about the possible reasons for discrepancies in ABT reporting with the uniform methods is still incomplete and needs to be pursued further in future prospective large scale studies. Future research should also focus on incorporating novel emerging technologies and mitigate discrepancies noted with current methods for antibody titration.

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## **Table 1: Proficiency Testing- Uniform Procedure (Tube and Gel methods) for Anti-A**

### **Anti-A**

#### **Summary of Uniform Procedure**

#### **Dilution of Sample**

Volume: 1 mL

Diluent: 0.9% NaCl, unbuffered

Technique: Calibrated pipet; new tip for each dilution

#### **Tube Technique: Anti-A**

First reading: (Defined uniform tube RT) (UT RT)

Incubation: Room temperature for  $30 \pm 1$  min and read without additional augmentation.

Second reading: (Defined uniform tube AHG) (UT AHG)

Incubation:  $37 \pm 1^\circ\text{C}$  for  $30 \pm 1$  min

Washes: Four times with at least ten-fold the volume of red cell + sample

Testing phase: Anti-IgG

Endpoint: w+, read macroscopically

#### **Gel Card Technique: Anti-A**

Two separate cards:

Gel only, incubated at room temperature for  $15 \pm 1$  min (Defined uniform gel RT) (UG RT)

Anti-IgG, incubated at  $37 \pm 1^\circ\text{C}$  for  $15 \pm 1$  min (Defined uniform gel AHG) (UG AHG)

Endpoints: 1+

#### **Provision of Reagent Red Cell**

For the purposes of ABT Survey testing, participants should use the titer cell provided with the ABT kit, and not a red cell supplied by your laboratory as defined in the uniform procedure.

**Table 2: Proficiency Testing- Uniform Procedure (Tube and Gel methods) for Anti-D**

**Anti-D**

**Summary of Uniform Procedure\***

**Dilution of Sample**

Volume: 1 mL

Diluent: 0.9% NaCl, unbuffered

Technique: Calibrated pipet; new tip for each dilution

**Tube Technique: Anti-D** (Defined uniform tube AHG) (UT IgG AHG)

Reagent red cell concentration: 3 - 5% in 0.9% NaCl

Volumes: 0.05 mL red cell suspension + 0.10 mL specimen, delivered by calibrated pipet

Incubation: 37±1°C for 30±1min

Washes: Four times with at least ten-fold the volume of red cell + sample

Testing phase: Anti-IgG

Endpoint: w+, read macroscopically

**Gel Card Technique: Anti-D** (Defined uniform gel AHG) (UG)

Card type: Anti-IgG

Reagent red cell concentration: 0.80% in 0.9% NaCl or diluent specified by manufacturer

Volumes: 0.05 mL red cell suspension + 0.025 mL specimen, delivered by calibrated pipet

Incubation: 37±1°C for

Endpoints: 1+

**Provision of Reagent Red Cell**

For the purposes of ABT Survey testing, participants should use the titer cell provided with the ABT kit, and not a red cell supplied by your laboratory as defined in the uniform procedure.

**Table 3: Abbreviations**

- 1) Antibody against A antigen- anti-A
- 2) Antibody against B antigen- anti-B
- 3) Antibody against D antigen- anti-D
- 4) Uniform Tube- UT
- 5) Other Tube- OT
- 6) Uniform Gel- UG
- 7) Other Gel- OG
- 8) Other Tube method with 6% albumin- OT 6% alb
- 9) Other Tube method with 22% albumin- OT 22% alb
- 10) Antibody Titration- ABT
- 11) Standard Operating Procedure- SOP
- 12) Anti-Human Globulin- AHG
- 13) Immediate Spin- IS
- 14) RT- Room Temperature
- 15) Hemolytic Disease of Fetus and Newborn- HDFN
- 16) American Association of Blood Banks- AABB
- 17) College of America Pathologists- CAP
- 18) Biomedical Excellence For Safer Transfusion- BEST
- 19) Proficiency Testing- PT
- 20) Rhesus- Rh
- 21) Transfusion Medicine Resource Committee- TMRC

**Table 4: Distribution of Titers Performed with Different Methods**

Proficiency Test Year	2009	2009	2010	2010	2011	2011	2012	2012	2013	2013	Median
	A	B	A	B	A	B	A	B	A	B	
<b>Anti-A</b>											
Total No Of Responses	672	682	564	578	509	570	565	560	584	578	574
UT RT (%)	22.03	21.99	26.06	25.61	23.38	25.26	23.72	25.36	27.23	25.78	25.31
UT AHG (%)	21.73	21.26	24.47	24.91	26.33	25.09	24.25	25	25.86	25.95	24.96
UG RT (%)	2.68	3.08	3.37	3.11	2.36	4.04	4.25	3.93	3.25	3.46	3.31
UG AHG (%)	4.02	3.52	4.26	3.63	4.32	4.04	4.6	4.64	4.11	3.98	4.08
OT IS (%)	10.57	9.97	7.27	6.75	2.95	5.96	5.31	5.18	5.14	5.19	5.64
OT RT (%)	17.41	17.01	16.13	16.26	14.34	17.02	15.93	17.32	15.41	16.61	16.44
OT IgG AHG (%)	16.52	17.01	14.54	16.09	22.2	16.49	17.88	16.79	16.61	16.78	16.70
OT Polyspecific AHG (%)	1.64	2.05	1.95	1.73	1.96		1.77				1.86
OG (%)		2.05	1.95	1.9	2.16	2.11	2.3	1.79	2.57	2.25	2.11
<b>Anti-D</b>											
Total No Of Responses	1211	1192	1098	1104	1109	1099	1059	1078	1101	1081	1100
UT (%)	38.15	36.41	37.43	36.41	35.8	36.58	36.07	38.5	36.15	37.56	36.50
UG (%)	12.63	12.5	11.02	11.32	12.35	11.83	12.46	12.7	13.17	12.86	12.48
OT AHG (%)	37.08	38.42	39.34	42.12	41.3	40.76	41.74	39.98	41.69	41.91	41.03
OT 6% Alb AHG (%)	6.52	6.46	5.74	5.07	5.32	5.55	4.91	5.1	4.72	4.63	5.21
OT 22% Alb AHG (%)	2.31	2.18	1.73	1.63	1.44	1.55	1.89	1.76	1.45	1.3	1.68
OT Poly Spec AHG (%)	2.23	2.52	2.09	2.08	2.34	2.37	1.89	1.95	1.91	1.76	2.09
OG (%)	1.07	1.51	1.73	1.36	1.44	1.36	1.04		0.91		1.36

Data is included in **Table 4** if 10 or more results were reported with individual method for every proficiency test. For Anti-D all methods were performed with IgG AHG phase, with one exception where polyspecific AHG was used.

**Abbreviations:** UT- Uniform Tube, UG- Uniform Gel, AHG- Anti Human Globulin, Alb- Albumin, RT- Room Temperature

**Table 5: Titer Variance between Uniform and Other Methods**

Survey	Methods Compared (n)	P value	Standard Deviation(SD)	Variance
<b>Anti-A</b>				
2010 A	UT RT (147) vs OT RT (91)	<b>0.001</b>	1.357 vs 0.944	1.84 vs 0.89
	UT AHG (138) vs OT AHG (82)	0.295	1.129 vs 1.068	1.27 vs 1.14
2010 B	UT RT (148) vs OT RT (94)	<b>0.001</b>	1.133 vs 0.810	1.28 vs 0.65
	UT AHG (144) vs OT AHG (93)	0.090	0.942 vs 1.068	0.88 vs 1.14
2011 A	UT RT (119) vs OT RT (73)	<b>0.001</b>	0.531 vs 0.353	0.28 vs 0.12
	UT AHG (134) vs OT AHG (113)	0.456*	1.085 vs 0.850	1.17 vs 0.72
2011 B	UT RT (144) vs OT RT (97)	0.062	1.357 vs 1.172	1.84 vs 1.37
	UT AHG (143) vs OT AHG (94)	0.289	1.123 vs 1.005	1.26 vs 1.01
2012 A	UT RT (134) vs OT RT (90)	0.099	1.078 vs 0.949	1.16 vs 0.90
	UT AHG (137) vs OT AHG (101)	0.304*	1.024 vs 1.212	1.04 vs 1.46
2012 B	UT RT (142) vs OT RT (97)	<b>0.032</b>	1.099 vs 0.920	1.20 vs 0.84
	UT AHG (140) vs OT AHG (94)	<b>0.035</b>	0.912 vs 1.082	0.83 vs 1.17
2013 A	UT RT (159) vs OT RT (90)	<b>0.002</b>	1.272 vs 0.965	1.61 vs 0.93
	UT AHG (150) vs OT AHG (97)	0.195	1.065 vs 1.152	1.13 vs 1.32
2013 B	UT RT (149) vs OT RT (96)	<b>0.048*</b>	1.274 vs 0.944	1.62 vs 0.89
	UT AHG (150) vs OT AHG (97)	0.131	1.192 vs 1.073	1.42 vs 1.15
<b>Anti-D</b>				

**Table 5: Titer Variance between Uniform and Other Methods (Continued)**

2010 A	UT (411) vs UG (121)	<b>0.002</b>	1.141 vs 0.917	1.30 vs 0.84
	UT (411) vs OT (432)	<b>0.003</b>	1.141 vs 0.997	1.30 vs 0.99
2010 B	UT (402) vs UG (125)	<b>0.001</b>	0.914 vs 0.661	0.84 vs 0.44
	UT (402) vs OT (465)	0.404	0.914 vs 0.925	0.84 vs 0.86
2011 A	UT (397) vs UG (137)	0.084	0.845 vs 0.765	0.71 vs 0.59
	UT (397) vs OT (458)	0.139	0.845 vs 0.891	0.71 vs 0.79
2011 B	UT (402) vs UG (130)	0.304	1.025 vs 1.061	1.05 vs 1.12
	UT (402) vs OT (448)	0.142	1.025 vs 1.080	1.05 vs 1.16
2012 A	UT (382) vs UG (132)	0.154	0.913 vs 0.846	0.83 vs 0.72
	UT (382) vs OT (442)	0.439	0.913 vs 0.906	0.83 vs 0.82
2012 B	UT (415) vs UG (137)	<b>0.033</b>	0.937 vs 0.820	0.88 vs 0.67
	UT (415) vs OT (431)	0.139	0.937 vs 0.988	0.88 vs 0.98
2013 A	UT (398) vs UG (145)	<b>0.006</b>	0.984 vs 0.824	0.97 vs 0.68
	UT (398) vs OT (459)	0.631	0.984 vs 1.00	0.97 vs 1.00
2013 B	UT (406) vs UG (139)	<b>0.002</b>	1.026 vs 0.833	1.05 vs 0.69
	UT (406) vs OT (453)	0.190	1.026 vs 1.071	1.05 vs 1.15

**Figure 1**

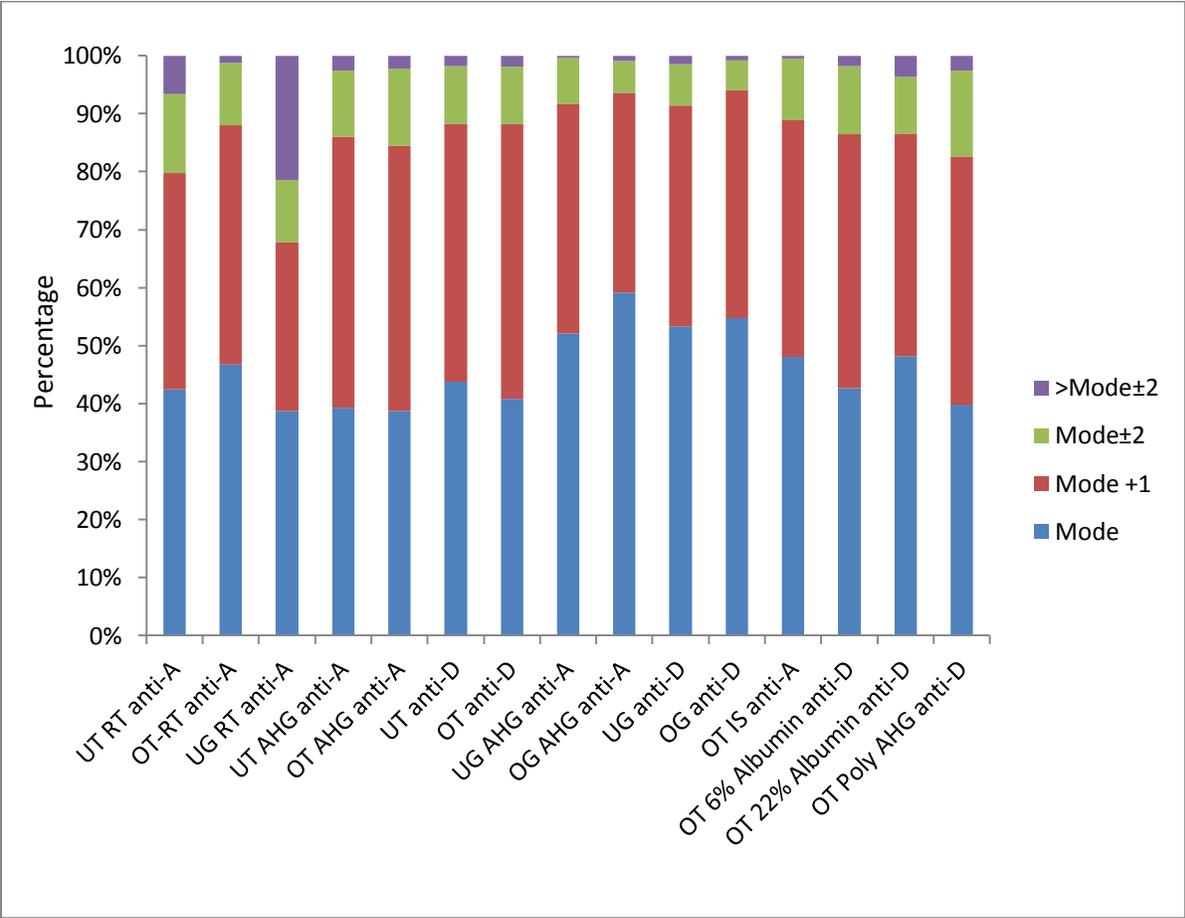
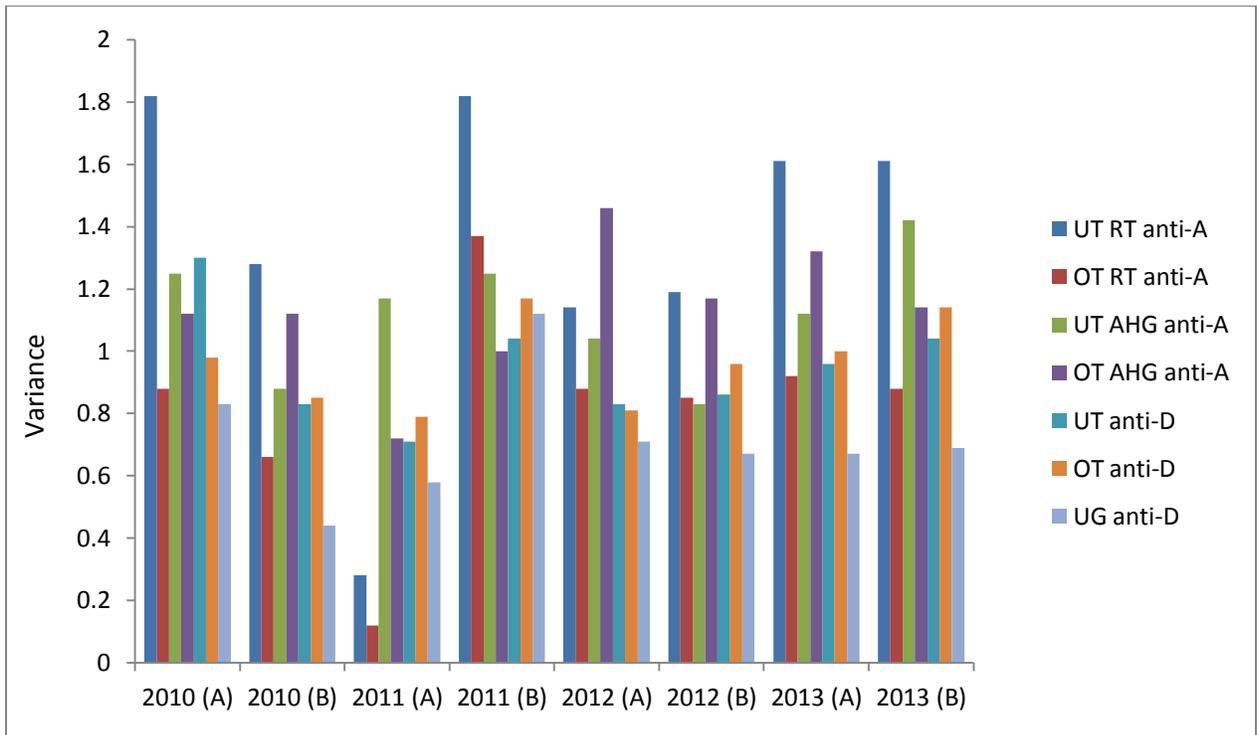


Figure 2



### **Figure Legends**

**Figure 1: Proportion of anti-A and anti-D titers around mode**

**Figure 2: Titer Variance with Different Techniques**