Antibody Comparison Between Two Solidphase Blood Bank Analyzers

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Background

A high volume blood bank changed Solidphase automated platforms in 2013 from Capture R®(CR) methodology on Galileo® Neo to Solidscreen® II (SSCII) methodology on the TANGO® due to high false positive antibody screen rates and excessive instrument downtime. Although the false positive rate and downtime decreased with implementation of the TANGO, the increasing volume of type and screens and difficulty in finding experienced blood bank staff led the laboratory to pursue assistance from their manufacturer to offer additional training, as well as review internal procedures to determine if additional improvements could be made to further reduce the number of send outs for antibody identification or confirmation. The purpose of this study was to evaluate the false positive rate of the TANGO by comparing results to those obtained by the outside reference lab used for antibody identification (ID) as well as to specify and quantify the improvements made in reducing antibody identification send-outs.

Methods

Retrospective audits were performed on all positive antibody screens and identifications performed over an 18 month period between 2013–2014 to determine the false positive rate with the Solidscreen® II methodology. False positives, commonly referred to as antibodies of undetermined significance (AUS), are specifically defined in this study as positive antibody screens that resulted in negative antibody identifications. Samples that could not be fully identified on the TANGO in-house using the 3/3 rule out procedure or due to samples having preferential reactivity of homozygous cells (antibody not reacting with homozygous cells) were sent to an outside reference lab for further workup, where testing was performed on the Galileo Neo (CR). The initial antibody identification results on the TANGO and the confirmatory results on the Neo were then compared to determine if there were any discrepancies in antibody identification. Discrepancies would include a positive result obtained on TANGO that was inconclusive, confirmed differently or found negative on the Neo, or an antibody identified by the reference lab and not identified on TANGO.

Results

A total of 77,284 antibody screens were performed on the TANGO and evaluated in this audit. Of the 77,284 antibody screens reviewed, 2,179 had a positive antibody screen, of which no false positives (AUS) were detected. Of the 2,179 positive antibody screens tested on the TANGO, 1,584 were completely identified in-house (62.4%); leaving 595 samples requiring additional workup (37.6%) and send-out to reference lab for confirmation. These 595 sample results were tested by both the SSCII and Capture R for antibody identification and compared. Three antibodies identified by the TANGO (anti-E, -M, and -K), were not detected on the Neo. There were no antibodies identified on the Neo that were not detected on the TANGO (Table 1).

Table 1.

	Volume of T&S	Total Antibody Positive Screens (In-house)	Positive Antibody (%)	# of Non-specifics (AUS) TANGO	Antibody ID Send-outs	TANGO Missed Antibodies (Relative Sensitivity) Total N=595	Neo Missed Antibodies (Relative Sensitivity) Total N=595
2013-2014	77284	2179	2.8%	0	595	0/595 (100%)	3/595 (99.5%)

The majority of the antibodies detected were directed against common blood group systems: Rh, Kell, Kidd, Duffy, MNSs. Of particular interest, there were no cold agglutinin antibodies (anti-I, auto anti-I, cold auto agglutinins) in the 2,179 positive screens.

Table 2 shows the specificities of the 595 samples tested with both SSCII and Capture R methods.

Table 2.

Specificity	# Antibodies	Detected with SS II	Detected with Capture R
Anti-D	101	101	101
Anti-M	113	113	112*
Anti-c	48	48	48
Anti-E	38	38	37*
Anti-Jka	25	25	25
Anti-K	16	16	15*
Anti-e	14	14	14
Anti-Jkb	8	8	8
Anti-Lea/Leb	17	17	17
Anti-Fyb	3	3	3
Anti-C	3	3	3
Anti-S	4	4	4
High (U, Tja)	4	4	4
Warm auto ab	29	29	29
Multiple , HTLA	172	172	172

**Antibodies detected by SS II but not Capture R.*

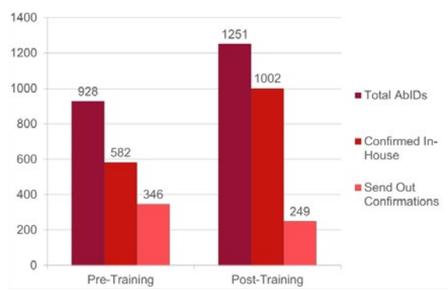
Policy Changes:

In an effort to reduce the number of samples sent to the reference lab for additional testing, the rationale for sending samples to the reference lab was evaluated. Record and policy review revealed many of the antibodies had already been conclusively identified based on the 3/3 rule out procedure. The previous policy would be to send out samples if an antibody did not demonstrate reactivity with all antigen positive cells (including heterozygous expressions), likely in place with the previous analyzer, wrought with false positive (AUS) reactions. With the implementation of the TANGO, the retrospective review showed no AUS antibodies out of 2,179 antibody positive samples reviewed. Typically sufficient antigen-positive cells were reacting to allow identification of the antibody and sufficient antigen-negative cells did not react to rule out other alloantibodies. Due to the high specificity with the SSCII method on the TANGO, policy changes related to antibody identification were identified.

- Staff was re-trained on specific blood group antibody nuances, antigen zygosity, panel interpretation approach, as well as following 3/3 rule out procedure.
- A panel with more homozygous cells was implemented as the first panel run on the TANGO to limit the need for additional cells at the bench.
- A PEG tube method was implemented for use on bench for selected cells and/or enhancing heterozygous non-reactive cells.

This training and adjustment to internal procedures demonstrated rapid and significant results. The laboratory reduced the total send outs from 37% to 20% (Figure 1). Pre-training and previous policy, there was a total of 928 positive antibodies identified with 346 (37%) sent out for confirmation. Two quarters post-training and policy change, there were 1,251 positive antibodies identified with 249 (20%) sent out for confirmation.

Figure 1.



Conclusion

The SSCII method employed by the TANGO demonstrated high relative sensitivity and specificity as demonstrated by no AUS detected (common blood bank measure of specificity) in 77,284 random patient antibody screens as well as no missed antibodies (common measure of sensitivity) in 595 positive antibody identifications.

The low rate of false positives reported on the TANGO (SSCII) has been cited in other publications (Mannherz1) and is due to the unique solidphase method employed by the TANGO platform(s). The microplate wells are coated with Protein A in the SSCII methodology. The Protein A has a high affinity to the Fc portion of the immunoglobulin allowing a direct method of detecting red blood cell antibodies (SSCII). In contrast, published reports of AUS detection with Capture R methodology have been reported to be as high as 39%. The high rate of false positive results with Capture R is probably due to the coating of the microplate wells with the red cell stroma and the requirement for indicator cells as an indirect method of detecting red blood cell antibodies. Red cell stroma exposes non-clinically significant internal red blood cell membrane antigens or cryptic antigens causing false positive results and the use of indicator cells introduces another red blood cell source that can cause AUS (Figure 3).

Figure 2. SolidScreen II Methodology

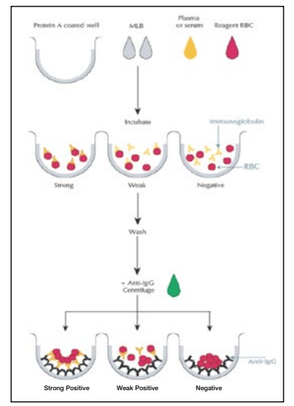
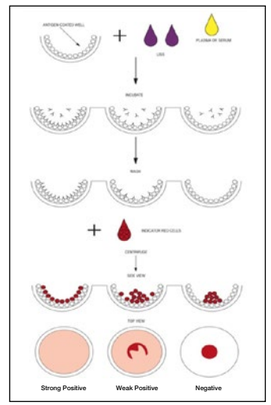


Figure 3. Capture R Methodology



Sensitivity was assessed through correlation of antibodies (N=595) identified/confirmed on each instrument (TANGO and Neo). Of the 2,179 samples with positive antibody screens, 595 were sent for confirmation to an outside reference lab. The TANGO demonstrated 100% sensitivity, identifying 595/595 antibodies accurately. The Neo demonstrated 99.5% sensitivity, due to three missed antibodies (anti-E, anti-K, and anti-M), two of which are typically considered clinically significant (anti-E, anti-K). There were no antibodies detected on the Neo that were not detected on the TANGO.

This study showed the impact that involving the vendor in data and procedural review, training, and minor policy changes can have on laboratory productivity and cost savings. By strengthening antibody identification capabilities, the laboratory significantly reduced the number of samples requiring outside reference laboratory workup. In this audit, antibody ID send-outs were reduced from 37% to 20%, resulting in significant cost savings for the laboratory.

1 O. Mannherz, B. Siebert (2008), Comparative Studies in Antibody Screening of Blood Donations with Three Different Red Blood Cell. DGTI, Presentation, 2008.

2 J. Olson, M. George. Using Flowcharts to Guide Workflow with Solid Phase Antibody Identification Technology. Penn State. Hershey Medical Center.

3 A.Ebrahim, JHunt2. Inconclusive Red Blood Cell Antibody Identification with Solid-Phase versus Manual Methods. AABB Abstract 2014 SP303