Commercial Gel Column Agglutination Technology Adapted to Estimate RBC Bound Acridine Adducts in Whole Blood Treated with the INTERCEPT Technology*

Anaïs ALTMEYER [1], Anne K NORTH [2], Melissa VON GOETZ [2], Elisa SAGE [1], Rudolf SCHWABE [1], Emmanuel RIGAL [3], Soraya AMAR EL DUSOUQUI [1] and Nina MUFTI [2]

[1] Swiss Transfusion SRC, Laupenstrasse 37, Bern, Switzerland [2] Cerus Corporation, Concord, CA, USA [3] Blood Transfusion Center – HUG, Geneva, Switzerland

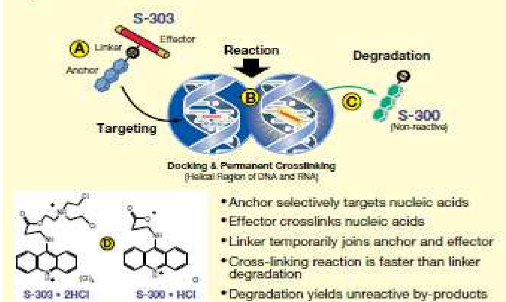
Aknowledgments: Niels LION & Michel PRUDENT, SRTS Vaud, R&D Unit, Route de la Corniche 2, 1066 Epalinges, Switzerland

AABB Annual Meeting 2015, October 24-27, Anaheim, CA

BACKGROUND: Effective pathogen inactivation (PI) in whole blood (WB) would address the overwhelming challenge of transfusion transmitted infections faced by countries with limited resources such as Sub-Saharan Africa (SSA). The INTERCEPT Blood System for Red Blood Cells (RBC) uses a nucleic acid cross-linking agent, amustaline (S-303) and glutathione (GSH) to achieve effective PI (Fig. 1). This technology was chosen by the "Whole Blood for Africa" research team of the Transfusion Swiss Red Cross to produce a WB PI system for developing countries. In the INTERCEPT RBC system, GSH is present to reduce non-specific binding of S-303 to the cell surface (acridine moiety) to minimize the potential for immune response. Binding is quantitated by flow cytometry which requires reagents, equipment and expertise not always available in SSA immunohematology centres. Therefore an easier, less expensive method is needed to assess the levels of bound molecules for WB PI process development.

AIM: Compare a modified gel column agglutination assay with quantitative flow cytometry to measure levels of surface-bound acridine in INTERCEPT WB and INTERCEPT RBCs.

Figure 1: S-303 Treatment Process Mechanism of Action



METHODS AND ASSAY CONTROLS

WB was treated with 0.2 mM S-303 and various concentrations of GSH (2 to 20 mM). Doubling dilutions of an anti-acridine monoclonal antibody and the BioRad (Hercules, USA) LISS/Coombs gel system were used to estimate acridine binding. Agglutination was scored using definitions supplied by the manufacturer and quantified as the highest titer (inverse of highest dilution) of antibody that gave positive (≥1+) agglutination. INTERCEPT RBCs carrying high and low levels of acridine were also included. In the flow cytometry assay acridine levels were expressed as geometric mean of fluorescence intensity (GMFI) from the same samples. INTERCEPT RBCs carrying high and low levels of acridine were also included as controls (**Fig. 2**). For each gel card assay, QC samples were prepared to produce specific agglutination scores (**Fig. 3**).

- Maximum (MAX) is the highest dilution that represents 4+ agglutination score
- Medium (MED) represents 2+ agglutination score
- Minimum (MIN) represents 1 doubling-dilution above the cut-point

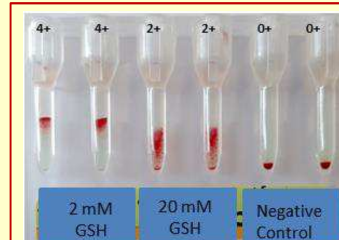


Figure 2: S-303 RBC controls

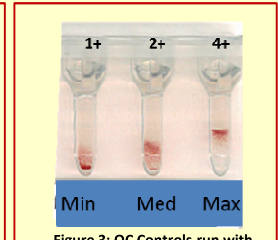


Figure 3: QC Controls run with each assay

RESULTS

Assay reproducibility was demonstrated by a consistent endpoint of 2-fold (doubling) dilutions of acridine specific monoclonal antibody. The highest titer that gave a positive result was reproducible with WB from 3 different donors (**Table 1**) in which each WB unit was treated with 0.2 mM S-303 and various concentrations of GSH. The differences between donors in cutpoint dilutions observed at 20mM GSH were in the accepted range (±1 doubling dilution).
Agglutination in the commercial gel system also correlated with acridine adduct levels from INTERCEPT treatment measured by indirect, quantitative flow cytometry.
As with RBCs, an increase in GSH resulted in a reduction of acridine-specific agglutination which also corresponded with the level of acridine from flow cytometry (**Table 2**).

Table 1: Reproducibility of assay cut-point with WB from different donors

[GSH]	Donor 1	Donor 2	Donor 3
2mM	1:32000	1:32000	1:32000
5mM	1:8000	1:8000	1:8000
7.5mM	1:4096	1:4096	1:4096
10mM	1:2048	1:2048	1:2048
20mM	1:512	1:256	1:1024

Table2: Acridine levels measured by flow cytometry

GSH (mM)	Acridine (GMFI) n=3	Highest positive titer n=3
2	1942 ± 90.2	16384
5	878 ± 24.2	8192
7.5	689 ± 93.6	4096
10	559 ± 27.5	2048
20	424 ± 41.0	256
RBC LOW	682 ± 37.7	512
RBC HIGH	1413 ± 22.5	4096

Stability of acridine adducts on S-303 treated RBC and WB:

Quantitative flow cytometry has shown that acridine adducts on INTERCEPT RBC are unstable during storage of RBC at 4˚C (Figure 4). The same is seen for acridine adducts on INTERCEPT WB when measured by gel agglutination following storage of WB at room temperature for 7 days in which the cutpoint is reduced from 36,000 to 8000 (Fig. 5).

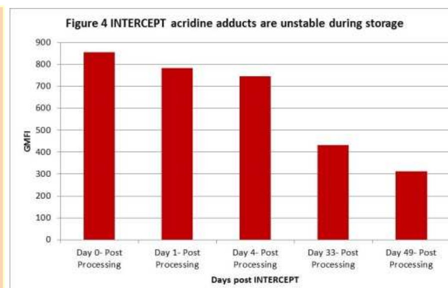
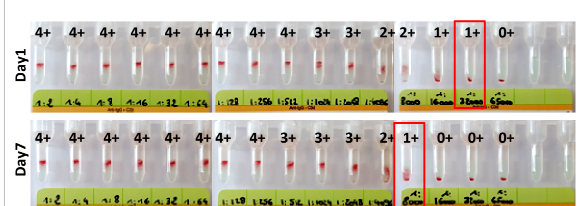


Figure 5: Antibody doubling dilutions ranged from 1:2 to 1:64000 for WB treated with 0.2mM S-303 and 2 mM GSH. The cut point is shown with red frame



CONCLUSION: Commercial gel column technology (routinely used in immunohematology labs to identify defined intrinsic RBC antigens) effectively measured acridine adducts in INTERCEPT WB and RBCs. Gel agglutination is a fast, robust and economical method to quantify acridine levels and can be used to guide development and optimization of INTERCEPT WB PI when reagents and specialized equipment for flow cytometry are not available.

*The INTERCEPT Blood System for Red Blood Cells and Whole Blood treated with the INTERCEPT Technology are in development and are not approved for sale.