ORIGINAL REPORT

Stability guidelines for dithiothreitol-treated red blood cell reagents used for antibody detection methods in patients treated with daratumumab

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Daratumumab (DARA), a drug used to treat patients with multiple myeloma, causes interference in pre-transfusion testing. Samples from patients receiving DARA exhibit panreactivity in antibody detection and identification tests with red blood cells (RBCs). Many hospitals are sending these samples to reference laboratories. Dithiothreitol (DTT), a sulfhydryl chemical treatment of RBCs, negates this reactivity. This study investigated the stability of the antigens on DTT-treated RBCs to determine if large quantities of RBCs could be treated at one time, stored, and used for testing at a later time. Panel cells were treated with DTT and then stored as three sets. Set 1 DTT-treated RBCs were stored in Alsever's solution at 2°C to 8°C, washed daily, and suspended in pH 7.3 phosphate-buffered saline (PBS) prior to antigen typing. Set 2 DTT-treated RBCs were stored in pH 7.3 PBS. Set 3 DTT-treated RBCs were stored in Alsever's solution. Sets 2 and 3 were inspected daily for 14 days for observation of hemolysis. In Set 1, all antigen reactivity remained at ≥2+ with both single- and double-dose cells for 14 days. The Rh antigens gave stronger reactions longer, compared with those tested in the Duffy, Kidd, and MNS blood group systems. Sets 2 and 3 were monitored for hemolysis. On day 3, Set 2 began displaying hemolysis, with complete hemolysis by day 8. Set 3 did not display hemolysis in 14 days. In conclusion, a large volume of RBCs can be treated with DTT and stored in Alsever's solution for use without deterioration of the RBC antigens, saving institutions tech time, resources, and money. ***Immunohematology* 2017;33: 105–109.**

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Daratumumab (DARA) is a human monoclonal antibody shown to recognize CD38.[1] DARA targets CD38, a glycoprotein found on the surface of many types of cells including B cells, which are overexpressed in patients with multiple myeloma.[2] This finding has been established as a successful treatment of patients with multiple myeloma and non-Hodgkin's lymphoma. In clinical trials, DARA has been proven highly cytotoxic to tumor cells via complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity, and apoptosis.[1,3] The success and efficiency in treatment for this patient population has resulted in a marked increase of use and application across the field.

During routine blood bank testing, serum/plasma samples from patients on DARA exhibit panreactivity across varying methods of antibody detection and identification, preventing blood banks from providing crossmatch-compatible red blood cell (RBC) units in a timely manner. With the increase in use of DARA, blood banks are sending increased numbers of these patient samples for antibody detection and identification to immunohematology reference laboratories (IRLs), causing delays in providing compatible RBC units and additional cost.

DARA binds with CD38 located on the reagent RBC membrane.[2] Chapuy et al.[2] reported that 0.2 M dithiothreitol (DTT) treatment of reagent RBCs can remove interference from DARA by disrupting the structure of the antigen. The DTT treatment then allows for the identification of underlying clinically significant antibodies.

DTT treatment of reagent RBCs requires precise technical detail and is also very labor-intensive. Current practice is to DTT-treat a few drops of the reagent RBCs required for patient testing. This practice uses at least 40–60 minutes of technologist time every time a sample from a patient on DARA is encountered, in addition to the screening, identification, and crossmatch testing that may be required. Each batch of treated RBCs also requires the use of quality control to ensure that the denaturation process has been successful. It is recommended to obtain the patient's RBC antigen phenotype or genotype prior to DARA use, but this testing is neither guaranteed nor accessible by all facilities.

Although the use of DTT-treated RBCs is recommended for testing current patients on DARA, the literature provides little information about the stability of the common RBC antigens when DTT-treated RBCs are stored. DTT-treated RBC panels are not commercially available. We investigated the stability of common clinically significant antigens on DTT-treated commercial panels by tube method with both commercial and human-source antisera. With these data, both

transfusion services and IRLs will have the necessary storage information for DTT-treated RBCs and their antigen stability.

Materials and Methods

DTT Preparation

One gram of DTT (Cleland's Reagent; Sigma-Aldrich, St. Louis, MO) is dissolved in 32.4 mL phosphate-buffered saline (PBS; Sigma-Aldrich) with a pH of 7.3. The DTT-to-PBS ratio may be adjusted depending on the desired volume of 0.2 M DTT.[4]

DTT Treatment

Two 11-cell panels (Panocell-10; Immucor, Norcross, GA) were DTT-treated. Each panel cell was placed into a labeled glass 12 × 75 mm test tube. Tubes were washed once with normal saline using a manual method. Four volumes of 0.2 M DTT were added to 1 volume of washed packed RBCs. The mixture was gently shaken, re-suspended, and incubated at 37°C for 30–45 minutes in a dry heat block. After incubation, the DTT-treated RBCs were washed four times manually with normal saline.

After the last wash, the DTT-treated RBCs were split into three sets of 11 donor RBCs for evaluation. All panels were DTT-treated at the same time and then separated into their designated testing sets. The DTT treatment was verified at time of treatment using an antigen known to be destroyed by DTT (Anti-k [Cellano]; Immucor) as the DTT control. Set 1 was reconstituted to a 3–5 percent solution with pH 7.3 PBS (Lonza, Walkersville, MD). Set 2 was reconstituted to a 3–5 percent solution with pH 7.3 PBS. Set 3 was reconstituted in an RBC preservative (Alsever's solution; Sigma-Aldrich).

Antigen Typing

Set 1 DTT-treated RBCs were tested with antisera by manual tube method. A double-dose RBC, single-dose RBC, and RBC known negative for the selected antigen were tested and the results documented daily. Antisera testing included all common, clinically significant antigens known not to be affected by DTT treatment (Anti-D Series 4, Anti-C, Anti-c, Anti-E, Anti-e, Anti-Jk^a; Anti-Fy^a, Anti-Fy^b; Immucor). Anti-M and Anti-S were generated via in-house protocols from human sources at a community blood center. Antigen testing was performed following package inserts for commercial antisera. For human-source Anti-S and Anti-M, indirect antihuman globulin testing was performed using polyethylene glycol to detect the antigens. Quality control for the human-source antisera and commercial antisera were documented on the day of testing. The antigen typing was completed daily on Set 1 for 14 days for comparison of antigen stability over time. The antigen testing was performed by the same technologist each day. The panel was stored in Alsever's solution at 2°C to 8°C. The panel was manually washed each day with normal saline and then resuspended in pH 7.3 PBS prior to antigen testing. Antigen testing was not viewed by a second reviewer; however, reactions were read and graded according to method 1–9 in the AABB Technical Manual.[4]

Set 2 DTT-treated RBCs were stored in pH 7.3 PBS. Set 3 DTT-treated RBCs were stored in Alsever's solution. Set 2 and Set 3 were visually assessed daily for 14 days for observation of any indication of hemolysis. All three sets were refrigerated (2°C to 8°C) between testing.

Results

Reactivity with double-dose RBCs of Set 1 is shown in Table 1. To demonstrate the stability of the DTT-treated double-dose RBCs, the number of antigens that maintained the baseline grade (day 0) at day 14 was compared with grades at day 1 (Table 2). These data show that there were no antigens with a grade difference greater than 2. This finding was not significant ($p = 1.0$). Reactivity with single-dose RBCs of Set 1 is shown in Table 3. To demonstrate the stability of the DTT-treated RBCs when only a single dose of the antigen being tested was present, the number of antigens that maintained the baseline grade (day 0) at day 14 was compared with grades at day 1 (Table 4). These data show that there were no antigens with a grade difference greater than 2. This finding was not significant ($p = 1.0$).

There were 130 data points for the 10 antigens for each of the two levels of antigen expression. To understand if there is a difference between single-dose and double-dose expression, the breakdown by grade difference compared with baseline is shown in Table 5. Although there was no statistical difference in the number of samples with a two-point grade differential from baseline, the difference for those with a one-grade differential was significant ($p < 0.05$). This observation is also reflected in the overall number of grades that are not equal to or greater than the baseline.

Rh blood group antigens displayed consistently stronger reactivity (3+ to 4+) throughout the study (14 days) in comparison with the other RBC antigens, which displayed more variation and weaker strengths of 2+ to 4+ (averaging 2+ to 3+). Nevertheless, following DTT treatment over 14 days, all antigen reactivity remained at a strength of 2+ or greater, with both amounts of antigen expression on the panel of RBCs. This

Table 1. Set 1: Reactivity with DTT-treated double-dose RBCs

	Day of testing														
Antisera	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
D	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
C	3+	3+	3+	3+	3+	4+	3+	3+	3+	3+	3+	3+	3+	3+	3+
c	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
E	4+	4+	4+	4+	3+	4+	4+	4+	4+	3+	4+	4+	4+	4+	4+
e	3+	4+	4+	4+	4+	3+	3+	3+	3+	3+	3+	3+	3+	2+	3+
Fy[a]	4+	3+	3+	3+	2+	3+	2+	2+	3+	2+	3+	2+	3+	3+	3+
Fy[b]	3+	3+	2+	3+	2+	3+	2+	2+	3+	2+	2+	2+	3+	2+	2+
Jk[a]	4+	3+	3+	3+	3+	3+	2+	2+	2+	3+	3+	3+	3+	2+	2+
M	4+	4+	3+	4+	4+	3+	3+	3+	3+	4+	3+	3+	3+	4+	3+
S	3+	3+	2+	2+	2+	2+	2+	2+	3+	2+	3+	3+	3+	2+	2+

DTT = dithiothreitol; RBCs = red blood cells.

Table 2. Set 1: Day 1 and day 14 reactivity comparison with baseline (day 0) on DTT-treated double-dose RBCs

	Number of antigens		
Reactivity	Day 1	Day 14	*p*
≥ baseline	8/10	5/10	0.35 (ns)
± 1 grade	10/10	9/10	1.0 (ns)
± 2 grades	10/10	10/10	1.0 (ns)

DTT = dithiothreitol; RBCs = red blood cells; ns = not significant.

finding suggests that the antigens tested are stable following DTT treatment when stored in Alsever's solution for up to 14 days. Further studies with a larger sample volume to include additional sources of antisera and RBCs of varied phenotypes and varied testing platforms will be needed to confirm this observation.

Aliquot Sets 2 and 3 were only monitored for hemolysis notation. On day 3, Set 2, which contained cells stored in PBS, displayed slight hemolysis (Fig. 1). By day 8, complete

Table 3. Set 1: Reactivity with DTT-treated single-dose RBCs

	Day of testing														
Antisera	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
D	4+	4+	4+	4+	4+	4+	4+	4+	3+	4+	4+	4+	4+	4+	4+
C	3+	3+	3+	2+	3+	3+	3+	3+	2+	3+	3+	3+	3+	3+	3+
c	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	3+	4+	4+	4+
E	4+	4+	4+	4+	3+	4+	3+	3+	3+	3+	3+	3+	4+	3+	4+
e	4+	4+	3+	4+	3+	3+	3+	3+	3+	3+	3+	3+	3+	2+	3+
Fy[a]	3+	2+	3+	2+	2+	3+	2+	2+	2+	2+	3+	2+	2+	2+	3+
Fy[b]	3+	3+	2+	3+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
Jk[a]	3+	2+	2+	3+	3+	3+	2+	2+	2+	2+	2+	3+	3+	3+	2+
M	4+	3+	3+	3+	4+	2+	3+	3+	3+	3+	2+	2+	3+	3+	2+
S	3+	2+	2+	2+	2+	2+	2+	2+	3+	2+	2+	3+	2+	2+	2+

DTT = dithiothreitol; RBCs = red blood cells.

Table 4. Set 1: Day 1 and day 14 reactivity comparison with baseline (day 0) on DTT-treated single-dose RBCs

	Number of antigens		
Reactivity	Day 1	Day 14	*p*
≥ baseline	6/10	5/10	1.0 (ns)
± 1 grade	10/10	9/10	1.0 (ns)
± 2 grades	10/10	10/10	1.0 (ns)

DTT = dithiothreitol; RBCs = red blood cells; ns = not significant.

Table 5. Comparison of antigen dosage effect on reactivity changes from baseline

		Number of antigens			
	N	≥ baseline grade	1 grade < baseline	2 grades < baseline	≥ 3 grades < baseline
Double-dose	130	72	48	10	0
Single-dose	130	51*	74*	5	0
Total	260	123	122	15	0

*$p < 0.05$ for comparison with double-dose reactivity.

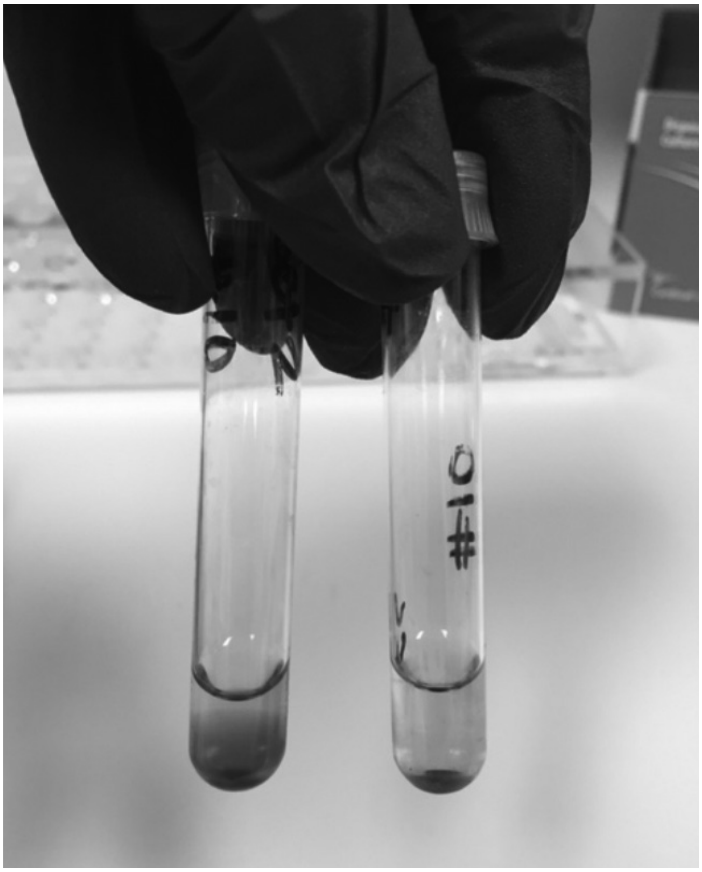

Fig. 1 Set 2 (right), Set 3 (left): On day 3, Set 2 (which contained cells stored in phosphate-buffered saline) displayed slight hemolysis.

Fig. 2 Set 2 (right) Set 3 (left): By day 8, complete hemolysis was seen in Set 2.

hemolysis was seen in Set 2 (Fig. 2). Aliquots of Set 3, which contained cells stored in Alsever's solution, showed no hemolysis throughout the 14-day investigation.

Discussion

There is a significant need to establish the antigen stability of DTT-treated RBCs to support laboratory efficiency. Antibody workups on patients taking DARA are becoming more common in IRLs and are time-consuming procedures requiring advanced technical skills. Some transfusion services are also considering the idea of bringing this work in-house. This study shows the ability to DTT-treat panel RBCs and to store them in Alsever's solution for up to 14 days; this would save institutions tech time, resources, and money.

Fung et al.[4] document the use of PBS with an 8.0 pH. In our study, the use of PBS with a pH of 7.3 was used. This decision was made based on the notation on the package insert stating that PBS pH of 6.5–7.5 should be used for antigen typing.

Commercial monoclonal and polyclonal antisera, as well as human-source antibodies, were used in this study. A variation in the source of antisera, however, did not suggest a significant difference in reaction strengths over time. Anti-Jk^b was not used because it was unavailable at the start of testing. All antigen reactivity remained 2+ or greater following DTT treatment for the remaining 14 days when stored in Alsever's solution.

Both single-dose and double-dose antigens displayed no significant change in stability over the 14-day time frame. This study suggests the ability to DTT-treat a large volume of RBCs and store them for later use. This practice would be beneficial to laboratories that receive samples from patients on DARA while also using the RBCs to resolve other complex antibody cases. This practice is not limited to the patient population being treated with DARA. When using DTT-treated RBCs, one must remember that Kell and Lutheran blood group antigens are destroyed[5,6]; a policy would be needed by each facility defining how to include and/or exclude their respective antibodies.

This study was conducted monitoring antigen strength and hemolysis. It was not performed on multiple occasions. One set of panel cells was monitored for the 14-day time frame. Strengths with other donors may vary.

References

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