An evaluation of storage time for dithiothreitol-treated reagent cells

CD38 monoclonal antibodies are therapeutic agents used in the treatment of patients with multiple myeloma who have not achieved remission with traditional treatment regimens. Daratumumab (Darzalex; Janssen Biotech, Inc.) is currently approved by the US Food and Drug Administration for use in patients with multiple myeloma. It is known that antibodies to CD38 (cluster of differentiation 38 [cyclic adenosine diphosphatase ribose hydrolase]) will interfere with serologic tests by causing positive indirect antiglobulin tests (IATs).[1] Because the drug will be pan-reactive with IAT screening and panel cells, clinically significant alloantibodies may be masked. Dithiothreitol (DTT) is a reducing reagent that effectively breaks disulfide bonds, disrupting the antigenic binding sites of the CD38 molecule on red blood cells. DTT-treated cells are a valuable reagent used to assess alloantibody production in patients undergoing anti-CD38 treatment. Some blood group systems, such as Lutheran, Kell, Cartwright, Scianna, Dombrock, Landsteiner Weiner, Cromer, Knops, Indian, and John Milton Hagen groups, are destroyed or weakened with DTT treatment.[2] Because Kell is a common clinically significant antibody and cannot be excluded using DTT-treated cells, we issue units that are negative for KEL1 or KEL2 based on the patient's phenotype. To have DTT reagent cells available routinely, we performed a validation study to determine the storage life of DTT-treated reagent cells when stored in red blood cell support solution (Hemo Bioscience).

The study was conducted over a period of 12 days. Twelve samples were treated with 0.2-M DTT for evaluation. The samples selected included three different reagent cells (C+c−E−, C−c+E+, and C−c+E−) from four different manufacturers (Ortho Clinical Diagnostics; Immucor, Inc.; Quotient; and Bio-Rad Laboratories, Inc.). Procedures used for the preparation of 0.2-M DTT reagent and the treatment of red blood cells are described in *Judd's Method in Immunohematology*.[3] Starting volumes of 10.5-mL treated cells and 0.6-mL untreated cells were diluted to 3 to 5% in red blood cell support solution. Samples were stored in a refrigerator set at 2 to 8°C. Daily evaluation of hemolysis was performed using a standard chart to visually grade hemolysis (Fig. 1).[4] Antigen integrity was assessed using routine antisera from Ortho Clinical Diagnostics: anti-C, anti-c, anti-E, and Immucor anti-k. Reagent cell performance was assessed with a positive control and a negative control. The positive control consisted of an Ortho confidence antibody diluted to 0.9% with normal saline, and a negative control was prepared from Immucor 22% bovine albumin diluted to 6 to 8% with phosphate-buffered saline, pH 7.1. Indirect antiglobulin testing was performed using Ortho column agglutination (gel), and tube testing was carried out with Immucor polyethylene glycol (PEG) additive and with no additive (saline method). The results were compiled, and these data were used to determine whether the reagent cells had maintained potency for the evaluation period. At Day 9, 1 mL of supernatant was removed to correct concentration of cells back to 3 to 5%.

The expected evaluation criteria for this study were:

1. Observable hemolysis less than 200 mg/dL;
2. k (KEL2) antigen tests negative;
3. D antigen test 2+ or greater;
4. Gel, PEG, and saline tests are 1+ or greater with dilute confidence antibody; and
5. Gel, PEG, and saline tests are negative with 6% albumin.

Our findings are summarized in Table 1. Although hemolysis levels for two samples reached 200 mg/dL on Day 7, we noted that hemolysis did not affect the expected performance of reagent cells. The study ended at Day 12 with one-third of the samples reaching 200 mg/dL hemolysis. Although the reagent cells tested from 0 to 1+ for k (KEL2) antigen after DTT treatment, the cells successfully avoided anti-CD38 reactivity when tested with patient plasma. Antigen testing for k (KEL2) remained 1+ or less throughout the study. Satisfactory D antigen reactivity remained on samples with 3+ to 4+ throughout the study. Gel and tube testing with PEG or with no additive was greater than 1+ with dilute confidence antibody (positive control) throughout the test evaluation. No false-positive reactions were observed with the 6 to 8% albumin negative control.

In conclusion, our study demonstrates that DTT-treated red blood cells stored in red blood cell support solution can maintain potency for up to 12 days. Because the number of samples that yielded unsatisfactory hemolysis results continually increased after 7 days, we decided to use a 7-day expiration for DTT-treated cells. Satisfactory results were demonstrated with positive and negative controls for all test methods (column agglutination and tube tests with PEG and no additive). D antigen integrity was maintained. As an adjunct to this validation, we evaluated Duffy antigens (Fy^a and Fy^b) after DTT treatment and observed that one of 10 samples had diminished antigen expression after treatment; thus, we recommend including tests for Fy^a and Fy^b antigens after preparing DTT-treated reagent cells. We have found that these reagent cells are

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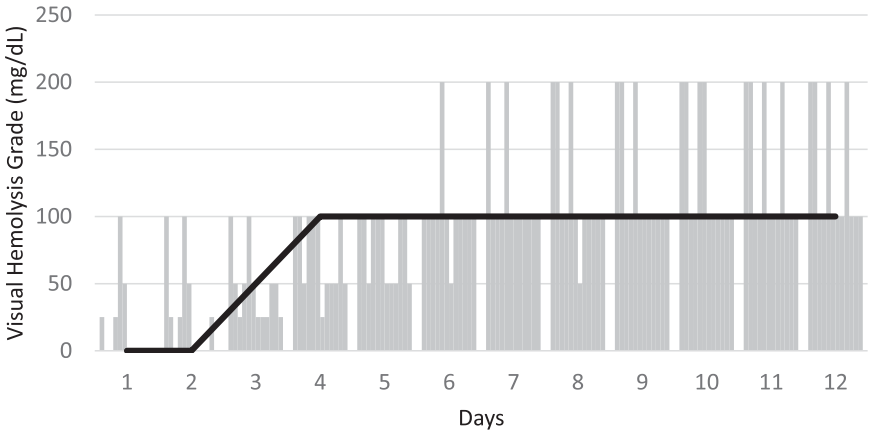

Fig. 1. This bar graph represents the visual grading of hemolysis with approximate levels of 0, 25, 50, 100, and 200 mg/dL for the 12 DTT-treated samples. The solid black line illustrates the mode for daily hemolysis.

TABLE 1. Results for data collected on the initial, middle, and final day reported

Test	Day 0	Day 7	Day 12
Hemolysis grading			
Untreated cells, mg/dL	0	0	0
DTT-treated cells, mg/dL	0	10 of 12 < 200	8 of 12 < 200
Antigen testing of DTT-treated cells			
Anti-k	8 of 12 = 0 4 of 12 ≤ 1+	8 of 12 = 0 4 of 12 ≤ 1+	8 of 12 = 0 4 of 12 ≤ 1+
Anti-C	12 of 12 = 4+	12 of 12 = 4+	3 of 12= 4+ 9 of 12 = 3+
Anti-c	12 of 12 = 4+	12 of 12 = 4+	12 of 12 = 4+
Anti-E	12 of 12 = 4+	12 of 12 = 4+	12 of 12 = 4+
IAT test performance of DTT cells with control sera			
Column agglutination (gel)			
Positive control	2+ to 4+	2+ to 4+	2+ to 4+
Negative control	0	0	0
Polyethylene glycol (PEG)			
Positive control	2+ to 4+	2+ to 4+	2+ to 4+
Negative control	0	0	0
No additive (saline method)			
Positive control	2+ to 4+	2+ to 4+	2+ to 4+
Negative control	0	0	0

useful in avoiding the pan-reactivity observed in patients who receive daratumumab when assessing allo-antibody formation.

CONFLICT OF INTEREST

The authors have no conflicts of interest or funding sources to declare.

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REFERENCES

1. Chapuy CI, Nicholson RT, Aguad MD, et al. Resolving the daratumumab interference with blood compatibility testing. Transfusion 2015; 55: 1545-554.
2. Reid ME, Lomas-Francis C, Olsson M. The blood group antigen factsbook. 3rd ed. London (UK): Academic Press; 2012.
3. Judd WJ, Johnson ST, Storry J; American Association of Blood Banks. Judd's methods in immunohematology. Bethesda (MD): AABB Press; 2008.
4. University of Iowa Health Care, Department of Pathology. Hemolysis chart. Laboratory services handbook: laboratory tests arranged alphabetically [cited 2017 Jun 27]. Iowa City (IA): University of Iowa Health Care; 2916. 2016. https://www.healthcare.uiowa.edu/path_handbook.