An evaluation of storage time for DTT treated reagent cells

Sheri Hugan, BS[1], Laura Cooling, MD[1], and Vivianne Larsson, BS[2]

Department of Pathology, University of Michigan, Ann Arbor, MI[1] and Eastern Michigan University, Ypsilanti, MI[2]

Conflict of Interest

The authors certify that they have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in this manuscript.

Corresponding author:

Sheri Hugan, BS, MLS(ASCP) SBB

IRL Coordinator

University of Michigan

Department of Pathology

UH2F225, Box 0054

1500 E. Medical Center Drive

Ann Arbor, MI 48109-0054

Phone: (734) 232-6283

FAX: (734) 936-6855

Email: slhugan@med.umich.edu

~~Word Count: 714~~

CD38 monoclonal antibodies are a therapeutic agent used in treating patients with multiple myeloma which have not achieved remission with traditional treatment regimens. Daratumumab (Darzalex, Janssen Biotech, Inc., Horsham, PA) is currently approved by the FDA for use in multiple myeloma patients. It is known that CD38 antibodies will interfere with serologic tests by causing positive indirect antiglobulin tests (IATs).[1] Since 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 that breaks disulfide bonds, which disrupts the antigenic binding sites of the CD38 molecule on red 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 are destroyed or weaken with DTT treatment.[2] Since Kell is a common clinically significant antibody and cannot be excluded using DTT treated cells, we issue units negative for KEL1 or KEL2 based on the patient's phenotype. In order to have DTT reagent cells available routinely, we performed a validation to determine the storage life of DTT treated reagent cells when stored in red cell support solution (Hemo bioscience, Research Triangle Park, NC).

The study was conducted over a period of 12 days. Three examples 12 samples were treated with 0.2M DTT for evaluation. Samples selected included 3 different reagent cells (C+c-E-, C-c+E+, and C-c+E-) from four different manufacturers (Ortho Clinical Diagnostics, Raritan, NJ), (Immucor, Inc., Norcross, GA), (Quotient, Newtown, PA), and (Bio-rad Laboratories, Inc., Hercules, CA) consisting of Rh phenotypes: C+c-E-, C-c+E+, and C-c+E- were treated with 0.2M DTT. Procedures used for preparation of 0.2M DTT reagent and treatment of red cells is found in Judd's Method in Immunohematology.[3] A starting volume of 10.5 mL treated cells and 0.6 mL untreated cells diluted to 3-5% in red cell support solution. Samples were stored in a refrigerator set at 2-8 degrees 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 negative control. made from The positive control consisted of Ortho confidence antibody diluted to 0.9% with normal saline and a negative control was prepared fromcontaining Immucor 22% bovine albumin diluted to 6-8% with phosphate buffered saline pH 7.1. Indirect antiglobulin testing was performed using Ortho column agglutination (Gel), and tube testing with Immucor Polyethylene Glycol (PEG) additive and no additive (saline method). The results were compiled on an excel

~~spreadsheet~~ and these data were used to determine whether the reagent cells had maintained potency for the evaluation period. At day 9, 1mL of supernatant was removed to correct concentration of cells back to 3-5%.

The expected evaluation criteria for this study were:

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

Our findings are summarized in (Table 1). Although hemolysis levels for 2 samples reached 200 mg/dL on day 7, ~~W~~we noted that hemolysis did not affect expected performance of reagent cells. The study ended at day 12 with 1/3 of the samples reaching 200 mg/dL hemolysis.~~However, this may have contributed to the decreased amount of available reagent which prompted the study to end at day 12.~~ Although the reagent cells tested 0 to 1+ for k (KEL2) antigen after DTT treatment, the cells successfully avoided anti-CD38 reactivity when tested with patient plasma~~were acceptable for use as CD38 negative cells~~. Antigen testing for k (KEL2) remained 1+ or less throughout the study. Satisfactory Rh antigen reactivity remained on samples with 3+ to 4+ throughout the study. Gel, and tube testing with PEG and no additive were greater than 1+ with dilute confidence antibody (positive control) throughout the test evaluation. There were no false positive reactions seen with the 6-8% albumin (negative control).

In conclusion, our study has shown DTT treated red cells stored in red cell support solution to maintain potency for up to 12 days. Since the number of samples with unsatisfactory hemolysis results continually increased after 7 days, we have 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, tube tests with PEG and no additive). Rh antigen integrity was maintained. As an adjunct to this validation, we evaluated Duffy antigens (Fy^a and Fy^b) post DTT treatment. We found 1 out of 10 samples had diminished antigen expression after treatment, and thus would recommend including testing for Fy^a and Fy^b antigens after preparing DTT treated reagent cells. We have found these reagent cells to be useful in avoiding pan-reactivity seen in patients receiving Daratumumab when assessing alloantibody formation. ~~Hemolysis did not interfere with either antigen or antibody testing. Having these cells~~

Formatted: Font: (Default) Times New Roman, Superscript

Formatted: Font: (Default) Times New Roman, Superscript

Formatted: Font: (Default) Times New Roman, Superscript

Formatted: Font: (Default) Times New Roman, Superscript

~~available provides a useful reagent for investigating anti-CD38, as well as, antibodies to Lutheran, Kell, Cartwright, Scianna, Dombrock, Landsteiner Weiner, Cromer, Knops, Indian, and John Milton Hagen.~~

References:

1. Chapuy, C., Nicholson, R., Aguad, M., et al. Resolving the Daratumumab Interference with Blood Compatibility Testing. Transfusion 2015; 55: 1545-554.
2. Reid, M., Lomas-Francis, C., and Olsson, M. The Blood Group Antigen FactsBbook. Third edition. Oxford: Academic, 2012.
3. Judd, W. John., Susan T. Johnson, Jill Storry, et al. *Judd's Methods in Immunohematology*. Bethesda, MD: AABB, 2008.
4. "Hemolysis Chart." *Laboratory Tests- Alphabetical*. N.p., n.d. Web. 21 Mar. 2016. <https://www.healthcare.uiowa.edu/path_handbook>

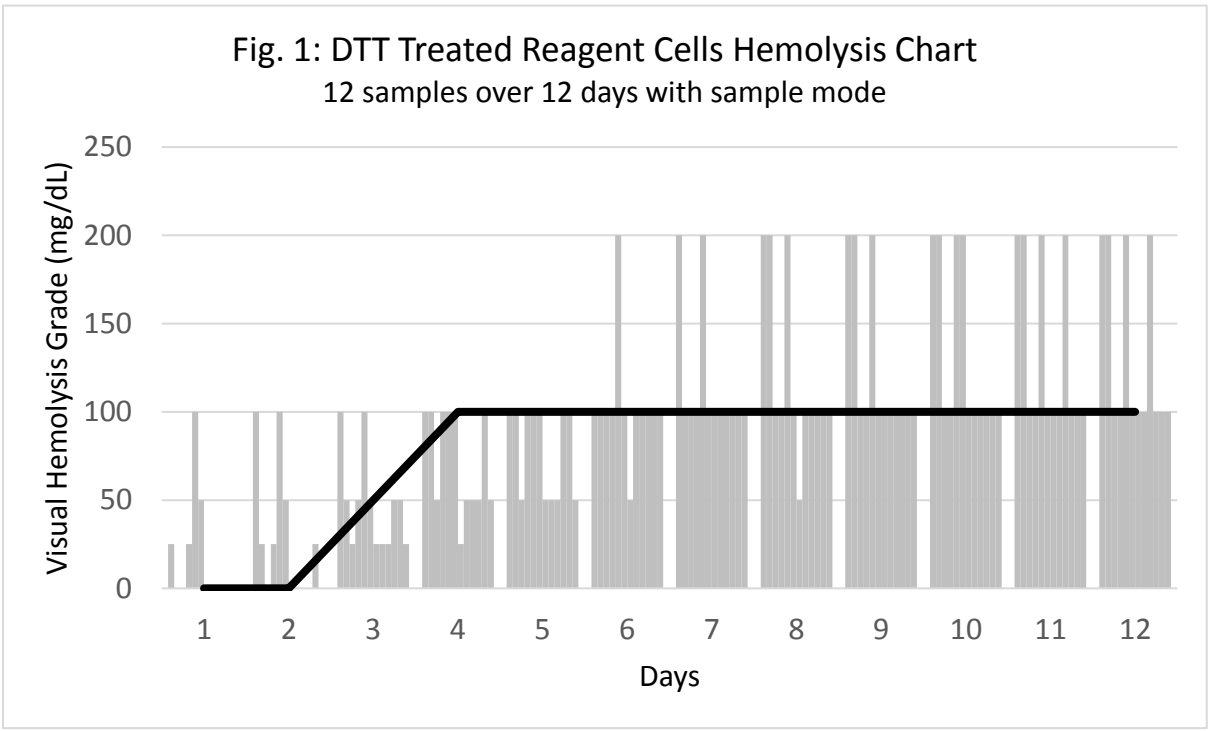

Fig. 1. The bar graph represents the visual grading of hemolysis with approximate levels of 0, 25, 50, 100, and 200 mg/dL for the 12 DTT treated samples. A solid black line illustrates the progression of the mode daily value of hemolysis.

Table 1: Results for data collected at initial, mid, and final days reported

	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 Contol	2+ to 4+	2+ to 4+	2+ to 4+
Negative Control	0	0	0
No additive (Saline method)			
Positive Contol	2+ to 4+	2+ to 4+	2+ to 4+
Negative Control	0	0	0