Beaumont

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DTT Treatment and Testing - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide policies and procedures that apply when using DTT (dithiothreitol) to prepare reagent red blood cells (RBCs) to aid in the identification of antibodies in patients undergoing treatment with CD38 monoclonal antibodies, Darzalex® (Daratumumab).

II. PRINCIPLE:

DTT is a water soluble protective reagent for sulfhydryl groups. It reduces disulfide linkages to free sulfhydryl groups in proteins and enzymes, thus cleaving the disulfide bonds and altering the structure of the RBC antigens. Treatment of RBCs with DTT destroys the structure of CD38; however it also may inactivate or weaken Kell-system antigens and certain high-prevalence antigens.

III. CLINICAL SIGNIFICANCE:

Daratumumab (DARA), a therapeutic agent for Multiple Myeloma, is an IgG1 kappa monoclonal antibody that recognizes CD38 on myeloma cells. DARA-treated patients can demonstrate a positive antibody screen and panreactivity on RBC panel testing due to the DARA binding in vitro to CD38 on the reagent RBCs. This interference can last up to 6 months after the last dose and result in delays in issuing RBC units to patients receiving these agents.

IV. POLICY:

A. Caregiver Notification of CD38 Drug
If the Blood Bank receives notification that a patient will begin treatment with the CD38 drug, request that a Type and Screen and one additional pink top tube be collected. Perform a base line type and screen and send the additional sample to the Blood Center of Wisconsin (BCW) for a molecular genotype.

B. Kell Blood Group

1. All patients must be given crossmatch compatible Kell negative RBCs, unless their molecular genotyping indicates they are Kell positive.
2. For Kell negative (or unknown) patients, the SIC_K antibody code should be added to the patient's internal demographics to ensure Kell negative RBCs are given. Additionally, an internal patient comment should be included indicating the SIC_K was added per policy due to the DTT treatment.

The SIC_K antibody code may be removed when the patient no longer requires DTT treatments during their workup.

C. DTT Treated Screens, Panels, and Crossmatches

1. A DTT treated antibody screen should be performed prior to any DTT treated panels. A Surgiscreen should be used for this initial screen, even if the same Surgiscreen was just used for 60-minute no-LISS testing as described in the Transfusion Medicine policy, *Warm Autoantibody Investigation*. If the patient has allo-antibodies in addition to the CD38 drug, a custom antigen negative antibody screen/ rule-out may be prepared instead. If reactivity is noted in the DTT treated antibody screen, a DTT treated panel should be performed to rule out underlying antibodies.
2. Only the initial DTT treated screen needs to be tested in parallel with untreated cells. Additional panel cells, including rule-out panels, do not need to be tested in parallel.
3. All DTT crossmatches must be tested in parallel with untreated cells, not just the initial crossmatches.
4. Only 3 – 5% reagent RBCs should be DTT treated. 0.8% reagent RBCs should not be used.

D. Positive to Negative Antibody Screen
If a patient's antibody screen returns to negative after the Darzalex treatment is complete and there is no history of an additional antibody for the patient, the NEXM antibody may be removed and electronic crossmatches may be performed.

V. DEFINITIONS:

Dithiothreitol (DTT): a small molecule redox reagent that cleaves disulfide bonds.

VI. SPECIMEN COLLECTION AND HANDLING:

A. The preferred specimen is a 6 ml EDTA sample with affixed identifying label. See Transfusion Medicine policy, Triaging and Identifying Acceptable Blood Samples for Testing, for acceptable alternatives.

B. If additional testing is required, including adsorption procedures or least incompatible crossmatches, then a large volume of serum, or multiple samples, may be required.

C. If a sample will be sent to a reference laboratory, then follow their sample requirements as indicated in Transfusion Medicine policy, *Submitting Samples to A Reference Laboratory*.

VII. REAGENTS:

A. Phosphate buffered saline (PBS)

B. Dithiothreitol (DTT)

C. 3 – 5% Reagent Red Blood Cells

D. AlbaQ-Chek® Controls

VIII. EQUIPMENT:

A. Heat Block

B. Centrifuge

C. Cell washer

D. Pipettes

E. Vortex

IX. SUPPLIES:

A. 12 x 75 tubes

B. Disposable pipettes

C. Parafilm

X. QUALITY CONTROL:

The below quality control must be completed each time a set of reagent or donor red blood cells are treated with DTT.

Reagent / Screen Cell	DTT Treated Kell Positive Cell	Untreated Kell Positive Cell	DTT Treated D Positive, Kell Negative Cell	Untreated D Positive, Kell Negative Cell
Anti-Sera	Anti-Kell (gel)	Anti-Kell (gel)	AlbaQ 1 (Anti-D)	AlbaQ 1 (Anti-D)
Expected Result	1+ or weaker	2+ or stronger	W+ or stronger	W+ or stronger

See Transfusion Medicine policy, *Quality Control of the Manual Gel System* and *Routine Quality Control of Blood Bank Reagents* for additional quality control information.

XI. PROCEDURE:

A. Determine which reagent RBCs will be DTT treated. A Surgiscreen should be used for the initial DTT treated antibody screen. If the patient has allo-antibodies in addition to the CD38 drug, a custom antigen negative antibody screen/rule-out may be prepared instead. If reactivity is noted in the DTT treated antibody screen, a DTT treated gel panel should be used to rule out underlying antibodies as described in the Transfusion Medicine policy, *Antibody Identification by Gel Card Test Method – Anti-IgG*

B. Prepare the DTT reagent by adding 250ul of PBS to the required number of DTT reagent vials. Ensure each vial is securely sealed with the vial cap or parafilm and vortex the DTT-filled vials to dissolve the DTT reagent. The reagent is ready for use once the DTT is fully dissolved.

   a. Example: To complete a Surgiscreen and two crossmatches, use four vials of DTT.

   b. NOTE: It may be necessary to use a pipette to dislodge the DTT reagent from the bottom of the vial. It is acceptable to continue through the procedure until step 8 while the DTT reagent is dissolving.

C. Label one 12 x 75 tube for each reagent RBC that will be treated with DTT.

   a. Example: To complete a Surgiscreen, label three 12 x 75 tubes SCI, SCII, and SCIII.

D. Add 12 drops of each reagent RBC into the corresponding 12 x 75 tube.

E. Label two 12 x 75 tubes for each donor unit to be crossmatched. Create a 2 – 4% red cell suspension for each donor unit in one of the corresponding tubes.

   a. The donor units should be Kell negative, in addition to antigen negative for any other allo-antibodies

that the patient possesses.

F. Add 12 drops of each donor unit's 2 – 4% red cell suspension to the other 12 x 75 tube with the corresponding donor unit number.

G. Wash the 12 drops of the 2 – 4% reagent RBCs and donor unit RBCs four times with normal saline by hand or using a cell washer.

   a. Note: If washing by hand, make sure the saline is decanted completely after the final wash, leaving only a dry cell button.

H. Use a disposable pipette to transfer four drops of DTT reagent to each 12 x 75 tube of washed reagent/ donor RBCs.

I. Resuspend the red cell button completely, place a cap or parafilm on each 12 x 75 tube and fully invert.

J. Incubate the tubes at 37°C for 30 minutes, fully inverting the tubes every 5 minutes.

K. After incubation, remove the cap or parafilm on each 12 x 75 tube and wash the cells four times with normal saline by hand or using a cell washer.

   a. If washing by hand, make sure the saline is decanted completely after the final wash, leaving only a dry cell button.

L. Resuspend the reagent/donor RBCs to a 2 – 4% suspension by adding 12 drops of normal saline.

M. After the RBCs have been resuspended, use the 2 – 4% cell suspension to make a 0.8% cell suspension, see Transfusion Medicine policy, Making a Test Red Cell Suspension.

   a. Cells must be reconstituted to a 2 – 4% cell suspension before being diluted to a 0.8% cell suspension for accurate results.

N. For each of the DTT treated reagent/donor RBCs prepared, label an additional 12 x 75 test tube for an untreated RBC cell suspension to be ran in parallel. Ensure the 12 x 75 test tubes are labeled in a way to differentiate which 0.8% cell suspension is treated and which is untreated. Create a 0.8% cell suspension of untreated cells in each of the corresponding tubes.

   a. Only the initial DTT treated screen needs to be tested in parallel with untreated cells. Additional panel cells, including rule-out panels, do not need to be tested in parallel.

   b. All DTT crossmatches must be tested in parallel with untreated cells, not just the initial crossmatches.

O. Perform quality control (QC), the antibody screen/panel, and patient crossmatches. Refer to Transfusion Medicine policy, *Antibody Identification by Gel Card Test Method – Anti-IgG* and *Serologic Crossmatching of Red Blood Cells* for additional information.

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P. Document the QC results on F-618C, DTT Treatment Quality Control. Document the patient results on the reagent RBC antigram (manufacturer prepared or selected cell panel).
   a. Document the DTT treated results and untreated results on the same antigram in separately labeled columns.

Q. Enter the untreated gel crossmatch results in SOFT as test of record. Add a test comment documenting the DTT treated gel crossmatch results. If the untreated gel crossmatches are incompatible, notify the patient's caregivers before issuing units. This notification should be documented as a unit comment in the Blood Bank computer system as directed in Transfusion Medicine policy, *Investigation of Incompatible Crossmatches.*

R. Bill the patient for the DTT treatment, action code ENZTC in the Blood Bank computer system.

S. Discard the open DTT vials in a biohazard bin.
   a. Ensure that the reagent RBCs being treated include RBCs that satisfy the quality control requirements for the DTT reagent. Refer to the *Quality Control* section of this document for additional information.

XII. EXPECTED VALUES:

The untreated antibody screen must demonstrate reactivity. If the untreated antibody screen is not reactive, the DTT treated antibody screen cannot be used for antibody identification. All antibody screening cells should be negative after DTT treatment. If reactivity is noted in the DTT treated antibody screen, perform a DTT treated gel panel to rule out underlying antibodies as described in Transfusion Medicine policy, *Antibody Identification by Gel Card Test Method – Anti-IgG.*

XIII. LIMITATIONS:

DTT treated cells must not be used for exclusion of antibodies to Kell system antigens. All patients must have crossmatch compatible, Kell negative RBC units available.

XIV. REFERENCES:

A. Chapuy C, Nicholson R, Aguad M, et al. Resolving the Daratumumab Interference with Blood Compatibility Testing. Transfusion 2015;55: 1545 – 54.

B. AABB, Technical Manual, current edition.

Attachments

F-618C, DTT Treatment Quality Control

Approval Signatures

Step Description	Approver	Date
	Ann Marie Blenc: System Med Dir, Hematopath	9/2/2021
	Craig Fletcher: System Med Dir, Blood Bank	8/27/2021
Policy and Forms Steering Committe (if needed)	Gail Juleff: Project Mgr Policy	8/18/2021
Policy and Forms Steering Committe (if needed)	Samantha Maynard: Medical Technologist	8/18/2021
	Billie Ketelsen: Mgr Laboratory	8/18/2021
	Samantha Maynard: Medical Technologist	8/16/2021

Applicability

Royal Oak