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Area	Laboratory-Blood Bank
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DTT Treatment and Testing - Blood Bank

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

A. This document will provide policies and procedures that apply when referring patient samples for testing with (dithiothreitol) DTT or when using DTT to prepare reagent red blood cells (RBCs) to aid in the identification of antibodies in patients undergoing treatment with CD38 monoclonal antibodies, such as Darzalex® (Daratumumab).

II. CLINICAL SIGNIFICANCE:

A. 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 pan reactivity 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.

B. Dithiothreitol (DTT) is a water soluble redox reagent that cleaves disulfide bonds and is a 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. POLICIES:

A. Caregiver Notification of CD38 Drug

1. 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 Versiti Wisconsin for a RBC molecular genotype.

B. 60-Minute No-LISS Screens, Panels, and Crossmatches

1. A 60-Minute No-LISS antibody screen should be performed prior to any DTT treatment.

C. Referral for DTT Testing

1. DTT testing will be performed only if all clinically significant antibodies can not be ruled out with the 60-Minute No-LISS testing. Note: Phenotypically matched units may be crossmatched with the 60-Minutes No-LISS method in lieu of DTT testing if phenotypically matched units are easily obtained.
2. DTT testing may only be performed at Beaumont sites for which validation testing has been proved and testing authorized.

D. 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 **KDTT** antibody code should be added to the patient's antibody file (Patient>Edit>Antibodies) to ensure Kell negative RBCs are given.

E. DTT Treated Screens, Panels, and Crossmatches

1. A DTT treated antibody screen should be performed prior to any DTT treated panels.
2. 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 Transfusion Medicine policy, Warm Autoantibody Investigation.
3. If the patient has alloantibodies 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.
4. 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.
5. All DTT crossmatches must be tested in parallel with untreated cells, not just the initial crossmatches.
6. Only 3 – 5% reagent RBCs should be DTT treated. 0.8% reagent RBCs should not be used.

F. Positive to Negative Antibody Screen

1. 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** code may be removed and electronic crossmatches may be performed. The Anti-CD38 antibody code will remain in

the patient's antibody display.

2. The **KDTT** antibody code (if applicable) may be removed when the patient no longer requires DTT treatments during their workup.

IV. 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 Samples For Testing for acceptable alternatives.

B. If additional testing is required, including reference lab testing or multiple crossmatches, then a large volume of plasma, 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.

V. REAGENTS:

A. Phosphate buffered saline (PBS)

B. Dithiothreitol (DTT)

C. 3 – 5% Reagent Red Blood Cells

D. AlbaQ-Chek® Controls

E. Ortho Anti-Kell antisera

VI. EQUIPMENT:

A. Heat Block

B. Centrifuge

C. Cell washer

D. Pipettes

E. Vortex

VII. SUPPLIES:

A. 12 x 75 tubes

B. Disposable pipettes

C. Parafilm or plastic caps

VIII. QUALITY CONTROL (QC):

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

Reagent /Screen	DTT Treated Kell Positive	Untreated Kell Positive Cell	DTT Treated D Positive, Kell Negative	Untreated D Positive, Kell

Cell	Cell		Cell	Negative Cell
Anti-Sera	Anti-Kell (gel)	Anti-Kell (gel)	AlbaQ1 (Anti-D)	AlbaQ1 (Anti-D)
Expected Result	1+ or weaker	2+ or stronger	w+ or stronger	w+ or stronger

B. Refer to site specific Transfusion Medicine policy, Quality Control of Routine Blood Reagents for additional QC requirements.

IX. PROCEDURE:

A. Referral of Samples for DTT Testing

1. Prior to sending sample confirm that testing with 60-Minute No-LISS is unresolved (does not exclude all clinically significant antibodies) and that phenotypically matched units can not be easily obtained from either general inventory or blood supplier.
2. Notify the testing site that DTT testing is required. The testing site will review results of 60-Minute No-LISS testing and consult with Medical Director/Supervisor if there is any concern with performing the testing.
3. Once approved for submission the referring site will:
    a. Order a miscellaneous Blood Bank test (LAB6339) in Epic and notify the phlebotomy area to request a stat blood draw, or notify the caregiver if phlebotomy does not collect the patient. Note: This miscellaneous Blood Bank test will remain pending until the samples have been collected and sent to the testing site, at which time the miscellaneous Blood Bank test may be canceled. Note: A properly labeled lavender from a prior collection (with band number) may be used in lieu of the BMISC sample.
    b. Prepare a properly labeled 2.5 mL plasma aliquot (minimum 1.0 mL) of the original sample for submission to the testing lab.
    c. Package the aliquot along with a second sample collected as BMISC for the testing lab.

B. DTT Testing (Dearborn/Royal Oak Only)

1. Determine which reagent RBCs will be DTT treated. A Surgiscreen should be used for the initial DTT treated antibody screen.
    a. Verify that the reagent RBCs being treated include RBCs that satisfy the quality control requirements for the DTT reagent. Refer to IX. *Quality Control* section for additional information.
    b. If the patient has alloantibodies in addition to the CD38 drug, a custom antigen negative antibody screen/rule-out may be prepared instead.
    c. 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 Transfusion Medicine policy, Antibody Identification. Make sure that the reagent RBCs being treated include RBCs that satisfy the quality control requirements for the DTT reagent. Refer to the *Quality Control* section for additional information.

2. Prepare the DTT reagent by adding 250 uL of PBS to the required number of DTT reagent vials. Typically four (4) vials of DTT are needed to complete the Surgiscreen and two crossmatches.
    a. Ensure each vial is securely sealed with the vial cap or parafilm and vortex the DTT-filled vials to dissolve the DTT reagent. Note: It may be necessary to use a pipette to dislodge the DTT reagent from the bottom of the vial.
    b. The reagent is ready for use once the DTT is fully dissolved. Note: It is acceptable to continue through the procedure until step 8 while the reagent is dissolving.
3. Label one 12 x 75 tube for each reagent RBC that will be treated with DTT.
4. Add 12 drops of each reagent RBC into the corresponding 12 x 75 tube.
5. Label two 12 x 75 tubes for each donor unit to be crossmatched.
    a. Create a 2 – 4% red cell suspension for each donor unit in one of the corresponding tubes.
    b. The donor units should be Kell negative, in addition to antigen negative for any other allo-antibodies that the patient possesses.
6. 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.
7. 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. Note: Make sure the saline is decanted completely after the final wash, leaving only a dry cell button.
8. Use a disposable pipette to transfer four drops of DTT reagent to each 12 x 75 tube of washed reagent/donor RBCs.
9. Resuspend the red cell button completely, place a cap or parafilm on each 12 x 75 tube and fully invert.
10. Incubate the tubes at 37°C for 30 minutes, fully inverting the tubes every 5 minutes.
11. 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. Make sure the saline is decanted completely after the final wash, leaving only a dry cell button.
12. Resuspend the reagent/donor RBCs to a 2 – 4% suspension by adding 12 drops of normal saline.
13. After the RBCs have been resuspended, use the 2 – 4% cell suspension to make a 0.8% cell suspension. Refer to Transfusion Medicine policy, Making a Test Red Cell Suspension. NOTE: Cells must be reconstituted to a 2 – 4% cell suspension before being diluted to a 0.8% cell suspension for accurate results.
14. 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.
    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.

c. Label the tubes such that there is a way to differentiate which 0.8% cell suspension is treated and which is untreated.

d. Create a 0.8% cell suspension of untreated cells in each of the corresponding tubes.

15. Perform quality control, antibody screens/ or panel, and patient crossmatches. Refer to Transfusion Medicine policies, Antibody Identification and Serologic Crossmatching of Red Blood Cells for additional information.

16. Document the QC results on the attached *DTT Treatment Quality Control*.

17. 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.

18. Enter the antibody identification results in the Blood Bank computer system.

19. Select the units for crossmatch using Inventory > Product Order Services > Select.

20. If the unit(s) are to be transfused at the testing hospital:

    a. Enter the untreated gel crossmatch test in the Blood Bank computer system.

    b. Add a free text test comment documenting the DTT treated gel crossmatch results.

    c. If the untreated gel crossmatches are incompatible notify the patient's caregivers before issuing units. This notification should be documented in the Blood Bank computer system using the **INCXM** comment code.

21. If testing is being performed on behalf of another laboratory:

    a. Access the untreated gel crossmatch test in the Blood Bank computer system but leave result and interpretation fields blank.

    b. Add a free text test comment documenting the untreated gel and DTT treated gel crossmatch results and the site and technologist performing the testing i.e. "Test Performed at Beaumont Dearborn" and use the Soft time stamp Keys (F6,F7, F8) to document the testing technologist.

        i. If the untreated gel crossmatches are incompatible the referring site will notify patients caregivers before issuing units and document this notification will be documented in the Blood Bank computer system using the **INCXM** comment code.

22. Bill the patient for the DTT treatment, by ordering the action code **ENZTC** in the Blood Bank

computer system.

23. Discard the open DTT vials in a biohazard bin.
24. If performing testing for another site:
    a. Prepare and package the crossmatched units for transfer to the referring facility in accordance with the Transfusion Medicine policy, Transfer of Blood Products to Outside Facilities.
    b. Retain the samples for potential add on crossmatches.
    c. Send copies of all testing results to the referring site.
25. Submit all original copies of testing for Supervisor review.
    a. Royal Oak: CABID (Consult Antibody Identification with the Blood Bank Medical Director) orders are not required for referred testing.

C. Documentation of Referral Testing

Upon transfer of units and return of patient samples the referring lab will:

1. Bring the transferred products into inventory in accordance with the Transfusion Medicine policy, Receiving Blood Components from Outside Source.
2. Perform a gel crossmatch for each unit transferred using the original patient sample in accordance with Transfusion Medicine policy, Serological Crossmatching of Red Blood Cells.
3. Enter the gel crossmatch results in the Blood Bank Computer.
    a. If the untreated gel crossmatches are incompatible, notify the patient's caregivers before issuing units. This notification should be documented in the Blood Bank computer system using the **INCXM** comment code.
4. Submit all paperwork for Supervisor review.

X. EXPECTED VALUES:

A. 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 for the gel method in Transfusion Medicine policy, Antibody Identification.

XI. LIMITATIONS:

A. 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.

XII. REFERENCES:

1. AABB, *Technical Manual*, current edition.

2. College of American Pathologists, *Transfusion Medicine Checklist*, current edition.
3. Chapuy C, Nicholson R, Aguad M, et al. Resolving the Daratumumab Interference with Blood Compatibility Testing. Transfusion 2015;55: 1545 – 54.

Attachments

DTT Testing Quality Control Form

Approval Signatures

Step Description	Approver	Date
	Vaishali Pansare: Chief, Pathology	10/18/2022
	Jeremy Powers: Chief, Pathology	10/12/2022
	Ryan Johnson: OUWB Clinical Faculty	10/7/2022
	Muhammad Arshad: Physician	10/7/2022
	Ann Marie Blenc: System Med Dir, Hematopath	10/4/2022
	John Pui: Chief, Pathology	10/4/2022
Policy and Forms Steering Committe (if needed)	Kelly Sartor: Supv, Laboratory	10/4/2022
Policy and Forms Steering Committe (if needed)	Gail Juleff: Project Mgr Policy	10/4/2022
	Kristen Lafond: Mgr Laboratory	10/4/2022
	Michael Rasmussen: Supv, Laboratory	10/4/2022
	Hilary Morey: Medical Technologist Lead	10/1/2022
	Ashley Beesley: Mgr Laboratory	9/30/2022
	Katherine Persinger: Mgr Laboratory	9/30/2022

Rebecca Thompson: Medical Technologist Lead	9/29/2022
Teresa Lovins: Supv, Laboratory	9/29/2022
Kelly Sartor: Supv, Laboratory	9/28/2022
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