PROCEDURE
Corewell Health East - DTT Treatment and Testing - Blood Bank - All Beaumont Hospitals

This Procedure is Applicable to the following Corewell Health sites:
Corewell Health Beaumont Grosse Pointe Hospital, Corewell Health Beaumont Troy Hospital, Corewell Health Dearborn Hospital, Corewell Health Farmington Hills Hospital, Corewell Health Taylor Hospital, Corewell Health Trenton Hospital, Corewell Health Wayne Hospital, Corewell Health William Beaumont University Hospital (Royal Oak)

Applicability Limited to: N/A

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Functional Area: Clinical Operations, Laboratory

Lab Department Area: Lab - Blood Bank

1. Principle
This document will provide policies and procedures that apply when referring patient samples for testing with DTT(dithiothreitol) 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).

2. Responsibility
A. Personnel who have completed the competency requirements will perform this testing.
B. DTT testing will be performed only if all clinically significant antibodies cannot be ruled out with the 60-Minute No-LISS testing. Note: Phenotypically matched units may be crossmatched with the 60-Minute No-LISS method in lieu of DTT testing if phenotypically matched units are easily obtained.
C. DTT testing may only be performed at Corewell Health sites for which validation testing has been proved and testing authorized.
D. 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 baseline type and screen and send the additional sample to Versiti Wisconsin for an RBC molecular genotype.

3. Definitions
A. **DARA -** Daratumumab, 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. **DTT –** Dithiothreitol, 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.

4. Specimen

A. The preferred specimen is a 6 mL EDTA sample with affixed identifying label. See Transfusion Medicine policy, Corewell Health East - Triaging And Identifying Acceptable Samples For Testing-Blood Bank - All Beaumont Hospitals 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, Corewell Health East - Submitting Samples to External Reference Laboratories - Blood Bank - All Beaumont Hospitals .

5. Reagent/Equipment Needed

A. Phosphate buffered saline (PBS)
B. Dithiothreitol (DTT)
C. 3 – 5% Reagent Red Blood Cells
D. ORTHO™ Daily QC Simulated Whole Blood Quality Control Kit
E. Anti-Kell antisera
F. Heat Block
G. Centrifuge
H. Cell washer
I. Pipettes
J. Vortex
K. 12 x 75 tubes
L. Disposable pipettes
M. Parafilm or plastic caps

6. Quality Control

A. Quality control must be completed each time a set of reagent or donor red blood cells are treated with DTT.
B. Select cells that meet the criteria in the table below must be included in each batch of DTT Treatment.
C. QC is completed as described in the table below and documented on the *DTT Treatment Quality Control Form* attachment.

Reagent /Screen Cell	DTT Treated K Positive Cell	Untreated K Positive Cell	DTT Treated D Positive / K Negative Cell	Untreated D Positive / K Negative Cell
Anti-Sera	Anti-K	Anti-K	ORTHO Daily QC Vial 4 (Anti-D)	ORTHO Daily QC Vial 4 (Anti-D)
Expected Result	1+ or weaker	2+ or stronger	w+ or stronger	w+ or stronger

D. Refer to site specific Transfusion Medicine policy, Corewell Health East - Quality Control of Blood Bank Reagents - All Beaumont Hospitals for additional QC requirements.

7. Procedure

A. Indications for DTT Testing (refer to attachment *CD38 Evaluation Flow Chart)*

1. DTT Testing is indicated if the following criteria are met:
   a. For any suspicion of a newly acquired Anti-CD38 Antibody where testing with 60-Minute No-LISS is unresolved (does not exclude all clinically significant antibodies) **-OR-**

b. Previous History of Anti-CD38 Antibody where testing with 60-Minute No-LISS is unresolved (does not exclude all clinically significant antibodies) and one of the following three (3) conditions exist:
   1) Last DTT workup was more than 3 months ago.
   2) Current reactions are inconsistent with expected reactivity.
   3) Current reactivity is stronger than previous results.

B. Crossmatching RBC for patient with active Anti-CD38 Antibody

1. DTT-compatible
   a. DTT treated cells must not be used for exclusion of antibodies to Kell system antigens. All patients utilizing DTT compatible units must receive K negative RBC units, when patient is not known to be K-antigen positive.
2. 60-Minute No-LISS Least Incompatible Phenotypically matched RBCs
   a. If DTT-workup is not indicated. **-OR-**
   b. If circumstances prevent timely completion of DTT-workup, phenotypically matched units least incompatible by 60-Minute No-LISS method may be provided.
      1) The DTT-workup will still be performed as indicated.
3. Consult Medical Director as needed for further direction.

C. Positive to Negative Antibody Screen

1. If a patient's current gel antibody screen returns to negative after treatment is complete and there is no history of an additional antibody for the patient, the 38 Antibody code may be inactivated and electronic crossmatches may be performed. The Anti-CD38 antibody will remain in the patient's antibody history in the extended typings tab.
2. The K negative RBCs Special Requirement (if applicable) may be removed when the patient no longer requires DTT treatments during their workup.

D. Referral of Samples for DTT Testing

1. Prior to sending the sample, the referring site **must** have completed testing with 60-Minute No-LISS and that criteria from **Indications for DTT Testing** section above are met.
   a. If circumstances prevent timely completion of DTT-workup, phenotypically matched units can be crossmatched using 60-Minute No-LISS method, least incompatible. A sample will still need to be sent for DTT workup.
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 Procedure, Blood Bank test (LAB1231200) 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 BB MISC sample. This specimen is to be used for the workup at the DTT Testing Site.
   b. Prepare a properly labeled 2.5 mL plasma aliquot (minimum 1.0 mL) of the original sample for submission to the testing lab. This is to be used for crossmatching at the DTT Testing Site.
   c. Use Inventory > Transfer > New Transfer to transfer the Type/Screen Specimen in SafeTrace to the Testing Site. Note the Transfer ID.
   d. Create a Blood Bank Packing List in LIS and send specimen to testing site Blood Bank (not Laboratory).
   e. Package the aliquot, BB MISC specimen, and a copy of the current workup for the testing lab.

E. DTT Testing (Only to be completed at CHE sites which have validated DTT Testing on site)

1. Determine which reagent RBCs will be DTT treated.
   a. Only 3 – 5% reagent RBCs should be DTT treated. 0.8% reagent RBCs should not be used.

   b. A Surgiscreen should be used for this initial DTT screen, even if the same Surgiscreen was just used for 60-Minute No-LISS testing.
   c. Verify that the reagent RBCs being treated include RBCs that satisfy the quality control requirements for the DTT reagent. Refer to Quality Control section of this document for additional information.
   d. If the patient has alloantibodies in addition to the CD38 drug, a custom antigen negative antibody screen/rule-out panel may be prepared instead.
   e. 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, Corewell Health East - Antibody Identification - Blood Bank - All Beaumont Hospitals . Make sure that the panel 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. Parallel Testing with untreated cells:
   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.
3. Prepare the DTT reagent by adding 250 µL of PBS to the required number of DTT reagent vials, the reagent may dissolve more quickly if it is dislodged from the bottom of the vial prior to addition of PBS. 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.
4. Label one 12 x 75 tube for each reagent RBC that will be treated with DTT.
5. Add 12 drops of each reagent RBC into the corresponding 12 x 75 tube.
6. 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.
      1) The donor units should be K negative when patient is not known to be K-antigen positive.
      2) The donor units should be antigen negative for any other allo-antibodies that the patient possesses.
7. 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.
8. Wash the 12 drops of the 2 – 4% reagent RBCs and donor unit RBCs four times with normal saline by hand.
   a. An automatic cell washer may be used, however there may be loss of RBCs.
   b. Make sure the saline is decanted completely after the final wash, leaving only a dry cell button.
9. Use a disposable pipette to transfer four drops of DTT reagent to each 12 x 75 tube of washed reagent/ donor RBCs. It is helpful to combine all prepared DTT vials to a 12 x 75 tube to ease in pipetting.
10. Resuspend the red cell button completely, place a cap or parafilm on each 12 x 75 tube and gently swirl to mix thoroughly.
11. Incubate the tubes at 37°C for 30 minutes, mixing the contents of the tubes every 5 minutes by gently swirling.
12. After incubation, remove the cap or parafilm on each 12 x 75 tube and wash the cells four times with normal saline by hand.
    a. An automatic cell washer may be used, however there may be loss of RBCs.

b. Make sure the saline is decanted completely after the final wash, leaving only a dry cell button.

13. Resuspend the reagent/donor RBCs to a 2 – 4% suspension with normal saline.
14. After the RBCs have been resuspended, use the 2 – 4% cell suspension to make a 0.8% cell suspension. Refer to Transfusion Medicine policy, Corewell Health East - Making a Test Red Cell Suspension - All Beaumont Hospitals . NOTE: Cells must be reconstituted to a 2 – 4% cell suspension before being diluted to a 0.8% cell suspension for accurate results.
15. 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. These can be prepared during the DTT incubation, to save time.
    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.
16. Perform quality control, antibody screens/ or panel, and patient crossmatches using Gel AHG method. Refer to Transfusion Medicine policies, Corewell Health East - Antibody Identification - Blood Bank - All Beaumont Hospitals and Corewell Health East - Serologic Crossmatching of Red Blood Cells - All Beaumont Hospitals for additional information.
    a. Example gel card set-up:

17. Document the QC results on the attached *DTT Treatment Quality Control* Form.
18. 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.
    b. In BBIS Patient Profile Orders Tab add on Extended AB Screen (ASCPOS).
19. In BBIS tests tab select pending ASCPOS Test -> Click Results -> Click Add -> Click Test. Select ABS3DTTG and ABS3GEL. Enter results for untreated and DTT-treated 3 cell gel screens.
20. Once antibody workup is completed, enter the antibody interpretation on the ABID test in the Blood Bank computer system, if applicable (a panel was run on the current specimen).
21. Select the units for the patient using Patient/Order > Product Selection.
22. If the unit(s) are to be transfused at the testing hospital:
    a. In Tests tab -> Select the pending Crossmatch test -> Results -> Add -> Test. Select XMGELAHG Crossmatch code and enter the untreated gel crossmatch test result in the Blood Bank computer system.
    b. In Tests tab -> Select the completed Crossmatch test -> Results - > Add -> Test. Select XMDTTGEL Crossmatch code and enter the treated gel crossmatch test result in the Blood Bank computer system.
    c. Do not enter both results at the same time. Use this process to ensure the compatible crossmatch is the crossmatch of record for issue.
23. If testing is being performed on behalf of another laboratory:
    a. Receive the transferred specimen in SafeTrace, Inventory > Transfer > Open Transfer.

b. In Tests tab -> Select the pending Crossmatch test -> Results -> Add -> Test. Select XMDTTGEL Crossmatch code and enter the treated gel crossmatch test result in the Blood Bank computer system.
c. Release unit(s) from patient using Inventory > Status Change > Available

24. Discard the open DTT vials in a biohazard bin.
25. 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, Corewell Health East - Transfer and/or Shipment of Blood Products - Blood Bank - All Beaumont Hospitals .
    b. Use Inventory > Transfer > New Transfer to transfer the Type/Screen Specimen ID in SafeTrace back to the original site. Note the Transfer ID.
    c. Retain the BB MISC sample for potential add on crossmatches or additional antibody workup.
    d. Send copies of all testing results to the referring site.
26. Submit all original copies of testing for Supervisor review.
    a. CABID (Consult Antibody Identification with the Blood Bank Medical Director), when applicable, should be ordered by the site of specimen origin.

F. Documentation of Referral Testing

1. Upon receipt of unit(s) the referring lab will:
    a. Bring the transferred product(s) into inventory in accordance with the Transfusion Medicine policy, Corewell Health East - Transfer and/or Shipment of Blood Products - Blood Bank - All Beaumont Hospitals.
    b. Receive Type/Screen specimen in BBIS and LIS.
    c. Perform a gel crossmatch for each unit transferred using the original patient sample in accordance with Transfusion Medicine policy, Corewell Health East - Serologic Crossmatching of Red Blood Cells - All Beaumont Hospitals .
    d. Enter the gel crossmatch results in the Blood Bank Computer.
        1) 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 Patient Profile Note.
    e. Submit all paperwork for Supervisor review.

8. Results/Interpretation

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.
B. 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, Corewell Health East - Antibody Identification - Blood Bank - All Beaumont Hospitals .

9. Limitations

A. DTT testing will be performed on day shift only.
    1. Royal Oak only: DTT testing may be performed on alternate shifts if workload and staffing allow.
B. DTT treated cells must not be used for exclusion of antibodies to Kell system antigens. All patients utilizing DTT compatible units must receive K negative RBC units, when patient is not known to be K-antigen positive.

10. Revisions

Corewell Health reserves the right to alter, amend, modify or eliminate this document at any time without prior written notice.

11. 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.

12. Procedure Development and Approval

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13. Keywords

Not Set