**PURPOSE**

To provide instructions for the treatment of reagent red blood cells with DTT for use in antibody identification

**PRINCIPLE & CLINICAL SIGNIFICANCE:**

Dithiothreitol (DTT) is an efficient reducing agent that can disrupt the tertiary structure of proteins by irreversibly reducing disulfide bonds to free sulfhydryl groups. Without tertiary structure, protein-containing antigens can no longer bind antibodies that are specific for them. Red cells treated with DTT are not reactive with antibodies in the Kell blood group system, most antibodies in the Knops system, or most examples of anti-LWa, -Yta, -Ytb, -Doa, -Dob, -Gya, -Hy, and -Joa.

DTT treatment of reagent cells is effective in resolving panreactivity of plasma from patients treated with Daratumumab (DARA). DARA is a monoclonal antibody that targets the CD38 antigen on erythrocytes and is used in the treatment of multiple myeloma. Treating reagent screening and panel reagent cells cleaves the CD38 antigen resolving panagglutination due to DARA and allowing identification of underlying clinically significant antibodies. Treatment with DTT also cleaves Kell antigens preventing the identification of underlying Kell antibodies

**POLICIES:**

* Kell negative red blood cell components should be provided to Kell negative or unknown patient when DTT-treated cells are used to rule out underlying clinically significant antibodies
  + DTT-treated cells must be quality control on day of initial DTT treatment and each day of use
  + QC and patient testing may be performed in parallel if QC is determined to be acceptable prior to reporting test results
* Remaining thawed DTT must be discarded after use
* DTT treated reagent red blood cells will be stored in a Alsevers solution and hemolysis check is required before use.
* **DTT treated reagent red blood cells stored in Alsevers solution at 2 to 8 0C and should be resuspended in blood bank saline prior to use.** 
  + Alsevers solution should be stored in the dark @ 2 to 8OC.
  + Expiration date of Alsevers solution is 42 days from open date or manufacturer’s expiration date if shorter than 42 days.

**SPECIMEN REQUIREMENTS:**

EDTA is preferred and if not tested soon after collection, should be stored at 2-6°C.

Red top tubes are acceptable.

See SOP *Specimen Acceptability*

**REAGENTS/SUPPLIES/EQUIPMENT:**

|  |  |  |
| --- | --- | --- |
| **Reagents:** | **Supplies:** | **Equipment:** |
| * DTT powder * Blood bank saline * Reagent Red Blood Cells * Anti-K antisera * Alsevers solution | * 12 x 75 mm glass and plastic tubes * Blood bank transfer pipettes | * Calibrated serologic centrifuge * Calibrated cell washer * 37°C Heat Block * Agglutination viewer |

**QUALITY CONTROL:**

Treated reagent red cells are quality control tested on day of use

Anti-K is quality control tested on day of use

DTT treated reagent cells are QC’d on day of use

**INSTRUCTIONS:**

**TABLE of CONTENTS**

[**Preparing 0.2M DTT**](#PrepDTT)

[**QC of 0.2M DTT**](#QCDTT)

[**Treating Red Blood Cells with 0.2M DTT**](#TreatingRBC)

[**Storage of DTT-treated Reagent Red Blood Cells**](#StorageDTTRBC)

[**Preparing Alsevers Solution Stored Reagent Cells for Patient Testing**](#PatientTesting)

**Preparing 0.2M DTT**

| **STEP** | **ACTION** |
| --- | --- |
| 1 | Document the following information on the ***Reconstituted 0.2M DTT Log***:   * DTT powder reagent manufacturer, lot number and expiration date * Blood Bank Saline manufacturer, lot number and expiration date * Reconstitute DTT lot number and expiration date  |  |  | | --- | --- | | **Lot Number** | Today’s date MMDDYY  Example: If today is July 26, 2016, the lot number is 072616 | | **Expiration Date** | 1 year from today or expiration of DTT powder reagent or saline, whichever is shorter | |
| 2 | Label a clean glass container with the following information:   * DTT concentration (0.2 M) * Reconstituted lot number * Reconstituted expiration date |
| 3 | Add the following to the labeled glass container and allow the powder to dissolve   |  |  | | --- | --- | | **Volume of Saline (mL)** | **Weight of DTT (g)** | | 32 mL | 1.0 g | |
| 4 | Go to section **QC of 0.2 M DTT** to perform quality control of the reconstitute DTT reagent |

**QC of 0.2M DTT**

| **STEP** | **ACTION** |
| --- | --- |
| 1 | Select a K positive reagent red cell and treat with 0.2 M DTT following Steps 1 thru 6 in section [**Treating Red Blood Cells with 0.2 M DTT**](#TreatingRBC) |
| 2 | Phenotype both the treated and untreated red blood cells for K antigen and record the results on the ***DTT Reagent QC*** form  **NOTE:** Perform K antisera QC if not already performed on the day   |  |  |  | | --- | --- | --- | | **UNTREATED cell is** | **TREATED cell is** | **Then** | | K Positive | K Negative | DTT QC is acceptable | | K Positive | K Positive | DTT QC is unacceptable   * Repeat step 4 in Treating RBC’s section * Notify Lead/Manager if QC is still unacceptable | |
| 3 | Label 12x75 mm plastic tubes with the following information   * Date of reconstitution * 0.2M DTT * Lot # * Expiration Date * Prepared by (tech ID) |
| 4 | Aliquot 1 mL of reconstituted 0.2M DTT reagent into each labeled tube |
| 5 | Record the number of aliquots made on the ***Reconstituted 0.2M DTT Log*** |
| 6 | Store labeled aliquots at ≤ -18°C after verifying QC on the reagent is acceptable. |

**Treating Red Blood Cells with 0.2 M DTT**

| **STEP** | **ACTION** |
| --- | --- |
| 1 | Add 1 volume of reagent red cells to a labeled 12x75 glass tube – no less than 2 mL |
| 2 | Manually wash the reagents red cells 1 time |
| 3 | Mix 4 volumes of 0.2 M DTT with 1 volume of washed packed RBCs in a labeled tube  **EXAMPLE:** Mix 200uL of 0.2 M DTT with 50uL of packed RBCs |
| 4 | Incubate at 37°C ±1 for 30 minutes mixing approximately every 5 minutes  **NOTE:** Time may be extended up to 45 minutes |
| 5 | Wash manually 4 times with saline  **NOTE:** Repeat steps 1 thru 4 using 2-3 volumes of 0.2 M DTT if RBC lysis occurs |
| 6 | Verify the cells are at an approx. 3-4% suspension and adjust if necessary with 0.9% saline |
| 7 | |  |  | | --- | --- | | **If** | **Then** | | Testing patient cells | Follow appropriate procedure | | Reagent screening red cells | * Go to section [**QC of 0.2 M DTT**](#QCDTT)step 2 * Can be performed in parallel with patient testing | |
| 8 | Record results of testing on the Extended Work-up form or antibody panel sheet. |
| 9 | Go to section *Result Reporting in Sunquest* |

**Storage of DTT-treated Reagent Red Blood Cells**

|  |  |
| --- | --- |
| **STEP** | **ACTION** |
| 1 | Centrifuge the DTT treated reagent RBCs for 1 minute |
| 2 | Decant and discard the supernatant |
| 3 | Resuspend the cells using Alsevers solution to a 3 to 4% cell suspension |
| 4 | Record the following on the treated reagent cell label:   * Lot number of DTT treated reagent RBCs * Cell number * Date of treatment * Expiration date (Alsevers solution or reagent RBCs, whichever is shorter) |
| 5 | Transfer the cell suspension to a clean labeled 12x75 glass tube  **NOTE:** Ensure the solution is clear, no precipitate and in-date |
| 6 | Record the following on the ***DTT Treated Reagent Red Blood Cell Log***:   * Reagent red cell manufacturer, Lot number and pre and post treatment expiration date * 0.2M DTT lot number, and expiration date * Alsevers solution manufacturer, lot number and expiration date |
| 7 | Store at 2-8 0C |

**Preparing Alsevers Solution Stored Reagent Cells for Patient Testing**

| **STEP** | **ACTION** |
| --- | --- |
| 1 | Label 12x75 glass test tubes for each reagent cell |
| 2 | Add 4 drops of DTT-treated reagent red cells to the appropriate labeled test tube  **NOTE**: A larger volume may be added if needed for volume of testing |
| 3 | Centrifuge for 1 minute |
| 4 | Decant supernatant |
| 5 | Resuspend the cells using saline to a 3 to 4% cell suspension |
| 6 | Perform daily reagent QC prior to patient testing |

**CALCULATIONS/INTERPRETATIONS/RESULTS REPORTING/NORMAL**

**VALUES/CRITICAL VALUES**

**Interpretation:**

NA

**Results Reporting in Sunquest:**

| **STEP** | **ACTION** |
| --- | --- |
| 1 | Access the sample accession in Blood Order Processing(BOP) |
| 2 | Enter the following:   |  |  | | --- | --- | | **In the “Add Spec. Test window enter test code** | **Then enter result** | | ABI | DARA | | DTT | Billed for services performed (autopopulates) |   **EXAMPLE:**  \\lapis\Home$\Q-Z\senn\Capture.PNG |

**CALIBRATION:**

NA

**PROCEDURE NOTES AND LIMITATIONS:**

* Treatment of red cells with 0.2 M DTT will denature or weaken all antigens of the Kell, Cartwright, LW, Dombrock, and Knops systems.
* It has been shown that this method does not affect RBC's coated with IgG and complement.

**REFERENCES:**

* Reid, ME. Autoagglutination dispersal utilizing sulfhydryl compounds. Transfusion 1978; 18:353-355
* Immunohematology Methods and Procedures, 1st Ed., American Red Cross National Reference Laboratory, Rockville, MD 1993
* Technical Manual. Bethesda, MD: AABB Press, current edition
* Judd’s Methods in Immunohematology. Bethesda, MD: AABB Press, current

**RELATED DOCUMENTS:**

FORM DTT Treated Reagent Red Blood Cell Log

FORM Reconstituted 0.2M DTT Log

FORM DTT Reagent QC

SOP Antibody Screen

SOP Antigen Typing

SOP Grading Reactions

**APPENDIX:**

NA

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| --- | --- | --- | --- |
| **UWMC SOP Approval:** | | | |
|  |  |  |  |
| **UWMC CLIA Medical Director** |  |  |  |
|  | Mark H. Wener, MD | Date |  |
|  |  |  |  |
| **Transfusion Service Manager** |  | Date |  |
|  | Nina Sen |  |  |
|  |  |  |  |
| **Compliance Analyst** |  | Date |  |
|  | Christine Clark |  |  |
| **Transfusion Service**  **Medical Director** |  | Date |  |
|  | Monica Pagano, MD |  |  |
|  |  |  |  |
| **UWMC Biennial Review:** | |  |  |
|  |  |  |  |
|  |  | Date |  |
|  |  |  |  |
|  |  | Date |  |
|  |  |  |  |

03/19/2018 – Minor edits made to SOP after validation to provide clarity. Results reporting and billing added. Forms added.