DTT’S DARA DILEMMA

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OBJECTIVES

- Define purpose of Daratumumab administration.
- Understand utility of DTT in the blood bank.
- Discuss long term storage of DTT treated red blood cells.

WHAT’S YOUR NAME?

- Daratumumab
  - DAR a TOOM u e mab
- Darzalex
  - DAR za lex
- Dara

  Just not DORA...
WHY DO BLOOD BANKS KNOW DARA?

- Multiple Myeloma patients
  - Multiple myeloma is the third most common blood cancer in the U.S., following only leukemia and lymphoma.

DARZALEX® is a prescription medicine used to treat a type of cancer called multiple myeloma in people who:

- Have received at least 3 prior medicines to treat multiple myeloma, including a proteasome inhibitor (PI) and an immunomodulatory agent, or
- Did not respond to a proteasome inhibitor (PI) and an immunomodulatory agent

HOW DOES DARA WORK?
WHAT DOES DARA LOOK LIKE IN BLOOD BANK TESTING?
- Positive Antibody Screen
- RBC Panel: Panreactivity
- Incompatible Crossmatches with all Units
- Unable to Adsorb Away

NOW WHAT?
- Autoantibody??
- Antibody to High Frequency Antigen??
- Underlying Alloantibodies??
- Enzyme treatment??
- Molecular Testing??

- WHICH DIRECTION DO WE GO???
DTT = Dithiothreitol
- DL-Dithiothreitol
- ≥98% (HPLC), ≥99.0% (titration)
- Synonym: threo-1,4-Dimercapto-2,3-butanediol, Cleland’s reagent, DTT

DTT USES
- Breaks disulfide bonds
- Distinguishes between IgM and IgG antibodies
- Elimination of Kell group antigens
- Alters JMH, McC\(^a\), Yk\(^a\), Lutheran, Yt\(^a\), Lw\(^a\), LW\(^a\), Dombrock and Cromer group antigens

DTT TREATMENT PROCESS
BLOOD BANK TECHNICAL MANUAL, 18TH EDITION
- Reagents Required
  - Dissolve 1 gram of DTT in 32.4mL of phosphate buffered saline with a pH of 8.0. Divide excess into 1 mL aliquots and freeze at -18C.
  - PBS at pH 7.3
  - Red blood cells known to be positive for the antigen to be disrupted as controls.
  - Control antisera from manufacturer or patient sample form (example, Anti-K)
**DTT TREATMENT PROCESS**
**BLOOD BANK TECHNICAL MANUAL, 18TH EDITION**

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Combine four volumes of the prepared DTT volumes with one volume of PBS-washed packed red cells to be treated.</td>
</tr>
<tr>
<td>2</td>
<td>Incubate at 37°C for 30-45 minutes mixing every 5 minutes</td>
</tr>
<tr>
<td>3</td>
<td>Wash four times with PBS. Hemolysis may occur; if hemolysis is excessive repeat procedure using fresh red blood cells and a smaller volume of DTT.</td>
</tr>
<tr>
<td>4</td>
<td>Resuspend the cells to a 2%-5% cell suspension</td>
</tr>
<tr>
<td>5</td>
<td>Test DTT treated cells with serum containing the antibody in question. Test K+ cells with Anti-K.</td>
</tr>
</tbody>
</table>

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**WHAT ARE YOUR CONCERNS WITH THIS PROCEDURE IN YOUR LAB?**

MY CONCERNS...

- 8.0 Saline
- Tech Time
- Technique
- Question.....STABILITY
MY SBB PROJECT

- Can DTT treated RBCs be stored??
- Storage Solution Options?
- DTT-treated RBC panels are not commercially available.
- Are common clinically significant RBC antigens stable long term?
- 7.3 Blood Bank Saline only?

MATERIALS/METHODS

- Antigen Testing
  - Set 1
    - DTT-treated RBCs tested with antisera.
    - Double-dose RBC
    - Single-dose RBC
    - Known negative for the selected antigens
    - Anti-D Series 4, Anti-C, Anti-c, Anti-E, Anti-e, Anti-Jk*, Anti-Fy*, Anti-Fy®, Immucor. Anti-M and Anti-S were obtained from human sources provided by a blood center.
    - Panel was stored in Alserver’s Solution at 2-8°C.
    - The panel was manually washed each day with normal saline, and then re-suspended in pH 7.3 PBS prior to antigen testing.
MATERIALS/METHODS

- **Antigen Testing**
  - Set 2: DTT-treated RBCs were stored in pH 7.3 PBS.
  - Set 3: DTT-treated RBCs were stored in Alsever's solution.

Set 2 and Set 3 were visually compared daily for 14 days for observation of any indication of hemolysis. All three sets were refrigerated (2-8°C) in between testing.

RESULTS

- Antigen testing was performed following package inserts for commercial antisera. For human sources, Anti-S and Anti-M, indirect antihuman globulin testing was performed using PEG to detect the antigens.

14 DAY STABILITY

<table>
<thead>
<tr>
<th>Antigen</th>
<th>DTT-treated Double-Dose Reactivity</th>
<th>DTT-treated Single-Dose Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0 1 2 3 4 5 6 7 8 9 1</td>
<td>0 1 2 3 4 5 6 7 8 9 1</td>
</tr>
</tbody>
</table>
RESULTS

- Rh blood group = higher reaction strengths for longer period.
- All antigen reactivity remained at a 2+ strength or greater.
- Antigens tested are stable following DTT-treatment when stored in Alsever’s solution for 14 days.
- Sets 2 and 3 monitored for hemolysis notation.
- By Day 8, complete hemolysis was displayed in Set 2. Set 3, never displayed any hemolysis throughout the 14 day investigation.

RESULTS

- Double dose and single dose antigens monitored.

- The baseline grade (day 0) at day 14 were compared to grades at day 1. These data show that there were no grades >2 difference. This was not significant (p=1.0).

| Day 1 and Day 14 Grade Comparison with Baseline Grade (Day 0) DTT-treated Single Dose Reactivity |
|----------------------------------|------------------|-----------------|-------------------|
| All 10 Antigens equal or greater than baseline | Day 1 | Day 14 | P value |
| All 10 Antigens within 1 Grade | 10/10 | 9/10 | 1.0 (n.s.) |
| All 10 Antigens within 2 Grades | 10/10 | 10/10 | 1.0 (n.s.) |

| Day 1 and Day 14 Grade Comparison with Baseline Grade (Day 0) DTT-treated Double Dose Reactivity |
|----------------------------------|------------------|-----------------|-------------------|
| All 10 Antigens equal or greater than baseline | Day 1 | Day 14 | P value |
| All 10 Antigens within 1 Grade | 8/10 | 5/10 | 0.35 (n.s.) |
| All 10 Antigens within 2 Grades | 10/10 | 10/10 | 1.0 (n.s.) |
RESULTS

Baseline Comparison of Grade Changes with Dosage.
*p<0.05 for comparison with double dose reactivity.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Equal or Greater than Baseline</th>
<th>1 Grade Less Than Baseline</th>
<th>2 Grades Less Than Baseline</th>
<th>More than 3 Grades Less Than Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double Dose</td>
<td>130</td>
<td>72</td>
<td>48</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Single Dose</td>
<td>130</td>
<td>51*</td>
<td>74*</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>260</td>
<td>123</td>
<td>122</td>
<td>15</td>
<td>0</td>
</tr>
</tbody>
</table>

Baseline Comparison of Grade Changes with Dosage.
*p<0.05 for comparison with double dose reactivity.

RESULTS

- Sets 2 and 3
- Day 3, Set 2 displayed slight hemolysis.
- Day 8, Set 2 displayed complete hemolysis.
DISCUSSION

- There is a significant need to establish antigen stability of DTT-treated RBCs.
- DARA patient antibody work-ups are becoming more common in IRLs and are very timely procedures.
- The literature by Fung et al documents the use of PBS with an 8.0 pH. In this study, the use of PBS with a pH of 7.3 was used. This decision was made based on the notation in the package insert stating that PBS pH of 6.9-7.5 should be used for antigen typing.
- Commercial monoclonal and polyclonal antisera, as well as human-source antibodies were used in this study. A variation in the source of antisera did not prove a significant difference in reaction strengths over time.
- Anti-Jk\(^\text{a}\) was not used due to being unavailable at the start of testing.
- Both single and double dose antigens display no significant change in stability over the 14 day time frame.
- This practice would be beneficial to laboratories seeing DARA patient while also using the RBCs to resolve other complex antibody cases. This practice is not limited to the patient population being treated with DARA.
- When using DTT-treated RBCs one must remember that the Kell and Lutheran blood groups are destroyed. A policy would be needed by the facility on how this situation would be honored.

REFERENCES


Darzalex.com

Transfusion
2016 Vol. 56 Supplement
SP261
DTT’s DARA Dilemma

Immunohematology
2017 Volume 33
Stability guidelines for dithiothreitol-treated red blood cell reagents used for antibody detection methods in patients treated with daratumumab
Questions? 
Concerns? 
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There are other drugs coming down the pipeline that also have the ability to interfere with some blood banking methods. Have you experienced any of them besides Anti-CD38?

ADVANTAGES OF MOLECULAR TECHNOLOGY

- Helps prevent mismatches that can cause potentially life-threatening reactions
- Enables routine characterization of donor units for the most relevant antigens
- Create detailed phenotypic profiles of patients to store as critical component of the medical health record
- Determines true antigen make-up of recently transfused patients

TEST OF RECORD

- FDA-approved IVD test eliminates need for confirmatory testing
  - Difficult to source serological reagents for some of PreciseType™ test antigens
- FDA guidelines state that RUO tests cannot be used for patient diagnosis
Run 96 samples in a single work shift

Provides 96 tests in two formats:
- 8 chip slides (12)
- 96-chip microplate

Automated read, interpretation, and reporting

- Identifies 35 red blood cell antigens and 3 phenotypic variants from 11 blood groups simultaneously
- Detects 24 gene mutations and one polymorphism associated with hemoglobinopathies (HgbS)*

*HgbS is not intended for diagnosis of sickle cell disease

- The first and only FDA approved in-vitro diagnostic for molecular typing of red blood cell antigens
- Rapidly detects genotypes for accurate prediction of phenotypes
- Simplifies the identification of rare antigens
- Tests for a wide range of genetic variants affecting RBC antigen expression
QUESTIONS/CONCERNS

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