Dithiothreitol treatment of red blood cells

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Dithiothreitol (DTT), a reducing reagent, has multiple applications in blood bank testing. DTT disrupts the bridging of the disulfide bonds between amino acid residues necessary for structural conformation of some proteins and the bonds holding an IgM molecule in the pentameric formation. DTT treatment of red blood cells (RBCs) can denature or modify certain blood group antigens—in particular, those in the Kell, Lutheran, YT, JMH, LW, Cromer, Indian, Dombrock, and Knops systems—and prevent recognition by the corresponding antibodies. It also destroys RBC CD38, allowing DTT-treated RBCs to be used to avoid testing interference by therapeutic anti-CD38 preparations. DTT treatment can be used to disperse spontaneous agglutination of RBCs caused by heavy IgM autoantibody coating that invalidates ABO/Rh cell grouping and direct antiglobulin tests. ***Immunohematology* 2017;33:170–172.**

Key Words: dithiothreitol (DTT), indirect antiglobulin test, Kell system, daratumumab

Principle

Dithiothreitol (DTT) is a reducing agent capable of irreversibly cleaving accessible disulfide bonds when the solution pH is >7. DTT treatment of red blood cells (RBCs) will modify the tertiary structure of protein-based erythrocyte membrane antigens if their confirmation depends on disulfide bonds.[1] Antigens of the following blood group systems are destroyed or weakened by 0.2 M DTT treatment: KEL, IN, JMH, YT, LU, MER2, KN, DO, CROM, and LW.[2,3] Antibodies directed at antigens in these systems will not react or will be significantly weaker with the treated RBCs.

DTT will also cleave the disulfide bonds that connect the monomeric subunits and the J chain of the IgM antibody pentameric form. When heavy coating of RBCs with IgM autoantibody causes spontaneous agglutination, DTT treatment will disrupt the IgM structure and disperse the agglutination.

Indications

DTT-treated reagent RBCs can be used in antibody identification to suggest the possible blood group specificity of an unidentified antibody based on whether reactivity is affected by the treatment. When a known antibody is directed at a DTT-sensitive antigen, treated RBCs may be used to detect or exclude underlying antibodies to DTT-resistant antigens.

Treatment of autologous RBCs with 0.01 M DTT can resolve spontaneous agglutination due to potent IgM autoantibodies that interfere with ABO/Rh and direct antiglobulin testing.[4]

Monoclonal anti-CD38 (daratumumab), approved by the U.S. Food and Drug Administration for treatment of multiple myeloma, targets the CD38 antigen on malignant plasma cells. CD38 is also weakly expressed on all normal RBCs, including those in RBC reagents used in pretransfusion testing. This expression complicates the identification of clinically significant RBC antibodies because the plasma/serum of such patients will react with most or all RBCs in antibody detection

Treatment to Destroy RBC Antigens

Reagents/Supplies

Reagents	Supplies
▪ PBS, pH 7.3	▪ Test tubes
▪ 0.2 M DTT	▪ Pipettes
▪ RBC test cell	▪ Centrifuge
▪ K+ RBCs (control cell)	▪ pH meter
▪ Anti-K (reagent or serum/plasma specimen)	▪ Water bath or 37°C incubator

RBC = red blood cell; PBS = phosphate-buffered saline; DTT = dithiothreitol.

Procedural Steps

0.2 M DTT Preparation

- Dissolve 1 g DTT in 32 mL PBS, pH 8.0. Adjust final pH of DTT to 8.0 with 0.1 M HCl/0.1 M NaOH as needed.
- Aliquot and store at –20°C or colder for up to 12 months.

Procedure

- Wash 1 volume of the test RBCs and control RBCs with PBS, pH 7.3. Decant.
- Add 4 volumes of 0.2 M DTT, pH 8.0.
- Incubate at 37°C for 30 minutes.
- Wash three to four times with PBS.
- Resuspend the cells to a 2–5 percent suspension in PBS.
- Test DTT-treated cells with serum containing the antibody in question. Test K+ treated RBCs with anti-K.

DTT = dithiothreitol; PBS = phosphate-buffered saline; RBCs = red blood cells.

Treatment to Disperse Spontaneous Agglutination

Reagents/Supplies

Reagents	Supplies
▪ PBS, pH 7.3 ▪ 0.2 M DTT ▪ 6% albumin	▪ Test tubes ▪ Pipettes ▪ Centrifuge ▪ Water bath or 37°C incubator

PBS = phosphate-buffered saline; DTT = dithiothreitol.

Procedural Steps

0.01 M DTT Preparation

▪ Dilute 1 volume 0.2 M DTT with 19 volumes PBS, pH 7.3.

Procedure

▪ Wash autologous RBCs at least three times with PBS and dilute to a 50% concentration in PBS.
▪ Add an equal volume of 0.01 M DTT to the RBC suspension.
▪ Incubate at 37°C for 15 minutes.
▪ Wash RBCs three to four times in PBS.
▪ Resuspend the RBCs to a 2–5 percent suspension.
▪ Test the treated RBCs with 6% albumin (immediate-spin test) to assess for dispersal of spontaneous agglutination.

DTT = dithiothreitol; PBS = phosphate-buffered saline; RBCs = red blood cells.

and identification tests performed by the indirect antiglobulin test.[5] Because CD38 configuration depends on disulfide bonds, DTT treatment of reagent RBCs is used to denature the red cell CD38 and avoid this interference.[6] A large volume of RBCs can be treated with DTT and stored in Alsever's solution, since the stability of common RBC antigens has been demonstrated for up to 14 days.[7] Serologic investigations within this time period are more efficiently performed when pretreated cells are available.

Procedure to Destroy RBC Antigens

In a properly labeled tube, place 1 volume of the patient's packed RBCs (approximately 50 µL). Wash the RBCs three times with phosphate-buffered saline (PBS), pH 7.3. Decant the supernatant completely after the last wash. Add 4 volumes of 0.2 M DTT (approximately 200 µL) to 1 volume of RBCs to be treated. Mix well, and incubate at 37°C for 30 minutes. Wash the RBCs four times with PBS. If marked hemolysis occurs, repeat the procedure with fresh RBCs and a smaller volume of DTT. Resuspend the RBCs to a 2–5 percent suspension if the testing is performed in the tube or to the concentration required for non-tube test methods. Assess the batch for completeness of treatment using anti-K as described in the quality control section. If the reactivity of the test serum is eliminated, appropriate RBC samples can be treated and tested to exclude other clinically significant alloantibodies.

Procedure to Disperse Spontaneous Agglutination

In a properly labeled tube, wash autologous RBCs three times in saline. Washing with warm saline can also aid in removing cold-reacting IgM autoantibodies. Dilute washed RBCs to a 50 percent suspension in PBS. Add an equal volume of 0.01 M DTT. Mix and incubate at 37°C for 15 minutes. Wash treated RBCs at least three times in PBS. Resuspend an aliquot of the RBCs to a 2–5 percent suspension or to the concentration required by the test method being performed. Test the RBCs for removal of the spontaneous agglutination as described in the quality control section.

Limitations

As in all serologic procedures, factors such as contaminated materials or inadequate incubation time, temperature, or centrifugation may produce false results. Preparation of the 0.2 M DTT at pH 8.0 is required for irreversible denaturation of antigens.[2] Reagent preparations at a lower pH may cause inadequate and reversible reduction of disulfide bonds. Treated RBCs cannot be typed for any antigens destroyed by DTT.

Antibodies to antigens destroyed or weakened by DTT cannot be excluded on nonreactive treated RBCs. Because anti-K is the most frequently encountered clinically important antibody in this group, K– RBCs should be selected for transfusion if the patient's RBCs are K– or the K antigen status is unknown, unless anti-K has been excluded in other tests.

Quality Control

When performing treatment to destroy RBC antigens, K+ RBCs should be DTT-treated with each test batch. The treated and untreated K+ RBCs are then tested with anti-K. The treated RBCs should be nonreactive; otherwise, the DTT treatment was not adequate. Other antigens of the Kell system can also serve as controls.[1]

If dispersal of spontaneous agglutination is being performed, test the treated RBCs using 6 percent albumin. No agglutination should be present if the treatment was successful.

Summary

DTT treatment of RBCs is an informative method to aid in antibody identification and to determine whether a serum contains additional alloantibodies when an antibody to a DTT-sensitive antigen is present. It also destroys CD38 on the RBC surface, thereby avoiding interference from anti-CD38 in pretransfusion testing. Donor units typed as K– must be transfused when antibody detection/identification has been performed using DTT-treated RBCs, unless anti-K is excluded by another method.

Spontaneous agglutination of autologous RBCs causing false-positive results in ABO/Rh and direct antiglobulin testing can be resolved by using DTT to degrade the IgM antibody coating the RBC.

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