1. **Principle**

The action of DTT on blood group antigens is unknown. It is felt that the DTT disrupts sulfhydryl bonds, and therefore alters the binding sites of some blood group antigens.

1. **Scope and Related Policies**
	1. DTT‑treated red cells will not react with antibodies in the Kell blood group system, as well as some examples of anti‑Ge, ‑Yta, -Doa, -Dob and other HTLA antibodies. This procedure may be helpful in identifying some of the above antibodies, and to determine if additional underlying alloantibodies are present.
2. **Specimen**

EDTA anticoagulated whole blood preferably less than 72 hours old.

1. **Material**

**Equipment:** Serological centrifuge

 Block for test tubes

Water bath/Heating block at 37°C

**Supplies:** Test tubes – 10 x 75 mm

 Serological pipettes

**Reagents:** 0.2M DTT (pH 8.0)

0.9% saline

1. **Quality Control**
	1. Test treated cells with the appropriate plasma (see 2.0 - Scope and Related Policies – plasma containing identified antibodies) to determine inactivation of selected antigen(s) and lack of deterioration of unaffected antigens.
	2. Anti-k may be used as a positive control as Kell system antigens are known to be destroyed by DTT.
2. **Procedure**

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| * 1. Wash one volume of red cells with saline.
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| * 1. To one volume of the washed, packed red cells, add 4 volumes of 0.2M DTT (pH 8.0).
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| * 1. Incubate at 37°C for 30 minutes.
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| * 1. Wash four times in normal saline.
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| * 1. Resuspend red cells to a 3% suspension in saline.
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| * 1. Test DTT‑treated red cells with the appropriate patient plasma and control anti-sera.
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1. **Reporting**

Interpretation:

* 1. The tests are read and recorded as usual.
	2. If used as a control the k+ cells should give negative reactions with anti‑k if the cells were treated properly.
	3. If the plasma reactivity is eliminated, test sufficient red cells to exclude the presence of most clinically significant alloantibodies.
1. **Procedural Notes**
	1. Slight hemolysis might occur. If excessive hemolysis does occur, decrease the concentration of DTT.
	2. Treat a control cell each time samples are treated. The control could be a k+ cell. The cells are adequately treated, if the DTT‑treated k+ cells are negative when tested with anti‑k.
	3. Reactivity with anti‑LW is eliminated when one drop of LW+ washed packed red cells is incubated with 2 drops of 0.1 M DTT at 37°C for 30 min. Longer incubations, or higher concentrations of DTT may be required for strong examples of the antibody.
2. **References**
	1. Roback, JD. ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: pg 912-913.
	2. Judd, WJ ed. Judd’s Methods in Immunohematology, 3rd ed., Bethesda, MD: pg. 273-274.
3. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual
* Revised wording of section 1.0
* Replaced “red cells” with “whole blood” in section 3.0
* Replaced “normal saline” with “0.9% saline” in section 4.0- *Reagents*
* Revised wording to include “as Kell system antigens are known to be destroyed by DTT” in section 5.2
* Revised wording to include “patient plasma and control anti-sera” in section 6.6
* Updated list of references to include most recent editions
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