1. **Principle**

Thiol reagents such as DTT cleave interchain disulfide bonds of IgM molecules and abolish the agglutinating ability of IgM antibodies while leaving IgG molecules unaffected.

DTT is used to differentiate between IgM and IgG antibodies by inactivating IgM antibodies allowing identification of any unaffected IgG antibody.

1. **Scope and Related Policies**
   1. DTT treatment of test serum, plasma or eluate can aid in antibody investigation where there may be a mixture of IgM and IgG antibodies.
2. **Specimen**

Plasma, serum or eluate

1. **Material**

**Equipment:** Cell Washer

Serological centrifuge

Block for test tubes

Microscope

Water bath/Heating block at 37°C

**Supplies:** Test tubes – 10 x 75 mm

Serological pipettes

SP.029F Plasma Treatment with DTT - Worksheet

**Reagents:** Phosphate buffered saline (PBS), pH 7.3

0.01 M DTT: 0.154 g DTT diluted to 100 mL in PBS. Alternatively, a 1 in 10 dilution in PBS of 0.1 M DTT (0.154 g DTT in 10 ml PBS) can be made. Aliquots of 0.01 M DTT can be frozen in glass at –20°C for up to 6 months.

Control IgM antibody (e.g. high titer anti-I)

Control IgG antibody

Appropriate test RBCs (determined by specificity of antibody under investigation)

Anti-IgG and IgG sensitized control cells

1. **Quality Control**
   1. A known IgM antibody and a known IgG antibody tested in parallel with the test plasma will provide assurance that the thiol reagent is working properly. The IgM antibody reactivity should be abolished and the IgG antibody should still be detected.
   2. Repeat treatment and testing of the plasma (test and controls) if the controls do not react as expected.
   3. Repeat the test if the antiglobulin control cells are nonreactive.
   4. Results are to be recorded on SP.029F- Plasma Treatment with DTT - Worksheet
2. **Procedure**

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| --- |
| For each sample and control, mix an equal volume of plasma and DTT in a tube (e.g. 2 drops of plasma + 2 drops of DTT). |
| * 1. For each sample and control,mix an equal volume of plasma and PBS as a dilution control in a separate tube (e.g. 2 drops of plasma + 2 drops of PBS). |
| * 1. Incubate at 37°C for 30 minutes. Treatment with DTT may be extended up to 2 hours. |
| * 1. Test 4 drops of the treated and dilution control plasma (in parallel) with 1 drop of the appropriate RBCs. |
| * 1. If possible test antigen negative RBCs with the DTT/plasma mixture if indirect antiglobulin testing is to be performed. |
| * 1. Incubate the tubes for 30-60 minutes at the temperature appropriate for the reactivity (e.g. room temperature or 37°C) centrifuge and read for agglutination. Record results. |
| * 1. If the antibody is 37°C and/or antiglobulin reactive, wash the RBCs x 4 for the antiglobulin test. Observe for agglutination prior to adding anti-IgG if agglutination was seen in a previous phase. |
| * 1. Add anti-IgG, centrifuge and read. Record results. |
| * 1. All negative reactions should be confirmed by the addition of IgG sensitized control cells. If a negative reaction is obtained with the control cells that tube must repeated prior to reporting the results. |

1. **Reporting**

# Interpretation:

# Equivalent reactivity in both the treated and dilution control plasma indicates the presence of an IgG antibody, but a mixture of IgM and IgG antibodies may not be obvious by direct testing and may require titration to detect a reduction in antibody activity.

* 1. Decreased reactivity of the treated plasma when compared to the reactivity of the dilution control plasma may indicate only partial inactivation of IgM rather than the presence of an IgG component.

Examples and interpretations of diluted plasma:

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| --- | --- | --- | --- | --- | --- |
|  | Plasma dilution | | | | Interpretation |
| 1/2 | 1/4 | 1/8 | 1/16 |
| Plasma + DTT | grade 3 | grade 2 | grade 1 | 0 | IgG |
| Plasma + PBS | grade 3 | grade 2 | grade 1 | 0 |
| Plasma + DTT | 0 | 0 | 0 | 0 | IgM |
| Plasma + PBS | grade 3 | grade 2 | grade 1 | 0 |
| Plasma + DTT | grade 1 | 0 | 0 | 0 | IgG + IgM or partial inactivation of IgM |
| Plasma + PBS | grade 3 | grade 2 | grade 1 | 0 |

* 1. A lack of reactivity in the treated plasma and reactivity in the dilution control plasma indicates the presence of an IgM antibody.
  2. No reactivity in either the treated or dilution control plasma indicates that dilution of the antibody has occurred and no conclusion can be made.
  3. For the test to be valid, the IgM antibody control should not demonstrate any reactivity and the IgG antibody control should demonstrate reactivity

1. **Procedural Notes**
   1. Gelling of plasma samples may occur during DTT treatment if the concentration is greater than 0.01 M or upon extended incubation. Gelled samples cannot be tested for antibody activity. If gelling occurs, try repeating treatment with 0.005 M DTT (dilute 0.01M DTT 1 in 2 in PBS); it is important to show that the IgM control antibody is denatured with this concentration of DTT.
   2. Test 4 drops of treated and dilution control plasma to compensate for the dilution effect.
   3. PBS-DTT loses its reducing ability upon storage at 4°C, but is stable when stored at –20°C for at least 6 months. Storage in glass improves stability when compared to plastic containers.9.2
   4. Thiol reagents diminish complement-binding activity of antibodies. If it is important to demonstrate that complement is bound, incubate the DTT-treated plasma with the appropriate RBCs, wash the RBCs and reincubate with fresh normal plasma as a source of complement, and proceed to the antiglobulin test with anti-C3 or anti-human globulin. Alternatively, dialyze the treated and dilution control plasma overnight, and test with and without the addition of fresh normal plasma as a source of complement.
2. **References**
   1. Roback, JD. ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011 pg. 912-913
   2. Pirofsky B, Rosner ER. A new method to differentiate IgM and IgG erythrocyte antibodies. Vox Sang 1974; 27:480-8.
3. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual * Revised sections 1.0 & 2.0 * Added “and IgG sensitized control cells” to section 4.0- *Reagents* * Added “e.g. 2 drops of plasma + 2 drops of PBS” in section 6.2 * Added the last row to the table in section 7.2 * Updated list of references to include most recent editions |