1. **Principle**

Characteristically, HTLA antibodies react at high dilutions in antiglobu­lin tests, but the reactions are not strong (grade 2 or less). In contrast, other undiluted antibodies (e.g. anti-D and anti-Fya that react grade 2 or less in antiglobulin tests) will usually have titres less than 8.

1. **Scope and Related Policies**
   1. As an initial screen test for HTLA antibodies a 1/10 dilution of plasma will be tested against weakly reactive red cells by IDAT.
2. **Specimen**

Patient plasma diluted 1/10 with bovine serum albumin (BSA)

1. **Materials**

**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

**Reagents:** Minimum of 3 test cells

0.9% saline

Anti-IgG

IgG-coated cells

6% BSA

1. **Quality Control**
   1. Ideally the three test cells will be selected on the basis of their reactivity with test plasma (e.g., grade 2, grade 1, w).
2. **Procedure**

**Screen Procedure**

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| * 1. For a quick screen procedure for HTLA antibodies, test a 1 in 10 dilution of patient's plasma in 6% BSA with the test cells by an antiglobulin technique. If reactions are the same as undiluted plasma, the presence of HTLA antibody is indicated. |

**Titre Procedure**

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| * 1. Prepare serial two-fold dilutions of test plasma in 0.9% NaCl from 1 in 2 to 1 in 4096 (12 tubes) with a minimum volume of 0.6 mL. Refer to SP.002 – Titrations. |
| * 1. Place 3 drops of each dilution into each of 3 appropriately labeled 10 x 75 mm test tubes. |
| * 1. Test each dilution against the three cells by adding 1 drop of a 3% suspension of cells to appropriate tube. |
| * 1. Mix contents and incubate at 37°C for 1 hour. |
| * 1. Wash red cells 4 times in 0.9% NaCl and test with anti-IgG. |
| * 1. Examine microscopically for agglutination. |
| * 1. Record results. |
| * 1. Add IgG coated check cells to any negative tubes, centrifuge and read. The test must be repeated for any negative tubes. |

1. **Reporting –** 
   1. Plasma that continues to react at 4 dilutions beyond that which gives a grade 1 reaction can be considered to contain an HTLA antibody.

If identification of the antibody is required additional testing specific for this type of antibody will be required.

* 1. Cells that give variable reactions with undiluted plasma should be tested to determine whether dilution has the same effect on all reaction strengths. If some red cells react only or preferentially with low plasma dilutions, the plasma may also contain non-HTLA antibodies. Additional testing should be performed with the dilute plasma against a commercial cell panel. See NRT.007- Antibody Identification of Warm Reactive Antibodies.

1. **Procedural Notes**
   1. HTLA antibodies will generally demonstrate titres of 64 or greater with consistently weak reactivity.9.1

1. **References**
   1. Roback, JD. ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011 pg. 480-481, 907-909.
2. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual * Revised wording in section 3.0 to include “bovine serum albumin” * Replaced “Normal saline” with “0.9% saline” in section 4.0- *Reagents* * Added “Refer to SP.002- Titrations” in section 6.2 * Revised and renumbered sections 7.0 & 8.0 * Updated list of references |