1. **Principle**

Treatment of red cells with 6% AET (2-aminoethylisothiouronium bromide) inactivates the Kell antigens (except KX) and artificially creates KoKo red cells. AET may also reduce the strength of the following high titer, low avidity (HTLA) antigens JMH, Kn(a), McC(a), McC(d), Hy, Gy(a), Yk(a), as well as Yt(a), Yt(b), Vel and LW(a) antigens.

AET treated cells may be used to aid in antibody investigation.

1. **Scope and Related Policies** 
   1. To determine whether antibodies reacting with a high incidence antigen have specificity within the Kell system.
   2. To help identify additional alloantibodies in plasma containing some HTLA or Kell system antibodies.
2. **Specimen**

EDTA anticoagulated whole blood

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

**Reagents:** Phosphate buffered saline

0.9% saline

Washed packed cells for treatment (or a cell panel)

Anti-IgG (see Procedural Notes 8.1)

IgG-coated cells

6% AET solution – prepare immediately before use

Anti-K or anti-k reagent

Alsevers Solution

1. **Quality Control**
   1. AET treated cells should be tested with a known anti-K (K+ treated cells) or with a known anti-k (k+ treated cells) to ensure the Kell antigens have been destroyed.
2. **Procedure**

**Treatment of red cells:**

|  |
| --- |
| * 1. Mix one volume of washed packed red cells with four volumes of 6% AET solution.   To treat panel cells, take 10 drops (3-5%), wash x 2, and add 2 drops of 6% AET to packed red cells. |
| * 1. Incubate at 37°C for 20 minutes, mixing every 5 minutes. |
| * 1. Reaction is stopped by washing cells with phosphate buffered saline at pH 7.0. Subsequent 3-4 washes can be done with 0.9% saline. Treated cells have a brownish colour. |
| * 1. AET treated cells can be stored in Alsevers solution for 7 days. |
| * 1. Prior to use prepare a 3% saline cell suspension. |

**Test procedure:**

|  |
| --- |
| * 1. Label the number of 10 x 75 mm test tubes required to test the patient's plasma against the AET treated red cells and the same red cells untreated. |
| * 1. Add 3 drops of patient's plasma to each tube. |
| * 1. Add 1 drop of 3% cell suspensions (treated and untreated) to the appropriately labelled tube. |
| * 1. Incubate at 37°C for 1 hour. |
| * 1. Wash a minimum of 3 times with saline. |
| * 1. To dry red cell button, add 2 drops of anti-IgG and mix. |
| * 1. Centrifuge at 3400 rpm for 10 -15 seconds, resuspend gently, read microscopically, 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 of Results:

|  |  |  |
| --- | --- | --- |
| Reactivity With: | | |
| RBC's-AET  Treated | RBC's  Untreated | Possible Antibody Specificity |
| + | + | Not within Kell system |
| O | + | Within Kell system |
| + or O | + | Anti-JMH, -Kna, -McCa, -McCd, -Gya,  -Yka, -Yta, -Ytb, -VEL |
| + | + | Anti-Kx, -S, -M |

+ = Reactivity

O = No reactivity

 = Reduced

 = Enhanced

See Table SP.009-1, on page 5, for a list of the effects of AET on some antigens.

1. **Procedural Notes**
   1. Red cells treated with AET bind complement components in a non-specific manner. Use anti-IgG reagents when performing antiglobulin tests with AET treated cells.
   2. To assure that AET is inactivating Kell system antigens, test the AET treated red cells with appropriate anti sera (e.g. k+ red cells test with anti-k).
   3. Tests may be performed by LISS - LIDAT methods.

1. **References**
   1. Judd, WJ ed. Judd’s Methods in Immunohematology, 3rd ed, Bethesda, MD: pg. 271-272
   2. Roback, JD. ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: pg 912-913.
2. **Revision History**

|  |  |
| --- | --- |
| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual * Changed document number from SP.010 to SP.009 * Revised sections 1.0 and 4.0 * Revised wording to include “EDTA anticoagulated whole blood” in section 3.0 * Revised and renumbered section 6.0 * Updated list of references to include most recent editions * Revised and renamed Table SP.009-1 * Revised list of references for Table SP.009-1 |

# Table SP.009-1: EFFECTS OF AET ON ANTIGENS

|  |  |  |
| --- | --- | --- |
| **UNAFFECTED** | **DENATURED or ALTERED** | **ENHANCED** |
| ABH | K, k | K15 |
| Rh, (C, Cw, D, c, E, e, Rh 29) | Kp(a) Kp(b) Kp(c) | Lea OCH findings |
| Lewis (Lea, Leb) | Ku | \*Leb |
| P1 | Jsa, Jsb | Duffy (Fya) |
| MNSs | K17, 11 | e |
| Kidd (Jka, Jkb, Jk3) | U1(a) | c |
| Fya, Fyb | K12, 13, 14, 18, 19 | C |
| Ii | K20, 22 |  |
| Sd(x) | MacLeod |  |
| Sp1 | Kna |  |
| Ge | Yka |  |
| Dia , Dib | Hy |  |
| Jra | Gy |  |
| Sc:1, SC:2 | McCa |  |
| Ata | LWa, LWb |  |
| Wra | Lutheran system antigens |  |
| Cha | Dombrock system antigens |  |
| Rga | Cromer system antigens |  |
| En(a) | JMH |  |
| Xg(a) | Yta, Ytb |  |
| Lan |  |  |
| Vel |  |  |
| Coa , Cob |  |  |
| Cs |  |  |

\* Enhancement and/or hemolysis noted with some samples.

Ref: 1. Berger, R.: National Reference Laboratory, CRCS, Toronto

2. Roback JD ed. AABB Technical Manual 17th ed. Bethesda MD; 2011:476.