1. **Principle**

Red blood cells coated with IgG are first thoroughly washed, then suspended briefly in an EDTA glycine-acid (EGA) solution to dissociate the bound antibody. The mixture is immediately brought to a neutral pH with TRIS Buffer; the cells are separated by centrifugation and further washed with three changes of saline. If the direct antiglobulin test (DAT) is negative, the washed red blood cells are ready to be tested for their surface antigens by the antiglobulin test (or with a reagent containing potentiators). The treatment inactivates the antigens of the Kell blood group system, as well as Era, Bg, and possibly others. This reagent does not denature enzyme-sensitive antigens such as those of the MNSs and Duffy systems. It has been reported that the EGA method is the procedure of choice for typing red blood cells coated with warm-reactive IgG alloantibodies or autoantibodies.

1. **Scope and Related Policies** 
   1. Red blood cells coated with immunoglobulin, as in autoimmune hemolytic anemia or hemolytic disease of the newborn, can be expected to have a positive DAT. Accordingly, they cannot be tested for their surface antigens by means of the indirect antiglobulin test, nor with reagents containing potentiators of agglutination if their IgG coating is sufficient to cause spontaneous agglutination under such conditions.
   2. The ability to dissociate immunoglobulin from the cells without impairing surface antigen reactivity is of great value in enabling the cells to be typed as an aid to the recognition and identification of one or more alloantibodies coexisting with warm autoantibodies in a patient’s plasma.9.1, 9.2 & 9.3
2. **Specimen**

EDTA anticoagulated whole blood (positive DAT due to IgG).

1. **Materials**

**Equipment:** Serological centrifuge

Block for test tubes

Water bath/Heating block at 37°C

Timer

**Supplies:** Test tubes - 10 x 75 mm

Serological pipettes

0.9% Saline

**Reagents:** EGA Solution 1: A concentrated solution of sodium EDTA.

EGA Solution 2: A low-pH glycine solution. This solution contains no preservative.

EGA Solution 3: A TRIS (hydroxymethyl)-aminomethane solution

6% BSA (Inert control reagent)

Blood grouping reagents needed for the appropriate test procedures to be performed on the patient’s cells from which IgG has been dissociated

1. **Quality Control** 
   1. Proper controls are essential in the performance of all laboratory procedures. As proof that the antigen(s) to be tested for on the treated cells are not destroyed by the treatment, it is recommended that appropriate known antigen-positive red blood cells be treated in parallel with the IgG-coated cells and tested with the relevant reagent(s) for positive control purposes. This control may be omitted if the laboratory has previously confirmed (and has convincing documentation) that the relevant antigen(s) are not destroyed by the treatment.
   2. In addition, it is recommended that a parallel indirect antiglobulin test be carried out with an inert control reagent, such as 6% bovine albumin, as confirmation that the immunoglobulin coating has been successfully removed from the cells.
2. **Procedure**

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| * 1. Wash the coated (patient’s) red blood cells three times in saline and resuspend them to a concentration of 3%. |
| * 1. Place 30 drops of the suspension of washed patient red blood cells into a clean labeled test tube. |
| * 1. Centrifuge to pack the cells as completely as possible and carefully remove the supernatant without disturbing the cells. As a guide, one minute at 3400 rpm should pack the cells sufficiently. |
| * 1. In a separate test tube, prepare the EGA solution by adding 4 drops of EGA Solution #1 to 16 drops of EGA Solution #2. |
| * 1. Immediately add the freshly prepared EGA solution to the washed, packed red blood cells and mix gently. |
| * 1. Start a timer and allow the mixture to stand at room temperature (23ºC+ 3ºC) for no longer than two minutes. If the cells become markedly tanned or clumped after this much exposure, the treatment time may need to be shortened (in 15 second increments) until a level of treatment is achieved that does not result in clumping or marked tanning of the cells. |
| * 1. Immediately add 4 drops of EGA Solution #3, mix thoroughly and centrifuge for 30 seconds at 3400 rpm. |
| * 1. Remove and discard the supernatant, then resuspend the treated cells in 0.9% saline. If at this point the treated cells are not markedly tanned or clumped (see step 6.6), proceed to wash the cells with at least three changes of saline.   **Note:** While the supernatant removed from the treated cells may contain eluted antibody, this is substantially diluted. Accordingly, it is NOT RECOMMENDED that this should be treated as an eluate (e.g. used to determine the specificity of the coating antibody). |
| * 1. Carry out a DAT on the washed, treated cells. If negative, proceed to test the cells for the desired antigens by the indirect antiglobulin procedure, following the directions of the reagent manufacturer(s).   See NRT.009 - Antigen Typing.  ***Caution:*** *The EGA treatment causes red blood cells to show an increased tendency to hemolysis upon standing. Accordingly, if there is any delay between washing the cells and testing them for antigen status, further washing may be required before use.*  **Note:** If the direct antiglobulin test on the treated cells is still positive after one treatment, the treatment procedure may be repeated, but not more than one time. It should be noted that further exposure to acid conditions may cause permanent irreversible damage to the cell membrane. |

1. **Reporting**

# Interpretation of Results:

* 1. If the DAT is negative on the red blood cells after treatment, the cells may be tested for antigen status (other than for antigens known to be destroyed by the treatment) by the indirect antiglobulin procedure.

1. **Procedural Notes**
   1. Limitations: Factors that may yield misleading results include the following:
      1. Treatment of the red blood cells must be limited to the number of times recommended in the package insert. Prolonging incubation of the red blood cells in the EGA medium will alter the cell membrane irreversibly. Visible signs of overtreatment may be the development of a maroon-tan coloration and clumping.
      2. The treatment of red blood cells by this procedure renders the cells incapable of being typed for antigens of the Kell system. This feature may be used to advantage in a situation where plasma being investigated contains a mixture of antibodies that is suspected to include a specificity belonging to the Kell system. In such cases, uncoated cells typed for other antigens may be treated for use in antibody identification procedures.
      3. The Era blood group antigen and Bg have also been reported to be rendered inactive by EGA treatment. Other antigens may similarly be impaired. The only antigens that have been demonstrated not to have been denatured or destroyed by the treatment process are: M, N, S, s, D, C, E, c, e, Fya, Fyb, Jka and Jkb.
      4. The treatment procedure may not be successful in removing IgG completely from coated cells in all cases. Sometimes, the strength of the DAT can only be reduced, but sufficiently as to make possible the reliable interpretation of an indirect antiglobulin test. In other cases, the strength of the DAT reaction may not be perceptibly reduced. In two published reports, cells treated one or two times with solutions similar to those comprising the EGA Kit could not be used for phenotyping in 15% 9.1 and in 18% 9.2 of cases, respectively.
   2. The solutions comprising the Gamma EGA Kit have been tested by the procedure detailed in this direction insert and found to dissociate IgG from coated red blood cells. Dissociation is sufficient in most cases to enable the treated cells to be tested for their surface antigens (except for those destroyed by the treatment) using the indirect antiglobulin test. Uncoated cells are also shown to lose the Kell system antigens K and k as a consequence of the treatment.
2. **References**
   1. Judd, WJ ed. Judd’s Methods in Immunohematology, 3rd ed, Bethesda, MD: pg. 167-168
   2. Roback, JD. ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: pg 892-893.
   3. For additional information refer to current manufacturers insert for Gamma EGA Kit.
3. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual * Separated SP.012 into two documents: SP.011 and SP.012 * Revised wording to include “EDTA anticoagulated whole blood (positive DAT due to IgG)” in section 3.0 * Specified “0.9% Saline” in section 4.0- *Supplies* * Renumbered section 5.0 * Revised and renumbered sections 6.0 & 8.0 * Updated list of references to include most recent editions |