1. **Principle**

Enzyme treatment of red cells enhances reactivity of an antibody with the corresponding antigen by increasing antibody uptake onto red cells due to either exposure of latent antigen sites or decreasing stearic hindrance through the removal of carbohydrates and polypeptides from the red cell surface.

Reduction of reactivity of an antigen, with the appropriate antibody, can be due to total cleavage of the antigen site or to the removal of a constituent close to the reaction site which affects the stearic or charge configuration of the antigen so that it is no longer recognised by the antibody.

1. **Scope and Related Policies**
	1. Both freeze dried papain (FDP) and ficin may be used for enzyme treatment.
2. **Specimen**

EDTA anticoagulated whole blood.

1. **Material**

**Equipment:** Water bath/Heating block at 37°C

**Supplies:** Test tubes – 10 x 75 mm

 Serological pipettes

**Reagents:** BCA Freeze dried papain (FDP)

0.9% Saline

Alsevers solution

Anti-c

Anti-Fya

1. **Quality Control**

5.1 Appropriate controls must be tested with the enzyme treated cells to validate adequate treatments.

* 1. To be performed on working volume of enzyme treated red cell panel

or screen cells:

|  |  |
| --- | --- |
|  Serum |  Expected Reactions |
|  Dilute anti-c (w - grade 1) SIDAT, LIDAT |  Positive 37°C PIDAT |
|  Anti-Fya > grade 2 SIDAT, LIDAT |  Negative 37°C PIDAT |
|  \* Inert Plasma Negative SIDAT, LIDAT |  Negative 37°C PIDAT |

SIDAT Saline Indirect Antiglobulin Test

LIDAT LISS Indirect Antiglobulin Test

PIDAT Papain Indirect Antiglobulin Test

\*Inert Plasma or 6% BSA

1. **Procedure**

**Two-Stage (Indirect) Method Pre-treatment of Red Cells**

|  |
| --- |
| * 1. Reconstitute vial of FDP with 2 mL of 0.9% saline.
 |
| * 1. Label 10 x 75 mm tubes for enzyme pre-treatment.
 |
| * 1. Add 10 drops of a 3% suspension of washed x 2 cells to be treated, to the appropriate tube (include patient's own cells).
 |
| * 1. Add 1 drop of the reconstituted FDP. Mix.
 |
| * 1. Incubate at 37°C for ten minutes. Incubation time critical.
 |
| * 1. Wash the cells with saline a minimum of three times.
 |
| * 1. Prepare a 3% saline suspension of each of the treated cells.
 |
| * 1. See addendum for stock preparation of panel and antibody screen cells.
 |

**NOTE:**The following will be performed if a panel or screen cells are to be treated:

**Stock Preparation**: **Panel**

|  |
| --- |
| * 1. Wash entire contents of vial (3 mL) once with saline.
 |
| * 1. Reconstitute to 3% with saline.
 |
| * 1. Add 6 drops of reconstituted FDP.
 |
| * 1. Incubate at 37°C for 10 minutes.
 |
| * 1. Wash 3 times with saline.
 |
| * 1. Prepare 50% suspension in Alsevers (panel is good until expiry date).
 |

To Prepare **Working Panel**:

|  |
| --- |
| * 1. Take 1 drop of stock and wash once with saline.
 |
| * 1. Resuspend to 3% suspension in saline.
 |
| * 1. Control with anti-c and anti-Fya sera (refer to 5.2 above).
 |

**Stock Preparation: Antibody Screen Cells**: (In current use) 2 sets

|  |
| --- |
| * 1. Take 3 mL of each set. Prepare stock and working suspensions as for panel cells above.
 |
| * 1. **Test procedure**
 | * + 1. Label test tubes with the first three letters of the patient family name and the corresponding papain treated panel cell number and one additional tube for an autologous control.
 |
| * + 1. Prepare papain treated autologous cells as described in 6.1 – 6.7.
 |
| * + 1. Place 2 drops of patient’s plasma into each of the labelled tubes.
 |
| * + 1. Add 1 drop of each of the treated reagent cells and 1 drop of the treated patient cells to the appropriately labelled tubes.
 |
| * + 1. Mix the contents of each tube and incubate the tubes at 37°C for 15 minutes.
 |
| * + 1. Wash a minimum of three times with saline.
 |
| * + 1. Add 2 drops of anti-IgG to each tube.
 |
| * + 1. Mix and centrifuge the tubes at 3400 for 10 - 15 seconds.
 |
| * + 1. Gently resuspend each red cell button and examine macroscopically for agglutination. Record results.
 |
| * + 1. Confirm validity of all negative reactions with IgG – sensitized control cells.
 |

1. **Reporting**
	1. Absence of agglutination is a negative test result and indicates the absence of an antibody/antigen reaction.
	2. Negative tests are verified by the addition of IgG – sensitized control cells.
	3. Presence of agglutination or hemolysis is a positive test result and indicates the presence of an antibody/antigen reaction.
2. **Procedural Notes**
	1. See Table SP.008-1 (page 5) for the reactions of some antibodies with papain-treated cells.
3. **References**
	1. Roback, JD. ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011 pg 902-905.
	2. Manufacturers insert (current version) for freeze dried papain (FDP) in use
4. **Revision History**

|  |  |
| --- | --- |
| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual
* Added section 2.1
* Revised wording to include “Add 10 drops of a 3% suspension…” in section 6.3
* Changed 5% to 3% in section 6.10
* Specified “place 2 drops of patient’s plasma…” in section 6.19.3
* Updated incubation temperature to 37ºC in section 6.19.5
* Renumbered section 6.0
* Specified “IgG- sensitized control cells” in section 7.2
* Updated list of references to include most recent editions
 |

**Table SP.008-1:** The reactions of some antibodies with papain-treated cells by the papain indirect antiglobulin test (PIDAT).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ANTIBODY | REACTIVE | NONREACTIVE | ANTIBODY | REACTIVE | NONREACTIVE |
| Ata | X |  | Kpa (K3) | X |  |
| Bga, Bgb, Bgc | X |  | Kpb (K4) | X |  |
| C | X |  | Ku (K5) | X |  |
| c | X |  | Lan | X |  |
| ces (V) | X |  | Lea | X |  |
| Ch |  | X | Leb | X |  |
| Coa | X |  | Lsa | X |  |
| Cob | X |  | Lua | X |  |
| Cote (K11) | X |  | Lub | X |  |
| Cw | X |  | Lu3 | X |  |
| D | X |  | Lu8 | X |  |
| Dia | X |  | Lu11 | X |  |
| Dib | X |  | LW | X |  |
| Doa | X |  | M |  | X |
| E | X |  | McCoy  |  | X |
| e | X |  | Mg |  | X |
| Ena\*\*\* |  | X | Mur\* | X |  |
| Fya |  | X | N |  | X |
| Fyb |  | X | P1 | X |  |
| f | X |  | Rg |  | X |
| G | X |  | S |  | X |
| Ge | X |  | s |  | X |
| Gya | X |  | Sc1 (SM) | X |  |
| H | X |  | Sda | X |  |
| He\* | X\* |  | Sgro (K13) | X |  |
| Hy | X |  | Swa\* | X\* |  |
| I | X |  | U |  | X |
| i | X |  | Ula | X |  |
| Jka | X |  | V (ces) | X |  |
| Jkb | X |  | Vel | X |  |
| JMH |  | X | Wb |  | X |
| Jra | X |  | Wra | X |  |
| Jsa | X |  | Wrb | X |  |
| Jsb | X |  | Wu | X |  |
| K | X |  | Xga |  | X |
| k | X |  | Yka |  | X |
| Kna | X |  | Yta\*\* |  | X |
|  |  |  | Ytb\*\* |  | X |

\* = Neg or trace reactions by PIDAT, strong reactions at RT.

\*\* = Reactions can be variable.

\*\*\* = May be sensitive, dependant upon molecular structure.

**Ref: Reid ME, Lomas-Francis C. Blood Group Antigen Factsbook. 2nd ed. San Diego, CA: Academic Press, 2004**