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. **Specimens**

EDTA anticoagulated whole blood

1. **Material**

**Equipment:** Serological centrifuge

Block for test tubes

Water bath/Heating block at 37°C

**Supplies:** Test tubes – 10 x 75 mm

Serological pipettes

**Reagents:**

Ficin treated red cell panel - commercial

Ficin solution - supplied with commercial panel

Ficin control solution - supplied with commercial panel

1. **Quality Control**
   1. The ficin control solution that is supplied with the panel must be used and the expected results obtained for the treatment to be considered successful.
   2. Manufacturer's instructions must be adhered to for the use and storage of ficin.
2. **Procedure**

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| * 1. Prepare ficin treated cells: | * + 1. To ficin treat the patient's red cells and/or any additional cells required, prepare 3% saline suspension of washed cells. |
| * + 1. Prepare a working solution of ficin by diluting 0.1 mL ficin solution in 0.9 mL saline. |
| * + 1. Label a 10 x 75 mm tube for each cell to be treated. |
| * + 1. To each labeled tube add 10 drops of the appropriate 3% cell suspension and 10 drops of the ficin working solution (Ratio 1:1). |
| * + 1. Mix and incubate tubes at 37°C for 10-15 minutes. See Procedural Notes 8.1. |
| * + 1. Wash the red cells at least 3 times with saline decanting thoroughly after each wash. Prepare 3% saline suspension of the treated cells. |
| * + 1. Label a 10 x 75 mm tube for a control test on each of the treated cells. |
| * + 1. To each tube add 1 drop of the appropriate 3% saline suspension of ficin treated cells and 2 drops of the ficin control solution. Mix and centrifuge at 1000 rpm for 1 minute. |
| * + 1. Gently resuspend the red cell button in the control tubes and examine for agglutination. Cells that have been adequately treated with the ficin solution should produce grade 3 to 4 reactions with ficin control. |
| * + 1. If weak reactions are obtained with the CONTROL, repeat ficin treatment. If control is valid proceed with testing patient plasma with ficin treated cells. |
| * 1. Test procedure: | * + 1. Label test tubes with the first three letters of the patient family name and the corresponding ficin treated panel cell number and one additional tube for an autologous control. |
| * + 1. Prepare ficin-treated autologous cells as described in 6.1 – 6.10. |
| * + 1. Place 2 drops of 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 to 30 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 rpm for 10 -15 seconds. |
| * + 1. Gently resuspend each red cell button and examine macroscopically for agglutination. Record results. See Procedural Notes 8.2. |
| * + 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. Incubation time for enzyme treated red cells should not be any longer than 15 minutes. Overtreatment will result in false positive reactions.
   2. Enzyme techniques can be especially prone to giving equivocal reactions. Increased sensitivity is not limited to clinically significant antibodies but extends also to auto-antibodies, both of the warm and cold types, which may be too weak to be detectable by conventional test methods. Microscopic examination is not recommended.
   3. Red blood cells that have a positive DAT must not be used for the indirect antiglobulin test.
   4. Improper technique may invalidate the results.
   5. An enzyme test procedure must not be used as the sole method of detecting unexpected antibodies.
3. **References**
   1. Roback, JD. ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011 pg 902-905
   2. Manufacturer’s Insert with ficin product/panel in use.
4. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual * Added section 5.2 * Revised section 6.0 * Revised and renumbered section 7.0 * Revised list of references to include most recent editions |