

STANDARD OPERATING PROCEDURE

Preparation Site: **Louis Pasteur**

SOPs Number : **LP06**

Title	OPERATING PROCEDURES FOR THE INDIRECT COOMBS
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Version Number:	Preparation Date:	Effective date:

Written By:

Signature:

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Signature: _____

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Purpose: This SOP details a procedure on how to perform a Direct Coombs test

Scope: All competent staff (Technicians, Technologists, Scientists and Pathologist) may perform the test. The test is used for antibody screening and identification of pregnant Rh-negative women, cases of haemolytic disease of the new-born and autoimmune haemolytic anaemias.

Responsibility: It is the responsibility of all staff members (Technicians, Technologists, Scientists and Pathologist) in the laboratory to follow procedures outlined in this document.

Principle of the Test: The Indirect antiglobin test is used to identify free antibodies in the serum of the patient. The patient's serum or plasma is incubated with commercial red cells at 37 °C, allowing sensitization of the red cells to occur. The red cells are then washed to remove unbound globulins. AHG is then added. If agglutination occurs, it means there is a reaction between the antibody in the serum and antigen present on the red cells. If red cells fail to agglutinate, the test is reported as negative.

Specimen Collection: Venous blood in the EDTA or Red top (without gel) tube is stable for seven days if kept at 2-8°C. Haemolysed specimens and specimens older than seven days should be rejected. Serum or plasma may be used.

EQUIPMENT AND REAGENTS:

Reagents: AHG, Screen cells 1 & 2, Sensitized red cells, Saline/0.9% NaCl

Equipment: Centrifuge, specimen rack, tubes 10 x 75mm or 12 x 75mm, Pasteur pipettes, 37°C waterbath

Environmental And Safety Controls: All specimens should be considered infectious.

Method:

- 1) Centrifuge test specimen at 2500 rpm for 5 minutes
- 2) Setup two glass tubes for each patient and label them with the requisition numbers.
- 3) Put the tubes in 2 rows with row 1 marked for Screen cells 1 and row 2 marked for Screen cells 2
- 4) Add one drop of serum/plasma in each tube
- 5) Add 1 drop of Screen cell 1 suspension in tubes in row 1 and mix
- 6) Add 1 drop of Screen cells 2 suspension in tubes in row 2 and mix
- 7) Incubate the tubes in a 37^o C water bath for 60 minutes
- 8) After 60 minutes was the red cells three times using saline (refer Direct Coombs SOP for washing instructions)
- 9) Add 1 drop of AHG to each tube after the last wash
- 10) Centrifuge at 2000 rpm for 30 seconds
- 11) Look for agglutination by gently agitation at the tip of the tube
- 12) Record the results on the raw data sheet as positive (agglutination present) or as negative (agglutination absent)
- 13) Control step: Add 1 drop of sensitized red to the negative tubes. Centrifuge at 2000 rpm for 30 seconds. If negative, repeat the whole test for the patient. If positive enter the results on Prelink.