HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER
MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank		
Document:	Internal Policy and Procedure		
Title:	Direct Antiglobulin Test "DAT" (Direct Coomb's Test "DCT")		
Applies To:	All Blood Bank Staff		
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1. PURPOSE:

1.1 Demonstration of in vivo coating of red cells with globulins, in particular IgG and C3d.

2. DEFINITONS:

N/A

3. POLICY:

3.1 Direct antiglobulin (Coomb's) test is used for detection of allo-antibodies present on the RBCs.
3.2 Direct antiglobulin test is used for diagnosis of:
- 3.2.1 Autoimmune haemolytic anaemia.
- 3.2.2 Drug induced hemolysis.
- 3.2.3 Haemolytic disease of newborn.
- 3.2.4 Alloimmune reactions to recent transfusion.
- 3.2.5 Transfusion reaction.

3.3 DAT will be performed on cord blood samples, all Transfusion reaction investigation, and blood samples which show positive auto control as well as upon request of the physician.

4. PROCEDURE:

4.1 **Principle:**
- 4.1.1 Washed red cells from a patient or donor are tested directly with polyspecific AHG (Coomb's reagent) and monoclonal anti IgG and anti-C3d reagents.
- 4.1.2 Monospeciflc antiglobulin reagents are needed to confirm which globulins are present especially if patient has a positive autocontrol.

4.2 **Reagent and materials:**
- 4.2.1 Coombs reagent (Polyspecific AHG)
- 4.2.2 Monospecific (IgG -C3d) AHG
- 4.2.3 Normal saline
- 4.2.4 IgG sensitized cells (CCC)
- 4.2.5 Test tubes and slide
- 4.2.6 Automatic cell washer
- 4.2.7 Disposable pipettes and slides
- 4.2.8 Marker, pencil or pen
- 4.2.9 Microscope

4.3 **Specimen used:**
- 4.3.1 Anti-coagulated (EDTA) freshly drawn blood to avoid in vitro deposition of complement
- 4.3.2 Avoid haemolysed specimens.

4.4 **Steps:**

4.4.1 Wash 1.0 ml of each sample 4 times with saline and complete decant of the final wash.

4.4.2 Add one drop of cell suspension to each of three tubes labelled for polyspecific AHG, IgG and C3d.

4.4.3 Add 2 drops polyspecific AHG (Coomb's reagent) to appropriately labelled tube and mix .

4.4.4 Centrifuge for 15 sec at 3000 rpm.

4.4.5 Gently resuspend the cell button and examine macroscopically and microscopically for agglutination or hemolysis.

4.4.6 Grade and record the results.

4.4.7 If negative, incubate at room temperature for 10 minutes, centrifuge, resuspend and read microscopically and record results.

4.4.8 Add one drop IgG sensitized cells (Coombs control cells) to all negative.

4.4.9 Centrifuge for 15 sec at 3000 rpm.

4.4.10 Gently resuspend the cell button and examine macroscopically and microscopically for agglutination or hemolysis.

4.4.11 If agglutination is not detected after adding Coombs control cells, the entire test must be repeated.

4.4.12 If polyspecific AHG test is positive, perform monospecific AHG test.

4.4.13 Add two drops of anti-IgG monospecific AHG into the appropriately labelled tube.

4.4.14 Mix well, centrifuge, resuspend and examine macroscopically and microscopically for agglutination.

4.4.15 To any negative IgG tests, add one drop of CCC, centrifuge, resuspend and examine and if negative, repeat the test.

4.4.16 To any negative C3d tests, add one drop of complement control cells, centrifuge, resuspend and examine and if negative, repeat the test.

4.4.17 If the DAT is positive with anti-IgG, anti-C3d and polyspecific AHG. you must perform 6% albumin control to rule out the presence of spontaneous agglutination.

4.5 **Interpretation:**

4.5.1 If agglutination is detected, it is a positive test.

4.5.2 Absence of agglutination indicates that tested sample does not contain antibody or complement-coated red cells.

4.5.3 IgG-coated red cells usually give immediate reactions, whereas complement coating (anti-C3d) may be more easily demonstrable after incubation.

4.5.4 No interpretation can be made if the results with all anti-sera used to perform a direct antiglobulin test and 6% bovine albumin (control) are reactive. This indicates spontaneous agglutination which must be resolved before further testing is performed.

4.5.5 Cells that are positive by DAT will also be positive in any indirect antiglobulin test (crossmatching).

4.5.6

Polyspecific	Control	Anti-IgG	Anti-C3	DAT Interpretation
Negative	Negative	Not tested	Not tested	Negative
Weakly Positive	Negative	Negative	Negative	Negative
Positive	Negative	Positive	Positive	Positive
Positive	Negative	Positive	Negative	Positive
Positive	Negative	Negative	Positive	Positive
Positive	Positive	Positive	Positive	Unable to report

4.5.7 **Neonatal reporting-only:**

Polyspecific	Control	Anti-IgG	Anti-C3	DAT Interpretation
Positive	Negative	Not tested	Not tested	Positive
Not tested	Negative	Positive	Not tested	Positive
Not tested	Negative	Negative	Not tested	Negative

4.5.8 Patient who develops a positive DAT after blood transfusion may be undergoing a delayed haemolytic transfusion reaction and the treating doctor should be notified.

4.5.9 A newborn who has positive DAT. may be undergoing a haemolytic disease of a newborn and the treating doctor should be notified.

4.6 **Causes of false negative test:**

4.6.1 Failure to wash cells adequately to remove all plasma.

4.6.2 Contamination of Coombs reagent by extraneous protein. Do not use finger or hand to cover tube during mixing.

4.6.3 High concentration of IgG Para proteins in test plasma. protein may remain even after multiple washes.

4.6.4 Bound IgG may dissociate from red cells and leave little IgG to be detected.

4.6.5 Agglutination of IgG-coated cells is weakening by time, centrifuge and read immediately.

4.6.6 Improper reagent storage

4.6.6.1 AHG may lose reactivity if frozen.

4.6.6.2 AHG may become bacteriological contaminated.

4.6.7 Improper procedures:

4.6.7.1 Under centrifugation.

4.6.7.2 Failure to add AHG reagent.

4.6.7.3 Too heavy red cell concentration may mask weak agglutination .

4.6.7.4 Improper serum / cells ratios.

4.6.8 Saline:

4.6.8.1 Low pH of saline can decrease sensitivity (optimal pH 7.0-7.2).

4.6.8.2 Some antibodies may require specific saline temperature. Use at both 37°C and 4°C.

4.7 **Causes of false positive test:**

4.7.1 Cells agglutinated prior to washing:

4.7.1.1 Agglutination may not disappear during washing. Observe cells prior to addition of AHG.

4.7.2 Particles or contaminants:

4.7.2.1 Dust or dirt in glassware may cause clumping.

4.7.3 Improper procedure:

4.7.3.1 Over centrifugation.

4.7.3.2 Complement components may bind to cells from clot of CPDA-1 during storage at 4 °C. Use fresh EDTA samples.

4.7.3.3 Complement may attach to cells in specimens collected from infusion lines.

5. MATERIALS AND EQUIPMENT:

5.1 **Forms and Records:**

5.1.1 Careware system of the laboratory Coomb's test register.

5.2 **Materials**:

5.2.1 As above mentioned in 4.2.

6. RESPONSIBILITIES:

6.1 It is the responsibility of the blood bank staff in the pre-transfusion area.

7. APPENDICES:

N/A

8. REFERENCES:

8.1 The Unified Practical Procedure Manual for Blood Banks in The Arab Countries, 1434-2013.

8.2 The Standard Policy for Blood Banks in The Kingdom of Saudi Arabia, 1st edition, 1435-2014.

8.3 National Standards for Clinical laboratories and Blood Banks, 1st edition, 2015.

8.4 AABB Technical manual, 18th edition, 2014.

8.5 AABB Standards for Blood Banks and Transfusion Services, 30th edition, 2016.

8.6 Mollison's Blood Transfusion in Clinical Medicine; 12th edition, 2014.

9. APPROVALS:

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