

DEPMEDS LABORATORY PROCEDURES
DEPARTMENT OF CLINICAL SUPPORT SERVICES
U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL
FORT SAM HOUSTON, TEXAS 78234-6137

MCCS-HCL

STANDING OPERATING PROCEDURE

1 November 01

ABO CELL GROUPING

1. PRINCIPLE:

Agglutination of red blood cells with a given antisera (Anti-A or Anti-B) indicates the presence of the corresponding antigen on the red cells (positive result). Absence of agglutination indicates the corresponding antigen is not present (negative result).

2. SPECIMEN:

- a. For unit verification: 1 unit segment.
- b. For patient verification: 1 tube non-anticoagulated blood or EDTA, less than 48 hours old. Follow reagents manufactures instructions. Generally clotted or anticoagulated blood may be used . Specimen should be tested as soon as possible after collection.
- c. Store at 1-6°C.

3. REAGENTS AND EQUIPMENT:

- a. Centrifuge.

NOTE: Use biohazard precautions and wear Personal Protective Equipment (PPE) when handling or pipetting body fluids or products.

- b. Anti-A: Store at 1-6°C.
- c. Anti-B: Store at 1-6°C.
- d. Normal (0.85%) Saline: Store at room temperature (25°C), avoid excessive heat, protect from freezing.

4. QUALITY CONTROL:

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- a. Centrifuge: See Blood Bank Quality Control SOP, Serological Centrifuge Calibration.
- b. Anti-A, Anti-B, Normal Saline: See Blood Bank Quality Control SOP, Reagent Quality Control.
- c. Technician Proficiency: See Blood Bank Quality Control SOP, Technician Proficiency.

5. PROCEDURE:

- a. Make a 2% to 5% cell suspension of red blood cells to be tested.

NOTE: | Use biohazard precautions and wear Personal Protective Equipment (PPE) when handling or pipetting body fluids or products.

- b. Label two tubes with specimen number or patient identification and then label one A and one B.
- c. To the tube labeled A, add one drop of Anti-A.
- d. To the tube labeled B, add one drop of Anti-B.
- e. To each tube add one drop of cell suspension.
- f. Mix gently and centrifuge at the optimum speed and time. (Usually 15-30 seconds at 900-1000 x g.)
- g. Gently resuspend the cell button and examine macroscopically for agglutination.

NOTE: Soak tubes and pipets in 10% bleach solution for 8 hours and discard in regular trash. Drain solution into biowaste pit.

- h. Record graded results.

6. RESULTS:

- a. Positive test -- agglutination or hemolysis of the rbc button.
- b. Negative test -- a smooth suspension of rbcs after resuspending the rbc button.

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c. Interpretation:

ANTI-A	ANTI-B	BLOOD GROUP
=	=	O
+	=	A
=	+	B
+	+	AB

= No agglutination

+ Agglutination

7. PROCEDURAL NOTES:

a. To make a 2% to 5% cell suspension: Mix one drop of packed red blood cells with 1 to 2 mL of saline (1 mL = 20 drops).

b. Grading reactions.

(1) Hold test tubes up to an overhead light or a mirror with reflected light. Shake the tubes gently so that the cell button is shaken loose from the bottom of the tube. Watch the cells dislodge from the cell button. Gently tilt tubes several times until an even suspension is obtained throughout. Tubes should be held at an angle so that the movement of the fluid over the button helps to dislodge the cells.

(2) Grade the agglutination pattern according to the following scheme:

(a) 4+: one large, tight clump, macroscopically.

(b) 3+: one large, loose clump with several small clumps, macroscopically; or several large clumps, macroscopically.

(c) 2+ : medium-sized clumps with clear background, macroscopically.

(d) 1+ : many small clumps with turbid red background, macroscopically.

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(e) +/- : 2-3 small (5-10 cells) clumps per field, microscopically.

(f) = : no agglutination; homogeneous suspension of red cell

(g) h : hemolysis.

8. REFERENCE:

- a. TM 8-227-3, AABB Technical Manual, Aug 1993.