

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

01 November 01

ABO SERUM GROUPING

1. PRINCIPLE:

Anti-A and Anti-B are antibodies which occur naturally in persons lacking the corresponding antigen. Testing against known A and B cells serves as a confirmation of the ABO cell grouping procedure.

2. SPECIMEN:

- a. For unit verification: 1 unit segment.
- b. For patient verification: 1 tube non-anticoagulated blood or EDTA tube, less than 48 hours old.
- 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. A Cells (Prepared Locally): Store at 1-6°C.
- c. B Cells (Prepared Locally): Store at 1-6°C.

4. QUALITY CONTROL:

- a. Centrifuge: See Blood Bank Quality Control SOP, Serological Centrifuge Calibration.

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- 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. Label two tubes with specimen number or patient identification, then label one AC and one BC.

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

- b. To both tubes add two (2) drops of patient serum.
- c. To the tube labeled AC, add one drop of A cells.
- d. To the tube labeled BC, add one drop of B cells.
- e. Mix gently and centrifuge at the optimum time and speed for a saline test.
- f. Gently resuspend the cell button and examine macroscopically for agglutination/hemolysis.

NOTE: Dispose of tubes and pipets into 10% bleach solution for 8 hours then drain into biowaste pit and put tubes and pipets into regular trash.

- g. Record graded results.

6. RESULTS:

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

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c. Interpretation:

A CELLS	B CELLS	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 reaction.

(1) Hold test tubes up to an overhead light or a mirror with reflected light. Shake the tubes gently so that the cell button shakes 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 cells.

(g) h : hemolysis.

8. REFERENCE:

- a. TM 8-227-3, AABB Technical Manual, Aug 93.