LESSON 4-2 Immunohematology—ABO Grouping
Student Performance Guide

LESSON 4-2 ABO Grouping—Slide Method
Worksheet

LESSON 4-2 ABO Grouping—Tube Method
Worksheet

LESSON 4-3 Immunohematology—Rh Typing
Student Performance Guide

LESSON 4-3 Rh Typing
Worksheet

LESSON 4-4 Slide Test for Infectious Mononucleosis
Student Performance Guide

LESSON 4-5 Slide Test for Rheumatoid Factors
Student Performance Guide

Student Performance Guide

LESSON 4-2 Immunohematology—ABO Grouping

Name _____ Date _____


INSTRUCTIONS

1. Practice performing ABO grouping following the step-by-step procedure.
2. Demonstrate your understanding of this lesson by:
   a. Completing a written examination successfully, and
   b. Performing ABO grouping satisfactorily for the instructor. All steps must be completed as listed on the instructor's Performance Check Sheet.

Note: Package inserts should be consulted for specific instructions before test is performed.

MATERIALS AND EQUIPMENT

- gloves
- hand disinfectant
- EDTA anticoagulated specimens
- stopwatch or timer
- wax pencil
- applicator sticks or stirrers
- physiological saline (0.85% or .15M NaCl)
- Pasteur pipets and rubber bulb or disposable plastic pipets
- anti-A (commercially available)
- anti-B (commercially available)
- A cells (2–5% suspension, commercially available)
- B cells (2–5% suspension, commercially available)
- serofuge or centrifuge capable of spinning 13 x 75 mm tubes at 2000–2500 rpm (optional)
- test tubes, 13 x 75 mm (disposable)
- test tube racks
- blood-grouping worksheets
- surface disinfectant (10% chlorine bleach solution)
- biohazard container
- puncture-proof container for sharps
- clean microscope slides or grouping slides

PROCEDURE

Record in the comment section any problems encountered while practicing the procedure (or have a fellow student or the instructor evaluate your performance).

S = Satisfactory
U = Unsatisfactory

You must:	S	U	Comments
1. Wash hands and put on gloves.			
2. Assemble equipment and materials			
3. Perform slide grouping following steps 4–14			
4. Obtain a clean slide, mark the slide into two halves using a wax pencil.			
5. Label the left side "A" and the right side "B"			
6. Place one drop of anti-A serum on the "A" side (do not allow dropper to touch slide)			

You must:	S	U	Comments
7. Place one drop of anti-B serum on the "B" side (do not allow dropper to touch slide)			
8. Add one drop of well-mixed blood to each side of the slide using the Pasteur pipet (the drop of blood should be no larger than the drop of antibody)			
9. Mix the blood and antiserum on side A and spread into a smooth round circle about the size of a nickel using a clean applicator stick.			
10. Repeat the same procedure on side B using a clean applicator stick			
11. Rock the slide gently for two minutes and look for agglutination using strong light			
12. Record agglutination results on worksheet: agglutination = +; no agglutination = 0			
13. Determine the blood group and record			
14. Repeat steps 4–13 on additional blood samples			
15. Perform ABO forward tube grouping following steps 16–25			
16. Place one drop of a well-mixed blood specimen into a test tube; add eighteen to nineteen drops of saline, and label the tube "patient cells" (2–5%)			
17. Label two test tubes "A" and "B"			
18. Place one drop of anti-A in tube "A"			
19. Place one drop of anti-B in tube "B"			
20. Place one drop of the 2–5% patient cell suspension in each tube and mix			
21. Place tubes in serofuge and spin thirty seconds. ***Note:*** balance the serofuge by placing tubes opposite each other. (If no serofuge is available, allow tubes to stand at room temperature for fifteen to thirty minutes and go to step 23)			
22. Allow the serofuge to come to a complete stop and remove the tubes			
23. Tap each tube gently to loosen cells from the bottom and observe cells for agglutination using good light. Grade agglutination (See Figure 4-12)			
24. Record results from each tube on worksheet			
25. Determine the blood group of the sample and record			
26. Perform ABO reverse grouping on the blood sample following steps 27–38			
27. Centrifuge the blood sample, remove plasma from the sample, and place it in a clean test tube			

You must:	S	U	Comments
28. Label three test tubes “a,” “b,” and “control”			
29. Place two drops of plasma into each of these tubes			
30. Place one drop of a 2–5% suspension of A cells into tube “a” and mix			
31. Place one drop of a 2–5% suspension of B cells into tube “b” and mix			
32. Place one drop of the patient’s 2–5% cell suspension into “control” tube and mix			
33. Place tubes in serofuge (be sure tubes are balanced) and spin thirty seconds (or allow tubes to sit at room temperature 15–30 minutes)			
34. Remove the tubes from the serofuge after it stops completely			
35. Tap each tube gently and observe cells for agglutination. Grade agglutination (See Figure 4-12)			
36. Record the results from each tube on worksheet			
37. Determine the blood group of the sample and record			
38. Compare results of forward grouping with results of reverse grouping of the same sample. Reverse grouping should agree with results of forward grouping.			
39. Repeat forward and reverse grouping on additional blood specimens if available			
40. Discard all specimens appropriately			
41. Soak reusable glassware in 10% chlorine bleach solution in a minimum of ten minutes and wash. Discard disposable tubes into biohazard sharps container			
42. Clean equipment and return to proper storage. Return all reagents to proper storage			
43. Clean work area with surface disinfectant			
44. Remove gloves and discard in biohazard container			
45. Wash hands with hand disinfectant			

Evaluator Comments:

Evaluator _____ Date _____

Worksheet

LESSON 4-2 ABO Grouping—Slide Method

Name _____ Date _____

	AGGLUTINATION RESULTS*		INTERPRETATION
Specimen I.D.	**Anti-A**	**Anti-B**	**ABO group**
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

*Record results as:
0 = no agglutination
+ = agglutination

Worksheet

LESSON 4-2 ABO Grouping—Tube Method

Name _____ Date _____

	DIRECT (FORWARD) GROUPING*		INTERPRETATION	INDIRECT (REVERSE) GROUPING*			INTERPRETATION
Specimen I.D.	**Anti-A**	**Anti-B**	**ABO Group**	**A Cells**	**B Cells**	**Control**	**ABO Group**
_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____

*Record results as:

0 = no agglutination
w+ = fine agglutinates, most cells not agglutinated
1+ = tiny clumps, cloudy background
2+ = several small clumps, clear background
3+ = several large clumps, clear background
4+ = 2-3 large clumps, clear background

Student Performance Guide

LESSON 4-3 Immunohematology—Rh Typing

Name _____ Date _____

INSTRUCTIONS

1. Practice performing Rh typing following the step-by-step procedure.
2. Demonstrate your understanding of this lesson by:
   a. Completing a written examination successfully, and
   b. Performing Rh typing satisfactorily for the instructor. All steps must be completed as listed on the instructor's Performance Check Sheet.

Note: Package insert should be consulted for specific instructions before test is performed.

MATERIALS AND EQUIPMENT

- gloves
- hand disinfectant
- centrifuge
- test tube racks
- serological tubes, 13 x 75 mm
- physiological saline
- disposable plastic pipets
- clean microscope slides
- applicator sticks or stirrers
- anti-D serum (anti-Rh_o)
- Rh control solution
- blood specimen
- lighted viewbox
- Rh typing worksheet
- wax pencil
- stopwatch or timer
- surface disinfectant (10% chlorine bleach solution)
- biohazard container
- puncture-proof sharps container

PROCEDURE

Record in the comment section any problems encountered while practicing the procedure (or have a fellow student or the instructor evaluate your performance).

S = Satisfactory
U = Unsatisfactory

You must:	S	U	Comments
1. Wash hands and put on gloves			
2. Assemble equipment and materials			
3. Turn on viewbox			
4. Label two clean microscope slides "D" and "C" (control)			
5. Place one drop of anti-D serum on the "D" slide			
6. Place one drop of Rh control solution on the "C" slide			

You must:	S	U	Comments
7. Place one large drop of well-mixed whole blood on each slide			
8. Mix blood and anti-D well with an applicator stick, spreading the mixture over at least one-half of the slide			
9. Repeat procedure for the control slide using a clean applicator stick			
10. Place slides on the lighted viewbox and start timer			
11. Tilt the viewbox slowly back and forth for two minutes			
12. Observe the slides for agglutination at the end of two minutes			
13. Record results on worksheet: agglutination = +; no agglutination = 0			
14. Determine the Rh type and record on worksheet			
15. Repeat steps 4–14 on other blood samples, as directed by instructor			
16. Perform tube typing on a specimen (or go to step 25)			
17. Prepare a 2–5% suspension of the blood specimen by adding 19 drops of saline to one drop of well-mixed blood			
18. Label two tubes "patient" and "control"			
19. Add one drop of patient cell suspension to each labeled tube			
20. Add one drop of anti-D to "patient" tube and one drop of control diluent to "control" tube			
22. Mix contents of tubes and centrifuge for 30 seconds			
23. Gently tap tubes to loosen cell pellets and observe for agglutination			
24. Grade reactions and record results on worksheet. ***Note:*** absence of agglutination in patient tube requires a test for weak D before the patient can be definitively typed as D negative			
25. Discard specimens, tubes, and slides in biohazard sharps container			
26. Clean equipment and return to proper storage			
27. Clean work area with surface disinfectant			
28. Remove gloves and discard in biohazard container			
29. Wash hands with hand disinfectant			
Evaluator Comments:			

Evaluator _____ Date _____

Worksheet

LESSON 4-3 Rh Typing

Name _____ Date _____

	AGGLUTINATION RESULTS*		INTERPRETATION**
Specimen I.D.	**Anti-D**	**Control Diluent**	**Rh Type**
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

*Record results as:

0 = no agglutination
w+ = fine agglutinates, most cells not agglutinated
1+ = tiny clumps, cloudy background
2+ = several small clumps, clear background
3+ = several large clumps, clear background
4+ = 2-3 large clumps, clear background

**Record interpretation as:

Rh D positive or
Rh D negative

Student Performance Guide

LESSON 4-4 Slide Test for Infectious Mononucleosis

Name _____ Date _____

INSTRUCTIONS

1. Practice performing the slide test for IM following the step-by-step procedure.
2. Demonstrate your understanding of this lesson by:
   a. Completing a written examination successfully, and
   b. Performing the slide test for IM satisfactorily for the instructor. All steps must be completed as listed on the instructor's Performance Check Sheet.

Note: Procedure given is for Monospot™ test by Meridian Diagnostics. Package insert should be consulted before test is performed. If another kit is used, the manufacturer's instructions should be followed.

MATERIALS AND EQUIPMENT

- gloves
- hand disinfectant
- test serum or plasma
- stopwatch or timer
- surface disinfectant (10% chlorine bleach solution)
- test kit for IM (kit should include instructions, slide, serum dispensers, stirrers, reagents, and controls)
- biohazard container

PROCEDURE

Record in the comment section any problems encountered while practicing the procedure (or have a fellow student or the instructor evaluate your performance).

S = Satisfactory
U = Unsatisfactory

You must:	S	U	Comments
1. Wash hands and put on gloves			
2. Assemble equipment and materials			
3. Place the Monospot™ slide on a flat work surface			
4. Mix the reagent vials several times by inversion			
5. Fill the capillary pipet to the top mark with indicator cells: a. Place the rubber bulb on the end of the capillary pipet with the heavy black line b. Insert the pipet into the vial of indicator cells c. Allow the pipet to fill to the top mark by capillary action			
6. Place your index finger over the hole in the bulb and squeeze gently to dispense one-half of the cells (10 µL) on a corner of square I of the slide (the level of the cells should now be at the lower mark on the pipet)			

You must:	S	U	Comments
7. Deliver the remaining cells (10 µL) to a corner of square II			
8. Place one drop of thoroughly mixed Reagent I in the center of square I			
9. Place one drop of thoroughly mixed Reagent II in the center of square II			
10. Add one drop of test serum to the center of each square using the disposable plastic pipet provided			
11. Use a clean applicator stick to mix Reagent I with the serum using *at least ten* stirring motions without touching the indicator cells			
12. Blend in the indicator cells in square I with the applicator stick using *no more than ten* stirring motions, and spreading the mixture over the entire surface of the square			
13. Repeat steps 11–12 using Reagent II in square II, using a clean applicator stick			
14. Start the timer after you have completed mixing of both squares			
15. Do not pick up or move the slide			
16. Observe both squares for agglutination at the end of one minute (no longer) without moving the slide or picking it up			
17. Record the agglutination in each square and interpret the results: If the agglutination pattern is stronger in square I than in square II, the test is positive for the heterophile antibody of infectious mononucleosis. Any other combination of reactions is negative			
18. Record test results as positive or negative			
19. Repeat test procedure (steps 3–18) using positive and negative control sera			
20. Discard contaminated materials in biohazard container			
21. Dispose of specimen appropriately and disinfect reusable materials by soaking in 10% chlorine bleach solution for at least ten minutes. Wash and rinse thoroughly			
22. Clean work area with surface disinfectant			
23. Remove gloves and discard in biohazard container			
24. Wash hands with hand disinfectant			

Evaluator Comments:

Evaluator _____ Date _____

Student Performance Guide

LESSON 4-5 Slide Test for Rheumatoid Factors

Name _____ Date _____

INSTRUCTIONS

1. Practice performing the slide test for RF following the step-by-step procedure.
2. Demonstrate your understanding of this lesson by:
   a. Completing a written examination successfully, and
   b. Performing the slide test for RF satisfactorily for the instructor. All steps must be completed as listed on the instructor's Performance Check Sheet.

Note: Instructions given are general. The procedure should be modified to conform to the manufacturer's instructions for the kit being used.

MATERIALS AND EQUIPMENT

- hand disinfectant
- gloves
- timer
- test tubes (13 x 75 mm)
- test tube rack
- serum samples
- pipets for delivering .05 mL (50 µL), 0.5 mL, 0.95 mL
- RF slide test kit that includes:
  RF latex reagent
  RF positive control serum
  RF negative control serum
  glycine diluent
  ringed black glass, plastic or cardboard disposable slide
  dispenser-spreaders
- surface disinfectant (10% chlorine bleach solution)
- biohazard container
- applicator sticks (if spreaders are not in kit)

PROCEDURE

Record in the comment section any problems encountered while practicing the procedure (or have a fellow student or the instructor evaluate your performance).

S = Satisfactory
U = Unsatisfactory

You must:	S	U	Comments
1. Wash hands and put on gloves			
2. Assemble equipment and materials			
3. Allow all reagents to reach room temperature before performing test			
4. Prepare a 1:20 dilution of the test serum: a. Pipet 0.05 mL (50 µL) of serum into a 13 x 75 tube b. Pipet 0.95 mL of glycine diluent into the tube and mix well			
5. Dispense one drop of positive control serum into ring on slide			

You must:	S	U	Comments
6. Dispense one drop of negative control serum into ring on slide			
7. Dispense one drop of diluted patient serum (from step 4) into ring on slide using dispenser-spreader included in kit (save dispenser for mixing specimen)			
8. Mix the RF latex reagent well by inversion			
9. Dispense one drop of well-mixed RF latex reagent into each ring containing a control or test serum			
10. Use the spreader end of the dispenser (used to dispense serum) to thoroughly mix serum with reagent, spreading the mixture over the entire surface of the ring. ***Note:*** Be sure to use a separate spreader-mixer for each serum or control sample. An applicator stick may be used if no spreaders are available.			
11. Start timer and rock the slide in a figure-eight motion for the appropriate time (usually one to three minutes) to continue mixing			
12. Observe the ringed areas for agglutination immediately at the end of the appropriate time period			
13. Record the results of the controls and patient serum (agglutination = positive; no agglutination = negative or titer less than 20)			
14. Perform the quantitative test (steps 15-18) if the patient sample is positive for agglutination; if it is negative, go to step 19			
15. Prepare a two-fold serial dilution of patient serum: a. Label five test tubes: 1 (1:40), 2 (1:80), 3 (1:160), 4 (1:320), and 5 (1:640) b. Pipet 0.5 mL of glycine diluent into each tube c. Pipet 0.5 mL of 1:20 dilution of patient serum (from qualitative test, step 4) into tube 1 (1:40) and mix contents of tube well d. Transfer 0.5 mL from tube 1 to tube 2 and mix well e. Transfer 0.5 mL from tube 2 to tube 3 and mix well f. Transfer 0.5 mL from tube 3 to tube 4 and mix well g. Transfer 0.5 mL from tube 4 to tube 5 and mix well			
16. Use each dilution (tubes 1–5) as a separate test specimen and perform the agglutination test as in steps 5–13			
17. Record the results for each tube			
18. Record the serum RF titer (the reciprocal of the highest dilution that shows agglutination)			

You must:	S	U	Comments
19. Disinfect glass slide with 10% chlorine bleach solution for at least ten minutes and wash. Discard disposable slides into biohazard container			
20. Return all reagents and materials to proper storage			
21. Discard specimens and contaminated items appropriately			
22. Clean and disinfect work area			
23. Remove gloves and discard in biohazard container			
24. Wash hands with hand disinfectant			

Evaluator Comments:

Evaluator _____ Date _____