

Investigating Positive DAT Results:
A Case Study Approach
(Questions Only)



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Preface

In the spirit of *Antibody Identification: Art or Science? A Case Study Approach*, this volume continues the presentation of cases designed to guide the learner to achieve mastery in the selection and use of the many serological procedures available for problem solving. As the complexity of a serological case increases, so to must the skill of the medical laboratory scientist in selection and interpretation of these tests.

Each case study begins with a clinical scenario and initial test results which then guide the learner through a sequence of multiple choice questions that offer testing options and protocols for resolution. As in *Art or Science*, as each problem unfolds, the reader is provided with detailed feedback designed to enhance reflection and critical thinking. The difficulty of the cases ranges from basic to very advanced, allowing use by multiple levels of students and practicing medical laboratory scientists and physicians.

The common factor for this series of cases is a positive direct antiglobulin test. Situations such as acute and delayed transfusion reactions, hemolytic disease of the fetus and newborn, warm and cold autoantibodies in both untransfused and transfused patients, drug-dependent antibodies, and passive antibodies are difficult to simulate in hands-on teaching. These cases allow the learner to logically proceed from initial findings to resolution of these complex scenarios.

Written by practicing serologists and educators, the processes presented represent practical and efficient paths to problem resolution. This book will supplement didactic learning in a variety of settings from formal education programs to on-the-job instruction to competency assessments. Each case is designed as a stand-alone lesson enabling the instructor/supervisor to use just those cases most appropriate for the learner. However, the book is equally suited for self-study since practice can take place without immediately accessing the feedback.

Through cases that present serological problem solving as a series of logical and systematic steps, the authors hope to demonstrate that even in more complex serological situations, the *art* of antibody identification, as well as the science, can be a learned skill.

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Key to Abbreviations

√	Reactive IgG-sensitized cells
AC	Autocontrol
AHG	Antihuman globulin
DAT	Direct antiglobulin test
DTT	Dithiothreitol
ER	Emergency Room
HDFN	Hemolytic disease of the fetus and newborn
IAT	Indirect antiglobulin test
IS	Immediate spin
LISS	Low ionic strength saline
NH	No hemolysis
NT	Not tested
PEG	Polyethylene glycol
RBC	Red Blood Cell (unit)
RT	Room temperature
SOP	Standard operating procedure
SP	Solid phase

Interpretation of Agglutination Reactions

Macroscopically Observed Findings	Designation	Score
One solid agglutinate	4+	12
Several large agglutinates	3+	10
Medium-size agglutinates, clear background	2+	8
Small agglutinates, turbid background	1+	5
Very small agglutinates, turbid background	1+ ^w	4
Barely visible agglutination, turbid background	w+ or +/-	2
No agglutination	0	0
Mixtures of agglutinated and unagglutinated red cells (mixed field)	mf	
Complete hemolysis	H	
Partial hemolysis, some red cells remain	PH	

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Case Study 1

Initial Data:

A 50-year-old female, DT, presented to her physician for pain in her extremities and bluish tinged fingertips and toes when exposed to cold. On a blustery, cold day after walking six blocks she reported that her face looked black and blue when she came inside. Blood samples were collected for the following: complete blood count (CBC), routine chemistry profile, and direct and indirect antiglobulin tests (DAT and IAT, respectively). On review of the patient’s history at your facility, you note she was previously typed as group A, Rh positive, and there is no history of unexpected antibodies. The patient denied being transfused in the past.

Laboratory Results*:

Parameter	Patient Result	Reference Value
White blood cells	$3.3 \times 10^3/\mu\text{L}$	$4.8\text{-}10.8 \times 10^3/\mu\text{L}$
Red blood cells	$3.2 \times 10^6/\mu\text{L}$	$4.2\text{-}5.4 \times 10^6/\mu\text{L}$
Hematocrit	30.3%	37-47%
Hemoglobin	10.0 g/dL	12-16 g/dL
Mean corpuscular volume	88 fL	80-100 fL
Mean corpuscular hemoglobin	27 pg	27-31 pg
Mean corpuscular hemoglobin concentration	31%	32-36%
Red cell distribution width	12.0%	11.5-14.5%
Platelet count	125,000/ μL	150,000-400,000/ μL
*Conventional units.		

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
4+	0	4+	3+	4+

1. How would you interpret DT’s ABO forward (cell) type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. How would you interpret the ABO reverse (serum) type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

3. **What is the BEST next step in this case?**

- A. Report DT's type as group A.
 - B. Report DT's type as group O.
 - C. Hypothesize that the reverse typing is incorrect and investigate.
 - D. Hypothesize that the forward typing is incorrect and investigate.
-

4. **How would you interpret the DAT results above?**

- A. Neither IgG nor complement is present on the patient's cells.
 - B. Only IgG is present on the patient's cells.
 - C. Only complement is present on the patient's cells.
 - D. IgG and/or complement are/is present on the patient's cells.
-

5. **What testing would you perform next?**

- A. DAT with anti-IgG.
 - B. DAT with anti-C3b,-C3d.
 - C. Inert control.
 - D. All of the above.
-

6. **Which of the following specimens would be the BEST source of red cells for the monospecific DAT?**

- A. Purple top (EDTA).
 - B. Red top (clot).
 - C. Camouflage top (serum separator).
 - D. Any of the above.
-

7. **Based on the DAT results above, one can conclude that the patient's red cells are coated with which of the following?**

- A. IgG.
 - B. C3b,C3d.
 - C. Both IgG and C3b,C3d.
 - D. Neither IgG nor C3b,C3d.
-

8. **Given the results of the ABO typing, DAT profile, and antibody detection test, which of the following is the BEST conclusion?**

- A. Warm-reactive autoantibody.
- B. Cold-reactive alloantibody.
- C. Cold-reactive autoantibody.
- D. Cold-reactive alloantibody and/or autoantibody.

9. What do the results of Antibody Identification Panel 1 suggest?

- A. Warm-reactive alloantibody.
 - B. Warm-reactive autoantibody.
 - C. Cold-reactive alloantibody.
 - D. Cold-reactive autoantibody.
-

10. What is the MOST LIKELY explanation for the positive results seen with Cells 3, 5, and 11?

- A. Anti-K is identified.
 - B. Anti-E is identified.
 - C. Carryover due to cold-reactive antibody.
 - D. Cold-reactive antibody is reactive at 37 C.
-

11. Given the serologic data collected to this point, are all potentially clinically significant alloantibodies ruled out?

- A. Yes.
 - B. No.
-

12. What additional testing can be performed to confirm the hypothesis that no clinically significant antibodies are present in DT's serum?

- A. Prewarmed IAT.
 - B. Full saline antibody identification panel including IS, 37 C, IAT.
 - C. Saline-IAT with the reactive panel cells.
 - D. Saline-IAT with full panel.
-

13. What additional testing may be performed to identify the specificity of this cold-reactive autoantibody?

- A. Test O, A1, A2, cord cells, and autocontrol at IS, RT, and 18 C.
 - B. Test O, cord cells, and autocontrol at IS, RT, and 18 C.
 - C. Perform saline antibody identification panel.
 - D. Perform prewarmed panel.
-

14. What autoantibody specificity is suggested by the results of Antibody Identification Cold Panel 4?

- A. Autoanti-I.
- B. Autoanti-IH.
- C. Autoanti-P.
- D. Insufficient evidence to determine.

15. Is the patient's history consistent with a diagnosis of cold agglutinin disease (CAD)?

- A. Yes.
- B. No.

16. Because the autoanti-I did not show reactivity at 37 C, the reverse typing was repeated using a prewarmed technique. Using the results above, how should the reverse typing be interpreted?

- A. Group O.
- B. Group A.
- C. Group AB.
- D. The ABO type cannot be interpreted without additional testing.

Case Study 2

Initial Data:

A 26-year-old male, SW, presented to his physician with complaints of jaundice, weakness, fatigue, and dark-colored urine that had been present for several days. His vital signs were: temperature, 100.2 F; respiratory rate, 26/minute; blood pressure, 116/53; pulse, 138 bpm.

Samples were collected for laboratory testing, at which time the medical laboratory scientist (MLS) noted severe aggregation of the red cells.

Results of Laboratory Testing*:

Parameter	Patient Result	Reference Value
Spun hematocrit	11%	37-47%
Reticulocyte count	4.4%	0.5-2.0%
White cell count	22.0 × 10 ³ /μL	4.8-10.8 × 10 ³ /μL
Platelet count	296,000/μL	150,000-400,000/μL
Creatinine	1.0 mg/dL	0.8-1.3 mg/dL
Blood urea nitrogen (BUN)	31 mg/dL	8.1-21 mg/dL
Lactate dehydrogenase (LDH)	1950 U/L	101.8-197.6 U/L
Bilirubin, total	4.9 mg/dL	0.3-1.2 mg/dL
Haptoglobin	Absent	40-360 mg/dL

*Conventional units.

SW was admitted to the hospital and 4 units of Red Blood Cells Leukocytes Reduced (LR-RBCs) were ordered for transfusion. There is no record of previous serologic testing of SW in the transfusion service. His family denies any prior transfusion.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
1+	1+	4+	4+	4+

1. How would you interpret SW’s ABO forward (cell) type?

- A. Group O.
- B. Group A.
- C. Group AB.
- D. Unable to interpret.

2. How would you interpret the ABO reverse (serum) type?

- A. Group O.
- B. Group A.
- C. Group AB.
- D. Unable to interpret

3. Which of the following is the BEST next step in this case?

- A. Report SW's type as group AB.
 - B. Report SW's type as group O.
 - C. Hypothesize the reverse typing is incorrect and investigate.
 - D. Hypothesize the forward typing is incorrect and investigate.
-

4. What is SW's Rh type?

- A. D+.
 - B. D-.
 - C. Weak D+.
 - D. Cannot determine with the data provided.
-

5. What additional testing should be performed to confirm the Rh type?

- A. Weak D test.
 - B. Inert control.
 - C. Repeat anti-D.
 - D. None of the above.
-

6. Given the results of the repeat testing and control, what is SW's Rh type?

- A. D+.
 - B. D-.
 - C. Weak D+.
 - D. Cannot determine with the data provided.
-

7. What would cause an individual's red cells to spontaneously agglutinate before or during centrifugation?

- A. Warm-reactive autoantibody.
 - B. Rouleaux.
 - C. Cold-reactive autoantibody.
 - D. All of the above.
-

8. What is the MOST LIKELY cause for the positive Antibody Detection Test 1 result?

- A. Cold-reactive alloantibody and/or autoantibody.
- B. Drug-dependent antibody.
- C. Warm-reactive alloantibody and/or autoantibody.
- D. Cannot determine with the data provided.

9. Which of the following is MOST consistent with the results of Antibody Identification Panel 1?

- A. Single-specificity alloantibody.
 - B. Multiple alloantibodies.
 - C. One or more warm-reactive autoantibodies.
 - D. Cold-reactive autoantibody.
-

10. Based on the DAT results, one can conclude the patient's red cells are coated with which of the following?

- A. IgG.
 - B. C3b,C3d.
 - C. Both IgG and C3b,C3d.
 - D. Cannot determine with the data provided.
-

11. Which of the admission laboratory test results would support a hypothesis of red cell hemolysis?

- A. Lactate dehydrogenase (LDH).
 - B. Bilirubin.
 - C. Haptoglobin.
 - D. All of the above.
-

12. Given the results of the ABO typing, monospecific DATs, and antibody detection and identification tests, which of the following is the BEST conclusion?

- A. Warm-reactive autoantibody.
 - B. Cold-reactive alloantibody.
 - C. Cold-reactive autoantibody.
 - D. Cold-reactive alloantibody and/or autoantibody.
-

13. The MLS is concerned that there may be alloantibodies masked by the strong reactions of the cold-reactive antibody. What additional testing could the MLS perform to avoid some or all of the reactivity of the cold-reactive antibody?

- A. Antibody detection test—prewarmed IAT.
- B. Full saline antibody identification panel including IS, 37 C, and IAT.
- C. Antibody detection test—saline-IAT only.
- D. Full antibody identification panel—saline-IAT only.

14. **Given the results of Antibody Detection Test 2, which of the following tests is MOST suitable to perform next in order to determine if alloantibodies are present?**

- A. Repeat antibody identification with potentiating agents.
- B. Warm autoadsorption.
- C. Elution studies.
- D. Prewarmed IAT.

15. **How would you interpret the results of Antibody Detection Test 3?**

- A. Cold-reactive autoantibody reactivity was not eliminated by prewarming.
- B. No alloantibodies are present.
- C. Cold-reactive autoantibody is reactive at 37 C.
- D. Autoantibody or alloantibody is warm-reactive.

16. **If the patient's doctor calls and demands blood now, what would be suitable to release?**

- A. Emergency release, group O Rh negative.
- B. Crossmatch incompatible, group AB Rh positive.
- C. Emergency release, group O Rh positive.
- D. Crossmatch incompatible, group O Rh negative.

17. **Which of the following tests should be performed next to determine if any alloantibodies are present?**

- A. Repeat the saline-IAT.
- B. Warm autoadsorption.
- C. Repeat the prewarmed IAT.
- D. Cold autoadsorption.

18. **Before adsorption, the patient's autologous cells are pretreated with a protease reagent. Which of the following is/are protease(s)?**

- A. Polyethylene Glycol (PEG).
- B. Dithiothreitol (DTT) and 2-aminoethylisothiuronium bromide (AET).
- C. Ficin and papain.
- D. None of the above.

19. After the patient's autologous cells are ficin treated and the patient's serum is added to the treated red cells, at what temperature should the adsorption be incubated?

- A. 37 C.
 - B. 4 C.
 - C. 22 C.
 - D. None of the above.
-

20. During review of the results of the antibody detection using autoadsorbed plasma, what conclusion(s) can be made?

- A. The cold autoantibody was removed from the plasma.
 - B. The presence of clinically significant alloantibodies is unlikely.
 - C. Both of the above.
 - D. None of the above.
-

21. What additional testing would be MOST suitable to identify the specificity of this cold-reactive autoantibody?

- A. Test group O, A1, A2, cord cells, and autocontrol at IS, room temperature, and 18 C.
 - B. Test group O, cord cells, and autocontrol at IS, room temperature, and 18 C.
 - C. Perform saline antibody identification panel.
 - D. Perform prewarmed panel.
-

22. What autoantibody specificity is suggested by the results of antibody identification cold panel?

- A. Autoanti-I.
 - B. Autoanti-IH.
 - C. Autoanti-i.
 - D. No specificity apparent.
-

23. Which of the following tests is/are NECESSARY to complete the serologic evaluation?

- A. Resolution of ABO discrepancy.
- B. Resolution of DAT results.
- C. Both A and B.
- D. Neither A nor B.

24. What is the BEST technique to begin resolution of the ABO forward typing discrepancy?

- A. Wash the patient's red cells with 37 C saline.
- B. Warm the patient's EDTA sample to 37 C.
- C. Treat the red cells with DTT.
- D. None of the above.

25. Which of the following is true regarding cold agglutinin disease?

- A. Symptoms are mediated by complement.
- B. Patients frequently complain of cold-associated acrocyanosis.
- C. Symptoms often include chronic fatigue.
- D. All of the above.

Case Study 3

Initial Data:

AP, a 17-year-old female of European ancestry is complaining of extreme fatigue. Basketball season has started. Although she knows she is out of shape, she just doesn't feel able to run up and down the court with the same energy as usual. Her mother notes she is sleeping more than 12 hours per day, going to bed as soon as she comes home from practice. She also has had a low-grade fever for several days. An appointment is made with her pediatrician, who orders some tests.

Results of Laboratory Testing*:

Parameter	Patient Result	Reference Value
White blood cells	$14.4 \times 10^3/\mu\text{L}$	$4.8\text{-}10.8 \times 10^3/\mu\text{L}$
Red blood cells	$2.8 \times 10^6/\mu\text{L}$	$4.2\text{-}5.4 \times 10^6/\mu\text{L}$
Hematocrit	29.3%	37-47%
Hemoglobin	9.2 g/dL	12-16 g/dL
Mean corpuscular volume	88 fL	80-100 fL
Mean hemoglobin concentration	27 pg	27-31 pg
Mean corpuscular hemoglobin concentrate	31%	32-36%
Red cell distribution width	12%	11.5-14.4%
Platelet count	125,000/ μL	150,000-400,000/ μL
Erythrocyte sedimentation rate	30 mm/hour	
*Conventional units.		

AP's peripheral blood smear reveals an atypical lymphocyte count of 15%, and anisocytosis is noted. Mononucleosis spot test results are positive. Concerned with AP's low hemoglobin/hematocrit, her physician orders direct and indirect anti-globulin tests (DAT and IAT), once referred to as "Coombs Panel."

DAT:

	Immediate Spin (IS)	10-Minute Room Temperature Incubation
Polyspecific antihuman globulin (AHG)	2+	3+

1. Which of the following interpretations is consistent with the results of the DAT?

- A. Neither IgG nor complement is present on the patient's cells.
- B. Only IgG is present on the patient's cells.
- C. Only complement is present on the patient's cells.
- D. IgG and/or complement are present on the patient's cells.

2. Which of the following tests would be BEST to perform next?

- A. DAT with anti-IgG.
- B. DAT with anti-C3b,-C3d.
- C. Inert control.
- D. All of the above.

3. Which of the following specimens would be a suitable source of patient red cells for the monospecific DAT?

- A. Purple top (EDTA).
 - B. Red top (no additive).
 - C. Camouflage top (serum separator).
 - D. Any of the above.
-

4. Based on the DAT results, one can conclude that AP's red cells are coated with which of the following?

- A. IgG.
 - B. C3b,C3d.
 - C. Both IgG and C3b,C3d .
 - D. None of the above.
-

5. How would you interpret Antibody Detection Test 1?

- A. One or more alloantibodies are present in the serum.
 - B. One or more autoantibodies are present in the serum.
 - C. No antibodies are present in the serum.
 - D. No antibodies have been detected in the serum.
-

6. Which of the following procedures could the MLS perform to determine if there are any alloantibodies or autoantibodies present in AP's plasma?

- A. Test a selected cell cold panel.
 - B. Test cord cells alone.
 - C. Test an antibody identification panel.
 - D. Repeat the antibody detection test using the same method (PEG).
-

7. Given the patient's history and laboratory findings, which of the following autoantibodies is MOST probable?

- A. Autoanti-I.
 - B. Autoanti-P.
 - C. Autoanti-IH.
 - D. Autoanti-i.
-

8. How would you interpret AP's ABO type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

9. **Why is it important to know the patient's ABO type when performing a cold panel?**

- A. To have the patient's ABO type on record if blood is ordered.
- B. Cold-reactive autoantibodies often have ABO specificity.
- C. Cold-reactive autoantibodies often have H specificity.
- D. None of the above.

10. **What autoantibody specificity is suggested by the results of antibody identification mini cold panel?**

- A. Autoanti-I.
- B. Autoanti-i.
- C. Autoanti-IH.
- D. Insufficient evidence to determine.

11. **What additional testing is necessary to detect clinically significant alloantibodies that may be present in the patient's serum?**

- A. Cold alloadsorption.
- B. Cold autoadsorption.
- C. Elution studies.
- D. None of the above.

Case Study 4

Initial Data:

BP, a 49-year-old female, arrived at the emergency department of her local hospital. Her chief complaint was fatigue that had worsened since she had left the hospital 2 weeks ago following treatment for anemia of unknown origin. During this hospitalization, she had received 2 units of Red Blood Cells Leukocytes Reduced (LR-RBCs). There were no unexpected serologic findings during the pretransfusion testing.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
0	0	4+	4+	4+

1. **How would you interpret BP's ABO type?**

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. **What is BP's Rh type?**

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine with the data provided.

3. **What can be concluded from the results of the initial antibody detection test?**

- A. A single-specificity alloantibody.
- B. Multiple alloantibodies.
- C. One or more warm-reactive autoantibodies.
- D. Insufficient data to form a hypothesis.

4. **Which of the following is MOST consistent with the results of Antibody Identification Panel 1?**

- A. A single-specificity alloantibody.
- B. Multiple alloantibodies.
- C. One or more warm-reactive autoantibodies.
- D. A cold agglutinin.

5. Given the results of Antibody Detection Test 1 and Antibody Identification Panel 1, which of the following antibodies CANNOT be ruled out?

- A. Anti-c.
 - B. Anti-E.
 - C. Anti-Fy^a.
 - D. All of the above.
-

6. Which of the following tests would be the BEST to perform next?

- A. Patient phenotyping.
 - B. Crossmatch.
 - C. Selected cell panel.
 - D. Enzyme panel.
-

7. Given the results of Antibody Detection Test 1, Antibody Identification Panel 1, and the Selected Cell Panel, which of the following is the MOST LIKELY alloantibody solution?

- A. Anti-c only.
 - B. Anti-c but cannot rule out anti-E.
 - C. Anti-c and anti-Fy^a.
 - D. None of the above.
-

8. Given the serologic data generated thus far, which of the following tests would be the BEST next step?

- A. Crossmatch.
 - B. Direct antiglobulin test.
 - C. Enzyme panel.
 - D. No additional testing is necessary.
-

9. What can be concluded from the results of the DAT profile?

- A. The cells are coated with IgG.
 - B. The cells are coated with complement.
 - C. Both of the above.
 - D. None of the above.
-

10. What is the MOST LIKELY explanation for the mixed-field reactions in the direct antiglobulin testing?

- A. The AHG was contaminated.
- B. Inadequate cell washing.
- C. The patient has a mixed cell population (transfused and autologous cells).
- D. Technical error.

11. **What additional testing could provide data to support the transfusion reaction hypothesis?**

- A. Chloroquine diphosphate treatment.
 - B. Elution studies.
 - C. Red cell phenotyping.
 - D. None of the above.
-

12. **What can be concluded from the results of the eluate panel?**

- A. Anti-c was bound to the red cells.
 - B. Anti-E has been ruled out.
 - C. There is no evidence to support a transfusion reaction hypothesis.
 - D. Both anti-c and anti-E are bound to the red cells.
-

13. **What can be concluded from the phenotyping results?**

- A. The patient is likely c-negative.
 - B. The transfusion reaction hypothesis is supported by these data.
 - C. Anti-c is implicated in the positive DAT result.
 - D. All of the above.
-

14. **Which of the following laboratory tests would support the clinical diagnosis of a delayed hemolytic transfusion reaction?**

- A. Comparison of pretransfusion DAT and posttransfusion DAT results.
- B. Haptoglobin.
- C. Bilirubin.
- D. All of the above.

Case Study 5

Initial Data:

AT, a 54-year-old male, underwent cardiac surgery 8 days ago. He had a history of anti-Fy^a and during surgery received 5 units of Fy(a-), crossmatch-compatible Red Blood Cells Leukocytes Reduced (LR-RBCs). His postoperative hemoglobin level was 12.3 g/dL. His hemoglobin has been steadily dropping over the last 3 days and is currently 7.2 g/dL; there is no clinical evidence of bleeding. His doctor has ordered a type and screen and crossmatch for 2 units of LR-RBCs.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
4+	0	4+	0	4+

1. How would you interpret AT's ABO type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. What is the patient's Rh type?

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine from the data provided.

3. Which of the following is MOST consistent with the serologic results of Antibody Detection Test 1?

- A. The anti-Fy^a is still reactive.
- B. Multiple alloantibodies are present.
- C. A warm-reactive autoantibody is present.
- D. Both A and B.

4. What alloantibody specificity is suggested by the results of Antibody Identification Panel 1?

- A. Anti-Fy^a.
- B. Anti-Fy^a and anti-Jk^a.
- C. Anti-Fy^a and anti-E.
- D. Anti-Fy^a and anti-Jk^b.

5. Given the combined results of the initial antibody detection test and Antibody Identification Panel 1, which of the following alloantibodies CAN BE EXCLUDED?

- A. Anti-C.
- B. Anti-E.
- C. Anti-K.
- D. Anti-S.

6. Of the following, which is the BEST next step in the resolution of this antibody problem?

- A. Test an antibody identification panel from a different manufacturer using solid-phase testing.
- B. Select units negative for the Fy^a antigens and perform a full serologic crossmatch.
- C. Select units negative for the Fy^a and Jk^b antigens and perform a computer crossmatch to confirm ABO compatibility.
- D. Test a panel of selected Fy(a-) cells.

7. Given the results of the antibody detection test and both Antibody Identification Panels 1 and 2, which of the following antibodies was/were ruled out?

- A. Anti-E.
- B. Anti-K.
- C. Both of the above.
- D. None of the above.

8. What additional testing should be performed based on the autocontrol results in Antibody Identification Panel 2?

- A. Direct antiglobulin test (DAT) with polyspecific antihuman globulin (AHG).
- B. DAT with anti-IgG.
- C. DAT with anti-C3b,-C3d .
- D. Both B and C.

9. Given the results of the DAT testing above, what is coating the patient's red cells?

- A. IgG.
- B. C3.
- C. Both of the above.
- D. None of the above.

10. Given the patient's history and serologic results, which if the following is the MOST LIKELY hypothesis?

- A. The patient has warm autoimmune hemolytic anemia (WAIHA).
- B. Transfused donor cells are coated with alloantibody.
- C. The DAT results are due to a technical error.
- D. The previous anti-Fy^a hypothesis was incorrect.

11. What might BEST explain why the DAT result is significantly weaker than the reactivity of the antibody in the serum?

- A. Technical error.
- B. Reagent deterioration.
- C. Fewer antibody-coated donor cells are circulating.
- D. All of the above.

12. Given the history and serologic findings, what is the MOST LIKELY specificity of the antibody coating the transfused cells?

- A. Anti-Fy^a.
- B. Anti-Jk^b.
- C. Both of the above.
- D. None of the above.

13. What additional testing should be performed to determine if anti-Jk^b was implicated in the hypothesized DHTR?

- A. Enzyme panel.
- B. Elution.
- C. Adsorption.
- D. Neutralization.

14. Given the results of Antibody Identification Panel 3, which of the following antibodies is/are coating the transfused cells?

- A. Anti-Jk^b.
- B. Anti-Fy^a.
- C. Anti-Jk^b and anti-K.
- D. Both A and B.

15. **What additional testing would be useful to provide confirmatory evidence of the hypothesized new serum antibody AND the cause of a DHTR consistent with the patient's decreasing hemoglobin level?**
- A. Patient phenotyping for Jk^b on a pretransfusion sample.
 - B. Antigen typing of previously transfused donor units.
 - C. Crossmatching of previously transfused units with posttransfusion serum or eluate.
 - D. All of the above.

Case Study 6

Initial Data:

MJ, a 42-year-old female of European ancestry, was transported to the Emergency Department (ED) of the hospital following a car accident on an icy road. Although the clinical signs did not suggest that her injuries were life-threatening, the physician could not rule out the possibility of internal bleeding and ordered a type and crossmatch for 2 units of Red Blood Cells Leukocytes Reduced (LR-RBCs). MJ reports having three children, but no other history is currently available.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
0	4+	4+	3+	0

1. **How would you interpret MJ's ABO type?**

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. **What is MJ's Rh type?**

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine with the data provided.

3. **Given the results of Antibody Detection Test 1, what is the BEST initial hypothesis?**

- A. A single-specificity warm-reactive alloantibody.
- B. Multiple warm-reactive alloantibodies.
- C. Warm-reactive autoantibodies.
- D. Insufficient data to determine.

4. **Which of the following would be MOST INFORMATIVE at this stage?**

- A. An additional antibody identification panel using low-ionic-strength saline (LISS) IAT.
- B. An additional antibody identification panel using polyethylene glycol (PEG).
- C. A direct antiglobulin test (DAT) using polyspecific antihuman globulin (AHG).
- D. A serologic crossmatch.

5. **Based on the results of the DAT using polyspecific AHG, which of the following tests should be performed next?**

- A. Anti-IgG.
 - B. Anti-C3b,-C3d.
 - C. Both of the above.
 - D. None of the above.
-

6. **Based on the results of the DATs, what is coating the patient's red cells?**

- A. IgG.
 - B. Complement.
 - C. Both.
 - D. Neither.
-

7. **Which of the following would be MOST INFORMATIVE at this point in the case?**

- A. Elution studies.
 - B. Autoadsorption.
 - C. Serologic crossmatch.
 - D. Patient phenotyping.
-

8. **After reviewing the combined serologic data, the medical laboratory scientist (MLS) concluded the patient had a warm autoantibody and determined the best procedure to perform next would be an autoadsorption. The supervisor cautioned that this may not be the most suitable approach at this point in the case. Which of the following would support the supervisor's concern?**

- A. Lack of a complete patient transfusion history.
 - B. Lack of a complete patient medication record.
 - C. Incongruence between the strength of the DAT and the plasma reactivity.
 - D. All of the above.
-

9. **Given all test results and patient history now available, which of the following is the MOST LIKELY hypothesis?**

- A. Warm autoantibody in plasma and eluate.
- B. Transfusion reaction due to multiple alloantibodies.
- C. Transfusion reaction due to alloantibody to an antigen of high prevalence.
- D. Drug-induced autoantibody.

10. Based on the patient's history and the results with the chemically treated reagent cells, what high-prevalence antibody is **UNLIKELY**?

- A. Js(b-).
- B. k-.
- C. Kp(b-).
- D. Sc:-1.

11. Based on the results of Antibody Identification Panel 4, which of the following is **MOST LIKELY** to be present in the patient's serum?

- A. Anti-k.
- B. Anti-Kp^b.
- C. Anti-Sc1.
- D. None of the above.

12. What important piece of information should still be determined to complete this investigation?

- A. Rh phenotype of the patient.
- B. Sc1 status of the patient's red cells.
- C. Sc1 status of the transfused units.
- D. DAT of the patient's pretransfusion red cells.

Case Study 7

Initial Data:

EW, a 32-year-old pregnant female of African ancestry, arrived at the emergency department (ED) of her local hospital with vaginal bleeding. Her gestation was assessed at approximately 36 weeks. She said that this was her fourth pregnancy but she had had only two live births. EW reported no prenatal care with any of her pregnancies. Her admission hemoglobin was 7.1 g/dL. The ED physician ordered a transfusion of 2 units of Red Blood Cells Leukocytes Reduced (LR-RBCs).

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
0	4+	4+	4+	0

1. How would you interpret EW's ABO type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. What is EW's Rh type?

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine with the data provided.

3. Given the results of the initial antibody detection, what is the MOST LIKELY hypothesis?

- A. A single-specificity alloantibody.
- B. Multiple alloantibodies.
- C. One or more warm-reactive autoantibodies.
- D. Insufficient data to form a hypothesis.

4. The patterns of reactivity in Antibody Detection Test 1 and Antibody Identification Panel 1 are consistent with which of the following antibody specificities?

- A. Anti-C.
- B. Anti-D.
- C. Anti-D plus anti-E.
- D. Warm autoantibody.

5. **Given the patient's serologic data and clinical history, which of the following hypotheses is MOST LIKELY?**

- A. Anti-D due to Rh Immune Globulin (RhIG).
 - B. Autoanti-D.
 - C. Partial D with alloanti-D.
 - D. None of the above.
-

6. **Which of the following antibodies might be associated with a similar serologic picture?**

- A. A warm autoantibody.
 - B. Anti-C exhibiting dosage (single specificity).
 - C. Autoanti-LW.
 - D. None of the above.
-

7. **What is the infant's apparent ABO/Rh type?**

- A. AB negative.
 - B. O negative.
 - C. O positive.
 - D. Cannot determine without reverse typing.
-

8. **The medical laboratory scientist (MLS) has hypothesized the infant's clinical condition represents a case of HDFN due to the maternal anti-D. Of the following, which does NOT support this hypothesis?**

- A. Maternal antibody specificity.
 - B. Strong positive neonatal DAT result.
 - C. Neonatal clinical signs and symptoms.
 - D. Neonatal Rh type.
-

9. **At this stage in the investigation, which of the following tests would be MOST INFORMATIVE?**

- A. Prepare and test an eluate from the cord blood sample.
 - B. Repeat the DAT on the neonatal sample.
 - C. Repeat the antibody identification panel on the maternal serum.
 - D. Repeat the neonatal ABO and Rh type with washed cells.
-

10. **Which of the following are consistent with the results of the eluate panel?**

- A. Maternal anti-D was bound to the newborn's red cells.
- B. The newborn's cells are Rh positive.
- C. Both of the above.
- D. None of the above.

-
11. If EW was found by genotyping to be a partial DIIIa, what Rh-type blood should be selected for transfusion?
- A. Rh negative.
 - B. Rh positive.
 - C. Partial DIIIa.
 - D. None of the above.

Case Study 8

Initial Data:

SH, a 32-year-old female of European ancestry, has been admitted to the maternity ward of her local hospital in active labor at 39 weeks' gestation. She has had two prior pregnancies and has received regular prenatal care throughout this pregnancy. Prenatal testing revealed her as being Rh-negative, and she received a dose of Rh Immune Globulin (RhIG) at 28 weeks' gestation. A type and antibody screen was ordered upon her admission with the following results.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
0	0	0	4+	4+

1. How would you interpret SH's ABO group?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

Feedback:

Response A is correct. Given the initial serologic data, SH's forward (red cell) typing would be interpreted as group O. Her red cells were not reactive with either anti-A or anti-B, indicating neither A nor B antigens are present on the red cells. The reverse grouping indicates the presence of both anti-A and anti-B in her plasma. This is consistent with the red cell grouping and with the reactivity in a group O individual.

2. What is SH's Rh type?

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine with the data provided.

3. Why was antibody detection testing performed only with Rh-negative reagent cells?

- A. Pregnant females do not require testing for anti-D.
- B. Rh-negative cells are used to avoid detection of anti-G.
- C. The medical laboratory scientist (MLS) made an error in selecting the antibody detection cells.
- D. Rh-positive reagent cells would be expected to be reactive due to RhIG.

4. **What is the newborn's apparent ABO/Rh group and type?**

- A. Group A positive.
 - B. Group B positive.
 - C. Group O positive.
 - D. Cannot determine without reverse grouping.
-

5. **Which of the following reagents should be used in DATs for the newborn?**

- A. Anti-IgG.
 - B. Anti-C3b,-C3d.
 - C. Both.
 - D. Neither.
-

6. **The MLS recognizes that the positive DAT result on the neonate's red cells is an unexpected finding. Given the serologic results, which of the following antibodies would be the MOST LIKELY to have coated the infant's cells, causing the positive DAT result?**

- A. RhIG anti-D.
 - B. Maternal anti-A.
 - C. Maternal anti-c.
 - D. Antibody to low-prevalence antigen.
-

7. **Which type of eluate is MOST APPROPRIATE for the recovery of ABO antibodies?**

- A. Acid eluate.
 - B. Freeze-thaw.
 - C. Heat.
 - D. Either B or C.
-

8. **The test results from the two eluates eliminate which of the following options as the cause of the positive DAT result?**

- A. Anti-D from RhIG.
- B. Maternal anti-A.
- C. Common red cell alloantibodies.
- D. All of the above.

9. **Why was the paternal sample requested?**

- A. The plasma will be a good source of antibody.
 - B. The red cells will carry low-prevalence antigens.
 - C. There are insufficient cord cells for testing.
 - D. The paternal cells can predict the ABO zygosity of the neonate.
-

10. **Which is the MOST LIKELY way that the mother might have become immunized to the low-prevalence antigen?**

- A. Prior blood transfusion.
 - B. Through a prior pregnancy.
 - C. Contaminant of RhIG.
 - D. Naturally occurring antibody.
-

11. **Which of the following is the BEST way to approach additional testing in this case?**

- A. Test rare red cells carrying low-prevalence antigens.
 - B. Perform genotyping for alleles that code for low-prevalence antigens.
 - C. Type the paternal cells for low-prevalence antigens.
 - D. Treat the paternal cells with ficin and dithiothreitol (DTT).
-

12. **Why was 6% albumin tested by PEG-IAT with the chemically treated cells?**

- A. To exclude a false-positive reaction due to nonspecific aggregation.
 - B. To detect complement binding.
 - C. To detect ficin- or DTT-dependent autoantibodies.
 - D. To exclude a false-positive reaction due to hemolysis of the test cells.
-

13. **What is the BEST source of the antibody to low-prevalence antigen for additional testing?**

- A. An eluate prepared from an additional aliquot of cord cells.
 - B. An eluate prepared from a neonatal blood sample.
 - C. Neonatal plasma.
 - D. Maternal plasma.
-

14. **How can the maternal plasma be evaluated for anti-Rd?**

- A. Locate a group O Rh-negative, Rd-positive cell in another IRL and request testing.
- B. Remove the maternal anti-A, anti-B, and anti-D by adsorption.
- C. Neutralize the maternal anti-A and anti-B, and remove the anti-D by adsorption.
- D. All of the above.

- 15. If transfusion of the neonate had been required, what is the best source of red cells for transfusion?**
- A. Directed donation from the mother.
 - B. Directed donation from the father.
 - C. Crossmatch compatible unit from inventory.
 - D. Choices A, B, and C are equally acceptable.

Case Study 9

Initial Data:

An 85-year-old male, LL, arrives in the emergency department (ED) of your hospital immediately following an office visit to his physician. His chief complaint is increasing tiredness and some shortness of breath upon exertion. His hemoglobin level determined in the office is 4.5 g/dL. In the ED, the patient is alert and responsive. After confirming the low hemoglobin value, the ED physician orders direct and indirect antiglobulin tests (DAT and IAT, respectively) and crossmatch of 2 units of Red Blood Cells Leukocytes Reduced (LR-RBCs) for transfusion. LL reports an unremarkable clinical history and no history of blood transfusion or transplantation. The negative transfusion/transplant history was confirmed by his family members.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
0	0	4+	3+	4+

1. How would you interpret LL's ABO type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. What is LL's Rh type?

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine with the data provided.

3. Given the results of Antibody Detection Test 1 above, what is the BEST initial hypothesis?

- A. A single-specificity warm-reactive alloantibody.
- B. Multiple warm-reactive alloantibodies.
- C. One or more warm-reactive autoantibodies.
- D. Insufficient data to determine.

4. The medical laboratory scientist (MLS) interpreted the combined results of Antibody Detection Test 1 and Antibody Identification Panel 1 as being due to a possible warm-reactive autoantibody. Which of the data listed below support this hypothesis?

- A. All cells were reactive at IAT and reactions were of similar strength.
- B. The patient did not have a history of transfusion/transplantation.
- C. The autocontrol was positive.
- D. All of the above.

5. **What additional testing would be MOST INFORMATIVE at this stage?**

- A. An additional antibody identification panel using low-ionic-strength saline (LISS) IAT.
 - B. An additional antibody identification panel using ficin-treated cells.
 - C. A DAT using polyspecific antihuman globulin (AHG).
 - D. A DAT using anti-C3b,-C3d.
-

6. **Which of the following conclusions is MOST LIKELY given the combined results of Antibody Detection Test 1 and the DAT?**

- A. A warm-reactive autoantibody remains the most likely hypothesis.
 - B. Serologic evidence confirms the presence of an underlying alloantibody.
 - C. The DAT and antibody detection results rule out the possibility of a warm-reactive alloantibody.
 - D. The DAT result is not consistent with the results of the antibody detection test and should be repeated.
-

7. **What testing would you do next?**

- A. DAT with anti-IgG.
 - B. DAT with anti-C3b,-C3d.
 - C. Inert control.
 - D. All of the above.
-

8. **Given the results of the monospecific DATs, which of the following is/are coating LL's red cells?**

- A. IgG.
 - B. C3b,C3d.
 - C. Both.
 - D. Neither.
-

9. **Given that a request was made to prepare units for transfusion, what additional testing would provide useful serologic data?**

- A. Additional panel cells using PEG-IAT.
- B. Additional panel cells using LISS-IAT.
- C. Antibody detection using saline-IAT.
- D. No additional testing is necessary.

10. Given the results of the Antibody Detection Test 2 (Saline-IAT), what additional testing is suitable to determine if alloantibodies are present?

- A. Warm autoadsorption.
- B. Warm alloadsorption.
- C. Elution studies.
- D. None of the above.

11. The MLS pretreated the patient's cells with ZZAP before using them for the adsorption. What chemicals make up this reagent?

- A. PEG and ficin.
- B. Dithiothreitol (DTT) and papain.
- C. DTT and trypsin.
- D. PEG and DTT.

12. What is the purpose of treating autologous cells with ZZAP before adsorption?

- A. Remove IgG coating the patient's cells.
- B. Make patient's cells more effective in the autoadsorption procedure.
- C. Both of the above.
- D. None of the above.

13. When reviewing the results of the antibody detection using autoadsorbed plasma, what conclusion(s) can be made?

- A. The warm autoantibody was removed from the plasma.
- B. The presence of clinically significant alloantibodies is unlikely.
- C. Both of the above.
- D. None of the above.

14. What additional testing should be performed using the patient's red cells?

- A. Elution studies.
- B. Chloroquine diphosphate treatment.
- C. Red cell phenotyping.
- D. All of the above.

Case Study 10

Initial Data:

AL, a 42-year-old female of European ancestry, was diagnosed with warm autoimmune hemolytic anemia (WAIHA) 4 months ago. Despite a course of steroids and rituximab, she has been unable to maintain her hemoglobin level above 7 g/dL without multiple red cell transfusions. A red cell genotype was performed on a previous sample, because her hemolysis on admission was brisk and it was likely she would continue to require transfusion. Her red cell phenotype predicted from the genotyping results is C+E-c-e+; K-k+; Jk(a-b+); Fy(a+b-).

She is scheduled for splenectomy tomorrow. Two units of Red Blood Cells Leukocytes Reduced (LR-RBCs) are ordered to be transfused before surgery. Pretransfusion testing is performed.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
0	4+	3+	4+	0

1. How would you interpret AL's ABO type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. What is AL's Rh type?

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine with the data provided.

3. Given the clinical history and the results of Antibody Detection Test 1, what is the BEST initial hypothesis?

- A. A single-specificity warm-reactive alloantibody.
- B. Multiple warm-reactive alloantibodies.
- C. Warm-reactive autoantibody.
- D. Insufficient data to determine.

4. Given the results of the DATs, which of the following is/are coating AL's cells?

- A. IgG.
- B. C3b,C3d.
- C. Both.
- D. Neither.

5. Which of the following hypotheses are MOST LIKELY given the combined results of Antibody Detection Test 1 and 2 and the DAT?

- A. A warm-reactive autoantibody remains the most likely hypothesis.
 - B. Given the DAT results, a warm-reactive alloantibody is more likely.
 - C. The DAT and antibody detection results rule out the possibility of a warm-reactive alloantibody.
 - D. The DAT result is not consistent with the results of the antibody detection test and should be repeated.
-

6. The MLS is concerned there may be an alloantibody masked by the reactions of the autoantibody. In this case, which of the following procedures is MOST suitable to detect/identify underlying alloantibodies?

- A. Warm autoadsorption.
 - B. Warm allogeneic adsorption.
 - C. Elution studies.
 - D. Dilution of the plasma.
-

7. The MLS student intern on rotation in the transfusion service suggested that in order to save time, an elution should be performed concurrent with the alloadsorptions. How should the supervisory clinical instructor respond?

- A. Congratulate the student and ask her to perform the eluate to confirm earlier IRL results.
 - B. Tell the student the adsorption should be performed first, and an eluate may be warranted depending on the results of the antibody detection using adsorbed serum.
 - C. Remind the student that in this case IRL eluate results are consistent with current assumptions, and additional elutions would be unnecessary and duplicative.
 - D. Tell the student that performing an eluate is not consistent with the results of the DAT.
-

8. What is the BEST phenotype of donor red cells to use for the allogeneic adsorption?

- A. c-E-, K-, Jk(a-).
 - B. e-C-, K-, Jk(a-), Fy(b-).
 - C. c-E-, K-, Jk(a-), Fy(b-).
 - D. D-C-E-, K-, Jk(b-), s-.
-

9. Based on the serologic results, how many adsorptions should the IRL MLS initially perform to remove the autoantibody from the patient's plasma?

- A. 1.
- B. 2.
- C. 3.
- D. 4.

10. What conclusions can be drawn from the results of Antibody Detection Test 3 using alloadsorbed serum?

- A. Warm autoantibody is directed to a ficin-sensitive antigen.
 - B. No underlying alloantibodies are present.
 - C. One or more alloantibodies are likely present.
 - D. Warm autoantibody is not completely removed.
-

11. Which of the following antibodies can be eliminated using the serologic results of Antibody Detection Test 3?

- A. Anti-c.
 - B. Anti-K.
 - C. Anti-Jk^a.
 - D. All of the above.
-

12. Based on the results of Antibody Detection Test 3, what is/are the MOST LIKELY alloantibody(ies) present?

- A. Anti-Jk^a and anti-K.
 - B. Anti-K.
 - C. Anti-Jk^a.
 - D. All of the above interpretations are equally feasible.
-

13. What additional testing must be performed to determine what alloantibodies are present in the patient's serum?

- A. Test alloadsorbed serum against selected cells that are positive for the antigens that correspond to the suspected alloantibodies.
 - B. Perform another adsorption on the c-E-, K-, Jk(a-) donor cells because positive reactivity remains.
 - C. Adsorb with cells of a different phenotype.
 - D. All of the above.
-

14. What can be concluded from the results of the antibody identification panel?

- A. Reactivity with Selected Cells 1 and 2 [Jk(a+), K-] identifies anti-Jk^a.
- B. No reactivity with Selected Cell 3 provides an additional cell negative for K and Jk^a, satisfying the "Rule of Three" to identify antibodies.
- C. Reactivity with Selected Cells 4 and 5 [Jk(a-), K+] identifies anti-K.
- D. All of the above.

Case Study 11

Initial Data:

JR, a 56-year-old female of European ancestry, was diagnosed with warm autoimmune hemolytic anemia (WAIHA) 1 month ago. She is being treated with steroids. Her direct antiglobulin test (DAT; polyspecific) was positive (4+). An eluate prepared from her red cells was positive with all panel cells tested. She received 4 units of Red Blood Cells Leukocytes Reduced (LR-RBCs) over the last several weeks. On her follow-up visit to the hematology clinic today, her hemoglobin level has decreased to 6.8 g/dL and she is experiencing shortness of breath upon exertion. Two units of LR-RBCs are ordered for transfusion. Her pretransfusion serologic testing follows.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
0	0	4+	4+	4+

1. How would you interpret JR's ABO type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. What is the patient's Rh type?

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine with the data provided.

3. Which of the following hypotheses is/are consistent with the patient's history, clinical findings, and antibody detection test results?

- A. Drug-dependent antibody.
- B. Warm-reactive autoantibody.
- C. Warm-reactive alloantibody.
- D. Either A or B.

4. What method could be tested with the patient's serum that may diminish reactivity of the apparent autoantibody?

- A. Polyethylene glycol (PEG) tube test.
- B. Solid-phase testing.
- C. Saline or low-ionic-strength saline (LISS) IAT.
- D. None of the above.

5. Which of the following conclusions are consistent with the results of Antibody Detection Test 2?

- A. No alloantibodies are present in the serum.
- B. An alloantibody to a high-prevalence antigen is probable.
- C. The antibody is most likely drug related.
- D. None of the above.

6. Because it remains probable that a warm autoantibody is causing the strong reactivity in tests without enhancement media, another approach must be used to exclude alloantibodies. Given the patient's history, which of the following procedures is best used to accomplish this?

- A. Warm autoadsorption.
- B. Warm allogeneic adsorption.
- C. Elution studies.
- D. Dilution of the plasma.

7. Based on the result of the DAT, what is coating the patient's cells?

- A. IgG.
- B. C3b,C3d.
- C. Both of the above.
- D. None of the above.

8. The results of the IRL eluate panel and other serologic findings are consistent with which of the following interpretations?

- A. Cold-reactive autoantibody.
- B. Drug-dependent antibody.
- C. Warm-reactive autoantibody.
- D. Warm-reactive alloantibody.

9. The IRL MLS performed an antibody detection test with each adsorbed serum sample. Was this the ideal approach to solve the problem?

- A. No, only one adsorbed serum (R_1R_1) is required for antibody detection; the testing of R_2R_2 and rr cells is not necessary unless the R_1R_1 results are positive.
- B. No, antibody detection tests using adsorbed serum are not required unless the patient has an adverse reaction to future transfusions.
- C. No, phenotyping the patient's red cells would have been a more efficient approach.
- D. Yes, all three antibody detection tests are necessary in order to effectively rule out most clinically significant antibodies.

10. How would you interpret the adsorbed serum results?

- A. Warm autoantibody is removed.
- B. Most underlying alloantibodies have been ruled out.
- C. The test is invalid.
- D. Both A and B.

11. What is the primary limitation when alloabsorptions with ficin-treated cells are performed?

- A. Antibodies to antigens destroyed by ficin would not be adsorbed from the serum.
- B. When cells are treated with ficin they do not adsorb antibodies as well.
- C. Antibodies to protease-resistant antigens of high-prevalence would not be detected.
- D. All of the above.

12. Which of the following is the next BEST test to perform in order to provide compatible LR-RBCs for this patient?

- A. Serologic crossmatch using unadsorbed serum.
- B. Serologic crossmatch using alloadsorbed serum.
- C. Computer (electronic) crossmatch.
- D. None of the above.

Case Study 12

Initial Data:

ET, a 64-year-old male of European ancestry with a diagnosis of chronic lymphocytic anemia, is scheduled for outpatient transfusion tomorrow. He received 2 units of Red Blood Cells Leukocytes Reduced (LR-RBCs) 3 weeks ago. His hemoglobin level is 7.0 g/dL. Two units of LR-RBCs are ordered for transfusion.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
4+	0	4+	0	4+

1. How would you interpret ET's ABO type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. What is the patient's Rh type?

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine with the data provided.

3. Given the results of the antibody detection test above, what is the BEST initial hypothesis?

- A. A single-specificity warm-reactive alloantibody.
- B. Multiple warm-reactive alloantibodies.
- C. Warm-reactive autoantibody.
- D. Cannot determine with data provided.

4. Given the combined results of Antibody Detection Test 1 and Antibody Identification Panel 1 in solid phase, what is your initial hypothesis?

- A. Warm-reactive alloantibody.
- B. Warm-reactive autoantibody.
- C. Method-dependent antibody.
- D. Insufficient data to support a hypothesis.

5. **Of the following, which would be the BEST test to perform next in this case?**

- A. Saline antibody identification panel.
 - B. Polyethylene glycol (PEG) antibody identification panel.
 - C. Warm autoadsorption.
 - D. Direct antiglobulin test (DAT).
-

6. **Which of the following is MOST LIKELY given the combined results of Antibody Detection Test 1, Antibody Identification Panel 1, and the DAT?**

- A. A warm-reactive autoantibody is likely.
 - B. A warm-reactive alloantibody is likely.
 - C. The possibility of a warm-reactive alloantibody has been ruled out.
 - D. The DAT result is not consistent with the results of the antibody detection and should be repeated.
-

7. **Which of the following tests would you perform next?**

- A. DAT with anti-IgG.
 - B. DAT with anti-C3b,-C3d.
 - C. Inert control.
 - D. All of the above.
-

8. **Given the results of the monospecific DATs, which of the following is/are coating ET's red cells?**

- A. IgG.
 - B. C3b,C3d.
 - C. Both.
 - D. Neither.
-

9. **What antibody detection/identification method could be utilized with the patient's serum to potentially avoid reactivity of the apparent warm autoantibody?**

- A. PEG tube test.
- B. Gel testing.
- C. Saline or low-ionic-strength saline (LISS) indirect antiglobulin test (IAT).
- D. None of the above.

10. **Because it remains probable that a warm autoantibody is causing the strong reactivity in tests without enhancement media, another approach must be used to exclude alloantibodies. Which of the following procedures is BEST used to accomplish this?**

- A. Warm autoadsorption.
- B. Warm allogeneic adsorption.
- C. Elution studies.
- D. Dilution of the plasma.

11. **The results of the IRL eluate panel and other serologic combined findings are consistent with which of the following interpretations?**

- A. Cold-reactive autoantibody.
- B. Drug-dependent antibody.
- C. Warm-reactive autoantibody.
- D. Either B or C.

12. **If the patient had an underlying anti-Fy^a, which of the cells selected for the alloadsorptions would remove the antibody from the serum?**

- A. Cell I.
- B. Cell II.
- C. Cell III
- D. None of the above.

13. **Which of the following is consistent with the results of Antibody Detection Test 3?**

- A. Warm autoantibody was removed.
- B. No underlying alloantibodies are present.
- C. Alloantibody is likely present.
- D. Both A and C.

14. **Which of the following antibodies was/were ruled out based on the results of Antibody Detection Test 3?**

- A. Anti-M.
- B. Anti-K.
- C. Anti-c.
- D. All of the above.

15. After reviewing the evaluations provided for each of the adsorbed sera, which of the following is the **MOST LIKELY** alloantibody present in the patient's serum?

- A. Anti-D.
- B. Anti-E.
- C. Anti-S.
- D. Anti-e.

16. Which of the following would generally **NOT** be detected when using alloadsorption procedures?

- A. Alloantibodies to antigens of high prevalence.
- B. Alloantibodies to antigens of low prevalence.
- C. Both would be undetected.
- D. Both would be detected.

17. Based on the "Rule of Three," which of the following tests is/are required to confirm the identity of the alloantibody?

- A. Test serum adsorbed on c-E-, K-, Jk(b-) donor red cells against suspected antigen-positive selected cells.
- B. Test serum adsorbed on e-C-, K-, Jk(a-) donor red cells against suspected antigen-positive selected cells.
- C. Test serum adsorbed on D-C-E-, Jk(b-) donor red cells against suspected antigen-positive selected cells.
- D. A and C.

18. Given the serologic findings, what is the **BEST** method to provide compatible blood for transfusion to this patient?

- A. Provide phenotypically matched uncrossmatched donor units.
- B. Perform a serologic crossmatch with random-donor units using adsorbed serum.
- C. Perform a computer (electronic crossmatch) with E- donor units.
- D. Perform a serologic crossmatch with E- donor units using adsorbed serum.

Case Study 13

Initial Data:

CS, a 79-year-old female of European ancestry has been spending the winter in Florida. While visiting her daughter over the holiday season in Minnesota, she was admitted to the emergency department (ED) complaining of chest pain. Her hemoglobin level on admission was 6.5 g/dL. Two units of Red Blood Cells Leukocytes Reduced (LR-RBCs) were ordered to be transfused as soon as available. There is no record of the patient in this facility.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
0	0	0	4+	4+

1. How would you interpret CS's ABO type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. What is CS's Rh type?

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine with the data provided.

3. Given the results of Antibody Detection Test 1, what is the BEST initial hypothesis?

- A. Single-specificity warm-reactive alloantibody.
- B. Multiple warm-reactive alloantibodies.
- C. Warm-reactive autoantibody.
- D. Cannot determine with the data provided.

4. Given the combined results of Antibody Detection Test 1 and Antibody Identification Panel 1, which of the following is the MOST PROBABLE initial hypothesis?

- A. Warm-reactive alloantibody.
- B. Warm-reactive autoantibody.
- C. Warm-reactive autoantibody with an underlying alloantibody.
- D. Method-dependent antibody.

5. Which of the following tests would be the BEST to perform at this stage of the investigation?

- A. Warm alloadsorption.
 - B. Polyethylene glycol (PEG) antibody identification panel.
 - C. Warm autoadsorption.
 - D. Direct antiglobulin test (DAT).
-

6. Which of the following conclusions is MOST LIKELY given the combined results of Antibody Detection Test 1, Antibody Identification Panel 1, and the DAT?

- A. The data are consistent with the previous warm-autoantibody hypothesis.
 - B. Given the DAT results, a warm-reactive alloantibody is likely.
 - C. A warm-reactive alloantibody has been ruled out.
 - D. The DAT results are not consistent with the results of the antibody detection test, and the DAT should be repeated.
-

7. What testing would you perform next?

- A. DAT with anti-IgG.
 - B. DAT with anti-C3b,-C3d.
 - C. Inert control.
 - D. All of the above.
-

8. Given the results of the monospecific DATs, which of the following is/are coating CS's red cells?

- A. IgG.
 - B. C3b,C3d.
 - C. Both.
 - D. Neither.
-

9. When the monospecific DATs were performed, the medical laboratory scientist (MLS) included a room temperature incubation stage with the anti-C3b,-C3d but NOT with the anti-IgG. What should the supervisor say to the MLS regarding this test protocol?

- A. Explain that this was a procedural error; a room temperature incubation should have been included with both anti-IgG and anti-C3b,-C3d.
- B. Explain that this was a procedural error; the room temperature incubation should have been performed with anti-IgG but NOT with anti-C3b,-C3d.
- C. Explain that this was a procedural error; the incubation should have been performed at 37 C.
- D. Provide positive reinforcement because this was a correct choice.

10. What procedure is BEST to use for detecting underlying alloantibodies?

- A. Warm autoadsorption.
 - B. Warm allogeneic adsorption.
 - C. Elution studies.
 - D. Dilution of the plasma.
-

11. Which of the following statements is consistent with the results of the eluate panel?

- A. A drug-dependent antibody is likely.
 - B. A cold-reactive autoantibody is likely.
 - C. The warm-reactive autoantibody hypothesis has been confirmed.
 - D. A warm-reactive autoantibody has been ruled out.
-

12. Suggesting that it would save time and conserve reagents, a rotating student asked the MLS why she did not pool the three adsorbed sera and test a single antibody identification panel using this pool. How should the MLS respond to the student?

- A. Thank the student for her insight and use pooled serum as suggested.
 - B. Tell the student that pooling sera for antibody detection/identification may result in dilution of antibodies, with subsequent reduction in reactivity.
 - C. Tell the student that each of the adsorbed sera must be tested independently because each of the cells used will adsorb out different specificities.
 - D. Both B and C.
-

13. Which of the following is supported by the results of the antibody detection tests with the three alloadsorbed sera?

- A. Warm autoantibody was not completely adsorbed.
 - B. No underlying alloantibodies are present.
 - C. One or more alloantibodies are likely present.
 - D. Both A and C.
-

14. What antibodies can be eliminated using Evaluation 1?

- A. Anti-E.
- B. Anti-D.
- C. Anti-c.
- D. Anti-Fy^b.

15. What additional antibodies can be eliminated using Evaluation 2?

- A. Anti-C.
- B. Anti-M.
- C. Anti-Fy^a.
- D. Anti-Jk^a.

16. Which of the following is the MOST LIKELY alloantibody present?

- A. Anti-Jk^a.
- B. Anti-Fy^a.
- C. Anti-K.
- D. Anti-D.

17. What additional testing must be performed to determine what alloantibodies are present in the patient's serum?

- A. Test serum adsorbed on c-E-, K-, Jk(a-) donor red cells against suspected antigen-positive selected cells.
- B. Test serum adsorbed on e-C-, K-, Jk(a-) donor red cells against suspected antigen-positive selected cells.
- C. Test serum adsorbed on D-C-E-, Jk(b-), s- donor red cells against suspected antigen-positive selected cells.
- D. All of the above.

18. For the testing to confirm the suspected anti-Fy^a, any adsorbed serum may be tested with additional Fy(a+) selected cells. Why is this the case?

- A. Anti-Fy^a is adsorbed on all donor red cells selected.
- B. All adsorbing red cells are Fy(a+) after ficin treatment.
- C. All adsorbing red cells are Fy(a-) after ficin treatment.
- D. All adsorbing red cells are Fy(a+) weak after ficin treatment.

19. What do the results of testing the selected cells with the adsorbed sera indicate?

- A. No reactivity with Selected Cell 1 vs serum adsorbed with c-E-, K-, Jk(a-) red cells allows for rule-out of anti-Jk^a.
- B. Selected Cell 2 is positive, providing confirmatory evidence for the anti-Fy^a hypothesis.
- C. No reactivity with Selected Cell 3 vs serum adsorbed with c-E-, K-, Jk(a-) red cells allows for rule-out of anti-K.
- D. All of the above.

20. Are all other clinically significant alloantibodies ruled out according to the laboratory criteria?

- A. Yes.
- B. No, anti-Jk^b cannot be ruled out.
- C. No, anti-s cannot be ruled out.
- D. No, anti-S was NOT ruled out with a double-dose antigen-positive cell, as stipulated in the laboratory protocol.

Case Study 14

Initial Data:

An 87-year-old female, JW, was transferred from her nursing care facility to the short stay unit of the local hospital. Examination by her physician had revealed a hemoglobin level of 5.7 g/dL. JW is of African ancestry and has a history of four pregnancies (four live births) but no known blood transfusions. Her medical history currently shows slight dementia, diabetes, and hypertension with a prior hysterectomy and cholecystectomy.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
4+	4+	4+	0	0

1. How would you interpret JW's ABO type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. What is the patient's Rh type?

- A. D+.
- B. D-.
- C. Weak D.
- D. Cannot determine with data provided.

3. Given the results of Antibody Detection Test 1, what is the MOST LIKELY initial hypothesis?

- A. Multiple alloantibodies.
- B. A warm-reactive autoantibody.
- C. An autoantibody with an underlying alloantibody.
- D. Cannot determine with the data provided.

4. The medical laboratory scientist (MLS) interpreted these results as being due to a possible warm-reactive autoantibody. Which of the following would support this conclusion?

- A. All cells reactive and of similar strength.
- B. Positive autocontrol in a patient not recently transfused.
- C. Reactivity at the antiglobulin phase of testing.
- D. All of the above.

5. Which of the following tests would be MOST SUITABLE at this stage?

- A. Crossmatch.
 - B. Enzyme panel.
 - C. Direct antiglobulin test (DAT) with polyspecific antihuman globulin (AHG).
 - D. Elution studies.
-

6. How would you interpret the results of the DAT above?

- A. Only IgG is present on the patient's cells.
 - B. Only complement is present on the patient's cells.
 - C. IgG and/or complement are/is present on the patient's cells.
 - D. Neither IgG nor complement is present on the patient's cells.
-

7. What testing would you perform next?

- A. DAT with anti-IgG.
 - B. DAT with anti-C3b,-C3d.
 - C. Inert control.
 - D. All of the above.
-

8. Based on the DAT results above, one can conclude that the patient's red cells are coated with which of the following?

- A. IgG.
 - B. C3b,C3d.
 - C. Both.
 - D. Neither.
-

9. Before transfusion, the plasma must be evaluated for the presence of underlying alloantibodies. Which of the following procedures is the BEST way to accomplish this?

- A. Warm autoadsorption.
- B. Warm alloadsorption.
- C. Elution studies.
- D. Dilution of the plasma.

10. Before conducting the adsorption procedure, the MLS pretreated the patient's cells with ZZAP reagent. Why was this done?

- A. To remove IgG coating the patient's cells.
- B. To enzyme treat the patient's cells.
- C. Both A and B.
- D. Neither A nor B.

11. The autoadsorbed plasma appears to have the same reactivity as the unadsorbed plasma. Which of the following is an UNLIKELY explanation for this finding?

- A. The serum reactivity is due to an alloantibody of high prevalence.
- B. An insufficient number of adsorptions have been performed.
- C. The target antigen was destroyed by the ZZAP pretreatment.
- D. The serum reactivity is due to multiple common alloantibodies.

12. Why was the K antigen status not determined after the treatment with EDTA-glycine?

- A. The red cells are assumed to be K-.
- B. Anti-K is not a clinically significant antibody.
- C. EDTA-glycine treatment aggregates K+ red cells.
- D. EDTA-glycine treatment destroys KEL system antigens.

13. Which of the following hypotheses are supported by these results?

- A. Plasma reactivity is due to multiple alloantibodies to common antigens.
- B. Results are consistent with an alloantibody to a high-prevalence antigen.
- C. Results are consistent with an alloantibody to a low-prevalence antigen.
- D. None of the above.

14. Given the results of Antibody Detection Test 3, what test cells should be tested next to identify the antibody to high-prevalence antigen?

- A. k-, Kp(b-), Sc:-1.
- B. Js(b-), Hy-, Jo(a-).
- C. Yt(a-), JMH-, Ge:-2.
- D. Lu(b-), At(a-), U-.

15. Which of the statements above could logically explain the test results in this case?

- A. Statements 1 and 5.
 - B. Statements 2 and 3.
 - C. Statements 3 and 5.
 - D. Statements 4 and 5.
-

16. What conclusions can be made about the reactivity seen in Antibody Detection Test 4?

- A. Reactivity is consistent with autoantibody.
 - B. There is no evidence to suggest that an underlying alloantibody is present.
 - C. Reactivity is consistent with multiple alloantibodies to common antigens.
 - D. Both A and B.
-

17. Which of the following is/are likely, given the previous serologic data in combination with the eluate results?

- A. Autoantibody is coating patient's red cell.
 - B. The autoantibody is directed to an antigen in the KEL blood group system.
 - C. Both of the above.
 - D. None of the above.
-

18. What would be the MOST APPROPRIATE blood to select for transfusion of JW?

- A. Randomly selected, ABO-compatible Red Blood Cells.
- B. Blood from donors who are least incompatible or crossmatch compatible with adsorbed plasma.
- C. Kp(b-) red cells from a rare donor registry.
- D. Phenotypically similar Red Blood Cells.

Case Study 15

Initial Data:

GV is a 64-year-old male of European ancestry. His reported diagnosis is autoimmune hemolytic anemia (AIHA). This is the first time the transfusion service has seen this patient. Four units of Red Blood Cells Leukocytes Reduced (LR-RBCs) are ordered for transfusion. His hematocrit is 21.6%.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
4+	1+	4+	4+	4+

1. How would you interpret GV's ABO forward typing?

- A. Group O.
- B. Group A.
- C. Group AB.
- D. Unable to interpret.

Feedback:

Response D is correct. Given the initial serologic data, GV's forward (cell) typing shows agglutination with anti-A and anti-B. However, the strength of reactivity with anti-B is weaker than expected. A and B antigens should be strongly reactive with commercial antisera; weak reactions such as noted in this case should be investigated before they can be interpreted with confidence.

2. How would you interpret the ABO reverse typing?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

3. Given the results of the forward and reverse ABO typing, what is the BEST next step in this case?

- A. Report GV's type as group AB.
- B. Report GV's type as group O.
- C. Hypothesize the reverse typing is incorrect and investigate.
- D. Hypothesize the forward typing is incorrect and investigate.

4. What is GV's Rh type?

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine with the data provided.

5. **What additional testing should be performed to confirm the Rh type?**

- A. Weak D test.
 - B. Inert control.
 - C. Anti-D with a second source of reagent.
 - D. None of the above.
-

6. **Given the results of the repeat ABO forward and Rh type, what is GV's Rh type?**

- A. D+.
 - B. D-.
 - C. Weak D+.
 - D. Cannot determine with the data provided.
-

7. **What would cause an individual's red cells to appear to spontaneously agglutinate before a centrifugation?**

- A. Warm autoantibody.
 - B. Rouleaux.
 - C. Cold autoantibody.
 - D. All of the above.
-

8. **Given the results of the antibody detection test above, what is the BEST initial hypothesis?**

- A. A single-specificity, warm-reactive alloantibody.
 - B. Multiple warm-reactive alloantibodies.
 - C. A warm-reactive autoantibody.
 - D. Insufficient data to determine.
-

9. **Given the results of the antibody detection test and antibody identification panel in solid phase, what is your initial hypothesis?**

- A. Warm-reactive alloantibody.
 - B. Warm-reactive autoantibody.
 - C. Method-dependent antibody.
 - D. Insufficient data to support a hypothesis.
-

10. **At this point in the investigation, which of the following tests would be MOST INFORMATIVE?**

- A. Saline antibody identification panel.
- B. Polyethylene glycol (PEG) antibody identification panel.
- C. Warm autoadsorption.
- D. Direct antiglobulin test (DAT).

11. **How would you interpret the results of the DAT?**

- A. Neither IgG nor complement is present on the patient's cells.
 - B. Only IgG is present on the patient's cells.
 - C. Only complement is present on the patient's cells.
 - D. IgG and/or complement is/are present on the patient's cells.
-

12. **Based on the results of the DATs, which of the following is/are coating the patient's red cells?**

- A. IgG.
 - B. C3.
 - C. Both IgG and C3.
 - D. Insufficient data to determine.
-

13. **At this point in the investigation, which of the following tests would be MOST helpful to clarify the cause of the previous serologic results?**

- A. Saline antibody identification panel.
 - B. PEG antibody identification panel.
 - C. Cold antibody identification panel.
 - D. Eluate.
-

14. **What do the results of Antibody Identification Panel 2 indicate?**

- A. A warm-reactive antibody is present.
 - B. No alloantibody(ies) is (are) present.
 - C. Cold-reactive alloantibody is present.
 - D. Insufficient data to interpret.
-

15. **Which of the following tests would be MOST INFORMATIVE at this point in the investigation?**

- A. PEG antibody identification panel.
 - B. Saline antibody identification panel.
 - C. Cold antibody identification panel.
 - D. Low-ionic-strength saline (LISS) antibody identification panel.
-

16. **Which of the following can be concluded from the results of the cold panel?**

- A. A cold-reactive autoantibody is present.
- B. Autoanti-I is present.
- C. Autoanti-IH is present.
- D. A warm-reactive alloantibody is present.

17. Which of the following hypotheses is/are consistent with the results of Antibody Detection Test 2 and the previous serologic results?

- A. A warm-reactive autoantibody is likely present.
- B. Alloantibodies have not been ruled out.
- C. A cold-reactive autoantibody is carrying through to IAT.
- D. All of the above.

18. What conclusion(s) can be made from the results of the antibody detection test using autoadsorbed plasma?

- A. Warm autoantibody was removed from the plasma.
- B. The presence of clinically significant alloantibodies is unlikely.
- C. Both of the above.
- D. None of the above.

19. Of the following tests using the patient's red cells, which would be MOST INFORMATIVE at this point in the investigation?

- A. Elution studies.
- B. Chloroquine diphosphate treatment.
- C. Red cell phenotyping.
- D. All of the above.

20. What additional testing should be performed to resolve this case?

- A. An antibody identification panel at 4 C using cold adsorbed plasma.
- B. An antibody identification panel at 37 C using warm adsorbed plasma.
- C. A repeat ABO reverse grouping using serum adsorbed at 4 C.
- D. No additional testing is necessary.

21. How would you interpret the ABO reverse (plasma) type using the autoadsorbed plasma?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

22. Which of the following chemicals would be the BEST choice to treat the patient's red cells to remove autoantibody?

- A. Dithiothreitol (DTT) or 2-mercaptoethanol (2-ME).
- B. Chloroquine diphosphate.
- C. Glycine acid-EDTA.
- D. None of the above.

23. How would you interpret GV's ABO forward (cell) and Rh typing using the DTT-treated red cells?

- A. Group O+.
- B. Group A+.
- C. Group AB+.
- D. Unable to interpret.

24. Based on the results of the DATs with DTT-treated cells, what is coating this patient's red cells?

- A. IgG.
- B. C3b,C3d.
- C. Both.
- D. Insufficient data to determine.

Case Study 16

Initial Data:

JC, a 51-year-old female of European ancestry, was diagnosed with endometrial cancer 3 weeks ago. She has a history of Type 1 diabetes and moderate-to-severe hypertension. She had a total hysterectomy 12 days ago. Her postsurgical hemoglobin level decreased to a low of 3.5 g/dL and she received 8 units of Red Blood Cells Leukocytes Reduced over the course of the last 3 days. Her current laboratory values are as follows: hemoglobin, 6 g/dL; lactate dehydrogenase (LDH), 700 IU/L; and total bilirubin, 14.0 mg/dL.

Her physician is investigating the cause of this apparent hemolytic event and, upon review of JC’s medication history, notes she received 1 g of cefotetan intravenously on arrival in the operating room. Drug-related hemolysis is suspected, and a sample is sent to the immunohematology reference laboratory (IRL) with a request to perform drug studies.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
4+	4+	4+	0	0

1. How would you interpret JC’s ABO type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. What is the patient’s Rh type?

- A. D+.
- B. D-.
- C. Weak D.
- D. Cannot determine with data provided.

3. Which of the following is/are consistent with the results of Antibody Detection Test 1?

- A. Antibodies may be present but were not detected using saline.
- B. The patient does not have any alloantibodies in her serum.
- C. A drug-dependent antibody may be present in the serum.
- D. All of the above.

4. Which of the following hypotheses is/are consistent with the results of Antibody Detection Tests 1 and 2 performed in the IRL using enhancement methods (PEG and ficin)?

- A. The patient has a warm autoantibody.
- B. The patient has a drug-dependent antibody.
- C. The patient has a cold autoantibody.
- D. Either A or B.

5. **Given the results of the monospecific DATs, what is coating this patient's red cells?**

- A. IgG.
 - B. C3b,C3d.
 - C. Both IgG and C3.
 - D. Neither IgG nor C3.
-

6. **What additional testing would be MOST INFORMATIVE at this point in the investigation?**

- A. Elution.
 - B. Antibody identification panel using a saline-IAT method.
 - C. Antibody detection test with an alternate method.
 - D. Red cell phenotyping.
-

7. **Which of the following hypotheses would be consistent with the results of Antibody Identification?**

- A. The eluate technique did not remove antibody bound to the cells.
 - B. The eluted antibody is drug dependent.
 - C. There was a technical error.
 - D. All of the above.
-

8. **Which of the following is consistent with the results of the drug study with the patient's serum and drug-treated red cells (Drug Study 1)?**

- A. A cefotetan-dependent antibody is likely.
 - B. A cefotetan-dependent antibody is not supported by the data.
 - C. The test cannot be interpreted because of the reactions with normal plasma.
 - D. None of the above.
-

9. **Which of the following would provide confirmatory evidence to support the cefotetan-dependent drug hypothesis?**

- A. An antibody identification panel using drug-treated cells.
 - B. A decrease in signs/symptoms of hemolysis after withdrawal of the drug.
 - C. Testing drug-treated cells with an eluate from the patient's cells.
 - D. Both B and C.
-

10. **Which of the following can be concluded given the combined serologic results?**

- A. A cefotetan-dependent antibody is present in the plasma.
- B. A cefotetan-dependent antibody is **NOT** bound to the red cells.
- C. An underlying alloantibody is likely.
- D. All of the above.

Case Study 17

Initial Data:

SL, a 49-year-old male of European ancestry with a diagnosis of cirrhosis, hepatitis C virus (HCV), and human immunodeficiency virus (HIV), received 2 units of Red Blood Cells Leukocytes Reduced (LR-RBCs) 3 days ago. No alloantibodies were detected at that time. His hemoglobin today is 3.6 g/dL. Four additional units of LR-RBCs are ordered to be transfused as soon as available.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
0	0	4+	4+	4+

1. How would you interpret SL's ABO group?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. What is the patient's Rh type?

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine from the data provided.

3. Given the results of the antibody detection test, what is the MOST LIKELY initial hypothesis?

- A. Autoantibody.
- B. Multiple alloantibodies.
- C. Single alloantibody.
- D. Cannot determine from data provided.

4. Which of the following tests would be the BEST choice to perform next?

- A. Antibody identification panel.
- B. Autocontrol.
- C. Direct antiglobulin test (DAT).
- D. Both A and B.

5. The results of Antibody Identification Panel 1 are MOST consistent with which of the following interpretations?

- A. A single alloantibody.
 - B. Multiple alloantibodies.
 - C. An autoantibody.
 - D. An autoantibody with underlying alloantibodies.
-

6. Do the reactions suggest any specificity?

- A. No evident specificity.
 - B. Anti-e.
 - C. Anti-i.
 - D. Anti-I.
-

7. Based on the autocontrol results in Antibody Identification Panel 1, what additional testing should be performed next?

- A. DAT with polyspecific antihuman globulin (AHG).
 - B. DAT with anti-IgG.
 - C. DAT with anti-C3b,-C3d.
 - D. Both B and C.
-

8. Based on the result of the DAT, which of the following is/are coating the patient's red cells?

- A. IgG.
 - B. IgG and C3.
 - C. IgA.
 - D. None of the above.
-

9. The combined panel results and DAT (pattern and strength) are suggestive of which of the following?

- A. Warm autoantibody is coating the patient's red cells.
- B. Cold autoantibody is coating the patient's red cells.
- C. Drug-dependent antibody is coating the patient's red cells.
- D. Cannot determine from the data provided.

10. What additional information should be considered when evaluating the clinical significance of the DAT result?

- A. Transfusion and drug history.
- B. Clinical findings.
- C. Laboratory findings.
- D. All of the above.

11. The results of the IRL Antibody Identification Panels 1 and 2 are MOST consistent with which of the following interpretations?

- A. A single alloantibody.
- B. Multiple alloantibodies.
- C. An autoantibody with relative anti-e specificity.
- D. An autoantibody with no specificity.

12. What additional testing would be MOST beneficial at this point in the investigation?

- A. Elution.
- B. Red cell phenotype.
- C. Antibody detection test with an alternate method.
- D. Serologic crossmatch.

13. Which of the following is consistent with the results of the eluate?

- A. The DAT results were incorrect; there is no IgG on the red cells.
- B. The IgG on the red cells could not be detected using the test method employed in the eluate panel.
- C. The antibody is drug-dependent.
- D. Either B or C.

14. Of those listed above, which drugs have been reported to cause DIIHA?

- A. Lorazepam.
- B. Esomeprazole.
- C. Vancomycin and Zosyn.
- D. Levofloxacin and Lorazepam.

15. **Given the timing of the suspected drugs, which is/are MOST LIKELY implicated in the hemolytic anemia?**

- A. Vancomycin.
- B. Zosyn (piperacillin/tazobactam).
- C. Both vancomycin and Zosyn.
- D. None of the above.

16. **Given the serologic testing performed in the presence of drug, along with the previous serologic findings, what is/are the MOST LIKELY conclusion(s)?**

- A. A piperacillin-dependent antibody is present in the eluate.
- B. A piperacillin-dependent antibody is present in the serum.
- C. Both of the above.
- D. None of the above.

17. **What is the MOST LIKELY explanation for the relative anti-e reactivity seen when testing the patient's serum in Antibody Identification Panel 1?**

- A. The patient likely has an alloanti-e.
- B. Results were likely due to piperacillin-dependent antibody with relative e specificity.
- C. Results were likely due to technical error.
- D. None of the above.

Case Study 18

Initial Data:

TT, a 38-year-old male of European ancestry, was admitted to the Emergency Department (ED) complaining of shaking chills, shortness of breath, headache, and orange urine. The ED physician also noted jaundice on the patient’s record. The physician ordered a complete blood count (CBC) and routine chemistry panel.

Selected Admission Laboratory Values*:

Patient Values	Reference Values
Hemoglobin: 4 g/dL	14-18 g/dL
Hematocrit: 12%	42%-52%
Spherocytes: Few noted on peripheral smear	Not normally seen
Lactate dehydrogenase (LDH): 37,000 IU/L	105-333 IU/L
Bilirubin: Total: 16.1 mg/dL Direct: 12.1 mg/dL	Total: 0.3-1.9 mg/dL Direct: 0.0-0.3 mg/dL
*Conventional units.	

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
0	4+	4+	4+	0

1. How would you interpret TT’s ABO type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. What is the patient’s Rh type?

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine with the data provided.

3. In the context of this case and evidence of hemolysis, which of the following interpretations is/are consistent with the results of the antibody detection test?

- A. Hemolysis may due to an antibody directed against a low-prevalence antigen.
- B. Hemolysis may be due to a drug-dependent antibody.
- C. Hemolysis may be due to antibodies that were undetected using this method.
- D. All of the above.

4. **Given the clinical and serologic data, which of the following laboratory tests would be warranted?**

- A. LDH.
 - B. Plasma hemoglobin.
 - C. Repeat bilirubin.
 - D. All of the above.
-

5. **Given the results of posttransfusion testing (Parts 1 and 2) and the clinical history, which of the following tests would be useful to help determine the etiology of the presumed hemolysis?**

- A. Elution.
 - B. Direct antiglobulin test (DAT).
 - C. Antibody panel.
 - D. All of the above.
-

6. **How would you interpret the results of the DAT?**

- A. Only IgG is present on the patient's cells.
 - B. Only complement is present on the patient's cells.
 - C. IgG and/or complement are/is present on the patient's cells.
 - D. Neither IgG nor complement is present on the patient's cells.
-

7. **What testing would you conduct next?**

- A. DAT with anti-IgG.
 - B. DAT with anti-C3b,-C3d.
 - C. Inert control.
 - D. All of the above.
-

8. **What is coating this patient's red cells?**

- A. IgG.
 - B. C3b,C3d.
 - C. Both.
 - D. Neither.
-

9. **What additional information should be considered during evaluation of the clinical etiology of the DAT result?**

- A. Serologic history.
- B. Medication history (current and past).
- C. Previous clinical evidence of hemolysis.
- D. All of the above.

10. What additional testing would be most beneficial at this point in the investigation?

- A. Elution.
- B. Antibody identification panel (serum).
- C. Antibody detection test with an alternate method.
- D. None of the above.

11. How would you interpret the results of the acid eluate?

- A. Positive.
- B. Negative.
- C. Inconclusive.
- D. Other.

12. Which of the following is consistent with the results of the drug studies?

- A. Drug-induced hemolysis due to Tolectin.
- B. Warm autoimmune hemolysis not related to Tolectin.
- C. Cannot be interpreted due to lack of reactivity in the diluent and Tolectin tube.
- D. Nonimmune hemolysis.

Case Study 19

Initial Data:

TS is an 18-month-old male of European ancestry. His mother reports a 7-day history of fevers, cough, and runny nose. He has become increasingly fatigued and his skin began to yellow over the last 2 to 3 days. His urine is now reported to be brown/black.

He has no history of transfusion, medications, or allergies. He has not been exposed to any communicable diseases and his immunizations are up to date. The physician ordered a complete blood count (CBC), urinalysis, and routine chemistry panel.

Selected Admission Laboratory Values*:

Laboratory Data	Pediatric Reference Ranges
Hemoglobin: 4 g/dL	10.4 - 12.5 g/dL
Hematocrit: 13.2%	30.5% - 36.4%
Urine: 3+ positive; large amount of blood	No blood
Lactate dehydrogenase (LDH): 37,000 U/L	160 - 370 U/L
*Conventional units.	

An order for type, screen, and crossmatch for 1 unit (100 mL) Red Blood Cells Leukocytes Reduced (LR-RBCs) is received. Given TS's age and presenting symptoms, a viral testing panel was completed. The results were as follows: mycoplasma, negative; parvovirus, negative; Epstein-Barr virus, negative; and respiratory virus, positive for parainfluenza virus.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
0	0	4+	4+	4+

1. How would you interpret TS's ABO type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. What is the patient's Rh type?

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine with the data provided.

3. **What do the results of the antibody detection test indicate?**

- A. No alloantibodies were detected.
 - B. Autoantibody is present.
 - C. A single alloantibody is present.
 - D. Insufficient data to interpret.
-

4. **Why did the physician order a DAT?**

- A. A DAT is routinely ordered for pediatric patients less than 2 years old.
 - B. TS's history is consistent with immune hemolytic anemia.
 - C. TS has evidence of a viral infection.
 - D. Both B and C.
-

5. **How would you interpret the results of the DAT?**

- A. Neither IgG nor complement is present on the patient's cells.
 - B. Only IgG is present on the patient's cells.
 - C. Only complement is present on the patient's cells.
 - D. IgG and/or complement is/are present on the patient's cells.
-

6. **Given the results of the polyspecific AHG test, which of the following tests would be warranted?**

- A. DAT with anti-IgG.
 - B. DAT with anti-C3b,-C3d.
 - C. Inert control.
 - D. All of the above.
-

7. **Based on the results of the DAT testing, which of the following is/are coating the patient's red cells?**

- A. IgG.
 - B. C3b,C3d.
 - C. Both.
 - D. Neither.
-

8. **What additional testing would be beneficial to determine the etiology of the positive DAT?**

- A. Saline antibody detection test (IS, 37 C, IAT).
- B. Polyethylene glycol (PEG) antibody detection test.
- C. Repeat gel antibody detection.
- D. Eluate.

9. Which of the following possible interpretations is/are MOST consistent with the results of Antibody Detection Test 2?

- A. Warm-reactive alloantibody is present.
 - B. No antibodies are present.
 - C. Cold-reactive autoantibody is present.
 - D. Insufficient data to interpret.
-

10. Which of the following tests would provide the MOST useful serologic data at this point in the investigation?

- A. PEG antibody identification panel.
 - B. Saline antibody identification panel.
 - C. Cold antibody identification panel.
 - D. Low-ionic-strength saline (LISS) antibody identification panel.
-

11. What do the results of the cold panel indicate?

- A. Cold-reactive autoantibody is present.
 - B. Cold-reactive alloantibody is present.
 - C. Warm-reactive autoantibody is present.
 - D. Warm-reactive alloantibody is present.
-

12. Which of the following is/are consistent with the serologic results and clinical signs and symptoms?

- A. Cold agglutinin disease (CAD).
 - B. Paroxysmal cold hemoglobinuria (PCH).
 - C. Paroxysmal nocturnal hemoglobinuria (PNH).
 - D. Both A and B.
-

13. Which of the following tests is diagnostic for PCH?

- A. CD59 test.
 - B. Donath-Landsteiner (D-L) test.
 - C. Cold agglutinin titer.
 - D. Monospot.
-

14. How would you interpret the results of the D-L test?

- A. Invalid, controls at 4 C are positive.
- B. Negative, no hemolysis detected in controls.
- C. Positive, no hemolysis detected in controls.
- D. Positive, hemolysis detected in controls.

15. What is the MOST common specificity for D-L antibodies?

- A. Autoanti-P.
 - B. Autoanti-P1.
 - C. Autoanti-I.
 - D. Autoanti-IH.
-

16. What is the patient's MOST LIKELY diagnosis?

- A. Paroxysmal nocturnal hemoglobinuria.
 - B. Paroxysmal march hemoglobinuria.
 - C. Paroxysmal cold hemoglobinuria.
 - D. None of the above.
-

17. The medical laboratory scientist (MLS) intern asked the clinical instructor to suggest a test to rule out possible underlying, clinically significant alloantibodies. Which of the following would be the MOST suitable response?

- A. Suggest that the student perform an eluate panel.
- B. Suggest that the student perform an antibody identification panel using gel.
- C. Suggest that the student perform an antibody identification panel using ficin.
- D. Explain to the student that all clinically significant alloantibodies were ruled out with the original gel antibody detection test and saline antibody identification panel.

Case Study 20

Initial Data:

KK, a 35-year-old female of European ancestry, has been hospitalized for the last week following a relapse of her idiopathic thrombocytopenia (ITP). While hospitalized, she experienced an episode of severe epistaxis during which her platelet count fell to 8000/ μ L; it has now stabilized at 15,000/ μ L. Her hemoglobin is currently 6.2 g/dL. Her physician has ordered a type and screen in the event that transfusion is required.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
0	0	4+	3+	4+

1. How would you interpret KK's ABO type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. What is the patient's Rh type?

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine with the data provided.

3. Given the results of the Antibody Detection Test, which of the following is the MOST LIKELY hypothesis?

- A. A single-specificity alloantibody.
- B. Multiple alloantibodies.
- C. A warm-reactive autoantibody.
- D. A warm-reactive autoantibody with an underlying alloantibody.

4. Given the results of the Antibody Detection Test and Antibody Identification Panel 1, what is the specificity of the antibody in the patient's plasma?

- A. Anti-D.
- B. Anti-D and anti-C.
- C. Anti-D and anti-E.
- D. Anti-D and anti-Lu^a.

5. **What issues must be resolved to make an interpretation of this case?**

- A. The autocontrol is positive.
 - B. Additional clinically significant antibodies must be excluded.
 - C. The patient's cells type as D+ but anti-D is in the plasma.
 - D. Both A and C.
-

6. **Which of the following tests would be MOST INFORMATIVE at this stage?**

- A. Crossmatch.
 - B. Enzyme panel.
 - C. Direct antiglobulin test (DAT) with polyspecific antihuman globulin (AHG).
 - D. Elution studies.
-

7. **How would you interpret the results of the DAT above?**

- A. Neither IgG nor complement is present on the patient's cells.
 - B. Only IgG is present on the patient's cells.
 - C. Only complement is present on the patient's cells.
 - D. IgG and/or complement are/is present on the patient's cells.
-

8. **What testing would you do next?**

- A. DAT with anti-IgG.
 - B. DAT with anti-C3b,-C3d.
 - C. Inert control.
 - D. All of the above.
-

9. **Based on the DAT results above, one can conclude that the patient's red cells are coated with which of the following?**

- A. IgG.
 - B. C3b,C3d.
 - C. Both.
 - D. Neither.
-

10. **What additional testing would be MOST INFORMATIVE at this point in the case?**

- A. Treating the patient's cells with chloroquine diphosphate.
- B. Elution studies.
- C. Autoadsorption.
- D. Alloadsorption.

11. **What is the MOST LIKELY conclusion from the results of Antibody Identification Panel 2?**

- A. There was insufficient washing of the cells before the eluate was prepared.
 - B. The findings indicate warm autoimmune hemolytic anemia (WAIHA).
 - C. Anti-D is coating the patient's cells.
 - D. Anti-D and anti-C are coating the patient's cells.
-

12. **Given the compiled serologic data and patient history, what is the MOST LIKELY explanation for the anti-D in the patient's plasma and eluate?**

- A. Autoanti-D.
 - B. Contamination by reagent anti-D.
 - C. Passive administration of anti-D.
 - D. Cannot determine without additional information/testing.
-

13. **If the patient requires Red Blood Cell (RBC) transfusion, what should be the Rh type of the product selected?**

- A. Rh positive.
- B. Rh negative.
- C. Either Rh positive or Rh negative.
- D. Should be determined in consultation with patient's physician.

Case Study 21

Initial Data:

GH, a 48-year-old male of Hispanic ancestry, was in treatment for a recurrence of colon cancer. He is near completion of his second round of chemotherapy. During the laboratory testing performed before his last treatment, his hemoglobin was found to be 6.2 g/dL. His physician has ordered transfusion of 2 units of Red Blood Cells Leukocytes Reduced (LR-RBCs).

Blood bank records show GH is known to be group B negative. Anti-E and anti-K were identified at the time of his last RBC transfusion 2 months ago. He has become refractory to random-donor platelets. HLA Class I antibodies were identified in his plasma. He has received several doses of HLA-matched platelets, although these products are difficult to locate because of his uncommon HLA type.

ABO and Rh Typing:

Forward (Cell) Typing			Reverse (Serum) Typing	
Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
0	4+	0	4+	0

1. How would you interpret GH's ABO type?

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.

2. What is GH's Rh type?

- A. D+.
- B. D-.
- C. Weak D+.
- D. Cannot determine with the data provided.

3. Which of the following is the MOST LIKELY cause of the positive antibody detection test result?

- A. Anti-E and anti-K are reactive.
- B. Warm-reactive autoantibodies are present.
- C. Anti-c is present.
- D. Insufficient data to form a hypothesis.

4. Which of the following is the MOST EFFICIENT test to perform next?

- A. Selected cell rule-out panel in gel.
- B. Antibody identification panel in gel.
- C. Selected cell rule-out panel in polyethylene glycol (PEG).
- D. Antibody identification panel in PEG.

5. **What do the results of the selected cells indicate?**

- A. Anti-E and anti-K caused antibody screen reactivity.
 - B. There are no additional alloantibodies present.
 - C. The positive autocontrol requires investigation.
 - D. All of the above.
-

6. **Based on the results of the DAT, what is coating GH's red cells?**

- A. IgG.
 - B. C3b,C3d.
 - C. Both.
 - D. Neither.
-

7. **What test should be performed next?**

- A. DAT with polyspecific antihuman globulin (AHG).
 - B. Elution studies.
 - C. Crossmatch with E-, K- donor units.
 - D. Red cell phenotype.
-

8. **Which of the following is NOT a plausible explanation for the nonreactive eluate?**

- A. Positive DAT result is due to nonspecific protein adsorption.
 - B. Antibody to a low-prevalence antigen is present.
 - C. Antibody to a high-prevalence antigen is present.
 - D. ABO antibodies are present.
-

9. **What conclusion can be drawn from the nonreactive eluate?**

- A. GH's DAT result is false positive.
 - B. No clinically significant alloantibodies to common antigens are present.
 - C. GH is likely having a delayed transfusion reaction.
 - D. The physician should be advised to discontinue HLA-matched platelets.
-

10. **Which of the following is/are appropriate actions for further investigation?**

- A. Test red cells carrying antigens of low-prevalence with the eluate.
- B. Test group B red cells with the eluate.
- C. Investigate GH's recent drug history.
- D. All of the above.

11. Which of the following is the **MOST LIKELY** hypothesis for the source of anti-B coating the patient's cells?
- A. Transfusion of Fresh Frozen Plasma of incorrect blood type.
 - B. Infusion of intravenous immunoglobulin (IVIG).
 - C. Contamination of blood sample with reagent anti-B.
 - D. Transfusion of group O or group A HLA-matched apheresis platelets.