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Objectives

- Discuss the prevalence and clinical significance of cold autoantibodies.
- Describe how to determine if cold autoantibodies are interfering with routine blood bank testing.
- Perform and interpret prewarmed testing to circumvent cold autoantibodies.



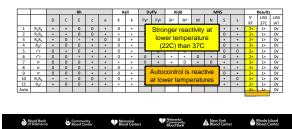
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What are cold autoantibodies?

- React better at lower than body temperature
 - May react at ~22C (room temperature)
 - React best at 4C
- Directed against antigens expressed by autologous cells
 - Autocontrol positive
- Often reacts as panagglutinin
 - All cells positive
 - Antibody against an antigen expressed on all/most cells (including autologous cells)

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Cold Autoantibodies: Example



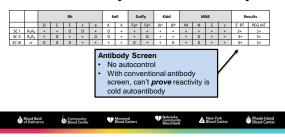
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Is this reactivity cold autoantibody?



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Is the reactivity cold autoantibody?



Is this reactivity cold autoantibody?

ABO/Rh Testing							
Front	Type: Patient cells test	Back Type: Patient plasma tested with					
Anti-A	Anti-A Anti-B		A ₁ cells B cells				
0	4+	4+	4+	2+			

Cold autoantibodies

- May cause ABO discrepancies
 - Extra reactivity in the back type (if reactive at 22C example above)
 Spontaneous agglutination of RBCs in the front type
- Additional testing would be needed to prove extra reactivity is due to cold autoantibody

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Who has cold autoantibodies?

- Most individuals have cold autoantibodies reactive at 4C
 - Routine blood bank testing not performed at 4C
- Stronger cold autoantibodies may be detected at 22C

- Reactivity may carry over to 37C, IAT

This is when cold autoantibodies affect us in the blood bank

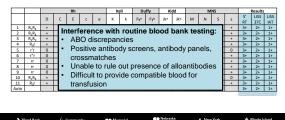
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Cold Autoantibodies: Clinical Significance



Cold Autoantibodies: Why do we care?



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Cold Antibody Screen

- · Method used to prove presence of cold autoantibody
 - Must be tested at <37C (usually 22C & 4C)
 - Must include an autocontrol
 - May include cord RBCs (or I-negative RBCs)
 - · Most cold autoantibodies have anti-I specificity



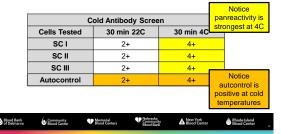
How to Perform a Cold Antibody Screen

- · Patient plasma + cells
 - SC I, SC II, SC III, Autocontrol
 - May include I-negative RBC
- 30 minutes 22C spin and read
- 30 minutes 4C spin and read



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Cold Antibody Screen: Example



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Circumventing Cold Autoantibodies · Prewarmed testing - With enhancement (LISS/PEG) - Without enhancement Don't allow plasma and red cells to interact at temperatures lower than 37C 16 Prewarmed Testing = All Testing at 37C 1. Warm all reactants to 37C prior to mixing · Patient plasma · Reagent RBCs · Enhancement media, if used (LISS, PEG) · Transfer pipets 2. Combine patient plasma, reagent RBCs and enhancement media quickly, using warmed pipet · Maintain tubes at 37C Memorial Blood Centers 17 Prewarmed Testing = All Testing at 37C 3. Incubate at 37C · Avoid 22C reading · Avoid centrifugation (at 22C) 4. Wash with 37C saline · Wash by hand - use saline warmed to 37C • Do not use automated cell washers (saline 22C) • Utilize heated centrifuge, if available - maintain 37C during centrifugation

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Prewarmed Testing = All Testing at 37C 5. Following washing, add anti-IgG · Avoid polyspecific antihuman globulin · Anti-IgG does not need to be warmed · Cold autoantibody removed from test system by washing 6. Centrifuge and read • 22C centrifuge acceptable 7. Add check cells to all negative reactions · Check cells should give positive result Blood Bank of Delmarva Community Blood Center Memorial Blood Centers A New York Blood Center 19 When to Use Prewarming Cold autoantibody is interfering in routine testing - 22C, 37C, IAT - Must *prove* cold autoantibody is present first 2. Prewarming cold reacting alloantibodies - Determine clinical significance - Must identify the cold reacting alloantibody first A New York Blood Center 20 Can you prewarm gel? NO! · 10 minute incubation at RT

A New York Blood Center

Memorial Blood Centers

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Some clinically significant alloantibodies *may not* be reactive in prewarmed testing!

Know what you are prewarming!

- · Prove cold autoantibody with cold antibody screen
- · Try prewarming using enhancement first



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Prewarming Caution



DO NOT EVER

- Assume weak IAT reactivity is due to cold autoantibody
 - Run cold antibody screen
- · Prewarm unidentified reactivity



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