



innovation • experience • expertise



### Cold Autoantibodies



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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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### 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

Cold Antibody Screen		
Cells Tested	30 min 22C	30 min 4C
SC I	2+	4+
SC II	2+	4+
SC III	2+	4+
Autocontrol	2+	4+

Notice panreactivity is strongest at 4C

Notice autocontrol is positive at cold temperatures

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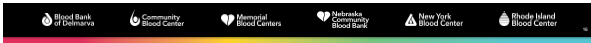


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### Circumventing Cold Autoantibodies

- Prewarmed testing
  - With enhancement (LISS/PEG)
  - Without enhancement

**Don't allow plasma and red cells to interact at temperatures lower than 37C**



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### 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



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### 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



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### 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



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### Can you prewarm gel?

- NO!
- 10 minute incubation at RT



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### Prewarming Caution



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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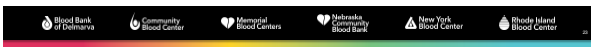
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### Prewarming Caution



#### DO NOT EVER

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



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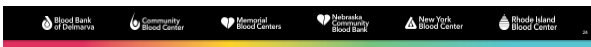
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