



Deviation From SOP

Date: 03/10/2023 Affected SOPs: Donath Landsteiner Test

Samples Involved: DL samples tested in January and February

Description:

Due to lack of source of ABO appropriate serum for DL testing, we used any serum within 24/72 hours for DL testing.

The DL testing serum is a control.

The control is tested with group O cells, so any type ABO is acceptable.

Possible Effects:

The sample may not have enough fresh complement. However, this should not affect the DL testing A1 tube, which is the primary interpretation of the test.

Accept this deviation from SOP for these samples, and for any other samples tested until the SOP can be used.

Due date for update to SOP 7/1/23


Technologist


Approved By



Indiana University Health

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Donath Landsteiner Test

Printed copies are for reference only. Please refer to the electronic copy for the latest version.

I. PURPOSE

Paroxysmal Cold Hemoglobinuria (PCH) is a rare form of autoimmune hemolytic anemia. The autoantibody has been shown to be an IgG antibody directed against the P blood group system. It is described as a biphasic hemolysin, in that it will bind to the red cells at cold temperatures, and as the test is warmed to 37C, complement is activated and lysis of the red cells occurs. This is the basis of the diagnostic test for PCH, the Donath-Landsteiner test (DL Test).

II. SCOPE

MT1, MT2 and Supervisors trained for the technique.

III. EQUIPMENT/RESOURCES

- Serum separated from a freshly collected patient blood sample (red top tubes)
- Freshly collected ABO compatible, normal sera (red top tubes)
- 50% suspension of washed group O red cells that express the P antigen (P1 positive reagent red cell)
 - Prepare washed 50% suspension by visual estimation compared to a standard 3-5% red cell suspension (BBT-006)
- Ice Bath with Crushed Ice or 1-6C Refrigerator
- 10 x 75-mm or 12 x 75mm test tubes
- Transfer pipettes
- Plastic pour-off tubes
- 37C water bath or dry heatblock
- Timer
- Centrifuge

IV. PROCEDURE

A. Collection of the Patient Sample

1. The patient sample should be collected and maintained warm (37C) using a Cryoglobulin Pack from Chemistry.
 - a. If a DL Test is ordered, then obtain a Cryoglobulin Pack from Chemistry.
 - b. Provide the materials and instructions for collection to the floor or clinic.
2. When the sample is received in the Blood Bank,
 - a. Allow tubes to separate without centrifugation for at least 2 hours in the 37C water bath or dry heatblock.
 - b. Allow tubes to fully clot, waiting for clot to retract. This will avoid loss of antibody by autoadsorption before testing.

- c. Using a transfer pipette, separate the serum from the red tops into labeled plastic pour-off tubes. **Warning:** Caution is advised when transferring serum to pour-off tubes, making sure not to transfer any red cells.
- d. Once the sample is separated, the tubes may be removed from the waterbath or heatblock.

B. Collection of Normal Serum

1. Obtain or Collect a Sample – Any ABO serum is acceptable as source of complement control
 - a. Identify a serum sample drawn within the last 24-72 hours, but the fresher the serum is preferable.
 - b. Alternately, one may collect 2-4 red top (serum) tubes from a lab volunteer
2. Centrifuge the tubes and place fresh serum in properly labeled plastic pour-off tubes.
3. Document the normal serum sample used for the testing on the BB Miscellaneous Test Form, indicating:
 - a. Unique identification of the sample
 - b. Draw date of the sample
 - c. ABO/Rh.
 - If the sample is a lab volunteer, then please test the ABO and document using the BB Miscellaneous Test form.
 - A print out from Cerner with this information is also acceptable.

C. Donath-Landsteiner Test

Step	Action
1	Label three sets of three test tubes as follows: A1-A2-A3; Test Set: Cold (1-6) for 30 Minutes and Warm (37C) for 60 Minutes B1-B2-B3; Cold Set: 1-6C for 90 Minutes C1-C2-C3; Warm Set: 37C for 90 Minutes 1 = patient serum Patient sample alone 2 = patient serum and normal serum Patient sample with complement 3 = normal serum Control normal serum
2	To tubes 1 and 2 of each set, add 10 volumes (eg, drops) of the patient's serum.
3	To tubes 2 and 3 of each set, add 10 volumes of fresh normal serum.
4	To all tubes, add one volume/drop of the 50% suspension of Group O, washed P-positive red cells (P1 positive cells are P positive) and mix well.
5	Place the three "A" tubes in a bath melting ice or 1-6C Refrigerator for 30 minutes and then transfer to the 37C waterbath or dry heatblock for 1 hour (60 min). Set a timer.
6	Place the three "B" tubes in a bath of melting ice or 1-6C Refrigerator for 90 minutes. Set a timer.
7	Place the three "C" tubes at 37C water bath or or dry heatblock for 90 minutes. Set a timer.
8	After incubations, gently mix and then centrifuge all tubes for 60-90 seconds.
9	After centrifugation, examine the supernatant fluid for hemolysis.

10	<p>Document the results on a Form: BB Miscellaneous Testing Form.</p> <ul style="list-style-type: none"> • H = Hemolysis present • NH = No Hemolysis present <p>Interpretation</p> <ul style="list-style-type: none"> • The DL Test is considered Positive if tubes A1 and A2 (patient sample and patient sample with normal serum) demonstrate hemolysis while tubes A3, B1-3 and C1-3 demonstrate no hemolysis. <div data-bbox="511 462 1079 798" data-label="Image"> </div> <ul style="list-style-type: none"> • The DL Test is considered Negative if no hemolysis is observed in sets A, B and/or C. • The A3, B3 and C3 test tubes are the control for complement activity and should not demonstrate hemolysis. • If hemolysis is observed in the B 1-3 tubes or C1-3 tubes, consult with management for interpretation.
11	<p>Complete Cerner Entry of the DL Testing Results. Positive = Hemolysis Negative = Non hemolysis</p>

V. CLINICAL SIGNIFICANCE/ SPECIAL CONSIDERATIONS

- This freshly collected normal serum will be used as a source of complement.
- This antibody has been shown to have specificity within the P blood group system (i.e., Anti-P); therefore, it will react with all cells except the rare p or P^K phenotypes.
- Active complement is essential for demonstration of the antibody. Because patients with PCH may have low levels of serum complement, fresh normal serum should be included in the reaction medium as a source of complement.

VI. REFERENCES

Technical Manual, AABB, Bethesda, MD: current edition

VII. FORMS/APPENDICES

[Form: BB Miscellaneous Testing Form](#)