1. **Principle**

The Donath Landsteiner indirect test detects bithermic hemolysis of paroxysmal cold hemoglobinururia (PCH).

The antibody produces hemolysis only when allowed to act first at a very low temperature and subsequently at a higher temperature (bithermic hemolysin). It is a non-agglutinating antibody that reacts almost exclusively with P+ red cells and is usually a transient condition secondary to viral infections (especially in children). It can also occur as an idiopathic chronic condition in older patients.

1. **Scope and Related Policies**
	1. The Fresh Normal Serum (FNS) used for this procedure MUST be fresh (less than four hours old) as it is used as a source of Complement.
2. **Specimen**

Serum separated from a freshly collected blood sample maintained at 37°C.

1. **Material**

**Equipment:** Water bath/Heating block at 37°C

**Supplies:** Test tubes – 10 x 75 mm

 Serological pipettes

 Ice bath

**Reagents:** Normal saline

Fresh group compatible serum (less than four hours old and known not to contain unexpected antibodies)

Approximately 10 drops 50% saline suspension of normal group O red cells that express the P antigen. (twice washed and suspended in 0.9% saline).

1. **Quality Control – N/A**
2. **Procedure**

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| * 1. Label three sets of three 10 x 75 mm test tubes as follows: A1-A2-A3; B1-B2-B3; C1-C2-C3. Refer to table below.

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| --- | --- | --- |
| Set A | Set B | Set C |
| A1 - pt sera & P+ cells | B1 - pt sera & P+ cells | C1 - pt sera & P+ cells |
| A2 - pt sera & FNS & P+ cells | B2 - pt sera & FNS & P+ cells | C2 - pt sera & FNS & P+ cells |
| A3 - FNS & P+ cells | B3- FNS & P+ cells | C3- FNS & P+ cells |
| Ice for 30 min., 37°C for 1 hour | Ice for 90 min. | 37°C for 90 min. |

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| * 1. To tubes 1 and 2 of each set, add 10 volumes (e.g. drops) of the patient's serum.
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| * 1. To tubes 2 and 3 of each set, add 10 volumes of fresh normal serum.
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| * 1. To all tubes, add one volume of the 50% suspension of washed P positive red cells and mix well.
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| * 1. Place the three "A" tubes in a bath of melting ice for 30 minutes, and then at 37°C for 1 hour.
 |
| * 1. Place the three "B" tubes in a bath of melting ice, and keep them in melting ice for 90 minutes.
 |
| * 1. Place the three "C" tubes at 37°C, and keep them at 37°C for 90 minutes.
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| * 1. Centrifuge all tubes at 3500 rpm for 10-15 seconds, and examine the supernatant fluid for hemolysis. Record results.
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**7.0 Reporting**

* 1. The Donath-Landsteiner test is considered positive when the patient's serum, with or without added complement, causes hemolysis in the tubes that were incubated first in melting ice and then at 37°C (i.e. tubes A1, A2) and there is no hemolysis in any of the tubes maintained throughout at 37°C (i.e. tubes C1, C2) or in melting ice (i.e. tubes B1, B2). The A3, B3 and C3 tubes serve as a control for complement activity and should not manifest hemolysis.
	2. A positive result must be telephoned to the patient’s physician.

**8.0 Procedural Notes**

* 1. This procedure will be done at the request of the attending physician.
	2. This test is indicated if the patient has a positive direct antiglobulin test (DAT) due to complement (C3) and is exhibiting hemoglobinemia and/or hemoglobinuria.9.1
1. **References**
	1. Roback, JD. ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: pg. 928.
2. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual
* Added “ice bath” to section 4.0- *Supplies*
* Specified “at 3500 rpm for 10-15 seconds” in section 6.8
* Added section 7.2
* Revised and renumbered section 8.0
* Updated list of references to include most recent editions
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