1. **Principle**
   1. The use of thrombin to accelerate clotting

If thrombin is added to a normal blood specimen, conversion of fibrinogen to fibrin will occur instantly and clotting results. Thrombin is also used to enhance clotting of samples from anticoagulated patients.

* 1. The use of protamine sulphate to promote clotting of heparinized blood

The addition of protamine sulphate to a blood specimen from a patient receiving heparin neutralises the anticoagulant heparin and allows clotting to occur.

1. **Scope and Related Policies**
   1. If a sample fails to clot or the serum gels, telephone the floor to determine whether the patient is on anticoagulants.
   2. For heparinized patients, use protamine sulphate first. If clot doesn’t form then thrombin may be used.
   3. For other anticoagulants, use thrombin first.
   4. If there is no obvious explanation for the failure of the sample to clot, the patient's physician must be notified immediately.
2. **Specimen**

Plain red stoppered tubes of blood.

1. **Material**

**Equipment:** Centrifuge

**Supplies:** Serological pipettes

Wooden applicator sticks

**Reagents:** 0.9% saline

Commercial thrombin - Prepare according to manufacturer's instructions

0.1% protamine sulphate. If lyophilized, prepare by adding 0.1 mL of a 1% concentrate of protamine sulphate to 0.9 mL of normal saline. Aliquot into test tubes (10 drops/tube) labeled with the name of the reagent and the date prepared. Store at -20°C or below for a maximum of 30 days.

(protamine sulphate may also be obtained in a liquid form from some hospital pharmacies.)

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

**The use of thrombin to accelerate clotting**

|  |
| --- |
| * 1. Add 2 drops of reconstituted thrombin to each 7 – 10 mL of blood. |
| * 1. Stir with wooden applicators. Clotting should occur immediately if blood specimen is normal. |
| * 1. Centrifuge at 3000 rpm for 3 - 5 minutes.   Separate serum by transferring to tube labeled with the patient’s full name. Transcribe the information from the patient specimen label (not from the request form).  Patient specimen labels may be used (ensure the information coincides exactly to the specimen label). |

**The use of protamine sulphate to promote clotting of heparinized blood**

|  |
| --- |
| * 1. Add 2 drops of freshly prepared or thawed 0.1% protamine sulphate to 7 – 10 mL of blood. |
| * 1. Mix well and incubate at 37°C. Allow to clot (5 to 10 minutes). |
| * 1. Centrifuge for 5 minutes at 3000 rpm.   Separate serum by transferring to tube labeled with the patient’s full name. Transcribe the information from the patient specimen label(not from the request form).  Patient specimen labels may be used (ensure the information coincides exactly to the specimen label). |

1. **Reporting – N/A**
2. **Procedural Notes – N/A**

1. **References**
   1. In house procedure, Ottawa Hospital.
   2. Roback, JD. ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011, 868-869
   3. Manufacturer’s package insert for thrombin and/or protamine sulphate.
2. **Revision History**

|  |  |
| --- | --- |
| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual * Revised wording of section 1.0 & 2.0 * Replaced “Normal saline” with “0.9% saline” * Updated list of references to include most recent editions |