Document Title:	**Euglobulin Clot Lysis Procedure**

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TITLE: Euglobulin Clot Lysis Procedure

I. PURPOSE

The purpose of this document is to provide guidance on how to perform the euglobulin clot lysis test.

II. SCOPE

This procedure will be used by the University of Rochester Medical Center, Strong Memorial Hospital, Hematology and Chemistry Laboratory staff.

III. RESPONSIBILITIES

Roles	Responsibilities
Quality	•Supports the development of this document. Review and approval of this document.
Medical Director	• Ensures that the procedure is followed. • Review and approval of this document.
Management	• Ensures that the procedure is followed. • Review and approval of this document.
Employees	• Follows the procedure.

IV. PRINCIPLE

The euglobulin clot lysis time is a test that measures overall fibrinolysis. The test is performed by mixing citrated platelet-poor plasma with acid in a glass test tube. This acidification causes the precipitation of certain clotting factors in a complex called the *euglobulin fraction*. The euglobulin fraction contains the important fibrinolytic factors fibrinogen, PAI-1, tissue plasminogen activator (tPA), plasminogen, and to a lesser extent alpha 2-antiplasmin. The euglobulin fraction also contains factor VIII.

In a hypotonic acid medium, in the cold, the "euglobulin fraction" of plasma forms a precipitate which contains fibrinogen, plasminogen, and plasminogen activator. It lacks the fibrinolytic inhibitors (anti-plasmins) present in whole plasma. Lysis of a euglobulin clot is normally more rapid than lysis of a whole blood clot. Differences in water solubility of euglobulins and inhibitors allow for separation and

precipitation of the euglobulins. Thrombin is added to the re-dissolved euglobulin precipitate to form a fibrin clot. During incubation, plasminogen is converted to plasmin which then cleaves fibrin to fdp's. The amount of time required for complete lysis of the fibrin clot is a rough measure of plasminogen activator activity

V. SPECIMENS

A. Citrated blood 9:1 (blood to anticoagulant) 3.2% sodium citrate. Follow

B. NCCLS guidelines H3-A3 and H21-A2. No other anticoagulant is acceptable.

C. Centrifugation: Centrifuge specimens for 12 minutes at 4000 rpm (RCF = 3756g). or 2 minutes at 16,000 RPM (Eppendorf High Speed Centrifuge).

D. Plasma Storage: 24 hours at 20 °C. **Do not store at 2 – 8 °C**

E. Unacceptable Specimens: Samples that are short, over draws, clotted or hemolyzed may yield incorrect results.

VI. QUALITY CONTROL

Pooled normal plasma is used for a normal control.

VII. SPECIAL SAFETY PRECAUTIONS

Use universal precautions when handling blood samples with precautions used for human blood. Follow Laboratory safety policy (SH.CP.AU.gen.0005.0001), CDC recommendations and Federal OSHA Blood Borne Pathogen Standard, 29 CFR part 1910.1030

VIII. MATERIALS

A. Equipment

1. Centrifuge
2. Waterbath

B. Supplies

1. 16X100 mm test tubes
2. glass stir rods

C. Reagents

1. 1% Acetic Acid
   (1 ml glacial acetic acid and 99ml distilled water.)

2. Working thrombin solution (stable for 24 hours at 2-8°C)
   (Prepared by the addition of 0.1 ml of 100 unit/ml thrombin to 3.9 ml Veronal buffer)

3 Stock thrombin solution (100 unit/ml thrombin) is stored in -75C freezer - prepared by adding 49 ml saline to 1 vial bovine thrombin 5000 NIH units.
(Recothrom Thrombin Topical 5000 units NDC:65293-006-41)
Inpatient Pharmacy

4. Veronal Buffer (Dade Owen's Veronal Buffer ref # B4234-25)
   In coag refrigerator.

5. CRYOcheck ^{TM} Pooled Normal Plasma (CCN-10 Precision Biologic) stored in -75°C freezer.

IX. PROCEDURE

A. Set up of patient and control.

1. **Patient** tubes (set up 2 of them)
   a. 0.5 ml of patient plasma
   b. 9.5 ml of <u>cold</u> distilled water
      (Set the 5 ml pipettor to 4.75 and dispense twice per test tube)

2. **Control** tube (set up only one)
   a. 0.5 ml of pooled normal patient plasma (CRYOcheck, -75 ° C freezer)
   b. 9.5 ml of <u>cold</u> distilled H_2O

B. 0.1 ml of 1% acetic acid is added to each tube (to adjust the pH to 5.3).

C. Cover with parafilm and mix by inversion. The mixture will appear cloudy.

D. Centrifuge the mixtures for 10 minutes at 1500 rpm.

E. Decant the supernatant from all tubes and thoroughly blot each inverted tube onto paper towels to remove all drops of supernatant.

F. Dissolve the precipitate by adding 0.5 ml of Veronal Buffer to each tube. Use a glass rod to scrape all the precipitate off the bottom.

G. **Slowly** add 0.5 ml of your thrombin solution [0.1 ml of 100 unit thrombin + 3.9 ml Veronal buffer] to the dissolved precipitate. DO NOT MIX!

H. Place the tubes on ice until they clot.

I. After the clot has formed place the tubes (capped) in the 37°C waterbath. Check the tubes every 30 minutes for clot lysis and record the status of the clot for a total of 2 hours. **Do not** report out the results until you check the control clot at 2 hours

X. LIMITATIONS

Normal euglobulin lysis time is greater than 2 hours**.** Falsely shorter times may be observed if plasminogen activator is released due to tourniquets, pumping of the hand, or excessive rubbing with alcohol swabs at the time of venipuncture. The lysis time will vary with age, sex, exercise, alcohol and smoking habits. Platelets contain activators which inhibit plasminogen and plasmin, so platelet-poor plasma should be used. The specimens are handled in the cold to decrease inactivation of plasminogen activator. The pH of the plasma-acid mixture is critical, and pH 6.2 yields maximal lysis by precipitation of the globulins. As the pH approaches 5.3, there is increasing prolongation of lysis times. The decanting and draining of the tube are also important inhibitors of lysis. Anti plasmins can drain back into the sediment and prolong the times, as will fibrinogen, thus increasing the amount of fibrin that has to be dissolved. It should be noted that only 50% to 60% of the plasma fibrinogen is in the precipitate, and the remainder is in the discarded supernatant.

The test cannot be performed if the fibrinogen is so depleted that no clot will form. Patients on fibrinolytic therapy or in primary fibrinolysis will have shortened times due to the excessive amounts of circulating plasmin and depleted stores of anti plasmins. If the fibrinogen levels drop so that no clot is formed, report out the results as no clot formed since no information can be gained from this test.

XI. CALCULATIONS

Not applicable

XII. INTERPRETATION

The reference range is greater than 120 minutes

XIII. RESULT REPORTING

The result is reported as Normal or Abnormal. If Normal, an automatic canned message is attached; “No lysis after 120 min”. If Abnormal, a result comment is added to indicate the time in minutes at which the clot lysis occurred, using the canned message list (F5) in the LIS seen below:

1	Complete lysis at 30 minutes incubation.
2	Complete lysis at 60 minutes incubation
3	Complete lysis at 90 minutes incubation
4	Complete lysis at 120 minutes incubation
5	Partial lysis at 30 minutes incubation.
6	Partial lysis at 60 minutes incubation
7	Partial lysis at 90 minutes incubation
8	Partial lysis at 120 minutes incubation

XIV. TRAINING

Role	**Training Needed**
Management	Knowledge Check
Designated Employees	Knowledge Check

XV. REFERENCES

A. Modified from Nilsson, I.M., Olow, B., Fibrinolysis induced by streptokinase in man. Acta Chirurgica Scandinavica, 123: 247, 1962.

B. Blix, "Studies on the fibrinolytic system in the euglobulin fraction of hyman plasma. A. A methodological study. B. Application of the methods." Scand. J. Clin. Invest. 58 (Suppl. 13): 3, 1961.

C. Kowalski, E.; M. Kopeć; S. Niewiarowski (1959). "An Evaluation of the Euglobulin Method for the Determination of Fibrinolysis". Journal of Clinical Pathology

Euglobulin Clot Lysis Procedure
Knowledge Check

In the event of a question answered incorrectly: Single-line through the incorrect answer, initial & date, then select the correct answer.

ALWAYS HAVE CHANGES INITIALED BY YOUR TRAINER.

Circle True or False for each of the following statements.

1. True or False The Euglobulin Clot lysis test is used to measure clot formation.
2. True or False CRYOcheck pooled normal plasma is used as an abnormal control.
3. True or False A normal result is no lysis after 120 minutes of incubation.
4. True or False Only complete lysis is reported as Abnormal.
5. True or False Stock thrombin solution (100 Unit/mL) is found in Mike's desk drawer.

Any incorrect answers I may have initially written have been discussed and corrected. I now understand the answers I may have gotten wrong.

PASSING GRADE IS 75% OR GREATER

_____
Employee name (print)

_____ _____
Employee signature **(Date)**

_____
Supervisor/Manager name (print)

_____ _____
Supervisor/Manager signature **(Date)**