

**OLIVE VIEW-UCLA MEDICAL CENTER  
DEPARTMENT OF PATHOLOGY  
POLICY & PROCEDURE**

**NUMBER: 11880  
VERSION: 1**

**SUBJECT/TITLE: SS 012 - COLLOIDAL IRON STAIN**

**POLICY:** The Colloidal Iron stain shall be performed at the request of the pathologist.  
**PURPOSE:** For the detection of acid mucopolysaccharides.  
**DEPARTMENTS:** **PATHOLOGY & LABORATORY SERVICES**

**DEFINITIONS:**

**PROCEDURE:** **FIXATIVE**  
10% Neutral Buffered Formalin is generally utilized, however tissue samples placed in other fixatives may also be accepted  
**Note:** If the tissue was fixed in a “B-5” solution, perform the Removal of Mercury Pigment procedure. (Outlined in HIS 76.)

**EQUIPMENT**

- |                    |                                  |
|--------------------|----------------------------------|
| Coplin Jars        | Graduated Cylinders              |
| pH measuring paper | Filter Paper                     |
| Erlenmeyer Flasks  | Heat Plate/ Stirrer and stir bar |
| Weigh Boat         | Portable weighing scale          |
| Brown Bottle       |                                  |

**SLIDE PREPARTIONS**

Section tissue blocks at 4-5 microns

**QUALITY CONTROL**

A section of small intestine, appendix or umbilical cord may be utilized as a positive control.

**REAGENTS**

**I. Stock Solutions**

- **29% Ferric Chloride (USP)**  
   Ferric Chloride.....29.0 gm  
   Distilled Water.....100 ml

It may take as long as one hour to dissolve the ferric chloride. If possible, utilize low heat and an ultrasonic dissolving instrument.

**SUBJECT/TITLE: SS 012 - COLLOIDAL IRON STAIN**

**Policy Number: 11880**

**Page Number: 2**

- **Colloidal Iron (Stock)**

Distilled Water.....250 ml

29% Ferric Chloride.....4.4 ml (Exactly)

Bring 250 ml of distilled water to a boil. While the water is still boiling, add (drop by drop) **exactly** 4.4 ml of 29% ferric chloride solution. Mix constantly until the solution turns brick-red. Remove from the heat and allow to cool. Place the solution into a brown bottle. This reagent is stable for one (1) year at room temperature. The stock solution is diluted just before use.

## **II. Working Solutions**

- Working Colloidal Iron Solution

Stock Colloidal Iron.....20 ml

Distilled Water.....15 ml

**Concentrated** Glacial Acetic Acid.....5 ml

The solution should measure pH 1.0. Utilize a pH measuring paper to confirm measurement.

- **12% Acetic Acid**

Glacial Acetic Acid.....24 ml

Distilled Water.....176 ml

Note: Prepare fresh before each use

- **Perl's Working Solution**

2% Aqueous Potassium Ferrocyanide.....25 ml

(Commercially prepared from Poly Scientific: S1872-16 oz)

2% Aqueous Hydrochloric Acid.....25 ml

(Commercially prepared from Poly Scientific: S2112-16 oz)

Note: Solution must be filtered.

Nuclear Fast Red (Kernechtrot)

- Commercially prepared from Poly Scientific: S248-16oz

## **PROCEDURE**

1. Deparaffinize and hydrate section(s) to distilled water.
2. Rinse the slides in 12% aqueous glacial acetic acid solution for 1 minute.
3. Place slides in working Colloidal Iron solution for 1 hour.
4. Place in 12% Acetic Acid solution. (4 changes at 3 minutes each.)
5. Stain in Perl's working solution for 20 minutes.
6. Rinse in distilled water (3 changes at 15 dips each)
7. Place in Nuclear Fast Red for 5 minutes
8. Wash in running tap water for at least 1 minute
9. Dehydrate in two changes each of 95% alcohol and absolute alcohol
10. Clear in three changes if Xylene
11. Mount with synthetic resin.

**SUBJECT/TITLE: SS 012 - COLLOIDAL IRON STAIN**

**Policy Number: 11880**

**Page Number: 3**

**RESULTS**

Acid Mucopolysaccharides and acidic epithelial mucin.....Deep Blue  
Background.....Pink to Red

References: Theory and Practice of Histological Techniques - sixth edition; John D. Bancroft and Marilyn Gamble	
Histotechnology: A Self-Instructional Text – third edition; Freida L. Carson and Christa Hladik	
Approved by: Holli Mason (Laboratory Director)	Date: 05/07/2020
Review Dates: 05/07/2020	
Next Review: 05/07/2022	
Distribution: Pathology & Laboratory Services	
Original Date: Not Set	