Status **Active** PolicyStat ID **12960796**

Beaumont

Origination	1/18/2023	Document Contact	Sharon Scalise: Supv, Laboratory
Last Approved	1/18/2023	Area	Laboratory-Histology
Effective	1/18/2023	Applicability	Royal Oak
Last Revised	1/18/2023		
Next Review	1/17/2025		

Histology Special Stain - Modified Gomori Trichrome - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of connective tissue, nerves, and the myofibrils in muscle biopsies. It also demonstrates mitochondria, though they are usually not seen in normal muscles. In ragged red fibers, the accumulation of bizarre mitochondria can be seen as a thin, reddish rim, often cracked, around the inside edge of each myofibril. This stain will demonstrate cytoplasmic bodies, whose composition is unknown. Nemaline rods are only demonstrated with modified Gomori trichrome.

II. PRINCIPLE:

The reaction is based on the acid/base (+/- charges) and the hardness/softness of the tissue and the dyes. The negative charged dyes will be attracted to the positively charged tissue components. Hard dyes will be attracted to hard tissue components, while soft dyes will be attracted to soft tissue components. Chromotrope 2R will stain cytoplasm and muscle. Fast Green FCF will stain connective tissues. Phosphotungstic acid is taken up by the collagen. Fast Green FCF is subsequently bound to the phosphotungstic acid. The acetic acid changes the charges/pH of the solution. The acetic acid rinse after the stain gives a more delicate stain but will not change the ratio. Hematoxylin stains the nuclei.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation

1. Fresh frozen tissue.

B. Processing

1. No processing.

C. Section Thickness

1. Cut frozen section muscle biopsies at 10µ.

D. Storage

1. Store slides in refrigerator.

E. Type of slide

1. Plus slides.

IV. REAGENTS:

A. **Modified Gomori Trichrome Solution**

Chromotrope 2R	**0.30 gm**
Fast Green FCF	**0.15 gm**
Phosphotungstic Acid	**0.40 gm**
Distilled Water	**50.00 ml**
Acetic Acid	**0.50 ml**

Dissolve together. Adjust pH to 3.4 with 1 N Sodium Hydroxide. Store in refrigerator; stable for several months or until weak.

B. **0.2% Acetic Acid**

Acetic Acid	**60.2 ml**
Distilled Water	**100.0 ml**

Stir together; store at room temperature; stable for several months.

C. **Hematoxylin**

Use hematoxylin from routine H&E set-up, Gill or Mayer preferable.

V. EQUIPMENT:

A. Balance

B. 60°C oven

C. Magnet stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Coplin jars

D. Forceps

E. Funnel

F. Filter paper

VII. SPECIAL SAFETY PRECAUTIONS:

A. Chromotrope 2R
    1. Is an irritant.

B. Fast Green FCF
    1. Is a carcinogen and an oxidizer.

C. Phosphotungstic Acid
    1. Is a corrosive.

D. Acetic Acid
    1. Is an acid.

VIII. QUALITY CONTROL(QC):

Frozen section of muscle with nerve and connective tissue.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Place slides in hematoxylin.	5 minutes	Incubate at room temperature.
2	Rinse in distilled water.	1 minute	
3	Stain in Modified Gomori Trichrome.	10 minutes	
4	Rinse in 0.2% Acetic Acid.		
5	Dehydrate through graded alcohols, clear with xylene.		
6	Coverslip.		

X. RESULTS:

A. Myofibrils - **green**
(Type I muscle may appear darker than Type II)

B. Connective tissue - **green**

C. Cytoplasm - **green**

D. Hypercontracted muscle fibers - **red**

E. Mitochondria - **red**

F. Intermyofibrillar sarcoplasmic reticulum - **red**

G. Nuclei - **blue**

XI. REFERENCES:

A. Carson FL: Histotechnology: A Self-Instructional Text. Chicago, IL, ASCP Press, 1990.

B. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.

Approval Signatures

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Applicability

Royal Oak