Status **Active** PolicyStat ID **13057120**

Beaumont

Origination	3/2/2023	Document Contact	Sharon Scalise: Supv, Laboratory
Last Approved	3/2/2023	Area	Laboratory-Histology
Effective	3/2/2023	Applicability	Royal Oak
Last Revised	3/2/2023		
Next Review	3/1/2025		

Histology Muscle Enzyme - Aldolase - Royal Oak

Document Type: Procedure

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I. PURPOSE AND OBJECTIVE:

Glycogen Storage Disease is a metabolic disorder that involves the enzymes that regulate glycogen metabolism. The purpose of this document is to provide a procedure for the demonstration of those enzymes that regulate glycogen metabolism. The symptoms, severity, and location of the storage of glycogen depends upon which enzyme is affected. Type VII, or Tauri's disease, is caused by a deficiency in muscle phosphofructokinase. Type XII is caused by a deficiency in muscle aldolase. In both, there will be an abnormally high level of glycogen stored in the muscles, because the cells cannot metabolize it.

II. PRINCIPLE:

Aldolase is a catalyst that is involved in the breakdown fructose 1, 6-diphosphate into dihydoxylacetone phosphate (ketose) and glyceraldehyde phosphate (aldose). Demonstration of Aldolase staining is carried out concurrently with Phosphofructokinase (PFK) staining to demonstrate the integrity of the metabolic pathway via fructose 1, 6-diphosphate. Phosphofructokinase is the enzyme involved in conversion of ATP and fructose-6-phosphate to ADP and fructose 1, 6-diphosphate.

Phosphofructokinase (tissue) | Aldolase (Tissue) |

Fructose-6-phosphate → ADP + Fructose 1, 6-diphosphate → Dihydroxyacetone Phosphate plus Glyceraldehyde Phosphate

| *ATP*

The Glyceraldehyde phosphate is oxidized in the presence of the coenzyme NAD, creating 1, 3-diphosphoglycerate plus NADH plus a release of H^+.

Glyceraldehyde 3-phosphate dehydrogenase (tissue)

NAD + Phosphate

|

Glyceraldehyde phosphate → 1, 3-diphosphoglycerate + NADH + H^+

The Hydrogen (H^+) reduces the tetrazolium salt, nitro blue tetrazolium (NBT), forming a highly colored formazan dye which is finely granular blue.

H^+ + Nitro blue tetrazolium (NBT) → reduced tetrazolium → formazan (blue)

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Unfixed tissue that has been frozen.

B. Processing
    1. Fresh tissue.
    2. No processing

C. Section Thickness
    1. Cut frozen sections at 8-12µ.

D. Slide Drying
    1. None.

E. Type of Slide
    1. Plus slides.

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IV. REAGENTS:

A. **0.01 M Hydrochloric Acid**

Hydrochloric acid, conc (HCl)	**1.7 ml**
Distilled water	**200.0 ml**

Slowly add hydrochloric acid, drop by drop, to water. Stir; store at room temperature; stable for months.

B. **0.01 M Sodium Hydroxide**

Sodium hydroxide (NaOH)	**4.0 gm**
Distilled water	**1000.0 ml**

Dissolve together. Store at room temperature; stable for months.

C. **0.2 M Sodium Cacodylate Stock (Same as "PFK Stock")**

Sodium cacodylate, sodium salt, trihydrate (Sodium arsenate) ($C_2H_6AsNaO_2i3H_2O$)	**4.28 gm**
Distilled water	**100.00 ml**

Dissolve sodium arsenate and water together; stable for months. *NOTE: Buy sodium cacodylate from Electron Microscopy Sciences (12300-25). Type of sodium cacodylate salt make a difference in the dissolvability of the salt. (NOTE: Buy in 25 gram bottles to avoid paying hazardous shipping fees.*

D. **Cacodylate Buffer, pH 8.6**

0.2 M Sodium Cacodylate Stock	**25.0 ml**
Distilled water	**75.0 ml**

JUST BEFORE USE, mix together. Adjust pH to 8.6 with either 0.1 M Sodium hydroxide or 0.1 M hydrochloric acid. *Warning – pH jumps quickly as it nears 8.6. Dilute 0.1 M NaOH or HCl solutions, if necessary.*

E. **10% Calcium Chloride**

Calcium chloride ($CaCl_2i2H_2O$)	**10.0 gm**
Distilled water	**100.0 mL**

Mix together. Use to make the Formol-Calcium.

F. **Formol-Calcium**

40% Formaldehyde (HCHO)	**100.0 ml**
Distilled water	**900.0 ml**
10% Calcium Chloride	**100.0 ml**

Mix together. Store at room temperature; stable for years.

G. **Incubating Solution**

Fructose 1, 6 diphosphate, tri-sodium salt	**0.08 gm**
Distilled water	**10.0 ml**

JUST BEFORE USE, dissolve together. Then add:

0.2 M Sodium Cacodylate Buffer, pH 8.6	**10.0 ml**
Nicotinamide Adenine Dinucleotide (NAD)	**0.005 gm**
Nitro Blue Tetrazolium (NBT)	**0.01 gm**
Magnesium chloride, anhydrous (1-2 small crystals)	**0.004 gm**

JUST BEFORE USE, mix together in first part of incubating solution. Adjust pH to 8.6 with 0.1 M Hydrochloric acid or 0.1 M Sodium Hydroxide. All NBT may not dissolve at room temperature but will dissolve upon incubation. *Warning – pH jumps quickly as it nears 8.6. Dilute 0.1 M hydrochloric acid or 0.1 M Sodium hydroxide, if necessary*. Use immediately.

H. **Negative Control Incubating Solution**

0.2 M Sodium Cacodylate Buffer, pH 8.6	**10.0 ml**
Distilled water	**10.0 ml**
Nicotinamide Adenine Dinucleotide (NAD)	**0.005 gm**
Nitro Blue Tetrazolium (NBT) (Sigma N-6876)	**0.01 gm**
Magnesium chloride, anhydrous (1-2 small crystals)	**0.004 gm**

JUST BEFORE USE, mix together in first part of incubating solution. Adjust pH to 8.6 with 0.1 M Hydrochloric acid or 0.1M Sodium Hydroxide. All NBT may not dissolve at room temperature but will dissolve upon incubation. **Warning – pH jumps quickly as it nears 8.6. Dilute 0.1 M Hydrochloric acid or 0.1 M Sodium hydroxide, if necessary**. Use immediately.

V. EQUIPMENT:

A. Metler balance

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B. 37°C oven

VI. SUPPLIES:

A. Erlenmeyer flasks
B. Graduated cylinders
C. Funnel
D. Coplin jars
E. Pipets
F. Filter paper

VII. SPECIAL SAFETY PRECAUTIONS:

A. Hydrochloric Acid
    1. Is corrosive.
    2. Store in an acid cabinet.

B. Sodium Hydroxide
    1. Is corrosive.

C. Sodium Cacodylate
    1. Is toxic, mutagenic, and can cause gastro-intestinal damage resulting in vomiting and diarrhea, and general vascular collapse leading to shock, coma and death.
    2. Muscular cramps, facial edema, and cardio-vascular reactions are also known to occur following oral exposure to arsenic.

D. Fructose 1,6 Diphosphate
    1. Is an irritant.

E. Nitroblue Tetrazolium
    1. Is an irritant.

F. Nicotinaminde Adenine Dinucleotide (NAD)
    1. Is an irritant.

G. Adenosine Triphosphate (ATP)
    1. Is an irritant.

H. Magnesium Chloride
    1. Is an irritant and is hygroscopic.

I. Calcium Chloride
    1. Is an irritant and is hygroscopic.

J. Formaldehyde

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1. Is a poison.
2. May be fatal or cause blindness if swallowed.
3. Cannot be made non-poisonous.
4. Possible cancer hazard. Irritating to eyes, skin, and respiratory tract.
5. Can cause severe eye burns.

VIII. QUALITY CONTROL:

A. Frozen section of muscle.

B. Normal muscle, frozen section, may be used as a positive control section for aldolase.

C. It is essential to incubate adjacent patient sections in “negative control” incubating solution, as the background on both normal and aldolase negative patients will be a pale blue staining.

IX. LIMITATIONS:

A. Procedure is very pH sensitive. Care must be taken to control pH.

B. The pH tends to rise quickly, so dilution of the 0.1 N Hydrochloric acid may be necessary.

C. Incubating medium must be made up just before use.

D. Not all the NBT will dissolve at room temperature. It will finish dissolving during the 37°C. incubation.

E. Make certain using **NAD, *NOT NADH*,** reduced.

F. Store NBT and NAD in freezer.

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X. PROCEDURE:

Step	Action	Time	Notes
1	Take 2 patient slides. On one, write “Negative”. This will be the negative substrate control.		
2	Pour Incubating Solution in plastic mailer over the patient’s slide without the writing. Cover to prevent evaporation.		Incubation time may range from 30-90
3	Pour Negative Control Incubating Solution in plastic mailer over the patient’s slide with “Negative” written on it. Cover to prevent evaporation.		
4	Incubate bother slides in their own solution in 37°C. oven.	1 hour	
5	Rinse with distilled water.	1 minute	Rinse carefully, as sections have a tendency to fall off.
6	Combine slides together.		
7	Fix in Formol-Calcium.	10 minutes	

8	Rinse in distilled water.	1 minute	
9	Mount with Apathy Gum Syrup.		
10	Seal with fingernail polish.		

XI. RESULTS:

A. Aldolase (found in cytoplasm of muscle fibers) - **blue**

B. Deficiency of Aldolase - **pale**

C. Background, normal and aldolase deficient - **pale**

D. Slides in “Negative Control Incubating Media” - **pale**

XII. REFERENCES:

A. Bancroft JD and Steven A: Theory and Practice of Histological Techniques, 3rd edition, New York, NY. Churchill-Livingstone, 1990.

B. Histochemical Archives of Neurology, 1971

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Approval Signatures

Step Description	Approver	Date
Medical Director	Kurt Bernacki: System Med Dir, Surgical Path	3/2/2023
Policy and Forms Steering Committee (if needed)	Sharon Scalise: Supv, Laboratory	3/1/2023
Policy and Forms Steering Committee (if needed)	Gail Juleff: Project Mgr Policy	2/21/2023
	Amy Knaus: Dir, Lab Operations C	2/21/2023
	Jennifer Lehmann: Mgr Laboratory	2/21/2023
	Sharon Scalise: Supv, Laboratory	1/30/2023

Applicability

Royal Oak