Washington University School of Medicine
Neuromuscular Lab
CAP: 1923316
CLIA: 26D0652044
NY: PFI 3499

Menadione–Linked Alpha Glycerophosphate Dehydrogenase

PRINCIPLE: Reducing body myopathy is a rare disease of skeletal muscle, often classified as a congenital myopathy (Dubowitz 1978), although adult onset is recognized. This condition was first described by Brooke and Neville in 2 unrelated children in whom muscle biopsy showed unusual structures that were termed "reducing bodies" (Brooke and Neville 1972). Over 25 cases have been reported in the medical literature, and the clinical presentation is variable.

Reducing bodies are defined by distinctive histochemical and ultrastructural abnormalities. Histologically, reducing bodies contain a substance that can reduce dihydroxydinaphthyl disulfide, confirming that sulfhydryl groups are present. They also stain black with menadione-linked alpha-glycerophosphate dehydrogenase and are able to reduce nitroblue tetrazolium even in the absence of the substrate alpha-glycerophosphate, giving the same intensive stain. By electron microscopy, reducing bodies appear as dense osmiophilic material that, at higher magnification, consists of closely packed variably and irregularly shaped particles mixed with fibrillar material, measuring 12 nm to 18 nm in width. Proliferation of cytoplasmic bodies and rimmed vacuoles is recognized.

SPECIMEN REQUIRED:

Snap frozen human striated muscle.

Controls:

Positive control: Stain several different muscles simultaneously. There is normally always enzyme activity present in muscle. The degree and location is of pathological interest.
Negative control: Follow incubation protocol, but without alpha-glycerophosphate

METHOD:

Use snap frozen tissue.

Technique:

Cut 10 - 16 micron (12 µm) sections in cryostat from snap frozen biopsy. Attach one or more sections to a No. 1½, 22 mm square coverslip.

Equipment:

Ceramic staining rack - Thomas Scientific #8542-E40
Columbia staining dish - Thomas Scientific #8542-C12
Columbia staining dish(jar) - Thomas Scientific #8542-E30
Forceps Latex gloves

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Reagents:

Acetone - Baxter #010-4 **FLAMMABLE**
deionized water
Alpha-glycerolphosphate – Sigma G6880
Menadione (vitamin K3)- Sigma M5625
Gelatin - 100 bloom -ICN 960317 store at room temperature
Nitro blue tetrazolium - Sigma N6876; Store desiccated at 0 - 5 ^{o}C
PBS 0.2 M

Incubation Solution:

0.2 M Phosphate Buffer, pH 7.6	10mL
Menadione	4 mg
Nitro blue tetrazolium (NBT)	10 mg
Alpha-glycerophosphate	30 mg

Adjust pH to 7.6

NOTE: Menadione is difficult to dissolve in aqueous solutions. Acetone (0.2 ml) may be used to dissolve the menadione. Alternatively, enough is dissolved in the aqueous solution to produce the desired stain.

Staining Procedure:

1. Incubate sections in incubation solution at 37^{0} C for 1 hour.
2. Wash in distilled water for 1 minute.
3. Dehydrate in 30%, 60%, 90% acetone, then rehydrate in 60%, 30% acetone
4. Rinse in distilled water for 1 minute.
5. Mount in glycerine jelly.

Results:

The blue-gray stain of the inter-myofibrillar aqueous phase is light in type I fibers and dark in type II fibers. It is useful in defining architectural disturbances in type II fibers. Reducing bodies and Dense bodies in acid maltase deficiency show positive staining.

REFERENCES:

1. Dubowitz V, Sewry C, Oldfors A. Muscle Biopsy. A Practical Approach. 4th Ed Saunders 2013 p 24.

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