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Neuropathy Phentoyping Protocols - Hematoxylin and Eosin Staining

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We use this protocol and it's working

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Abstract

Summary:

Phenotyping of Rodents for the Presence of Diabetic Neuropathy
In man, the development of diabetic neuropathy is dependent on both the degree of glycemic control and the duration of diabetes. Diabetic neuropathy is a progressive disorder, with signs and symptoms that parallel the loss of nerve fibers over time. Consequently, assessments of neuropathy in mice are not performed at one time point, but are characterized at multiple time points during a 6 month period of diabetes. The degree of diabetes is evaluated in 2 ways: tail blood glucose measured following a 6 hour fast and glycated hemoglobin levels. The initial degree of neuropathy is screened using the methods discussed below. Detailed measures of neuropathy are employed when the initial screening instruments indicate a profound or unique phenotypic difference. This document contains protocols used by the DiaComp staff to examine and measure diabetic neuropathy at the whole animal, tissue and cellular levels.

Diabetic Complication:

Neuropathy

Reference:

AFIP Laboratory Methods in Histotechnology, 1994, p. 16, 53-55.

Materials

Solutions:

Mayer's Hematoxylin:

50 g Ammonium alum
1000.0 ml Distilled water
1.0 g Hematoxylin
0.2 g Sodium iodate
1.0 g Citric acid
50.0 g Chloral hydrate

1. Dissolve the alum in distilled water using magnetic stirrer.
2. When alum is dissolved, add hematoxylin. Dissolve completely.
3. Add sodium iodate and let stir for approximately 10 minutes.
4. Add citric acid and stir for another 10 minutes.
5. Add chloral hydrate and stir until completely dissolved.

Resulting solution is a deep wine color.
One ml of solution dropped into tepid water will turn blue.
This progressive stain stains nuclei only.

Eosin:

Eosin Stock:

1.0 g Eosin Y, water soluble
100.0 ml Distilled water

Phloxine Stock:

1.0 g Phloxine B
100.0 ml Distilled water

Eosin-Phloxine Working:

100.0 ml Eosin stock
10.0 ml Phloxine stock
780.0 ml 95% EtOH
4.0 ml Acetic acid, glacial

Troubleshooting

H&E Procedure:

1 For paraffin sections and fixed cryosections, deparaffinize and hydrate to water.

2 For fresh cryosections, place in alcoholic formalin for 45 seconds and rinse in water.

3 Stain in Mayer's hematoxylin for 15 min.

4 Wash in lukewarm running water for10 minutes.

5 Rinse in distilled water.

6 95% EtOH for one minute, agitating.

7 Counterstain in eosin-phloxine solution for 2 minutes.

8 Dehydrate and clear.

9 Coverslip and mount.