PREPARATION OF FRESH FROZEN SECTIONS OF FREE-LIVING AND
PLANT PARASITIC NEMATODES WITH A COLD MICROTOME

BY

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A technique is described for the preparation of fresh frozen sections for histochemical studies. This technique fulfills most requirements for histochemical work, and the quality of the sections is satisfactory.

Paraffin sections are not always suitable for the histochemical detection especially of enzymes, and frozen sections of fixed or unfixed tissues must be prepared. The preparation of frozen sections of nematodes, however, poses technical difficulties because of the small size of the objects. The current paper describes a method to obtain fresh frozen sections suitable for the detection of enzymes.

Fresh frozen sections of nematodes are prepared with a rotary microtome (Lipshaw Standard Model 50-AB) mounted in a cryostat (Lipshaw Cryotome Model 1500, Lipshaw Manufacturing Co., Detroit, Mich.). Before cooling the microtome, it is advisable to remove the thermometer from its holder on the back-wall of the freezing chamber, and bend it towards the microtome. Temperature readings thus obtained come closer to the temperature of the microtome than with the thermometer in its original position. Open dishes containing an absorbent are placed on the bottom of the freezing chamber to reduce frost formation when the humidity is high. Before sectioning, the microtome and knife should be cooled to —15° to —20° C.

Mounting of the nematodes on the object disc

Nematodes are mounted in "M-1 Embedding Matrix for Frozen Sectioning", available from Lipshaw. One drop of this viscous fluid is frozen to an object disc, flattened with a warm spatula, and the disc cooled for at least 15 minutes on the quick freeze stage. Transfer of the nematodes is accomplished by use of a small device easily made of copper wire less than 1 mm in diameter (Fig. 1). The loop is filled with a small amount of embedding matrix which should never reach the upper surface of the wire. The nematodes are transferred into the embedding matrix, and killed specimens oriented without difficulty. With living nematodes, the loop is placed on the cold microtome for one or two minutes (the matrix should not freeze). The cold object disc is then pushed with its shaft into a block of insulating material to retard warming up, and the block is placed under a
dissecting scope. At the same time, the loop with the nematodes is placed on top of the block, with the nematodes about 2 mm above the object disc to keep the embedding matrix cool. The nematodes can now be oriented by use of a needle. The loop then must be lowered towards the upper surface of the frozen matrix. Once contact with the frozen matrix is established, the liquid matrix which contains the nematodes freezes within 10-15 seconds, and the loop can be removed.

This procedure requires skill and patience, however, the following hints will help in becoming familiar with it.

![Fig. 1. Loop made of copper wire to mount nematodes on the object disc. The wire should be 0.7-0.8 mm thick, and the inner diameter of the loop should not exceed 3 mm.](image)

1. To fill the loop with embedding matrix, pour some matrix into a shallow dish, and barely touch its surface with the loop. If the loop is completely immersed in the matrix, the matrix also adheres to the upper side of the wire, and, once frozen, it is almost impossible to remove the loop without cracking the frozen matrix containing the nematodes.

2. Once the nematodes are oriented, the loop should be lowered towards the upper surface of the frozen matrix on the object disc only up until the point where contact between frozen and liquid matrix is established. The loop should never rest on the frozen matrix, because it would partly freeze in, rendering its removal difficult.

3. To remove the loop, turn its handle slightly to the left and right. The wire should loosen from the frozen matrix and can then be lifted. If not, touch the loop with the finger tips from two sides to warm the wire.

4. After the loop has been removed, fill the center of the frozen, round block with some liquid matrix.

5. Mark the direction in which the nematodes are arranged.

**Sectioning**

The object disc with the frozen nematodes is cooled on the quick freeze stage for 30 minutes. It is then attached to the microtome with the nematodes parallel to the edge of the knife. With the anti-roll plate in the correct position upon the knife, sections 10 μ in thickness can be made without great difficulty.

Sections should be removed from the knife with a fine, dry, cold brush. To avoid distortion, individual sections may be lifted on one side under the edge of the knife. Short ribbons of 3-5 sections may be handled in the same way.