Express technique to prepare permanent collection slides of nematodes

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A simple method of 1-2 hours technique to prepare permanent collection slides is described and illustrated (normally the slide preparation takes 1-2 weeks). The method may be recommended for cases when the express identification is needed: in quarantine and plant protection services, and as an auxiliary method in molecular and applied investigations, to prove reliability of the species identification after years and future taxonomic changes. The method includes killing, fixation, processing in glycerin, and preparation of slides using an Eppendorf tube at high temperature (60-95 °C).

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Permanent slides of nematodes (used for different purposes: molecular, ecological or physiological investigations) serve as documents to prove the correctness of identification, especially after years and following taxonomical changes.

Reliable identification of nematode species can be done mainly on specimens mounted on permanent collection slides. Classical methods (e.g., Seinhorst, 1959, 1962, 1966, 1974), their modifications (Netscher & Seinhorst, 1966; De Grisse, 1969; Hooper, 1986, 1990; Sulston & Hodgkin, 1988; De Ley et al., 1995; Hall, 1996) give excellent results, but the procession in glycerin takes several days. Here is proposed a 1-1.5 hour technique of preparation with the use of routine molecular laboratory equipment or a hot plate.

1. **Killing**
(Figs 1-2)

It is important to maintain a high temperature for killing and fixation. Because of this, it is better to use only plastic vessels (Eppendorf tubes and pipettes) and not the glass ones, because glass is a good thermal conductor as compared with plastic, and the fixative liquid becomes cool more quickly.

For killing two equal Eppendorf tubes are used: one, with alive nematodes in a minute drop of water and a second one, filled with 4% formalin. Equal volumes of tubes are important, because nematodes in the first tube are to be killed by hot formalin from the second tube. The smallest size of tube (0.5 ml) is recommended: this type of tube has the thinnest walls, and because of this, temperature changes from outside reach the inner liquid quickly. It is also minimizes the quantity of chemicals and the possible formalin evaporation during the procedure (this evaporation is dangerous for the breathing organs of lab workers and has to be minimum!).

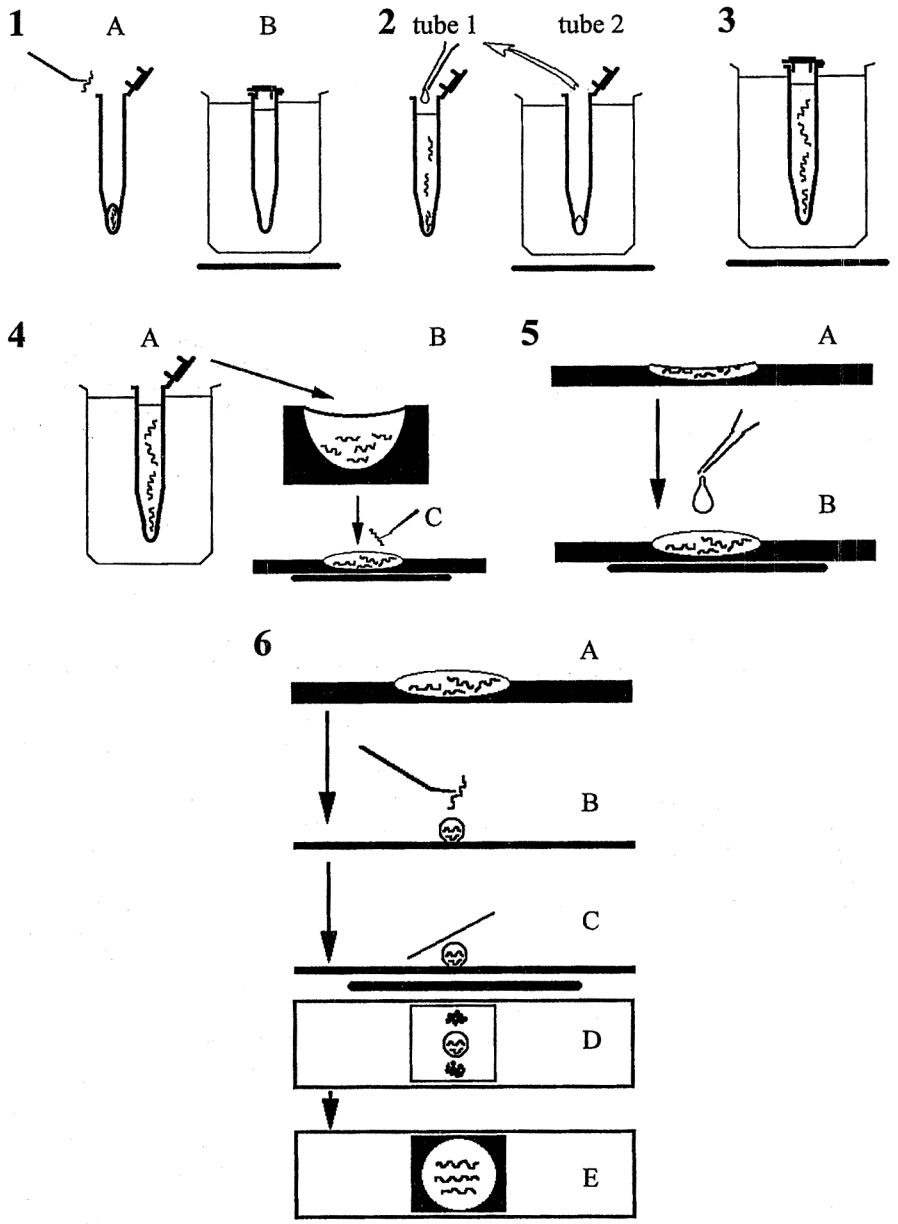
The technique is to be applied only to actively moving nematodes.

A drop of 10 µl distilled water is to be placed to the bottom of a plastic 0.5 ml Eppendorf tube. Thereafter the nematodes may be transferred to the drop with a needle. Tube with alive nematodes may be stored for several days in a refrigerator at 4-8 °C before fixation. Another 0.5 ml Eppendorf tube filled with 4% formalin is to be put into a beaker placed on a 95 °C hot plate or in a PCR machine adjusted to the same temperature.

The first tube with alive nematodes from the fridge is to be exposed at a room temperature. After the tube containing nematodes reaches the room temperature, it is to be filled quickly with hot formalin from the second tube. A plastic pipette is used to transfer formalin. The tube with nematodes is to be closed and shaken to prevent worms stick to the tube walls.

2. **Fixation**
(Fig. 3)

The tube is immediately placed in a programmable thermal controller with a program: 95 °C for 2 minutes, 65 °C for 10 minutes, 75 °C for 10 minutes, 85 °C for 10 minutes, 95 °C for 10 minutes. If the thermal controller is not available and



Figs 1-6. 1-2, killing; **3,** fixation, **4-5,** procession to glycerin; **6,** slide preparation. **1**: **A,** 0.5 ml Eppendorf tube with alive nematodes in the small drop of water at the bottom, nematodes are put into a drop by needle (above); **B,** the Eppendorf tube filled with 4% formalin placed in a beaker on a 95 °C hot plate. **2**: Hot 95 °C formalin from tube 2 is transferred by a plastic pipette to tube 1 with alive nematodes. **3**: 20-40 minutes fixation of the nematodes in the Eppendorf tube placed in a beaker with water at 80 °C. **4**: tube 1 is rinsed with distilled water (**A**), and water with nematodes is dropped to a container slide (**B**) (the procedure is repeated several times to transfer all nematodes into a cavity with water); nematodes are picked out by a needle and transferred to a drop of the mixture of glycerin and distilled water (1 : 20) on a glass slide with cavity (**C**); the latter is placed on the hot plate at 70 °C for 15-20 minutes. **5**: **A,** water is evaporated from the mixture, and after that, nematodes are in anhydrous glycerin; **B,** a drop of glycerin is added to the cavity with nematodes. **6**: **A-B,** nematodes are transferred into a minute drop of pure glycerin on a slide glass; **C** (lateral view) & **D** (view from above), a cover slide is placed at an angle above the drop of glycerin with nematodes; it is supported by two paraffin pieces placed at both sides of the drop laterally; slide is placed on a hot plate at 80-85 °C till the paraffin melts and seals the glycerin drop with nematodes; **E,** collection slide, nematodes in the drop of glycerin surrounded by the paraffin edging.

you use a simple hot plate, you may keep the tube in a beaker with water at 80 °C for 20-40 minutes depending on the nematode size (for large nematodes, the time of fixation is longer). After fixation the tube is kept at room temperature.

Note. Different temperature levels in the thermal controller program correspond to the finish of killing (95 °C), fixation of the soft tissues (65 °C), fixation of the rigid and sclerotised tissues (75° and 85 °C), final fixation and hardening of the body structure (95 °C).

3. **Processing in glycerin**
(Figs 4-5)

When the tube has reached a room temperature, it is shaken and its content is transferred to a glass container. Rinse the tube several times with distilled water and add the water to the container. Nematodes are picked out and transferred to a glass slide with a cavity filled with a mixture of glycerin and distilled water in proportion 1 : 20. The cavity slide with nematodes is placed on a hot plate at 70 °C for 15-20 minutes.

During this time the surface of the drop transforms from wave-like at the beginning to smooth when the water is evaporated from the mixture. Nematodes are now in anhydrous glycerin. One more drop of anhydrous glycerin may be added if the nematodes are not completely covered by liquid. To compensate the possible shrinkage of the nematode body surface, the temperature of the plate has to be increased to 75-80 °C. The glasshole slide with nematodes is moved quickly 3-5 times to the room temperature surface and back again to the thermal plate for a better impregnation by glycerin. When you see under the binocular that the possible partial shrinkage or deformation of the nematode body is completely disappeared, you can start to prepare the permanent slide.

Important note. Glycerin has the chemical features of alcohol which are activated at high temperature. This effect is used here to make the preparation more quick. It is not recommended to expose the cavity slides to a temperature of 80 °C and higher for more than 30 minutes: at this temperature glycerin can evaporate.

4. **Slide preparation**
(Fig. 6)

Slide preparation is done according to the standard technique for glycerin collection slides: nematodes are transferred into a minute drop of pure glycerin on a slide glass with the paraffin ring (or simply with small pieces of paraffin: Fig. 6.4), then covered by a cover glass. The cover glass is placed at an angle to the slide glass above the drop of glycerin with nematodes; it is supported by two paraffin pieces situated at both sides of the drop laterally. The slide glass is gently heated on a thermal table at 80-85 °C till the paraffin melts and seals the glycerin drop with nematodes. Because of the slope of the cover glass, the liquid paraffin moves first to the lowest edge of the cover glass and then spreads gradually around the paraffin drop. Because of this, all minute air bubbles are ejected towards the upper edge of the glass and thus removed from the paraffin seal. The collection slide is finished (Fig. 6.5) in 1-1.5 hour from the moment of nematode killing.

Note. A mixture of natural wax and paraffin in proportion 1 : 5 (generally used to prepare histological sections) is recommended for sealing. Wax transforms the crystallic structure of paraffin into amorphous one. Crystallic paraffin has the slow effect of a filter paper: after 5-10 years glycerin can flow away from nematodes causing the impossibility of morphological and taxonomical examination, according to the author's 23 years experience in work with the paraffin-sealed slides in the Nematode Collection of Zoological Institute.

5. Results

Outer and inner structures including nuclei and fine details of glands, genital system, musculature are perfectly visible. Slides made by the described method are stored in the nematode collections of the following institutions: Zoological Institute (St.Petersburg, Russia), the Department of Zoology (University of Gent, Belgium), Swedish Museum of Natural History (Stockholm, Sweden; used by Dr. B. Sohlenius), Institute of Parasitology (Abo Akademi University, Abo/Turku, Finland; used by Dr. H.-P. Fagerholm), Institute for Experimental Pathology (University of Iceland, Keldur/Reykjavik, Iceland; used by Dr. K. Skirnisson).

References

De Grisse, A.T. 1969. Redescription ou modification de quelques techniques utilisees dans l'etude des nematodes phytoparasitaires. *Meded. Rijksfakulteit Landbowwetenschappen Gent*, 34: 351-369.

De Ley, P., Blaxter, M. & Giuliano, D. 1995. Extracting, fixing and mounting nematodes. WORMWOOD WWW Home Page (A resource for nematode phylogeny). *http://helios.bto.ed.ac.uk/mbx/fgn/worm/extrafix.html*

Hall, G.S. (Ed.) 1996. *Methods for the examination of organismal diversity in soils and sediments.* Wallingford, U.K.: CAB International. xii + 307 p.

Hooper, D.J. 1986. Handling, fixing, staining and mounting nematodes. *In*: Southey, J.F. (Ed.) *Laboratory methods for work with plant and soil nematodes*: 59-80. London: MAFF.

Hooper, D.J. 1990. Extraction and processing of plant and soil nematodes. *In*: Luc, M., Sikora, R.A. & Bridge, J. (Eds.). *Plant parasitic nematodes in subtropical and tropical agriculture*: 45-68. Wallingford, U.K.: CAB International.

Netscher, C. & Seinhorst, J.W. 1969. Propionic acid better than acetic acid for killing nematodes. *Nematologica*, **15**: 286.

Seinhorst, J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*, **4**: 67-69.

Seinhorst, J.W. 1962. On the killing, fixing and transferring to glycerin of nematodes. *Nematologica*, **8**: 29-32.

Seinhorst, J.W. 1966. Killing nematodes for taxonomic study with hot f. a. 4 : 1. *Nematologica*, **12**: 175.

Seinhorst, J.W. 1974. How small is a small drop of water? *Nematologica*, **19**: 121.

Sulston, J. & Hodgkin, J. 1988. Methods. *In*: Wood, W.B. (Ed.) The nematode *Caenorhabditis elegans*: 587-606. *Cold Spring Harbor Laboratory, Cold Spring Harbor.*

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