THE USE OF AGAR AS A COVER-GLASS SUPPORT FOR MOUNTING NEMATODES

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Morphometric and taxonomic studies of nematodes involve the handling and measurement of large numbers of specimens. During the preparation of permanent slides, glass-wool is widely used as a cover-glass support to avoid any distortion or flattening of specimens (Hooper, 1986). Though glass-wool is very useful, difficulties can arise during the handling and selection of fibres of the correct diameter (i.e. those that exactly suit the nematode body diameter). When studying morphological variations among different nematode populations or the effects of methods of killing, fixing or mounting nematodes on their measurements hundreds of nematode specimens may be used and the glass-wool process can become tedious and time-consuming. For such studies and for other routine observations of nematodes, a simple method for quick preparation of mounts of nematodes is described here.

Preparation of agar

2 g of agar (Technical grade No. 3, Oxoid Ltd.) and 50 mg of cupric sulphate ($CuSO_4$, $7H_2O$) is dissolved in 100 ml of distilled water by heating gently for 2 to 3 min. It is then autoclaved at 121 °C for 15 min., allowed to cool and whilst still warm, poured into Petri-plates.

Preparation of nematode mounts

1. Place narcotised or killed specimens in a drop of mountant (narcotic, e.g. sodium azide, water, fixative, e.g. formalin or TAF, lactophenol or anhydrous glycerol) on a glass slide.
2. Take a small quantity of the agar (prepared as above) from the Petri-plate with clean forceps and place it on the slide adjacent to the drop of mountant containing nematodes.
3. Divide the agar into three to five similarly-sized portions with forceps and arrange them at equal intervals around the outer margin of the drop of mountant.
4. Carefully place the cover glass on the drop of mountant so that it rests evenly on all the pieces of agar.
5. While observing the slide under a low power stereoscopic microscope press the cover-glass gently with forceps, to the extent that the cover-glass almost touches the nematodes.
6. A permanent preparation may now, if desired, be made by sealing the cover-glass with Thorne's cement (" Zut " or " Glyceel "), nail varnish, or " Araldite " resin (Hooper, 1986).

Using the above technique, 20 females each of *Caenorhabditis elegans* and *Tylenchus davainei* were mounted in glycerol (processed according to Seinhorst, 1959). Measurements of body diameter at the anus and at the vulva showed that the two techniques did not differ significantly (Tab. 1).

Table 1

Comparative mean body diameters (µm) at anus and vulva of twenty adult female *Caenorhabditis elegans* and *Tylenchus davainei* mounted in glycerol using glass-wool or agar as cover-glass supports

Nematode	Body diam.	Cover-glass support	
		Glass-wool	Agar
C. elegans	at anus	20.6 ± 3.2* (19.0 — 28.4)**	20.5 ± 2.9 (18.6 — 29.0)
	at vulva	59.8 ± 7.4 (48 — 72)	60.1 ± 6.9 (50 — 73)
T. davainei	at anus	16.1 ± 3.4 (12.4 — 20.2)	15.9 ± 3.5 (12.2 — 20.6)
	at vulva	24.3 ± 5.5 (20 — 33.4)	24.4 ± 5.6 (19.8 — 34.5)

* Mean width ± standard deviation, ** Range.
Differences in mean width were not significant at $p < 0.01$ with glass-wool and agar as cover-glass supports.

Commonly used media including Malt Extract Agar, Nutrient Agar, Yeast Extract Agar, Potato Dextrose Agar (at recommended concentrations) where tried and were found equally effective for routine preparation of temporary nematode mounts. But there is the danger of microbial contamination if the slides are to be kept for long. Distilled water agar did not give the required degree of gel as the pieces of agar slipped out of the cover-glass rather than spreading below. So a number of salts including copper sulphate, sodium ethylmercurithiosalicylate (" Thimerosal ") and sodium hypochlorite were tried to break the agar gel to the extent required and at the same time be toxic to microbes. In these respects copper sulphate proved ideal.

Esser (1988) embedded nematode cysts in agar blocks to examine the vulva area and the present paper provides an additional use of agar that overcomes some commonly-encountered problems during preparation of slides of nematodes. The wax-ring method of sealing mounts (De Maeseneer & D'Herde, 1963) is very useful for lactophenol or glycerol based permanent mounts, and can probably be adapted for temporary mounts (in water or fixatives) but the heat required to melt the wax could be detrimental to specimens especially narcotised ones; it might also affect comparative studies of methods of killing and fixing.

Other advantages of the described agar method are that it removes the need for additional cover-glass supports when the area occupied by the mountant exceeds a quarter of the cover-glass area (Hooper, 1986) as it often does in the case of aqueous or fixative mountants. Furthermore, oil-immersion objectives may be more easily used as thick mounts, which can result from the wax-ring method (Hooper, 1986), can be avoided using agar pieces.

Permanent nematode mounts prepared by the agar method were very successful. Esser (1973) recommended Thorne's cement (" Zut ") as a cover-glass support for preparation of permanent nematode mounts. Agar would be more useful because of the problems associated with the viscosity of cement (which is a critical factor). Also cement firmly seals the cover-glass to the slide making it difficult to recover/remount specimens. Agar did not provide any such problems to remounting. Moreover, this method is simple, quick and inexpensive.

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