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A Water Agar En Face Technique

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ABSTRACT: A method is described whereby en face views of live or fixed nematodes are prepared using water agar suitable for study, photography, or camera lucida drawings. Specimens subjected to this method can be fixed and remounted permanently.

A number of methods have been devised to prepare nematodes for en face examination (Cobb, 1920; Buhrer, 1949; Tromba and Douvres, 1953; Anderson, 1958; Lee, 1964). The most prevalent mode entails fixation, decapitation, and finally, orientation in glycerine-gelatin.

Workers who have used the glycerine-gelatin technique are very likely familiar with the agony and frustrating experiences associated with this procedure. Problems include cutting the head too long, or at an acute angle that prevents vertical presentation, or losing the head entirely in the cutting process. New problems arise when the head is placed in the glycerine-gelatin. Premature hardening may occur prior to orienting the head properly. Correct orientation can take considerable time, and sometimes the substrate must be melted several times to correct improper orientation.

A new method was devised to prevent some of the cumbersome problems inherent in the glycerine-gelatin technique.

Method

Subject fixation: Nematodes were fixed in either 2% formalin or lacto-phenol (Esser, 1973). In some cases, live immobile females or males were used.

Procedure: A 12 × 12 × 3-mm square of 1.7% water agar is cut very evenly with a razor blade (Fig. 1A) and placed on a microscope slide. A 3- to 4-mm piece is precisely cut from the square (Fig. 1A) and laid with the outer face down (Fig. 1B). Nematodes (at least 3 specimens per subject taxon) are placed on the upper side of the cut piece with the longitudinal axis of the head parallel with the outer edge of the cut piece (Fig. 1B). The cut piece is then placed back into the same orientation it occupied in Figure 1A, then gently pushed into its original position against the parent back (Fig. 1C). A small (4-mm) drop of water is applied to a 15-mm cover slip that is then placed waterside down over the cut line (Fig. 1C). A drop of immersion oil is applied to the center of the cover slip at the junction of the cut pieces. When the body of the nematode is properly aligned, the en face appears as in Figure 1D. If the en face is off-center or below the field of focus, the cover slip is removed, the cut piece placed backside down, and the specimen reoriented. Water must be added to the cover slip each time it is placed on the agar block. It takes 10–15 min to prepare an en face ready for viewing using this technique. Locating the en face is rather easy because it lies within the cut line.

Discussion

The method has been employed successfully and camera lucida drawings made using males, females, and larvae of *Verutus volvingentis* Esser, 1981; females of *Butlerius* sp., *Criconema* sp., *Criconemoides xenoplax* Raski, 1952, *Fictor* sp., *Helicotylenchus* sp., *Labronema* sp., *Mononchus* sp., *Rhabditis* sp., *Tripyla* sp., *Xiphinemella es-*

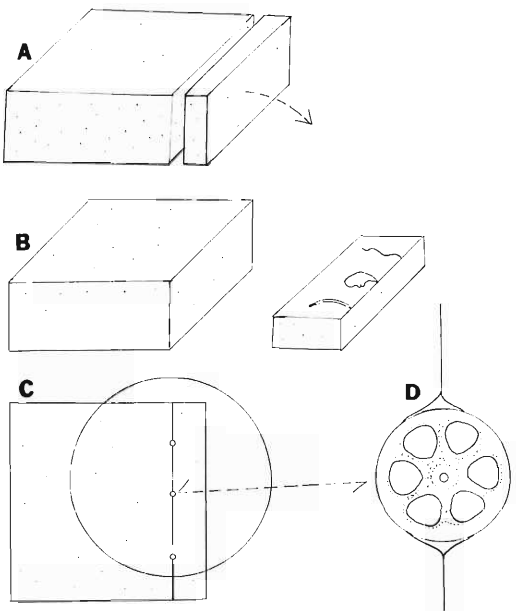

Figure 1. Water agar en face method: A) 12 × 12 × 3-mm square of water agar with a 3- to 4-mm piece cut off; B) specimens aligned on outer edge of the inner face of the cut piece; C) re-alignment of the separated agar pieces, with cover slip in place; D) close-up of en face in junction line of re-aligned agar.

seri Chitwood, 1957, and males only of *Meloidogyne* sp. Since initiation of the method, it has been 100% effective. An en face has been demonstrated at each trial of a subject taxon. Three specimens or more per trial insures success of at least one excellent en face. If the slide on which the en faces are mounted is placed in a petri dish with a small, moist piece of absorbent tissue, it will remain in good condition, ready for re-examination, for several days. When desirable, the specimens can be recovered for permanent mounting.

Swollen females and acutely curved forms in which the head lies far below the tail tip in the death curvature may have to be severed below the esophageal gland area to employ this technique.

Advantages of this method are basically its simplicity, speed, and high potential for success. En face drawings are also possible from live immobile specimens which preclude fixation artifacts. The total specimen can also be permanently mounted after the en face drawings are complete. The principal disadvantage of this method is that the en face mount cannot be permanently preserved.

Literature Cited

Anderson, R. C. 1958. Methode pour l'examen des nématodes en vue apicale. Annales de Parasitologie 33:173–175.

Buhrer, E. M. 1949. Technique for beheading and *en face* examination of nematodes and similar animal types. Proceedings of the Helminthological Society of Washington 16:3–6.

Cobb, N. A. 1920. Micro-technique, Sec. III. To obtain an end view of a nema, rotifer, or other similar small object. Transactions of the American Microscopical Society 39:236–237.

Esser, R. P. 1973. A four minute lactophenol fixation method for nematodes. Plant Disease Reporter 57: 1045–1046.

Lee, S. H. 1964. A technique for studying *en face* view of moderate-sized nematodes without decapitation. Proceedings of the Helminthological Society of Washington 31:137–138.

Tromba, F. G., and F. W. Douvres. 1953. A modified *en face* technique. Proceedings of the Helminthological Society of Washington 20:59.

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