SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD FOR PREPARING PERMANENT SLIDES OF THE OVA OF PARASITIC WORMS

THE aqueous media commonly used to prepare microscopic slides of ova of parasitic worms give rather fragile preparations which withstand classroom use poorly.[1] Yet these specimens must be mounted in water-soluble materials because dehydration and "clearing" either distort them beyond recognition or render them invisible.

In a limited series of tests of available water-soluble substances that might be used with some hope of improving durability of mounts, one formula of the gum acacia-chloral hydrate medium[2] has given satisfactory results, when used as indicated by the following directions.

(1) Prepare a series of dilutions of gum-chloral in 10 per cent. formalin starting with a 10 per cent. solution and increasing concentrations by 2 per cent. for each subsequent step, or make up each dilution as it is needed.

(2) Concentrate fecal suspensions that have been thoroughly fixed in neutral formalin until each drop of fluid contains 10 to 12 ova. Each cubic centimeter of this will yield about 10 slides.

(3) Pipette 5 cubic centimeters of the concentrated fecal suspensions into vaccine bottles of 15 cubic centimeter capacity. Next add an equal quantity of 10 per cent. gum-chloral-formalin, tilting the bottles to allow this fluid to run in along the lower side. In all dilutions of the series, gum-chloral is heavier than any fecal suspension tested and will occupy the lower half of the column of fluid. Do not shake the bottles or stir the contents.

(4) Cap the bottles and leave them at room temperature or in an incubator at 37° C. until the fecal material has completely settled. Then remove the clear fluid above the feces with a fine-pointed pipette attached to a vacuum pump, and add an equal volume of the next higher concentration of gum-chloral solution.

(5) Continue this process until the ova are suspended in full-strength gum-chloral medium. When the last sedimentation is complete and excess fluid removed, mix the contents of each bottle thoroughly before starting to prepare mounts.

(6) Use clean slides and cover glasses. Circular cover glasses 12 to 15 millimeters in diameter give better mounts than larger sizes. Squares are unsatisfactory for most specimens. Mounts are prepared by two persons working as a team; one transfers drops of the mixture to the slides, the other adds the cover glasses. With some practice one will come to judge the size of drop which will spread completely under the cover glass without excess. For transferring the material to slides, a heavy platinum loop about 4 millimeters diameter is superior to a pipette. The loop delivers a drop of about the correct size with few air bubbles. Cover glasses must be applied quickly to the mounts. Otherwise the medium hardens and spreads poorly.

(7) Dry the mounts in a horizontal position in an incubator at room temperature. Any air bubbles under the cover glass usually are extruded during drying. Thereafter, slides may be treated as if prepared with balsam or clarite. The medium is readily soluble in water, however.

This schedule requires several months for completion, but actually the time given to the specimens is only the matter of minutes for each change of solution. Results amply compensate for the effort. By this method I have prepared mounts of the ova of *Schistosoma mansoni, Clonorchis sinensis, Fasciola hepatica, Diphyllobothrium latum, Hymenolepis nana, Taenia saginata, Ascaris lumbricoides, Trichocephalus trichiurus* and *Enterobius vermicularis*. In these slides small numbers of the ova of some species are distorted, but the majority remain intact and are seen in greater detail than in temporary mounts of formalin-fixed feces. The only failure thus far has been with hookworm eggs, all of which became badly distorted.

Adult *Necator americanus* and other small nematodes, mites, ticks, lice and the larvae and pupae of flies are easily infiltrated and mounted by this method. These organisms remain soft and are easily arranged on slides. When mounted under small round cover glasses, supports do not seem to be necessary.

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[1] E. V. Cowdry, "Microscopic Technique," Baltimore, 1943.

[2] W. Morrison, *Turtox News*, 20: 157, 1942.

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