https://doi.org/10.1017/S1551929500063185 Published online by Cambridge University Press

Collecting Material For Specimen Preparation

J.B. Sanderson
Sir William Dunn School of Pathology, Oxford, UK

Most workers wishing to prepare material for microscopy will study a limited range of organisms, and already be familiar with raising, culturing or collecting the species in question because of their research interests or adopted field of study. For those new to microscopy who have not yet defined a field of interest, it is suggested that they read a practical introductory text such as that of Gravé (1991).

The diversity and abundance of animal, plant and microbial life available for collection means that gathering material can be a relatively simple task. Nevertheless, a methodical approach ensures that specimens are less likely to suffer damage and full details of their natural habitat are known, which will place any serious study into scientific context. Concomitant with the proper collection of material is an understanding of taxonomy. Readers wishing to know more about this subject are advised to consult Jeffrey (1989) and Margulis and Schwartz (1988). For our purposes, we can regard specimens as aquatic, static terrestrial (in general, plants) and mobile terrestrial forms. These notes are confined to remarks on collecting microbial, herbaceous or invertebrate life from the wild. Subculturing and propagating research material, or raising chordate populations, requires special facilities and is beyond the scope of this text.

A variety of microbes can be cultured using s simple hay infusion. A handful of chopped grass can be added to tap water that has previously been allowed to stand for a day or so to remove the chlorine, and after a few days bacteria will accumulate. The culture can be further enriched by the addition of horse manure. Likewise, animal pellets and soil samples can be collected and dissected into water or buffer to provide material for investigation.

Botanical specimens can be collected into polythene bags, or kept pressed between two lightweight boards lined with paper. In humid climates collection in alcohol vapour is preferred to prevent decay. Alternatively, specimens can be dissected and immersion-fixed in the field. Likewise, fungi can usually be dissected into small cubes for fixation in the field. Spore samples can be taken as imprints from the fruiting body by placing the hymenial surface directly onto the slide and fixed by air drying. Further details for collecting botanical specimens can be found in Forman and Bridson (1992).

Many insects live and feed on plants; they can be beaten or shaken into an umbrella or net, or else picked or sucked off with an aspirator. Insects are best killed using a bottle containing a swab soaked in ethyl acetate, or cyanide, or by immersion into 70% alcohol (which also fixes the specimen). Some insects are phototropic and can be caught using a light trap, while others respond to chemical repellents or attractants. Those insects which inhabit woodland floor detritus can be sifted using Tüllgren or Berlèse funnels. Further details are given in Borror *et al.* (1989), in addition to the guides published by the Natural History Museum for collectors of insects and other invertebrates.

Aquatic invertebrate species can be collected directly in glass vials or screw-top jars, or dredgings from plankton nets taken to provide species trapped in the algal weed. Benthic animals can be dislodged by stirring the water and overturning stones upstream of the net. Empty the contents of the net into a white dish, or translucent container with a white sheet or paper background. The animals will at once crawl out from the detritus, and can be identified and selected. Sorting is much easier if living forms are sorted; when dead they resemble the dredgings and, lacking movement, are much harder to discriminate. Many invertebrates will survive transport amongst damp weed kept in an air-tight tin better than they will in overcrowded bottles of water. If bottles are used, they should be cleaned with only a small amount of detergent and rinsed several times with tap water. Just prior to use, rinse out the bottle with pond water before sampling. When filling bottles, they should be left two thirds empty to provide a sufficiently high surface area to volume ratio between the water and air.

Whatever the species collected, a hard-backed notebook should be used to

record in pencil (later written up in ink) details of the collection, identified and suspected species, locality, weather, temperature and date. Details of suitable field record sheets, and further details of collection can be found in Needham (1962), Garnett (1965) and Knudsen (1972).

For initial identification and selection of material a good 10X hand lens (preferably with an achromatic doublet) is indispensable. Where it is imperative to keep both hands free (e.g. dissection) use an eye loupe, or magnifier fitted to a head band. Should higher magnifications be required there are two portable field microscopes on the market. The classical McArthur microscope is manufactured by W. Kirk and Sons, and can be adapted for use with phase, fluorescence and other contrast techniques. The Lensman microscope (Alltek Precions Plastics) is constructed of plastic and, while not of the same quality as the McArthur design, is much cheaper. Besides these instruments, it is possible to use microscopical video equipment, and miniature microscopes with long working distances. For further details, see Watt(1993). ■

Reprinted from Section 1.1 of the RMS Handbook "Biological Technique." This RMS Handbook is available from Microscopy Today, by check, Visa/MasterCard, or company purchase order for $36.00 plus $4.00 shipping

Editor's Note:
We particularly appreciate readers' contributions such as the one to the right - on any microscopy-related topic. Please help?
. . .Don Grimes, Editor

USING KODAK 4489 SHEET FILM TO MAKE PROJECTION ELECTRON MICROGRAPHS

Cindy L. Lewis
Children's Hospital Medical Center, Cincinnati, OH

We are submitting this in response to two technical notes in two previous issues of *Microscopy Today* (issues 94-2, March 1994 and 94-4, June 1994). We have been using Kodak 4489 sheet film rather than emulsion coated glass slides for some time to produce 2" by 2" projector slides. The method was taught to us by Dr. Carole Vogler, a former trainee in our department, now staff pathologist at Cardinal Glennon Hospital For Sick Children and Professor of Pathology at St. Louis School of Medicine in St. Louis, Missouri. She was taught the technique by the technical staff in the Department of Pathology at the University Of Texas. The individual originating the technique is unknown to us.

The film is exposed with a Durst enlarger with 150 mm lens and 240/240R condensers. The area for exposure (approximately 1 ½" x 1 ½" or 35 mm x 35 mm) is framed with the easel and the film is placed for exposure with the notch in the right lower corner. I expose the film at the range of 40-50 volts for 5, 7, and 9 seconds, bracketing the variable negative conditions. Develop the film in D-19 for 2 ½ minutes at 68° F, rinse, fix and dry as usual. The unexposed edges of the finished exposures are trimmed and the exposures are mounted in Gepe (c) 35 MM glass projection slide mounts.

This method is fast, easy and reproducible. It yields high resolution projection slides of a much higher quality than can be obtained by other methods, such as photographic reproduction of prints.

Hopefully this will be of help to electron microscopists in the future as an alternative to using Kodak projection slide plates.