Name: _____ Date: _____ Period: _____ BIN: _____

How to Prepare Microscope Slides

When providing the best possible answer to the following questions please apply all learned scientific techniques and procedures, do not use abbreviations, use proper scientific terminology, show work for all mathematical calculations, use all significant figure and scientific notation rules, apply S.E.E.C. writing strategies, and note that at all times spelling counts. Your ability to meet these and all established classroom expectations, including labeling of BINs, providing heading information, and your ability to follow directions may be included in computation of grade.

When preparing microscope slides for observation, it is important first to have all necessary materials on hand. This includes slides, cover slips, droppers or pipets and any chemicals or stains you plan to use.

There are two different types of microscope slides in general use. The common flat glass slide, and the depression or well slide. Both are rectangular and measure approximately 1 x 3 inches (25 x 75 mm). Depression slides have an indentation in the center to hold a drop of liquid, cost considerably more than the flat variety, and are usually used without a cover slip.

Standard slides are made of glass or plastic. For most purposes, glass slides of 1 to 1.2 mm thick are used. When working with high power objectives and condensers, the slide thickness should be reduced to 0.8 to 1 mm. When ordering slides, always order more than you expect to use. They usually are packaged in increments of 72 (which, incidentally, is 1/2 gross!).

A cover slip or cover glass is a very thin square piece of glass (or plastic) that is placed over the water drop. Because of surface tension, the water drop alone tends to sit in a thick dome. With a cover slip in place, the drop is flattened out allowing the investigator to focus with high power very close to the specimen. The cover glass also confines the specimen to a single plane and thereby reduces the amount of focusing necessary. Finally, the cover glass protects the objective lens from immersion into the water drop.

Glass cover slips should be handled carefully as they are very fragile and break easily. Cover slips measure 18 or 20 mm square and the glass variety is available in two thicknesses, Number 1 and Number 2. Number 1 cover glasses are 0.13 - 0.17 mm thick and are recommended for oil immersion or high resolution work. Number 2 slips are 0.17 - 0.25 thick and are used for general applications. They are sold by the ounce and there are about 120 cover slips per ounce (20 x 20 mm, Number 2). With *extreme* care, glass cover slips can be rinsed and reused many times.

For most general applications, a "pipet" is nothing more than a medicine dropper. They are inexpensive and can easily be cleaned or sterilized (after removing the rubber bulb). Longer pipets are available (for sampling the bottom of a deep jar) and you can make your own micro pipets with glass tubing.

When preparing a well slide, simply transfer one to four drops from the sample container to the depression slide. Focus carefully and do not use the higher power objective lenses as they will likely get wet when focusing too close to the drop.

The most common slide preparation is called the "wet mount" slide and utilizes a flat slide and a cover slip. To make one, place a drop of the sample in the middle of a clean slide and lower a cover slip gently over the drop at an angle, with one edge touching the slide first (See Figure 1, below). Allow the liquid to spread out between the two pieces of glass without applying pressure. It takes some practice to determine just how much liquid to use. If too much is placed on the slide, the cover slip will "float", creating a water layer that is too thick (allowing protozoans to swim up and down, in and out of focus). If too little liquid is used, the organisms may be crushed by the cover glass and evaporation will dry up the specimens quickly. A well prepared slide will last for 15 -30 minutes before it dries up.

To extend the life of a wet mount slide, scrape petroleum jelly onto each of the four edges of the cover slip (Figure 2). Place the cover slip over the drop of water ("jelly side down" - Figure 3), and press *lightly* to seal it to the slide. This sealed slide may last for several days.

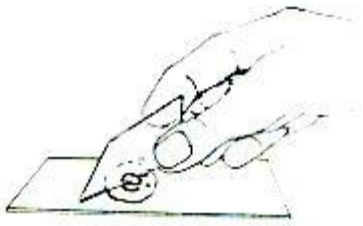

Figure 1

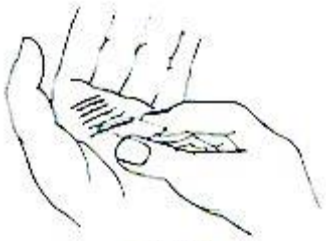

Figure 2

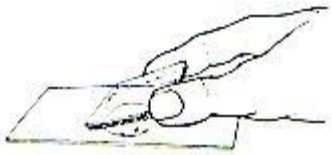

Figure 3

To use a cover slip with larger specimens, add a few broken bits of cover glass to the water drop. When the cover slip is placed over it, the solid particles will add considerably to the thickness of the channel (this is called a "raised cover slip").

To observe *Daphnia*, use a well slide and carefully hold a tiny piece of twisted paper towel to one edge of the drop. Soak up water with the towel until the *Daphnia* becomes "grounded" and is unable to move. Use a low power scope if possible when doing this to avoid soaking up *Daphnia* in the towel.

Many protozoans move too quickly for accurate observations. Larger ones (i.e., *Paramecium*) can be "corralled" by adding a few strands of cotton fiber from a cotton ball or swab to the drop of the sample before lowering the cover slip. Commercial chemicals called "proto-slow" or "quieting solution" are available from supply companies and work very well, particularly on ciliates. Usually one drop of quieting solution is added with one drop of sample and the protozoans slow to a stop within ten minutes. When working with these solutions be careful not to contaminate the sample container with the chemical dropper!

Staining techniques can be employed to aid in the observation of cell parts. "Non-vital staining" is the staining of dead cells or tissues. "Vital staining" is the staining of live cells.

Staining is a very complex subject, but there are many simple staining techniques that can easily be performed. Both pre-stained live samples and pre-stained permanent slides (dead organisms) can be inexpensively acquired from science supply companies.

When ordering permanent slides, look for the following abbreviations: "st" is a stained preparation; "wm" means whole mount (the complete organism); "cs" is a cross section, such as a thin wafer of a worm; "ls" is a longitudinal section, a section cut lengthwise; "sq" is a squash preparation; and, "sm" is a smear, such as a blood smear.