STAINS, REAGENTS, AND MOUNTING MEDIA

Water

Sections prepared for temporary observation should be mounted in distilled water. Measurements, and observations on color and granulation, should be made in water.

Glycerin

Bland (1971) recommended mounting sections in 10% glycerin, which is closer to the refractive index of the tissues and makes it easier to see structures clearly.

Application of Reagents, etc. to Microscopic Preparations

Replacement of one mounting medium, stain or reagent can be accomplished simply by adding a drop or two of the new substance to the edge of the cover slip and drawing it under by placing a square of filter paper, paper towel or "bibulous paper" (which is made for this purpose) at the opposite edge.

Stains

Lactophenol Cotton Blue (LCB)

This stain is essential for clearly seeing the lumina of hyphae, paraphyses, etc. It can also be useful in examination of the walls and ornamentation of spores.

Empty algal cells in a pseudocortex can also sometimes be detected in cotton blue.

According to Brodo (1984), the lactic acid in LCB can act similar to nitric acid, in dissolving some kinds of granular or crystalline inspersions (e.g., many of the large amphithecial crystals that occur in the Lecanora subfusca group and often the small medullary granules).

Lactophenol cotton blue can be cleared from the tissues by drawing a drop of the lactophenol withtout the cotton blue under the coverslip.

Hoyer's Solution

Brodo (1984) used this solution as one of his mounting media, to

help resolve certain types of tissue structures. He reported that it acts like a strong base, dissolving the same types of granules or crystals that KOH does.

Hoyer's solution contains chloral hydrate and therefore is very poisonous.

Chlorzinciodine (CZI)

Empty algal cells in a "pseudocortex" can be detected by staining with "chlorzinciodine", which turns a violet color in the presence of the cellulose walls of the algae, but does not react to the chitinous walls of the fungus (Poelt, 1958 and pers. comm.).

This stain has been used in a number of studies, including those of Galun? (19see "Characters" notebook), Baumgartner (1979), Timdal (1984)

Timdal (1984) recommended a slight modification of the pretreatment described by Baumgartner (1979): the sections should first be stained in chlorzinciodine for a few minutes, then both washed and soaked in Lugol's solution for a few minutes, and finally both washed and stained in chlorzinciodine again. Timdal found that washing the sections with water, as done by Baumgartner (1979) greatly diminished the effect of the pretreatment.

Poelt (pers. comm.) recommends that in species heavily inspersed with granules of usnic acid, it is usually necessary to first flush the cortex with KOH and then water, to remove obscuring granules.

This stain should be stored in bottles made of glass, not polyethylenes.

Other stains

McWhorter (1921) recommended safraninanalinblue stain for seeing cell walls and haustoria.

An 0.1% aqueous solution of phloxine increases contrast by staining cytoplasm more than walls (Rhoades, 1981, unpublished mycology lab notes).

Neutral red and TCC can be used to determine if the algae are alive or not (LeBlanc, et al., 1971).

Some structures (e.g. hyaline epispores) can be seen in black India ink (Mycol. Handbook).

Reagents

The following reagents can be used either for making spot tests or for making various tests or observations in microscopic preparations.

Potassium Hydroxide (KOH or K)

A solution of 10% (2025% according to Taylor, Lichens of Ohio v. 1) potassium hydroxide in microscopic preparations is used to 1)dissolve all granules except the calcium oxalate crystals (i.e., to clear the tissues and facilitate the observation of these crystals), 2) to free and dissociate hyphae and hymenial elements, 3) to make a final attempt at locating spores in a rather sterile apothecium, and 4) to observed any color change in tissues.

The 10% potassium hydroxide solution usually will stay active about a half year if kept stoppered (Dahl & Krog, 1973).

Paraphenylenediamine (Pd or P)

This reagent is prepared by making a saturated solution, using a few crystals in ethanol (7095%). A fresh solution must be prepared every few hours. According to Taylor (19 , Lich. of Ohio v. 1), Pd solution will be effective as long as it is clear, even if it has become colored.

The Pd reagent should not be inhaled.

Material contaminated by Pd should be destroyed, or it will discolor everything in the vicinity.

The formula for Steiner's stabilized Pd (see Thomson, 1967; Taylor, Lich. of Ohio; and article by _____), which will keep at least 6 months, is as follows:

Water	100 ml
Anhydrous sodium sulphite (Photographer's hypo)	10 gm

Paraphenylenediamine	1 gm
Liquid detergent	1020 drops
or	
Wetting agents (Saturated solution of Pril or Teepol)	40 drops

Hypochlorite Solution (C)

An undiluted, liquid commercial bleach preparation containing hypochlorite is used for this reagent.

Sometimes a C+ reaction is best seen if the specimen is pressed with a probe after the C is applied, or if the C is put on filter paper and the sample is squashed and smeared around in the C (Noble, 19 , Dissert.)

Generally a hypochlorite solution is still active as long as it smells strongly of chlorine, which is about a week or two (Dahl & Krog, 1973).

Iodine Reagents (I)

Timdal (1984) recommended studying asci in modified Lugol's solution (with water replaced by lactic acid), which can be used for semipermanent preparations.

The reaction of asci to iodine can be studied both with and without pretreatment with K; the former reaction is denoted the KIreaction, the latter the Ireaction.

Rossman (1981) pointed out that Mycologists dealing with freeliving fungi traditionally use Meltzer's Reagent, while lichenologists use IKI (or Lugol's), and caution must be exercised because in some cases the former may act somewhat differently than the latter two, which lack chloral hydrate. Rossman also pointed out that all Iodine solutions degrade with time and should be used when relatively fresh. A comparison of the compositon of the three reagents are given below.

	Meltzer's	IKI	Lugol's
Chloral hydrate	100 gm		
Potassium iodide	5 gm	1 gm	2 gm

Iodine	1.5 gm	1 gm	1 gm
Distilled water	100 ml	100 ml	300 ml

Meltzer's reagent is very poisonous, and chloral hydrate cannot be purchased without a drug license.

Swinscow (freshwater Verrucaria) measured spores in Meltzer's.

Nitric Acid (HNO3)

Concentrated nitric acid in microscopic preparations is used to 1) test the solubility of granules, 2) dissolve calcium oxalate crystals, and 3) to observe any color change in pigmented structures.

Timdal (1984) used 50% nitric acid.

Sulfuric Acid (H2SO4)

Timdal (1984) used 25% sulfuric acid to dissolve calcium oxalate.

Hydrochloric Acid (HCl)

Some workers recommend hydrochloric acid instead of nitric acid or sulphuric acid.

ChloramineT

Thomson (1967) used a 5% solution of chloramineT in alcohol as a test for usnic acid (which gives a yellow reaction).

Combinations of several reagents

Some workers recommend various combinations of several reagents.

Potassium hydroxide & Hypochlorite solution

According to Taylor (Lich. of Ohio v. 1), not more than a few seconds should elapse between application of K and C

Nitric acid & Potassium hydroxide

Makarevich (1971) recommended examination of tissues first in nitric acid and then in potassium hydroxide.