

Mycology Stains and Reagents

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Note – all of these fluids should be treated as though they are extremely toxic and/or carcinogenic. If you get any on you, immediately set down whatever you were doing, go to the nearest sink, and thoroughly wash the affected area with water for at least a few minutes.

Acetocarmine – test for siderophilous granules (Lyophyllaceae). Take a couple gill fragments and apply a couple drops of Acetocarmine. Heat the section of the slide with a flame to boil off almost all of the stain. Apply more stain and boil it off several more times, until the gill fragments are nearly black. Rinse the specimen with water to remove as much of everything but the gill fragments as you can, then make a squash mount with water.

Alcohol – used as a rehydrating medium and for cleaning implements.

Ammonia – used both as a macroscopic color test for many fungi, and also as a mounting and rehydrating medium.

Baral's – iodine test for Ascomycetes (particularly the Helotiales), similar to Melzer's, which should also be done. Do not pretreat the specimen with a strong base. Color changes are typically red or blue, and need to be compared to the results of Melzer's staining.

Congo Red – stains cell walls, used to see if spores (or other features) are smooth or ornamented, or to highlight clamps. Allow it to stain for 5 or more minutes, then rinse and blot with water or GSM. When this solution begins to rapidly form crystals on the slide it should be replaced.

Cotton Blue – cyanophilic test. Place a drop of Cotton Blue on the slide and allow it to sit for 10 – 20 minutes. Blot as much as you can off with a tissue, then rinse and blot with Lactoglycerol until the rinse is clear. Use Lactoglycerol as the mounting medium.

FeSO₄ – macroscopic reagent for many fungi. Apply a drop to the tissue and watch for color changes. Also compare to a control spot with water. Watch for this solution to discolor eventually, at which time it will need to be replaced.

GSM – basic mounting and rehydrating medium. Replaces KOH and Ammonia solutions for mounting, and evaporates very slowly, giving you more time to examine the slide.

Gum Guaiac – macroscopic reagent for many fungi. Apply a drop to the appropriate surface of the specimen and watch for color changes within several seconds.

HCl (2%) - microscopic reagent for testing for acid resistant incrustations on hyphae in *Russula*. Take a small piece of cap cuticle and soak it in HCl for a couple minutes, rinse with water, then stain with a general stain such as Methylene Blue.

KOH (10%) – macroscopic reagent for many fungi. Apply a drop to the tissue and watch for color changes. Also compare to a control spot with water. Can also be used as a mounting medium. When this solution becomes cloudy it needs to be replaced.

KOH (3%) - rehydrating reagent for many fungi. Soak the specimen to be rehydrated in a generous amount of reagent. Can be used as a mounting medium. When this solution becomes cloudy it needs to be replaced.

Lactoglycerol – acidic mounting medium. Used after Cotton Blue to remove excess dye and provide a clear mounting medium.

Melzer's – amyloid test. Sometimes you will need to allow the staining to proceed for 15 – 30 minutes. A negative reaction is reddish brown, an amyloid reaction is bluish black, and dextrinoid is bright purplish. Avoid contact between this and strong bases (e.g., KOH, GSM); rinse the specimen with water thoroughly before using the other reagent. Pretreating a specimen with a strong base will often intensify the staining; i.e., soak the specimen in KOH, NaOH, or GSM for at least several seconds, then thoroughly rinse the specimen with water before applying the Melzer's.

Methylene Blue – general microscopic stain. As it is actually a mixture of several different dyes, it will often produce differential staining in many specimens.

PDAB – macroscopic reagent for many fungi. NOTE: only works on fungi that are still fresh. Take a piece of the mushroom to be tested and place it in a depression on a white spot test plate. Apply enough PDAB solution to immerse the specimen. A positive reaction is obvious blue staining.

Schultze's – iodine test similar to Melzer's (experimental). Pretreatment with strong bases often seems to produce results similar to Melzer's. This reagent is experimental to see if interesting different and useful reactions result, or if it can be used as a substitute for Melzer's in some circumstances.

Sulfopiperonal – stain for visualizing pileocystidia in *Russula* and lactiferous hyphae in *Lactarius*. Mix one drop of water and one drop of concentrated H₂SO₄ on a slide, then add a small crystal of piperonaldehyde and stir. You should get a bright yellow liquid. Place a small piece of cap cuticle into the solution and squash. Pileocystidia will stain blackish. The water/ H₂SO₄ mixture can be premade to simplify the process – when it begins to discolor it must be replaced.