



Healthy-appearing spores from an extract are manually transferred to a watchglass in tap water and then checked carefully under a stereomicroscope to remove any hyphae or debris that may be present. The watch-glass is placed in a sealed petri dish (to reduce evaporation) or in a capped vial and stored at 4°C for at least 48 hrs .

Spores are examined closely again and ALL of those with any visual departure from the norm are removed and discarded. *Examples:* spore discoloration, spotting, disorganization of contents, excessive opaqueness, signs of bacterial or hyphal growth on spore surfaces.

Water is changed and spores are stored for another 24-48 hrs at 4°C. Any additional atypical spores are removed and discarded. The spores then are washed and transferred to sterile distilled water in screw-top vials for short-term storage (generally no longer than 30 days). Prior to any DNA extraction, spores are checked again for atypical specimens.

SHIPMENT OF SPORES: Dry autoclaved coarse Quartzite sand (90% of particles are > 1 and < 2 mm dia) is added to 2-ml plastic screw-top vials and filled to 3/4 of total volume. Spores are collected with a pipette in approximately 0.1 ml sterile distilled water and transferred to the vial. The vial is capped and the sand within is shaken vigorously to evenly distribute spores. Vials usually are mailed on a Monday to avoid sitting in a post office for longer than one day.

After the vial is received, it can be stored at 4°C for several weeks if necessary, but it is best to collect spores and use as soon as possible. Contents of the vial are added to a flask containing sterile distilled water. The water is swirled vigorously and quickly decanted into a petri dish. The sand particles are heavy and will remain on the flask bottom while spores are suspended in the water. Spores should be examined again for any signs of deterioration or parasite contamination before disrupting to extract DNA.