

STANDARD OPERATING PROCEDURE:

Physarum polycephalum (slime mould) care and use

Note: To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment.

1. Introduction

Physarum polycephalum is a slime mould that grows in dark humid conditions under the bark of decaying trees and amongst leaf litter on the forest floor. It is used as a tool for demonstrating cytoplasmic streaming* locomotion, and plasmodial fusion* to students. *Physarum polycephalum* is purchased as a living organism and needs to be fed daily and subcultured to prevent it from outgrowing the petri dish. The plasmodium is the active feeding stage of the organism and consists of a mass of multinucleate protoplasm. In moving, the plasmodium may move along many fronts that are connected by veins. Streaming of protoplasm is easily seen within the veins.

2. Context

- These instructions are for teachers and technicians for the use of *Physarum polycephalum* for demonstration purposes only.

3. Safety notes

- *Physarum polycephalum* is a Risk Group 1* microorganism that is suitable for use in schools. It is not known to be toxic.
- The petri dish should remain closed during class demonstrations.
- *Physarum polycephalum* is a living culture that, if allowed to starve or dry out, may begin to sporulate*. The spores* are unlikely to generate microbial aerosols*.
- Wear gloves, safety glasses and lab coat/apron when handling.
- Regard all microorganisms as potential pathogens, and treat them accordingly.

4. Regulations, licences and permits

Not applicable

5. Equipment

For handling and cultivating

- PPE: safety glasses, gloves, lab coat, closed shoes
- A clean, non-traffic area to feed and subculture
- 70% ethanol (flammable)
- Rolled oats
- Sterile scalpel blade
- Sterile forceps
- Fresh sterile plain agar plate
- Lab sealing tape
- *Physarum* should be stored at room temp away from bright light.

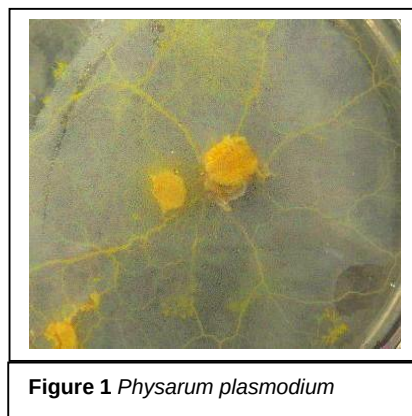


Figure 1 *Physarum plasmodium*

For class demonstrations

- PPE: safety glasses, gloves, lab coat, closed shoes
- Light microscope or stereo microscope
- Optional: Digital display camera for whole class viewing.
- Viable sample of *Physarum polycephalum* in a lidded petri dish.

6. Operating procedure

Feeding the *Physarum* culture – daily

1. Disinfect the bench and equipment with 70% ethanol (flammable)
2. Using clean forceps, place 1-2 rolled oats onto the plain agar on the bright yellow area/s.
3. Immediately replace the lid.
4. Store in a dark area at room temperature. Petri dish may be wrapped in foil if no dark area is available.
5. Disinfect the bench and equipment with 70% ethanol (flammable)

Subculturing *Physarum polycephalum*

Physarum polycephalum may be subcultured approximately every 5-7 days to prevent the culture from outgrowing the petri dish and to increase the number of petri dishes available for classroom use.

1. Disinfect the bench and equipment with 70% ethanol (flammable)
2. Using a sterile scalpel, select an area that has a colonised oat flake. Cut an agar cube (approx 1cm³) encompassing the colonised area and carefully lift the section out of the petri dish with sterile forceps. Replace the lid.
3. Immediately place the agar cube onto a fresh plain agar plate with the colonised oat flake in direct contact with the agar. Close the lid and tape closed with lab sealing tape.
4. Repeat steps 2 and 3 if desired, using a fresh sterile plain agar plate each time.
5. Feed the sub-cultured sample after 24 hours as per instructions above.
6. Close and seal the petri dish with lab sealing tape.
7. Disinfect the bench and equipment with 70% ethanol (flammable)

Cytoplasmic streaming class demonstration

1. Setup a light microscope or stereo microscope.
2. Place a petri dish containing the *Physarum polycephalum* on the microscope stage and remove the lid.
3. Close the diaphragm or lower the sub-stage condenser of the microscope.
4. Focus on the yellow branches of the *Physarum* using the 4x objective (40x magnification). Observe the back and forth flow of the cytoplasm within the yellow branches. The flow will change direction every few minutes.

7. Trouble shooting/emergencies

- First aid: See latest SDS for more detailed information
 - **If swallowed:** Do not induce vomiting. Rinse mouth with water, and then give water to drink. Seek medical attention.
 - **If in eyes:** Hold open and irrigate with copious quantity of water for at least 15 minutes. Seek medical attention.

- **If on skin/clothes:** Wash affected area with copious quantities of water immediately. Remove contaminated clothes and wash before reuse. If swelling, redness, blistering or irritation occurs seek medical attention.
- **If inhaled:** Remove to fresh air and seek medical attention if symptoms persist.
- For further advice contact the Poisons Information Centre on 131126
- *Any health concerns should be referred to the school first aid officer for assessment, accompanied by the relevant latest SDS if applicable. Follow your school's accident and incident policy and reporting procedures.*

8. Waste disposal

- *Physarum polycephalum* can be maintained after class use for an extended period of time. However, the ongoing commitment required to feed and subculture the culture is time consuming.
- Cultures should be sterilized by autoclave or pressure cooker at 121°C, 15psi for 20 minutes prior to disposing with general waste.

9. Related material

- Manufacturer's Safety Data Sheet
- Risk Assessment

*Glossary:

- **Cytoplasmic streaming** - the movement of the fluid substance (cytoplasm) within a plant or animal cell. The motion transports nutrients, proteins and organelles within cells.
- **Risk group 1 organism** - a microorganism that is unlikely to cause human or animal disease
- **Sporulate** – produce spores
- **Spores** – a minute organic body that develops into a new individual.
- Microbial aerosols – particles of microbes distributed in a finely divided state through a gas, usually air. In this instance, spores are liberated into the air which is undesirable.
- **Plasmodium** - a shapeless mobile multinucleate mass of cytoplasm without a firm cell wall.
- **Plasmodial fusion** - refers to the feeding stage of macroscopic slime moulds

References:

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