Culturing Amoeba

Live Material Care Guide

Background

Amoeba can be found among decaying vegetable matter and aquatic plants in ponds, puddles and eddy areas of streams. They are not found in high quantities in waters where crustaceans are present, since many crustaceans feed on *Amoeba*. Their abundance in natural water sources is not as high as one might guess. Those who have tried to locate *Amoeba* in the wild will agree that they are difficult to find. For this reason, it is advantageous to culture *Amoeba* under lab conditions. In culture, *Amoeba* are whitish-grey in appearance and are approximately the size of a pinhead to the naked eye. When healthy, they tend to be very active, although disturbances may cause them to stop moving and retract their pseudopods to "hide." Fortunately, *Amoeba*, are relatively simple to culture from purchased stock cultures and easy to locate using a dimly lit stereoscope. *Amoeba*, popular and often remembered protists, move around by means of pseudopodia (flowing cytoplasm) for locomotion. (Movements can best be observed under 400 X.)

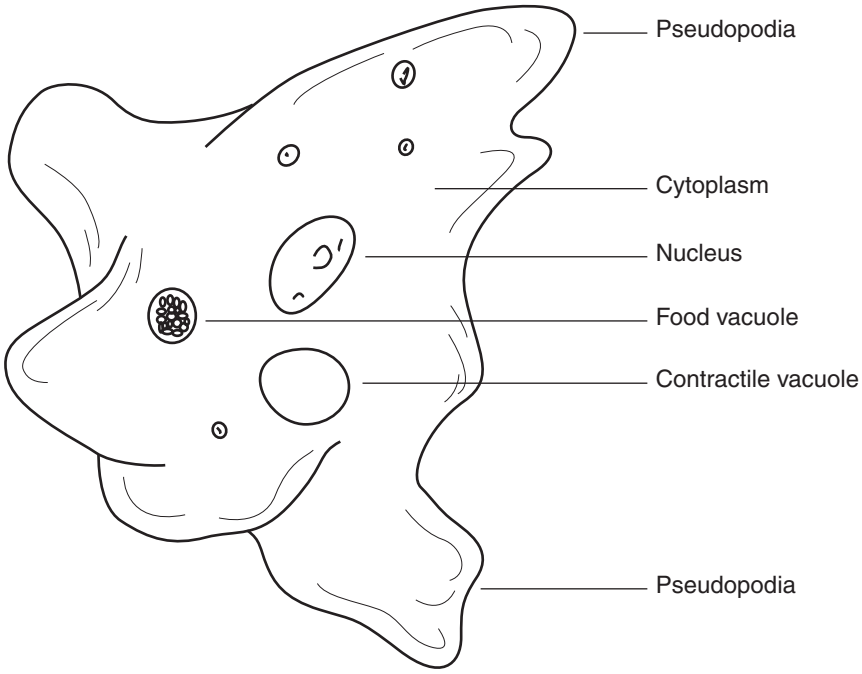

Figure 1. *Amoeba*

Culturing/Media

Upon arrival of *Amoeba* stock cultures, loosen the caps and immediately aerate the cultures by forcing air into the liquid using a clean pipet. Cultures should be kept at 18–22 °C (64–72 °F) and placed out of direct sunlight as they avoid light (negative phototaxic). Avoid moving the culture! A certain amount of experimentation may be necessary to find a suitable place to house stock cultures. It is wise to initially place multiple subcultures in a variety of sites while trying to determine the best culturing environment in a particular classroom. Observe sample cultures regularly using a dimly lit stereoscope. Placing black paper under the culture dish makes *Amoeba* easier to view.

The *Amoeba* population will increase for 21 days and may then require additional wheat seeds. Split a culture when 50 or more *Amoeba* are seen in one field of view on low power of a stereoscope. Stir the media in the dish, and divide the culture evenly into three clean culture dishes. Add enough liquid media solution to each dish to restore the volume to that of the original culture (approximately ⅔ full). Add two new boiled wheat seeds to each subculture. Dishes should be covered loosely and stacked to limit evaporation and contamination. Fresh media should be prepared monthly if the *Amoeba* will be maintained long term.

If *Amoeba* are to be cultured for extended periods of time, the following recipes and procedures should be followed to ensure that cultures remain viable.

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Hay Infusion

- Boil 100 mL of spring water for 10 minutes and then let it cool.
- Add eight lengths of Timothy hay stalks (~ 3 cm long) or about 10 g of pesticide-free dry grass clippings, and let stand uncovered for 24 hours.
- Transfer the mixture to shallow, stacking culture dishes and then add the *Amoeba* culture to the dishes. *Amoeba* tend to congregate on the bottom and around the edges where the sidewalls and bottom come together. Use a plastic pipet to forcefully squirt water into those areas to dislodge any *Amoeba* that might still be clinging to the container.
- Finally, add two grains of uncooked rice or wheat for every 50 mL of *Amoeba* culture. If the rice grains begin looking fuzzy, mold is beginning to grow on them, but the culture should be okay as long as it is kept aerated to prevent a surface scum from forming due to excess bacterial growth.

Chalkley's Stock Solution 10x (Synthetic Pond Water): This is the easiest medium to make and is recommended for culturing most types of protozoa, including *Amoeba*. This stock solution should be diluted by a factor of 10. In other words, every 100 mL of this solution should be diluted to 1 litre using spring water.

$CaCl_2$	0.06 g
NaCl	1.00 g
KCl	0.04 g

Add each of these amounts to one litre of spring water and mix thoroughly.

After pouring the media into small culture dishes or a large jar, add two rice or wheat grains per 50 mL of *Amoeba* culture to encourage the growth of the bacteria. Small ciliates feed on the bacteria, and the *Amoeba* feed on the ciliates. If the rice grains start to look fuzzy, mold is beginning to grow, and that is okay!

Tips

- To ensure positive transfers of *Amoeba* to student slides, do collection transfers while looking through a stereoscope. *Amoeba* tend to be on the bottom of the culture dish and can be viewed as they are being drawn into the transferring pipet. The transferred *Amoeba* should be obvious on the student slide.
- Students skilled in microscope use should view *Amoeba* in a depression slide. Looking for *Amoeba* should *not* be a first microscope practice exercise. Do not allow students to randomly take pipets full of water from an *Amoeba* culture. Continual agitation of the culture will cause *Amoeba* to "ball up" and become inactive. Slides of *Amoeba* can be kept viable for hours if slide gel is used and the sample is kept sealed around the coverslip.
- Allow students to view prepared microscope slides of *Amoeba* before working with the live specimens. This will eliminate some initial frustration.
- Monitor the culture regularly using a stereoscope. Have extra media already mixed and ready when subculturing is necessary. If ~50 specimens can be seen in one field of view, it is time to split the culture.
- For long-term culturing, fresh media should be prepared and changed monthly.

Disposal

It is recommended that you consult your local school board and/or municipal regulations for proper disposal methods that may apply before proceeding.

Materials for *Culturing Amoeba* are available from Flinn Scientific Canada, Inc.

Catalogue No.	Description
AB1264	Culture dish, medium
ML1378	Depression Slides, Single
AP2253	Wide-stem Pipets

Consult www.flinnsci.ca or your *Flinn Scientific Canada Catalogue/Reference Manual* for current prices.