Investigating Microlife

Teacher's Manual

87 W 9057

Kit contents

1 Jar, Microlife Dry Mix™ (87 W 9055)
1 Bottle, Detain (37 W 7950) — Protist slowing agent
1 Polystyrene cover
1 Dropping bottle, methylene blue stain
15 Dropping pipettes
1 Pad, Student Worksheets
1 Teacher's Manual
1 Activities Sheet — "Exploring the World of the Little"

Required but not supplied:
Microscope slides
Coverslips
India ink

Medium Rehydration

Use distilled water in rehydrating Microlife Dry Mix™. Open the lid of the smaller plastic jar. Empty the Microlife Dry Mix™ into the larger plastic jar. Add distilled water to a depth of about ⅔ of the jar. The amount of water added is not critical. Use a spoon (or similar instrument) to thoroughly mix and wet the dry material. Cover the jar using the polystyrene cover. Place the rehydrated mixture in an area that will receive northern light. Be careful not to expose the mixture to direct sunlight!

Microlife Dry Mix™ is a mixture of timothy hay and dry soil taken from the edge of an established pond. Its rehydration will produce a wealth of microlife forms ready for student observation in three to five days.

Microscopic Techniques

It should be noted that the study of dried infusions can be both exciting and frustrating for students. Most microlife forms are stagnant water types; most are also quite small. With support and encouragement your students will be able to readily observe them. Microlife observations will provide endless hours of fascination for you and your students. The dichotomous key included on the student worksheet will aid them in finding out just "who" these tiny creatures are, and a little about their microlifestyles.

The Activity Sheet included in this kit provides you with three microscopic activities to enhance the study of microlife forms. These activities may be used in conjunction with this kit or as separate activities. It is suggested that the dark-field activity be used to aid students in observing motile bacterial forms as "light shimmers". The following techniques can be used by students (with a little help and support from you) to study microlife forms usually encountered.

Staining Bacteria Student Activity

1
Use a plastic dropping pipette to sample for bacteria types. Take samples from the top (surface), middle, or along the bottom sediment.

2
Place a drop of water on a clean microscope slide.

3
Allow the drop of water to air dry on the slide.

4
Use a clothespin (or similar holding device) to hold the slide. Now pass the slide, smear side up, using slow, deliberate motions, through the flame three times. This action will heat-fix the bacteria cells.

5
Allow the slide to cool.

6
Add a drop of methylene blue stain to the area of the dried smear. Allow it to stand 1 minute.

7
Rinse the slide gently with tap water. Do not let the stream of water strike the smear directly or you will wash off the stained cells.

8
Blot the slide carefully using a paper towel. The slide can be observed at 430X (high-dry magnification). The slide may also be observed under an oil immersion lens (1000X). If this is desired, blot the entire slide dry. Apply immersion oil to the smear area.

Of course, greatest visual resolution of bacterial cells will occur at 1000X, but students can readily observe the basic shape and groupings of bacteria using high-dry magnification, just as the early micronaturalists did using their single-lens microscopes. The most commonly observed bacteria will be the rod-shaped bacilli, usually *Bacillus spp.*

Microscopy Hints
Students should use the flat mirror surface for microscopes that have condenser; convex mirror surface for microscopes without. Open the iris diaphragm, allowing in more light, when using higher power objective lenses. Students should scan the smear's outer edges to observe single cells that are not overstained.

Negative Staining Demonstration
The use of India ink as a negative stain will allow students to observe an outline of the bacterial cell. In negative staining, the organism itself is not stained, the surrounding glass surface is. In this way the organism is seen clearly as a "window" against a dark gray background. The size of the organism is better estimated because the cell is not heat fixed, with little shrinking encountered.

1
Mix one drop of the water sample with a drop of India ink on a clean microscope slide. Be sure to spread the water film over as much of the slide as possible. The water film should appear gray rather than black.

2
Allow this film to air-dry on the slide.

3
Examine the air-dried slide under an oil immersion lens. (You may wish to make this a demonstration slide that students can compare with their own wet mounts.) Students should look for "halos" or "ghosts" which will appear colorless against a gray background. These ghosts are bacteria cells. Again, rod-shaped forms will almost exclusively be observed.

Making Hanging Drop Preparations Demonstration
A technique frequently used to observe bacterial motility is the hanging drop procedure. In this preparation, microlife forms are seen in their natural living state, capable of independent movement. It is suggested that this technique be used as a demonstration.

Additional Materials
Concavity slide
Toothpicks
Petroleum jelly or Ward's Silicone Culture Gum (37 W 9810)

1
Apply a thin layer of petroleum jelly around the edge of the concavity.

2
Place a drop of the water sample in the center of a clean coverglass.

3
The concavity slide is then inverted and brought down over the drop on the coverglass. The entire preparation is then quickly turned right side up again. Check the edges of the coverslip to make sure a good seal was made.

If you followed this procedure correctly, your drop of microbial suspension will now be hanging on the underside of the coverglass within an air space created by the concavity of the microscope slide.

Microscopy Hints
Place this preparation on the microscope stage so that the edge of your suspension drop is located in the center of the field under low power (100X). Reduce the amount of light by using the iris diaphragm until the field of view becomes dull. Then swing the high-dry (430X) objective into place without moving anything else. The suspension should nearly be in focus. Observe microorganisms in motion. Remember that they will be quite small and will appear to your eye as ghost-like shimmers.

A variation of this technique is to mix a drop of the water sample with a drop of methylene blue stain. Then place a part of this larger drop on the coverglass. This can be done by using the end of the dropping pipette to suck up the colored drop then dabbing it on the coverglass surface. This action should give you a very small drop, the best for any hanging drop preparation. This stain will give a very faint color to the bacteria without killing them. These vitally-stained cells should be much easier to see. You can also use India ink — be sure to dilute it heavily before use.

Observing other Microlife

Protists can be observed by making simple wet mounts. Most of these larger forms are quite fleeting! You will almost always need to slow them down to identify them. The use of Detain (37 W 7950), our proprietary protist slowing solution, is recommended.

Microscopy Hints

Wet Mount — Surface Scum
Probably the richest hunting area for students will be the scum layer. This skin-like covering of protein and mucilage offers a microhabitat for many organisms.

Students should carefully extract a couple of pieces of floating debris and place them on a clean microscope slide. This action will also provide enough water — there is no need to add additional drops from a pipette. Now add two or three drops of Detain directly over the debris. Adding Detain will slow motile forms as well as provide a support medium that will fill in the natural voids which create air spaces when the coverglass is laid down.

Scan this wet mount using the low power (100X) objective. Begin searching around the smaller pieces of debris. Once an interesting form is encountered, switch to high power (430X) and use the worksheet to identify the microorganism.

Wet Mounts — Water
To prepare wet mounts from "open waters" using Detain, simply add a drop of Detain to a clean microscope slide. Next add a drop of the water sample directly on top of the drop of Detain. Use a toothpick to mix the two drops. Apply a coverglass in the standard fashion to complete the wet mount preparation.

Have students use the low power (100X) objective to scan the wet mount for signs of microlife activity. Since all wet mounts will have Detain in them, students should look for objects exhibiting limited movement. The students should then be directed to switch to the high-dry (430X) objective for specific study. Students should follow the directions of the key (included on their worksheets) identify microlife forms.

You may wish to demonstrate protist movement by having students observe wet mounts without the benefit of Detain. They will soon gain an appreciation for its effect!

Wet Mounts — Mud Sediments
In sampling mud sediments, have students use their pipettes as vacuum cleaners to withdraw a very small amount of sediment. Mix a small drop of sediment with Detain on a clean microscope slide to make a wet mount.

Microscopy Hints

Wet Mounts — Sediments
Students will observe the particles of soil as irregularly-shaped "boulders" under low power (100X) magnification and use their worksheets to identify the microorganism.

Have students make a number of samples from this microhabitat. Some may observe wiggling, snake-like creatures — nematodes, in addition to protists.

Studying Microlife Forms

The table below provides a guide as to "when" and "where" microlife forms can be expected. Again it must be emphasized that most microlife forms will be small (usually below 100 μm in size). This fact should not deter you or the students. With practice you (and they) will become expert in identifying the more common stagnant water forms usually encountered. The dichotomous key included on the student worksheets can be also used in other stagnant water locales — the ubiquitous mud puddle or at the pond's edge. Good hunting!

Microlife Notes

Bacteria
Almost always, rod-shaped forms are encountered. These forms are usually *Bacillus,* occurring singly or in chains. In addition to them, another bacterial form, spirilla, may also be observed. These microorganisms are usually larger and can be observed swimming in hanging drop preparations in a distinctive corkscrew fashion.

Protists
Will usually make their appearance in about two to three days. All must emerge from cysts. Usually the first to emerge are *Colpods, Urotrichia,* and *Protochrysis.* As bacterial populations grow, so do that of protists. Many times students will observe hundreds of *Protochrysis* engorging on clumps of dead bacteria. *Tillina* and *Vorticella* usually make their appearance later on, about Day 5.

It is important to note that as time passes a more varied protist population will emerge. The dichotomous key provides a limited means of identifying the most commonly present stagnant water forms, not in any manner all of them!

Stagnant water protists are ubiquitous, and can be found in almost any standing water condition. The dichotomous key may be used to identify them in these other, natural locales.

Nematodes
These almost macroscopic forms are invariably present in soil sediments. Look for them after Day 5. They will appear most frequently in the mud sediments formed on the bottom of the jar.

Guide to Microlife

	Surface	Open Water	Bottom Debris	Bottom Sediment
Day 1	Bacteria	Bacteria	Bacteria	Bacteria
Day 3	Bacteria	Bacteria	Bacteria	Bacteria
	Urotrichia *Tetrablepharis* *Chlamydomonas*	*Urotrichia* *Tetrablepharis* *Pyramimonas* *Chlamydomonas* *Peranema* *Euglena*	*Protochrysis* *Colpoda*	*Colpoda* *Protochrysis* *Peranema*
Day 5	Bacteria	Bacteria		Bacteria
	Urotrichia *Protochrysis* *Colpoda* *Tillina*	*Urotrichia* *Protochrysis* *Colpoda* *Tetrablepharis*	*Vorticella*	*Protochrysis* *Colpoda* *Tillina* *Chilodenella*
	Tetrablepharis *Pyramimonas* *Euglena* *Chlamydomonas* *Metapus* *Oxytricha*	*Pyramimonas* *Tillina* *Euglena* *Peranema* *Chlamydomonas* *Oxytricha*		Nematodes

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