

USER GUIDE

With our instructions, constructing the Armadillo Portable incubator is a fairly simple process and should take 4-5 hours and around \$200 to complete. All the required materials are readily available. Constructing the incubator involves wiring a simple circuit with the thermostat and resistive heating pads, cutting the foam and cardboard to the right sizes, drilling holes in the cooler and inner chamber, and packing all the parts inside the cooler. You can find detailed assembly instructions with resources for how to obtain materials in our [instructable](#).

Pre-operating instructions

The specified battery pack fully charges in 24 hours. To use the Armadillo in-country away from grid based AC power, simply charge the battery pack ahead of time in a place where you have power. If multiple 48-hours incubations are required, you may transport spare batteries or find a way to charge the battery in-country.

Sample-handling

It is critically important to collect and handle samples correctly before incubation. If it is not possible to directly inoculate samples from source onto the Petrifilm Count Plates, water samples should be collected in sterile containers and transported out of direct sunlight for a minimal amount of time. Each *E. coli* Petrifilm requires 1 milliliter of water. A convenient container for sample collection is small 5 ml vials.

Vials should be clearly numbered by a permanent marker with the source, condition, and time of sample collection should be recorded. Furthermore, samples should be kept cool and dark, and not exposed to bright sunlight or excessive heat. Finally, we recommend inoculating samples within 24 hours of collection.

We find it convenient to write identifying information for each sample with a permanent marker directly on the top of each petrifilm (outside of the circle where the growth medium is found) as it is inoculated.

Inoculation

Operators should practice inoculation and incubation with the Armadillo at home to be confident of procedures in the less comfortable environment in the field.

Inoculation Materials:

- Individually wrapped 1mL sterile transfer [pipettes](#),
- 5mL (or larger) sterile glass sample vials,
- Enough 3M Petrifilm plates for all water samples,
- 3M Petrifilm spreader, and
- A sharp-tipped permanent marker.

Inoculating and incubating Petrifims is easy, but a little practice helps. Please read over these instructions before you begin. You may want to practice an inoculation and your sample handling

process in a “dry run” before you use up your Petrifilms.

1. Obtain sterile pipettes with volume measurements on the sides as well as small (~5mL), sterile sample vials for samples.
2. Use sterile pipettes to take samples from different locations and store in the containers. Ideally, you should collect the samples and perform the inoculation and begin incubation within one hour, or as soon as possible, but not more than 24 hours. Store the samples in a room temperature environment away from sunlight.
3. You may find it useful to label each vial/jar with a Sharpie. Carefully record the date, time, and place of sample collection and name of the person sampling to associate with the mark on the vial. Because that is too much to write on a vial or a Petrifilm, you will want to to associate each sample with a short, abbreviated codename like “A1” or “TUR-3”.
1. When you are ready for inoculation, place the 3M petrifilm plate on a level surface. Use a permanent marker to label the corner of the petrifilm with the codename of the sample that it will be used for.
2. Shake the sample gently to make sure the bacteria are not at the top or bottom of the sample. With a sterile pipette, draw 1mL of samples from the chosen sample vial. (See Figure 2.
3. Lift the clear top film and with the pipette perpendicular to the inoculation area slowly dispense 1mL of samples onto the center of the bottom film. (see Figure 3.)

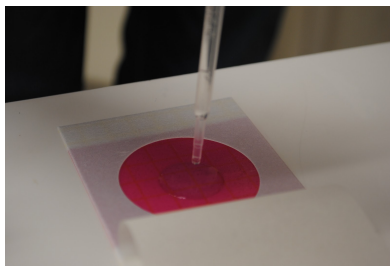


Figure 3: Lift cover and dispense 1 ml

4. Carefully roll the top film down onto the sample to prevent trapping air bubbles. (see Figure 4.)

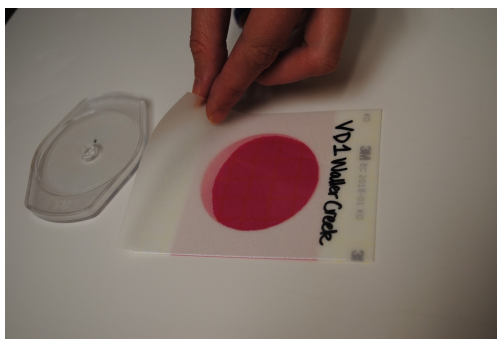


Figure 4: Roll down the cover

5. Place the 3M Petrifilm spreader with the flat side down on the center of the 3M Petrifilm plate. Press gently on the center of the 3M Petrifilm spreader to distribute the sample evenly. Do not slide the 3M Petrifilm Spreader across the film.
6. Remove the 3M Petrifilm spreader and leave the 3M Petrifilm plate undisturbed for at least one minute to permit the gel to form.
7. Your samples are ready to be placed in the Armadillo for incubation. Be sure to handle the Petrifilms carefully when transferring to the Armadillo.

8. You are allowed to stack as many 20 petrifilms together, but not more than that. The Armadillo allows you to incubate 40 petrifilms with a small cardboard stand for a second stack of 20.

For field work, the inexpensive, individually wrapped, sterile plastic 1ml transfer pipette and 5ml water vials are essential aids. They can be easily ordered by mail.

Samples should be inoculated in less than 24 hours after collection. Inoculation is as simple as dispensing 1 ml of the sample onto the petrifilm plate and ensuring that the sample is sealed cleanly on the film.

Incubation instructions

To incubate, place a fully charged battery in the incubator, plug it in and turn it on.

Place the petrifilms in stacks of up to 20 on each of up to two cardboard shelves created from the provided templates. Screw the inner chamber lid down onto the gasket with the four provided screws and the small screwdriver.

Keep the Armadillo more or less upright. After an hour, check the analog thermometer that the temperature has risen to approximately 37°C. This check is only to confirm that there is no loose connection or some other problem.

Remember to open the Armadillo in approximately 48 hours and proceed to post-incubation sample handling.

Post-Incubation Sample Handling

Incubated petrifilms are not completely stable, so after incubation, the films should be immediately photographed. Photographing them can be done conveniently with a cell phone with a camera. Care should be taken to have diffuse lighting so that glare does not obscure the labeling or growth medium of the petrifilm. Petrifilms change their appearance slowly after incubation, so it is important to take photos immediately.

Once you safely have photos, we recommend you dispose of the Petrifilms as they are a slight biohazard.

The E. Coli colonies should be counted from the photographs and the counts carefully recorded, for example in a spreadsheet. Figure 5 shows the same petrifilm immediately after incubation and the 5 days later, demonstrating that the gas bubbles critical to the E. Coli colony identification degrade or migrate over time.

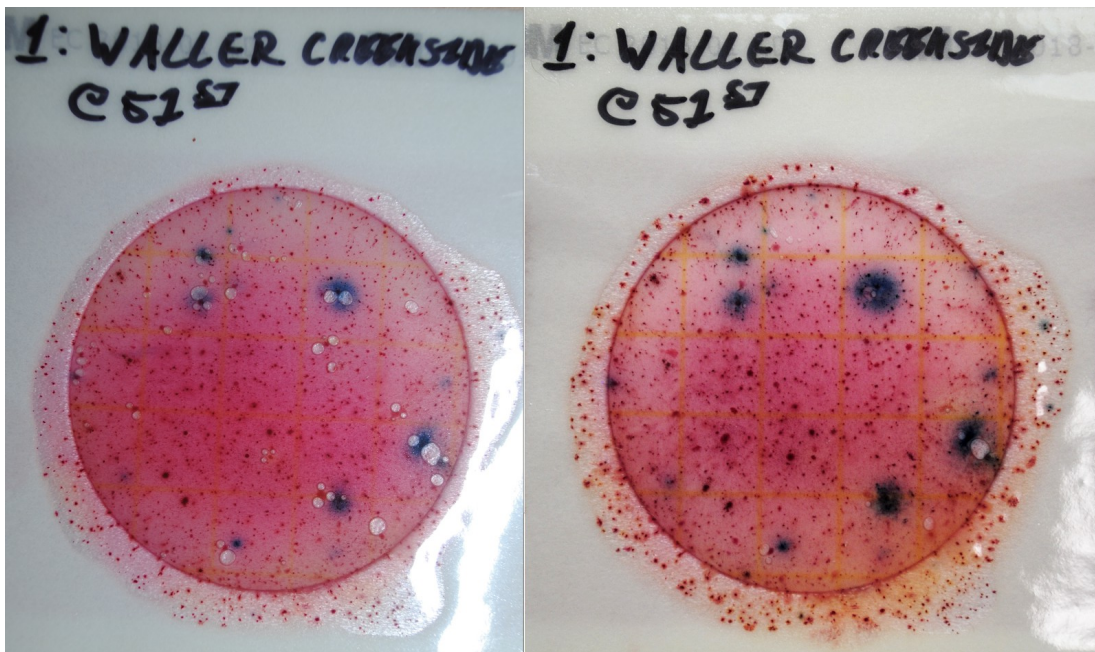


Figure 5: the same plate after 5 days of elapsed time

After incubation, ensure that the petrifilms stay outside of the inner chamber. Moisture from the petrifilms will condense on the inner surface of the chamber which might cause problems with future experiments.

Storage During Incubation instructions

The Armadillo should be stored upright and level in a location out of direct sunlight at a temperature less than body temperature. In general any place where a human being is comfortable will be warm enough to ensure a successful 48-hour incubation on a single battery charge. In temperatures near freezing the battery may not last 48-hours.

Field Use and Interpretation

One of the major advantages of portable Petrifilm incubation is that the incubated films provide visually striking and easy-to-understand results that can help the target community understand the clearly understand the problem. Numerical data returned from a distant laboratory may not have the same impact.

The Petrifilm approach allows comparison of different water sources. For example, one can clearly compare water run through a sand-filter, or UV-filter with unfiltered water. As is often pointed out, the mere presence of E. Coli does not directly indicate pathogenic organisms in water, but rather is a reliable indication of a sources of fecal contamination.

Limitations

Petrifilms are probably not useful when the water is either too clean or dirty. That is, some samples will produce “too many to count” colonies, or will generate no E. Coli colonies at all, as does sterile water and even tap water in the U.S.A. The WHO has recommended testing 100 ml of water, which is difficult to do with petrifilms, which test 1 ml at a time.

The presence of E. Coli does not directly indicate pathogenic organisms or pathogenic E. Coli, but

rather indicates fecal contamination, from human or non-human sources. Although this is an indirect measure of the the pathogenicity of the water, it is useful for comparing water sources and water treatments.