The Design and Usage of a Portable Incubator for Inexpensive In-Field Water Analysis

Summary Paper

Brian T. Heligman  
Department of Material Science, University of Texas, USA  
briantheligman@gmail.com

Alice Zhao, PhD  
alicezhao@gmail.com

Evan Bartilson  
PLH Advanced Engineering  
ebartilson@gmail.com

Abhay Gupta  
Department of Material Science, University of Texas, USA  
abhay.gupta@utexas.edu

Joshua Hannan  
Modular, Inc.  
hannanjoshua19@gmail.com

Maimouna Diop  
Mechanical Engineering Graduate, University of Texas  
maidio18@gmail.com

Robert L. Read, PhD  
Public Invention, Austin, TX, USA  
read.robert@gmail.com

Keywords: Bacterial Analysis, Off-grid functionality, Petrifilm, Water Quality, Portable Instrumentation, E. coli.

1 TARGET AUDIENCE

The audience for this paper is humanitarian workers or others who may benefit from a two-day turnaround of bacterial water quality analysis achieved using a portable, rugged, and battery-powered method ideal for field use in remote developing communities (see Figure 1.)
2 BACKGROUND

3M Petrifilm Count Plates (Petrifilms) can quantify bacterial contamination (see Figure 2.) They are easily understood by local community leaders despite potential language barriers due to their visual nature. They need to be incubated at close to body temperature (35°C). They are sometimes incubated using the body heat of field workers, which is another viable approach to incubation that addresses challenges frequently encountered in field settings without grid electricity. An inexpensive, application-specific battery-powered incubator would complement the existing incubation options and would significantly improve one’s ability to do water analysis testing in a wider variety of settings, especially low resource and off-grid settings.

Figure 1: The Armadillo incubator

Figure 2: Petrifilms from the same source incubated in the Laboratory Incubator (left) and the Armadillo (right) showing purple E. coli colony-forming units (CFUs)
3 PURPOSE
The portable Armadillo Petrifilm incubator (the Armadillo) (see Figure 1) described herein is a robust, reliable, and convenient incubation method which has been tested in the laboratory and field environments. Free, open-source construction plans for the Armadillo are published to encourage independent replication and co-development (http://www.instructables.com/id/Portable-Petrifilm-Incubator-for-Inexpensive-In-Fi/). The Armadillo is easily constructed from commercially available components. Total cost of one unit is less than US$200.

5 METHOD
The Armadillo was compared to an industrial laboratory incubator by enumerating bacterial counts in identical water samples. The temperature holding performance was measured at ambient temperatures of 25°C (65 hours) and 4°C (40 hours.)

6 RESULTS
The Armadillo can incubate for the full period on one battery charge in most ambient temperatures. Bacterial counts from water samples enumerated with the Armadillo were benchmarked against those with an industrial 110V AC laboratory incubator (estimated cost: US$1500), which produced comparable results.

7 IMPLICATIONS FOR TARGET AUDIENCES
The Armadillo provides a reliable, inexpensive method to measure bacterial water quality in poorly electrified locations with a turnaround time of 48 hours. The Armadillo is designed specifically for incubating Petrifilms, which provide intuitive, visually quantitative results of bacterial contamination that is understandable with minimal language dependence.
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Brian T. Heligman
Department of Material Science, University of Texas, USA
briantheligman@gmail.com

Alice Zhao, PhD
alicezhao@gmail.com

Evan Bartilson
PLH Advanced Engineering
ebartilson@gmail.com

Abhay Gupta
Department of Material Science, University of Texas, USA
abhay.gupta@utexas.edu

Joshua Hannan
Modular, Inc.
hannanjoshua19@gmail.com

Maimouna Diop
Mechanical Engineering Graduate, University of Texas
maidiop18@gmail.com

Robert L. Read, PhD
Public Invention, Austin, TX, USA
read.robert@gmail.com

Abstract: Humanitarian engineers need an inexpensive, fast, visually compelling way to assess bacterial water quality in remote locations. One way to do this is with 3M Petrifilm E. coli/Coliform (EC) Count Plates to detect E. coli in water samples. These require incubation at close to body temperature. To meet this need, we provide a free, open-source design of a battery-powered incubator capable of maintaining 35°C ± 1°C for up to 65 hours in ambient temperature of 25°C. Our incubator, called the Armadillo, can be replicated by an ordinarily skilled person in five hours for under US$200 in materials cost. We summarize our reference documentation on construction, sample handling, inoculation, and incubation using Petrifilms and the Armadillo. Colony-forming unit (CFU) counts generated by the Armadillo are compared side-by-side with a laboratory-grade incubator. Incubation performance at ambient temperatures of 25°C and 4°C shows that a single battery charge reliably powers a full incubation period of 48 hours under normal ambient temperatures.
Keywords: Bacterial Analysis, Off-grid functionality, Petrifilm, Water Quality, Portable Instrumentation, E. coli.

1. INTRODUCTION

Animal and human fecal contamination are a primary source of pathogens in water, and pose a serious health risk for millions of people around the world. One of the great challenges in promoting and implementing safe water programs is the difficulty in measuring and communicating the microbiological properties of water while on-site in rural developing communities. Many remote communities lack the equipment and know-how to measure bacterial quality of water. Although many microbial organisms are pathogenic, testing for all of them is cumbersome. E. coli is often treated as a key indicator organism. While the presence of E. coli is not directly indicative of pathogenicity, it is a reliable and possibly superior indicator of recent fecal contamination, and directly indicates bacterial contamination (Allen et al., 2015, Edberg et al., 2000, Bain et al., 2012, Vail et al., 2003). A simple, low-resource technique for quantifying E. coli utilizes 3M Petrifilm E. coli/Coliform (EC) Count Plates (Product #6414) (from hereon simply referred to as Petrifilms) (Wholsen et al., 2006.) Petrifilms are manufactured for quantification of bacteria in foodstuff and dairy products. 3M does not officially endorse the use of Petrifilms for water quality analysis. Because the World Health Organization (WHO) standards call for no observable E. coli in 100 mL of water and Petrifilms test only 1mL of water per film, they may be impractical for testing relatively clean water or final potability (World Health Organization, 1996). However, when used to assess current water quality and to plan remediation, they provide visually compelling, language independent bacterial quantification after only 48 hours of incubation. A major barrier to their proper utilization is the lack of incubation methods in resource scarce environments.

Although several researchers have demonstrated that it may be possible to complete the Petrifilm tests at ambient temperature for a longer period of time (Brown et al., 2011, Thaemert and Andrews, 2014), that technique remains non-standard. Commonly occurring environmental conditions, including low temperatures and rapid temperature variability has the potential to reduce reliability of Petrifilm tests without incubation.

This paper describes a low-cost approach to quantifying E. coli in water samples on-site within 48 hours. The technique utilizes Petrifilms in combination with a custom designed portable incubator, the Armadillo v1.0, henceforth referred to as the Armadillo. Petrifilms generally cost between about US$1 and US$3 per film, and 3M generously donates limited samples to educational institutions. The Armadillo can incubate up to 40 Petrifilms simultaneously. We have published free, open-source instructions for the construction of the Armadillo, which can be accomplished with less than US$200 in cost of materials (EWB-USA-Austin, 2017.) This battery-powered approach enables in-field, visually compelling, and quantitative microbiological water sample testing at low cost. Section 7 presents a side-by-side comparison between the Armadillo and a laboratory incubator.

2. BACKGROUND

Field experience in Latin America by volunteers based in Austin, Texas, USA associated with
Engineers Without Borders USA, Greater Austin Chapter (EWB-USA-Austin) resulted in the discovery of five requirements associated with the effective measurement and communication of water quality information to local community leaders in the developing world. First, the test must provide intuitive visual results, transforming bacteria which is invisible to the naked eye into a visibly compelling readout. Second, the readout must be understandable with minimal language dependence to allow broad communication across language barriers. Third, the readout must be quantitative enough to support meaningful comparisons, allowing for illustration of the microbial-diminishing effects of adopting clean water practices. Fourth, the complete methodology must be convenient and inexpensive to enable practical usage in developing countries. Lastly, the strategy must be feasible in-field, in poorly electrified communities and rough terrains. With these strategic considerations, we selected the 3M Petrifilm E. coli/Coliform Count Plates as the microbiological testing platform and developed a portable battery powered incubator.

2.1. E. coli Testing Kit

Petrifilms are simple, portable, and inexpensive water quality tests that quantitatively indicate colony-forming units (CFUs) of both E. coli and other coliform bacteria in 1 mL water samples. Although Petrifilms are commercially available for the detection of various types of bacteria with various speeds and sensitivities, following Edberg et al. (Edberg et al., 2000) we recommend the E. coli/Coliform version and E. coli in particular as the most indicative of fecal contamination and infection risk. While not specifically designed for water-quality analysis, the Petrifilm has many qualities that make it attractive. Bacterial colonies growing on the Petrifilm activate a dye in the medium, making CFUs directly visible to the naked eye. However, Petrifilms require incubation. Due to difficulty in transport in developing countries, laboratory analysis of water samples may not begin for hours or days after sample collection, exceeding recommend “holding times” for biological samples (EPA, 2016), creating a serious risk of underreporting due to decreased viability of bacteria in the samples. On-site incubation eliminates significant costs and delays associated with refrigeration, transportation, and laboratory analysis and processing. Petrifilms are easily inoculated in the field and report visually striking results within 48 hours of incubation at 35°C.

Laboratory incubators are not portable and relatively expensive, as are commercial portable incubators. Field engineers have resorted to using body heat to incubate Petrifilms on-site, simply taping baggies of Petrifilms against an operator’s skin for 48 hours. This method is physically irritating, quantity-limiting, unreliable, and extremely error-prone due to the need to count bubbles associated with E. coli CFUs (3M, 2018.) Although it is possible to construct a vest (Adegbite, 2015) to provide more comfortable body-heat incubation, this has not become widespread practice within the EWB community. A low cost, application-specific, battery-powered incubator would complement the Petrifilm testing platform to significantly improve the quality of results.

2.2. Incubator Design Goals

The Armadillo was designed with several important criteria to ensure its applicability and widespread adoption.

Ease of assembly and usage: We aimed to design an incubator whose construction could be
accomplished within hours using basic tools and fabrication skills. The incubator usage must also be as simple and intuitive as possible. No regular maintenance is required.

**Low-cost:** The Armadillo comprises less than US$200 in materials that are readily procurable online through several major retailers and distributors. To our knowledge, it is the most cost-effective portable incubator available that suits in-field incubation (Schwarz and Ward, 2015.)

**Portability:** The Armadillo is designed to be operable without dependence on an electrical grid and capable of completing at least one full round of incubation (48 hours) uninterrupted on a single battery charge. Its handle and size allows for hand-carry or transport inside a large backpack for maximum portability.

**Robustness:** In-field application demands robustness and the ability to withstand falls and bumps when handling and travelling amid extreme conditions. The Armadillo’s durable exterior shell and firm interior insulation provides maximum protection against impact from drops and accidents. Its inner chamber holds samples in place securely. Its leak-resistant and rugged design also provides protection against all weather conditions. Importantly, it also protects the Petrifilms from sunlight, a potential threat of sanitizing the sample or film.

**Reliability:** The constant target temperature (35 ± 1°C) is reliably sustained for at least 48 hours without the need for monitoring or operator intervention.

**Versatility:** The durable construction and flat top of the Armadillo doubles as a seat, and adjustable tie downs on the incubator lid functions to carry additional tools and supplies. Additionally, the incubator battery may deliver power to any USB-chargeable device, including many personal cell phones and cameras.

**Open-source:** In order to foster learning and improvement, the Armadillo’s design and documentation are published under free-libre open-source licenses. We encourage widespread independent replication and unrestricted alterations with no restrictions on- or of- product use. We also welcome the development of a strong technical support community and encourage open channels for discussion, collaboration, and expansion of similar instrumentation needs.

4. **ARMADILLO V1.0 DESIGN AND ASSEMBLY**

Figure 1: The Armadillo incubator
Figure 2: Exploded View of Armadillo Components

The Armadillo housing is constructed by modifying a 7-quart Stanley cooler (EWB-Austin-Github1, 2017.) The Petrifilms are suspended in an inner chamber constructed from a standard electrical junction box. These components are sturdy. Air inside the inner chamber is heated using resistive heating pads and maintained at 35°C using a thermostat. Space between the inner chamber and the cooler is filled with high R-value foam to minimize heat loss. Early prototypes showed that the junction box and insulation was needed to stay warm for 48 hours. The resulting design is very rugged. Detailed, free, step-by-step instructions, schematics, and construction templates are available at our Instructable (EWB-USA-Austin, 2017).

Constructing the Armadillo has been performed twice by teams of college students with no experience in assembling electronics in less than five hours. Materials are readily available online from common retailers such as Amazon for under US$200. (Below we compare to the Sheldon Manufacturing laboratory incubator requiring 110V AC, approximate cost US$1500.) Assembly involves (1) wiring a simple circuit with the thermostat and resistive heating pads, (2) cutting foam and cardboard to match our printable templates, (3) drilling holes in the cooler
and inner chamber for threading the thermometer, and (4) packing all the parts, including the battery, inside the cooler.

5. USAGE SUMMARY

Detailed instructions for inoculation and sample-handling are provided in the User Guide at our permanent GitHub repository (EWB-Austin-Github2, 2017).

5.1. Pre-operating instructions

The specified battery pack fully charges in 24 hours and has a charge-level indication light. To use the Armadillo off-grid, simply charge the battery pack ahead of time. If multiple 48-hours incubations are required in an area where grid power is not reliable, consider purchasing spare batteries, as the battery is easily swappable in the field.

5.2. Sample-handling

It is critically important to collect and handle samples correctly before incubation (EPA, 2016.) If it is not possible to directly inoculate samples from a source onto the Petrifilms, water samples should be collected in sterile vials and transported out of direct sunlight and stored for as little time as possible, but no more than 8 hours. Each Petrifilm requires 1 milliliter of water. Samples should not be exposed to direct sunlight or excessive heat as this may cause sterilization and produce false negative results. Identifying information for each sample may be recorded on vials during sample collection and on the top of Petrifilms during inoculation.

Figure 3: 1 mL of water is collected from a vial using a disposable pipette.
5.3. Sample Inoculation

Samples should be inoculated as soon as possible but not more than 48 hours after collection (EPA, 2016.) Inoculation is as simple as using a disposable, sterile pipette to transfer 1 mL of the water sample onto the Petrifilm and gently lowering the clear cover.

5.4. Incubation

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

Place the Petrifilms in stacks of up to 20 onto cardboard shelves created from the provided templates and secure the inner chamber with screws.

After an hour, check the analog thermometer and ensure that the temperature has risen to approximately 35°C.

5.5. Storage during incubation

The Armadillo should be stored upright and level in a location out of direct sunlight at a temperature less than body temperature. In temperatures near freezing, the battery may not last 48 hours.

5.6. Post-Incubation Sample Handling

Incubated Petrifilms are not fixed and stable, but rather will continue to undergo changes post-incubation. Figure 6 show the same Petrifilm immediately after full incubation and left for 5 days at room temperature, and demonstrates that gas bubbles critical to E. coli CFU
identification can disappear or migrate over time. Therefore, Petrifilms should be photographed upon completion of incubation. Care should be taken to minimize glare from the Petrifilm’s glossy top cover so critical information on the Petrifilm is not obscured in the image.

Figure 6: the same plate before and after 5 days of elapsed time

Once a photographic record is established, it is recommended to follow current local and industry standards for potential biohazard disposal of the Petrifilm.

5.7. Field Use and Interpretation

*E. coli* CFUs should be counted from the photographs following the 3M Petrifilm Interpretation Guide (3M, 2018.) One of the major advantages of portable Petrifilm incubation is the visually striking and easy-to-understand results that can help the target community clearly understand the problem. For example, one can compare Petrifilms for water run through a sand filter or UV filter in contrast to untreated water both visually and quantitatively based on *E. coli* CFU counts. Text-based reports returned from a distant laboratory may not have the same visual and emotional impact.

5.8. Limitations

Petrifilms have limitations for water quality analysis. Heavily contaminated water samples will produce “too many to count” CFUs, and clean water may generate no *E. coli* colonies. In cases with too many CFUs to quantitatively count, the test may be considered qualitative, or the user may attempt to dilute the sample until *E. coli* concentration is in a measurable range. In cleaner water samples with fewer than 100 CFU/100 mL many Petrifilms must be used to get a reliable count, imposing a practical limitation on this approach. Alternative testing techniques that test higher volumes should be used with cleaner water or to verify successful remediation and/or potability.

6. ARMADILLO V1.0 TECHNICAL EVALUATION

Table 1: Physical Specifications

<table>
<thead>
<tr>
<th>Physical Characteristic</th>
<th>Specification</th>
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<tbody>
<tr>
<td>Temperature Display</td>
<td>Analogue to 1°C</td>
</tr>
<tr>
<td>Temperature Accuracy</td>
<td>±1°C</td>
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<tr>
<td>-----------------------</td>
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</tr>
<tr>
<td>Overall dimensions W x D x H (cm, in)</td>
<td>31 cm x 34 cm x 22 cm (additional 44 cm for doors when open)</td>
</tr>
<tr>
<td></td>
<td>13.3 in x 11.1 in x 8.6 in (additional 17.4 in for doors when open)</td>
</tr>
<tr>
<td>Internal chamber dimensions W x D x H (cm, in)</td>
<td>10 cm x 10 cm x 10 cm</td>
</tr>
<tr>
<td></td>
<td>4 in x 4 in x 4 in</td>
</tr>
<tr>
<td>Internal chamber volume</td>
<td>1 litre (1000 cm³)</td>
</tr>
<tr>
<td>Weight</td>
<td>2.26 kg (5 lbs)</td>
</tr>
<tr>
<td>Max. power output</td>
<td>12 Watts</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Performance Characteristic</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient outside air temperature for guaranteed performance</td>
<td>15°C to 35°C</td>
</tr>
<tr>
<td>Time to heat to 35°C, minutes*</td>
<td>1 hour</td>
</tr>
<tr>
<td>Recovery time after door opened for one minute at 35°C</td>
<td>20 minutes</td>
</tr>
<tr>
<td>Battery lifetime in a 25°C environment</td>
<td>65 hours</td>
</tr>
<tr>
<td>Battery lifetime in a 4°C environment</td>
<td>40 hours</td>
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* Measurements in an empty chamber at an ambient temperature of 20°C

As shown in Table 2, several trials indicated a single battery charge powers the Armadillo for 65 hours at an ambient outside air temperature of 25°C (“room temperature”) and for 40 hours at an ambient outside air temperature of 4°C (tests performed inside a refrigerator), ensuring complete 48 hour incubation on a single battery charge except under very cold ambient temperatures.
7. **COMPARISON TO LABORATORY INCUBATION**

To demonstrate the validity of this approach, the Armadillo was compared to a 110V AC laboratory incubator (a Sheldon Manufacturing SM14E, approximate cost: US$1500) On August 7th, 2018, 15 ml of water were collected from five sources:

1. Waller Creek (WC) -- A polluted stream running through the city, with a much lower flow rate than Barton Springs.
2. Shoal Creek (SC) – A small creek running throughout the city.
3. Turtle Pond (TP) -- A small pond home to small fish and turtles.
5. Sterile water (SW) – Sterile water was used as a negative control.

The samples were analysed as described in Section 5. A total of 10 Petrifilms were prepared from each sample, of which 5 were incubated with the Armadillo and the other 5 with the laboratory incubator. After 48 hours of incubation at 35°C, all samples were removed from the incubators and photographed. We attempted to use the OpenCFU (Geissmann, 2013) software to analyse the images. However, *E. coli* colonies are indicated by the presence of a gas bubble associated with the colony, and this complexity led us to count the colonies by hand. Each Petrifilm was manually scored for the number of CFUs by two independent observers (see Figure 7.) The average number *E. coli* CFUs per Petrifilm is reported in Figure 8. No *E. coli* CFUs were observed in either the BS, TP or SW samples in either incubator. The sterile water showed no bacterial colonies of any kind. The Turtle Pond water, as expected, created many bacterial colonies, but no definitive *E. coli* CFUs, as indicated may occur with high overall bacterial counts (3M, 2018, see Figures 8 and 9.) The Barton Springs sample, also shows coliform CFUs but zero *E. coli* CFUs. The performance of the Armadillo closely matches that of the laboratory incubator (see Figure 8.)
Figure 7: Samples from two different water sources incubated in the Laboratory Incubator (left) and the Armadillo (right), showing 5 *E. coli* CFUs in sample WC3C and 3 *E. coli* CFUs in sample WC2A.

![Chart showing comparison of E. coli CFUs between commercial incubator and Armadillo](chart.png)

Figure 8: Comparison of the Armadillo to a laboratory incubator. The error bars represent the standard deviation in observed CFUs per Petrifilm for each water source.

7. CONCLUSION AND FURTHER CONSIDERATIONS

The Armadillo offers an inexpensive, easily constructible, portable platform for the field use quantification of *E. coli* in the field. The EWB-USA Greater Austin Chapter actively supports those wishing to borrow one of our Armadillos or to build their own. Documentation on the Armadillo may be found at [https://github.com/EWB-Austin/petrifilm-incubator](https://github.com/EWB-Austin/petrifilm-incubator) (EWB-Austin-Github3, 2018.) The Armadillo has been successfully used on an expedition to Iraq.

We have demonstrated that incubation with the Armadillo produces CFU counts similar to that with a laboratory incubator (see Figure 8.) The battery life is documented at “room temperature” of 25°C and at a much colder temperature of 4°C (see Table 2.)

Although usable as currently designed, further improvements may be possible, and our open-source design may be freely improved upon and shared.

*Travel Note:* The Armadillo contains a removable, off-the-shelf lithium ion battery. National, international and airline rules may vary about transport of lithium ion batteries. At the time of this writing when flying from the U.S.A., the most judicious course of action is to simply
remove the battery and carry it on board with you rather than attempting to transport it in checked luggage.

8. ACKNOWLEDGEMENTS

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9. REFERENCES


