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

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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 USD $200.00 in materials cost. This paper summarises the reference documentation for 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 faecal 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 whilst 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 faecal 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 utilises 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. As the World Health Organization (WHO) standards call for no observable E. coli in 100 mL of water and Petrifilms test only 1 mL 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 utilisation 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 & 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 utilises Petrifilms in combination with a custom designed portable incubator, the Armadillo v1.0, henceforth referred to as the Armadillo. Petrifilms generally cost between about USD $1.00 and USD $3.00 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 USD $200.00 in cost of materials (EWB-USA-Austin 2017). This battery-powered approach enables in-field, visually compelling, and quantitative microbiological water sample testing at a 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 that are 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 faecal contamination and infection risk.

Whilst 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) and creating a serious risk of underreporting of bacteriological count 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 polyethylene zip-lock food storage 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 a 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 USD $200.00 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 & 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 it to be carried by hand or to be transported 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 sanitising 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 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.

3 ARMADILLO V1.0 DESIGN AND ASSEMBLY

The Armadillo housing is constructed by modifying a 7-quart (6.62 L capacity) Stanley cooler (EWB-Austin-Github 2017a.) 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 minimise 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 university 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 USD $200.00, (in comparison to a 110 V AC Sheldon Manufacturing laboratory incubator with an approximate cost of USD $1,500.00).

Assembly involves: 1) wiring a simple circuit with the thermostat and resistive heating pads, 2) cutting foam and cardboard to match the 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.

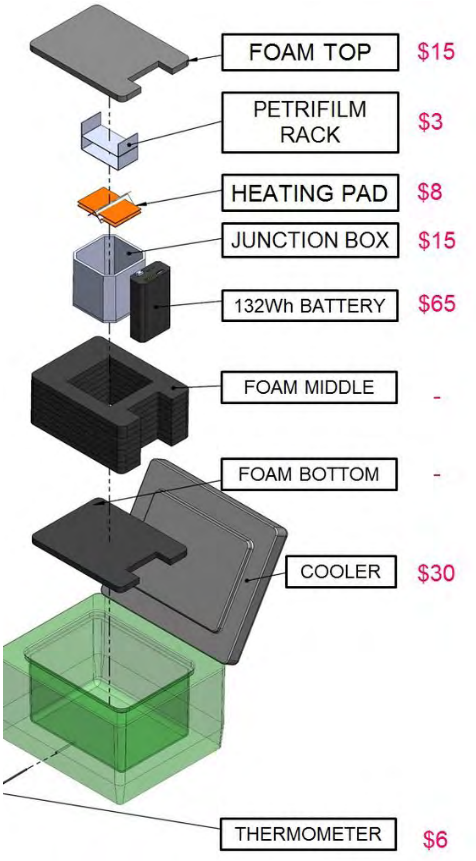
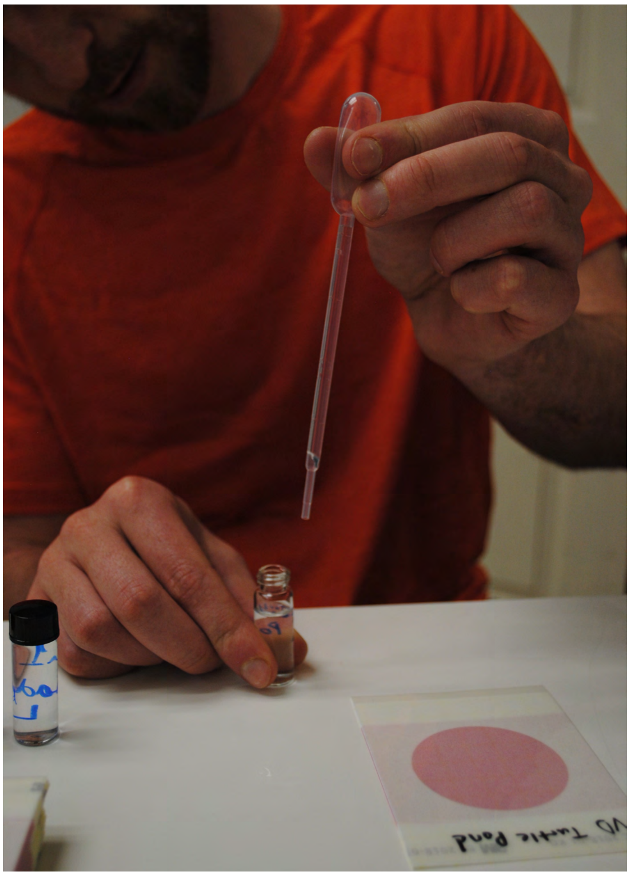


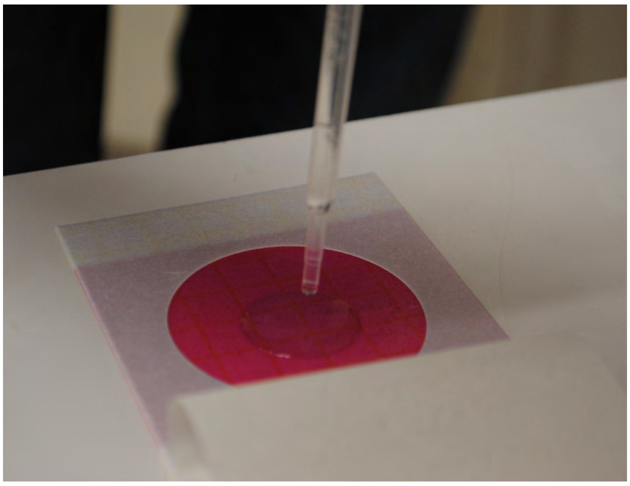
Figure 1 (*top left*): *The Armadillo incubator*

Figure 2 (*bottom left*): *Exploded View of Armadillo Components*

Figure 3 (*top* (*right*): 1 *mL of water is collected from a vial using a disposable pipette*

Figure 4 (*bottom right*): 1 *mL of water sample is dispensed onto the Petrifilm*



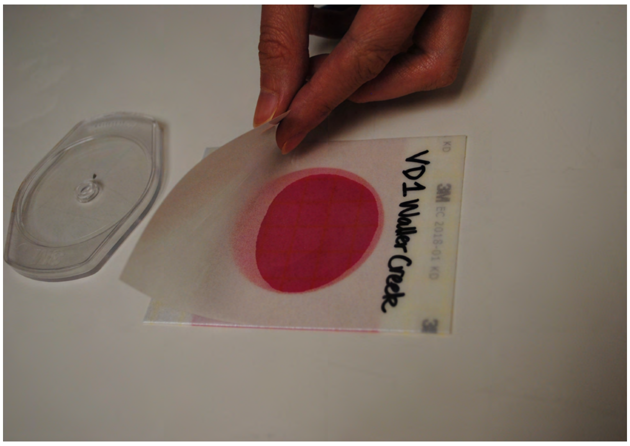


4 USAGE SUMMARY

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

4.1 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 hour incubations are required in an area where grid power is not reliable, consider purchasing spare batteries, as the battery is easily replaceable in the field.



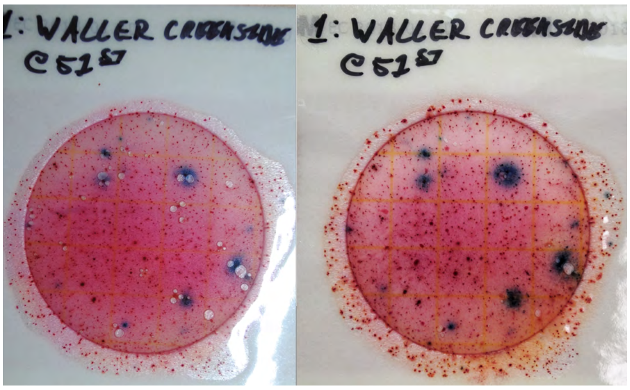


Figure 5 (top): Gently roll the top covering down onto the sample to avoid entrapping air bubbles

Figure 6 (bottom): Identical plate immediately post incubation (left) and after five days of exposure to room temperature post incubation (right)

4.2 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 mL of water.

Samples should not be exposed to direct sunlight or excessive heat as this may cause sterilisation 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.

4.3 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.

4.4 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.

4.5 Post-Incubation Sample Handling

Incubated Petrifilms are not fixed and stable, but rather will continue to undergo changes post-incubation. Figure 6 shows the same Petrifilm immediately after full incubation and the result of leaving the plate for 5 days at room temperature. This 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 minimise glare from the Petrifilm's glossy top cover so critical information on the Petrifilm is not obscured in the image.

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

4.6 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.

4.7 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.

5 ARMADILLO V1.0 TECHNICAL EVALUATION

As shown in Table 2, several trials indicated a single battery charge powers the Armadillo for 65 hours at

Table 1: *Physical specifications*

Physical characteristic	Specification
Temperature display	Analogue to 1°C
Temperature accuracy	±1°C
Overall dimensions W x D x H (cm, in)	31 cm x 34 cm x 22 cm (additional 44 cm for doors when open) 13.3 in x 11.1 in x 8.6 in (additional 17.4 in for doors when open)
Internal chamber dimensions W x D x H (cm, in)	10 cm x 10 cm x 10 cm 4 in x 4 in x 4 in
Internal chamber volume	1 litre (1000 cm^3)
Weight	2.26 kg (5 lbs.)
Max. power output	12 Watts

Table 2: *Performance specifications*

Physical characteristic	Specification
Ambient outside air temperature for guaranteed performance	15°C to 35°C
Time to heat to 35°C, minutes*	1 hour
Recovery time after door opened for one minute at 35°C	20 minutes
Battery lifetime in a 25°C environment	65 hours
Battery lifetime in a 4°C environment	40 hours

Notes:
* *Measurements in an empty chamber at an ambient temperature of* 20°C

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.

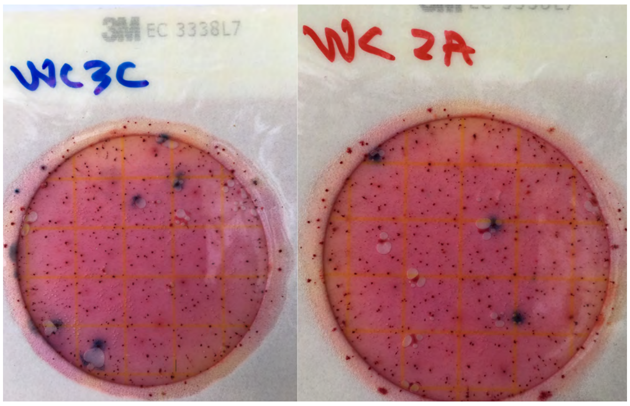
6 COMPARISON TO LABORATORY INCUBATION

To demonstrate the validity of this approach, the Armadillo was compared to a 110 V AC laboratory incubator (a Sheldon Manufacturing SM14E, approximate cost: USD $1,500.00) 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.
4. Barton Springs (BS) -- A spring-fed, untreated, natural swimming pool.
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 (Figure 7). The



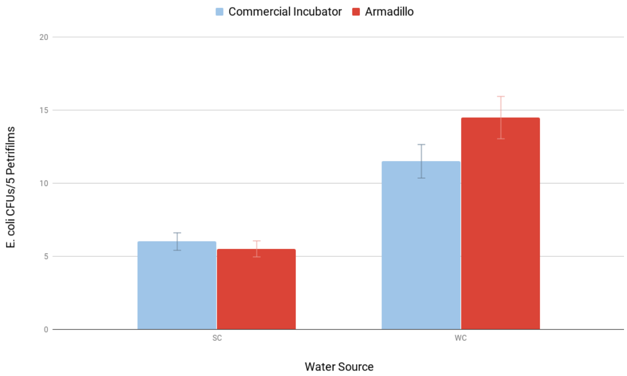


Figure 7 (top): Samples from two different water sources incubated in the Laboratory Incubator (left) and the Armadillo (right), showing 5 E. coli CFUs in sample WC3C and 3 E. coli CFUs in sample WC2

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

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 (Figures 8 and 9 in 3M 2018). 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).

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 (EWB-Austin-Github 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 (Figure 8) The battery life is documented at "room temperature" of 25°C and at a much colder temperature of 4°C (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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