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A Robust Incubator to Improve Access to Microbiological Culture in Low Resource Environments

To help address the limitations of operating conventional microbiological culture incubators in low resource environments, a new incubator design was developed and tested to meet the requirements of operation in laboratories without reliable power (power outages up to 12 contiguous hours) or climate control (ambient indoor temperatures from $5\,°C$ to $45\,°C$). The device is designed to enable adherence to incubation temperatures recommended for growth detection, identification, and drug susceptibility testing (DST) of human pathogenic bacteria. During power outages, stable temperatures are maintained in the device's internal sample compartment by employing phase change material (PCM) as a bi-directional thermal battery to maintain incubation temperature. Five prototypes were tested in a laboratory setting using environmental test chambers and programmable power supplies, and three were field tested in the Lao PDR in situations of intended use. The prototypes successfully held their temperature to within $\pm 1\,°C$ in both laboratory environmental chamber testing as well as during the field test. The results indicate that the device will maintain stable culture temperatures across periods of intermittent power supply, while enabling normal workflow of this could greatly increase the availability of microbiological culture for diagnosis and antimicrobial resistance (AMR) monitoring. [DOI: 10.1115/1.4042206]

Introduction

Microbiological culture remains important for diagnosis and surveillance of certain diseases through controlled growth of pathogens, and for determining antimicrobial drug resistance to inform patient treatment and/or integrated disease surveillance and response. Despite the increasing availability of other detection methods such as immunoassays and nucleic acid amplification technologies, the need for accurate phenotypic drug susceptibility testing (DST) will continue, and microbiological culture is likely to remain the mainstay and gold standard of laboratory diagnosis and DST for the foreseeable future. It remains essential for monitoring and managing the global rise in antimicrobial resistance (AMR).

Culture-based diagnostics and screening tools are very poorly accessible at lower levels of the tiered laboratory diagnostic network of most low-resourced health systems. This results partly from the high cost of incubators, the necessity of reliable mains power to maintain culture temperatures, supply of commodities, and availability of technicians trained in microbiological techniques. The availability of incubators that maintain constant temperatures on poor electrical grids and have low maintenance requirements would partially address this problem and allow health systems to begin developing more comprehensive microbiological diagnostic and surveillance programs.

Temperature control is critical for microbiological culture. Without continual power for conventional incubators, cultures held at lower than optimal temperatures may show false-negative results due to slow growth. Incorrect incubation temperatures will also provide misleading DST results. Additionally, if laboratory climate control is not provided and the ambient temperature rises, cultures may die off, again resulting in false-negative results. The use of water jac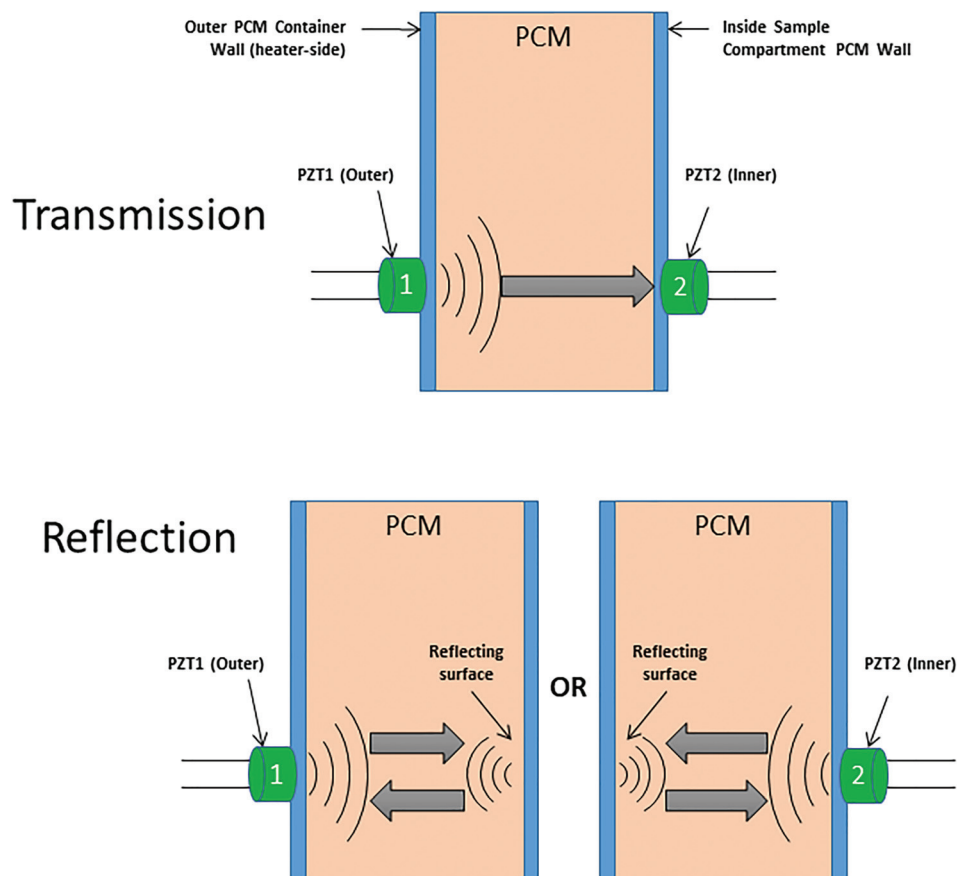kets to provide thermal mass in some incubators delivers limited holding capability, but as the jacketed water undergoes sensible heating (rather than latent heating), it changes temperature rapidly as energy is added to or removed from the system.

Global Good/Intellectual Ventures Laboratory designed and built five prototype incubators to meet the challenges of performing microbiological culture and DST in low resource settings. The target product profile included a hold time minimum of 8 contiguous hours (with an optimal goal of 12 contiguous hours) in an ambient temperature range of 5–45 °C, minimal maintenance requirements, and a minimal bill of materials cost. The devices were then evaluated in a setting of intended use with the Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU), Microbiology Laboratory in Vientiane, Laos.
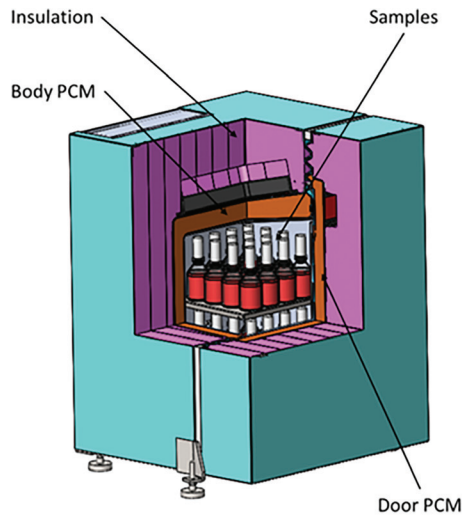
Incubator Concept

A conventional engineering solution to maintain temperature during power outages would rely on electrical batteries and a charging system to maintain input power. This approach has several drawbacks: batteries are expensive, heavy, and difficult to transport and degrade over time, necessitating maintenance or replacement. As conventional incubators do not have internal cooling systems, they also require a temperature-controlled room to maintain correct incubation temperatures in hot climates.

A more optimal approach is to use the latent heating of a material that has been tailored to undergo a phase change at the incubation temperature(s) of interest. All materials undergo phase change, but often at temperatures that are not in suitable temperature ranges. For example, carbon dioxide undergoes a phase change from solid to gas at -78 °C and water to ice at 0 °C. Some materials can undergo supercooling or superheating, where the

Fig. 1 Ultrasonic TOF sensing through PCM. For the transmission case, reciprocal behavior is observed if the transmitting element is changed to be the inner lead–zirconate–titanate transducer.

Fig. 2   Cutaway view of the incubator structure and an as-built prototype

temperature falls or rises prior to the onset of latent heating if a nucleation source is not present.

Certain hydrocarbon chain-based materials are amenable to tailoring of their bulk melting point. By distilling hydrocarbons, a purified blend that undergoes phase change at a desired temperature range may be obtained [1]. If the distillation were to proceed indefinitely, a uniform chain length could be obtained and thereby provide phase change within a very tight temperature range. However, economical production results in a range of latent heat enthalpies over a related range of temperatures [2,3]. As the incubator needs to buffer against both hot and cold temperature excursions, this property can be used to one's advantage. Hydrocarbons are also generally safe, unlike many other materials with phase transitions at the temperature(s) of interest (e.g., hydrated salts, modified alcohols, etc.). The nature of hydrocarbon chains also results in self-nucleation, preventing supercooling and superheating effects. For these reasons, a paraffin hydrocarbon blend was selected as the phase change material (PCM) for the incubator prototypes.
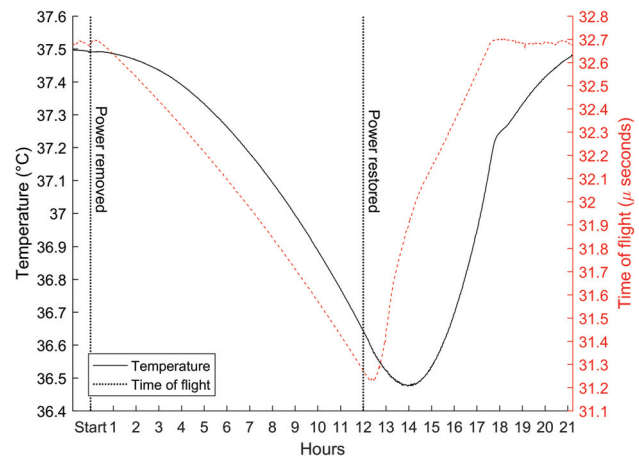
Thermal Management

To maintain the incubator's sample compartment at an optimal temperature for microbiological culture, a means of thermal management is necessary. We initially evaluated a control methodology based on direct measurement of the sample compartment temperature due to its advantages of simplicity and widespread use. As the temperature of the sample compartment drops, heaters would be engaged to return to the incubation set point. However, with the very large thermal mass of the PCM, this approach results in a very slow response to temperature change, providing poor temperature control. If power is lost and the exterior PCM begins to solidify, the temperature sensor will not observe a change for many hours. This delays turning the heaters on even if power is available, increasing the time required to recharge the PCM. External temperature sensors will provide even less control.
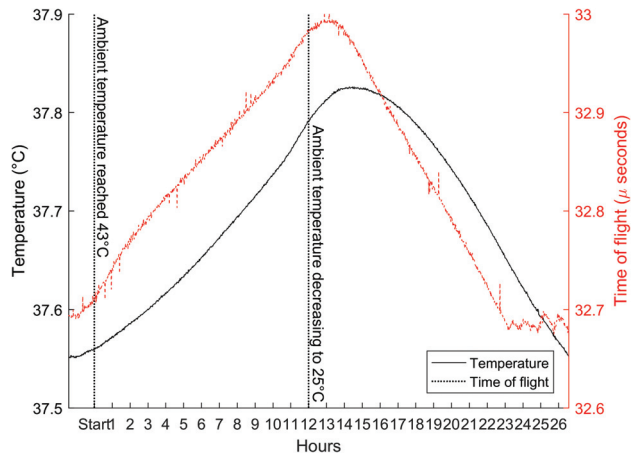
The issues presented by using temperature measurement for control resulted in an investigation of other physical properties that could provide more rapid and responsive inputs [4]. This approach was investigated in a benchtop experiment with simple ultrasonic transducers in a test cell filled with PCM. We observed that temperature and time of flight (TOF, the time required for an ultrasonic signal to travel through media to a receiver) correlated with one another, providing confidence that this approach would be successful [5,6]. This approach also provided an integrated measurement across the entire thickness of the PCM rather than discrete measurements obtained using the temperature probe

approach. As we applied heat to the test cell, the TOF responded instantaneously, while the temperature probes responded more slowly (Figs. 1 and 2).

The TOF measurement may be implemented in two basic methods, transmission, or reflection. In transmission, one lead–zirconate–titanate transducer acts as the sender while another on the far face of the PCM compartment acts as the receiver. This has the advantage of higher signal strength due to the shorter travel distance and associated dispersion and attenuation, but at the cost of an additional transducer and associated wiring and pulse timing chip. In reflection mode, the ultrasonic pulse is reflected off a hard surface (either on the PCM compartment wall if made of a flat, reflective material or else on a reflector attached to the wall) and the signal is collected after traveling double the distance of the transmission mode setup. We investigated both modes during development of the incubator,

Fig. 3   Sample compartment temperature (left axis) and TOF of an ultrasonic pulse through the PCM (right axis) during a cold hold test. During this test, power is removed (at start) while the incubator sits in 10 °C ambient conditions. Power is restored after 12 h. During the power-off condition, the internal sample compartment temperature drops 0.9 °C. Parameters of greatest interest: (*a*) the sample compartment temperature and (*b*) the TOF. "Power removed" indicates the point at which power was removed from the incubator, and "Power restored" indicates a point (i.e., 12 contiguous hours after power removal) when power was restored. Note that the minimum temperature occurs after the reapplication of power. The right axis shows the TOF measurement in microseconds.

Fig. 4 Hot hold test. For 12 h after the ambient temperature reached 45 °C the sample compartment temperature rose by only 0.3 °C, after which the ambient temperature dropped to 25 °C. At 23 h after the start of the test, heaters began regulating temperature again to maintain TOF and temperature against a lower ambient temperature.

and transmission mode was chosen for the field test while development of reflection mode continued in the laboratory (and was later successfully implemented, allowing for a cost reduction).

We constructed the incubator prototypes as similarly as possible to an envisioned future low-cost commercial product. ABS plastic (eplastics, San Diego, CA), was the primary construction material, being low cost, readily formable, and is compatible with the PCM used. An aluminum frame provided the structural load paths for the ABS components. Expanded polystyrene (Midland, MI) foam insulation minimized the heat leak into the incubator. Thermistors were incorporated for on-board temperature measurement.

Alternative current electrical heaters were installed on each of the five sides and the door, surrounding the entire PCM (Rubitherm GmbH, Berlin, Germany) and sample compartment assembly. The control circuit applied constant average power to the heaters using AC phase control switching to maintain power level regardless of incoming voltage level.

The incubators were equipped with on-board circuitry for incoming AC voltage metrology and protection from constant over-voltages. Two types of over-voltage scenarios were considered: constant over-voltages (lasting >1 s) and fast transient surge-type over-voltages (such as those caused by lightning, large inductive load switching, etc.). The former was prevented by an AC cut-off relay to completely isolate the equipment from constant over-voltage, while the latter was mitigated with sacrificial surge protection devices.

Sample compartment temperature was maintained by commanding heaters based on the measured TOF of the ultrasonic pulses. If the TOF is below the set point time, the PCM is more

solid and at a lower temperature than desired, and the heaters are engaged. As the PCM warms and liquefies, the TOF will increase, and when it exceeds the set point time the heaters are disengaged. The long-time constants of the system allow stability without excessive heater cycling (leading to eventual failure of the power switching hardware) or the need for deadband.
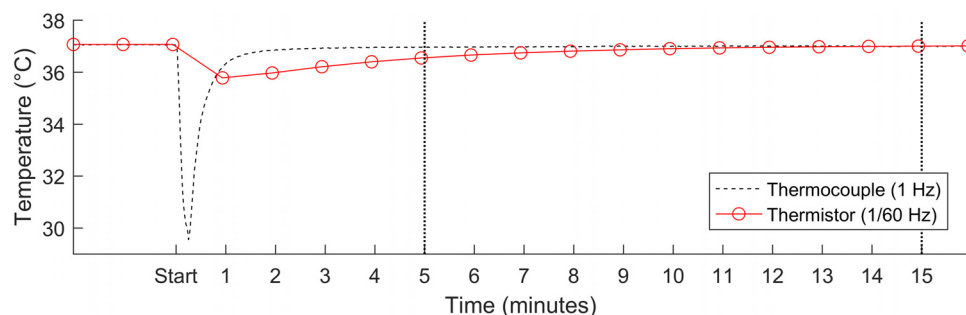
Laboratory Testing

The incubator was tested the prototypes in a laboratory setting, using adjustable AC power supplies to simulate power anomalies, and environmental test chambers to simulate a variety of ambient conditions. A laboratory data acquisition system using thin gauge thermocouples recorded higher resolution temperature data than the on-board sensors for validation of the on-board sensors. The laboratory test consisted of several phases, including power-off events during cold ambient temperatures, hot ambient temperatures, the placement of cold samples in the sample compartment, depletion testing, and a "week in the life" test that featured a wide variety of power conditions to simulate field conditions and engage the on-board power system software.
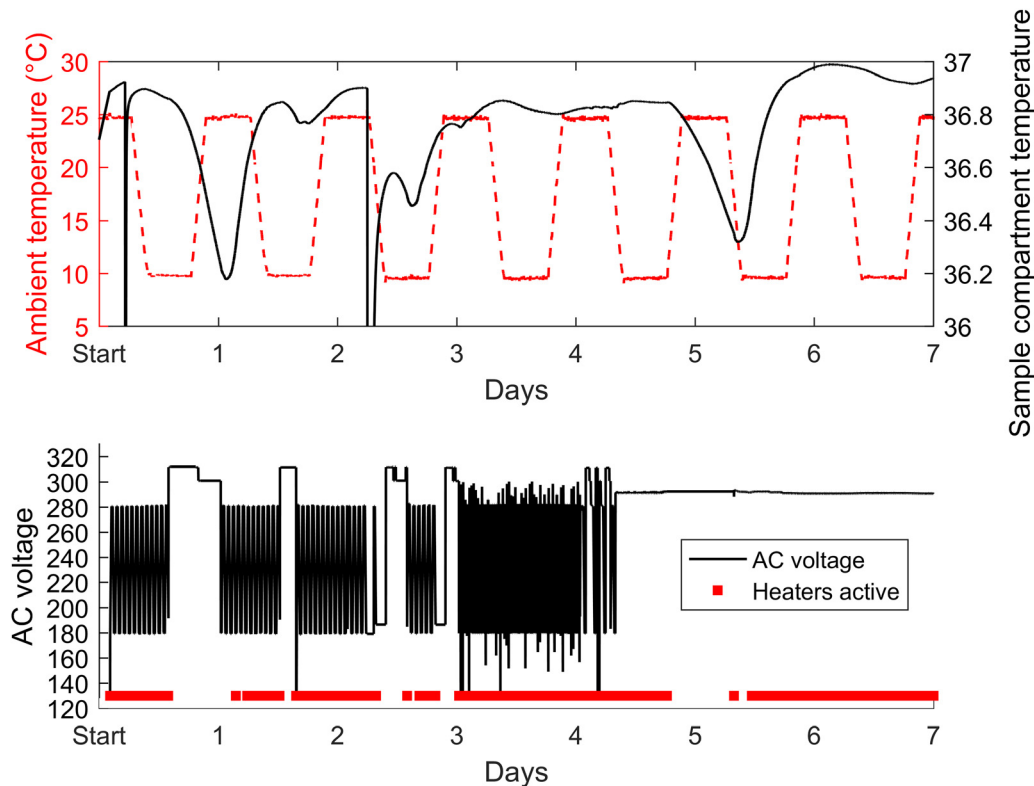
Cold Hold Test

Hot Hold Test

A cold hold test (Fig. 3) and a hot hold test (Fig. 4) verified that the PCM provided a thermal buffer against ambient temperature fluctuations. The lower ΔT of the hot test (ambient temperature of 45 °C—incubation temperature of 37 °C = 8 °C) allows for purely passive recovery after the ambient environment cools below 37 °C. The rate of recovery will be a function of the ambient temperature, and no recovery is possible at ambient temperatures greater than 37 °C. Fortunately, mean daily ambient temperatures in our targeted low-income countries are lower than 37 °C (even though peak temperatures may be higher during the day), making this a practical approach to recovery and avoiding the expense and complexity of powered cooling equipment. Also, of note in the cold hold test, the ultrasonic TOF measurement shows the effect of heaters being turned on several hours prior to the temperature sensor showing the resulting rise. The faster response time allow much more aggressive recharging than could be achieved with temperature sensors alone.

The sample compartment temperature as measured both by the on-board thermistor and the external thermocouple (Fig. 5). The thermistor samples once per minute while the thermocouple samples once per second. The thermocouple also is much less massive than the thermistor and thus better captures the drop-in temperature as the door is opened. The large amount of PCM at the incubation temperature allows the sample compartment air temperature to rapidly return to an optimal incubation temperature. The recovery behavior provides rapid return to ideal incubation temperatures after door openings, keeping the average temperature of many biological samples inside close to their optimal growth temperature.

Fig. 5 Door opening recovery test. After opening the inner and outer doors for 10 s the sample compartment temperature rapidly recovered once the door were closed.

Fig. 6 The top subgraph shows the environmental chamber temperature profile and internal sample compartment temperatures for one prototype, and the bottom subgraph shows the AC voltage provided to all prototypes in both environmental testing chambers and when heaters were active (when acceptable input power was available and the internal PCM state required heat to be added)
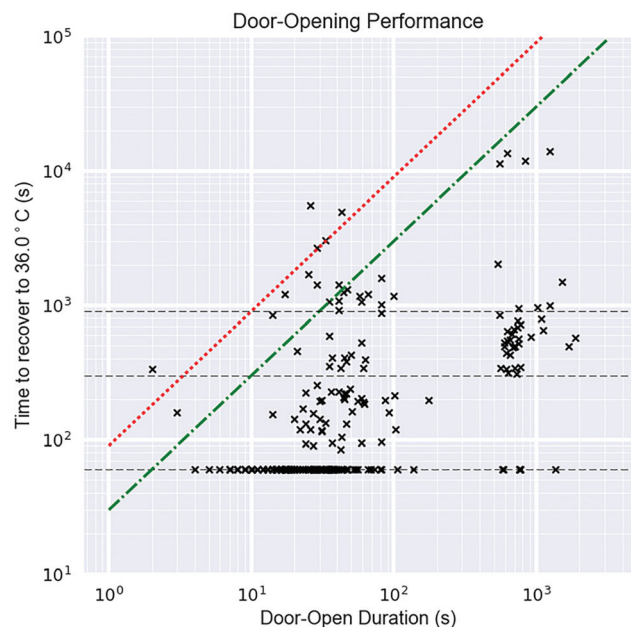
While the first test phases provided important verification of design requirements, the "week in the life" laboratory-based test was designed to assess the prototypes under conditions to which they would be exposed in typical field environments. With the exception of transients (resulting from door openings associated with sample insertions and subsequent removals), the sample chamber temperature remained within limits ($\pm 1\,^{\circ}$C of the set point) (Fig. 5).

The prototypes operated through significant voltage swings and voltage extremes (operational within 100–300 V, with power disconnected automatically when outside of that range for component protection) that would prove damaging to conventional incubators without external power protection. The rapid drop in temperature seen near the start of testing and on day 2 are from opening the sample compartment door for access during testing (Fig. 6).
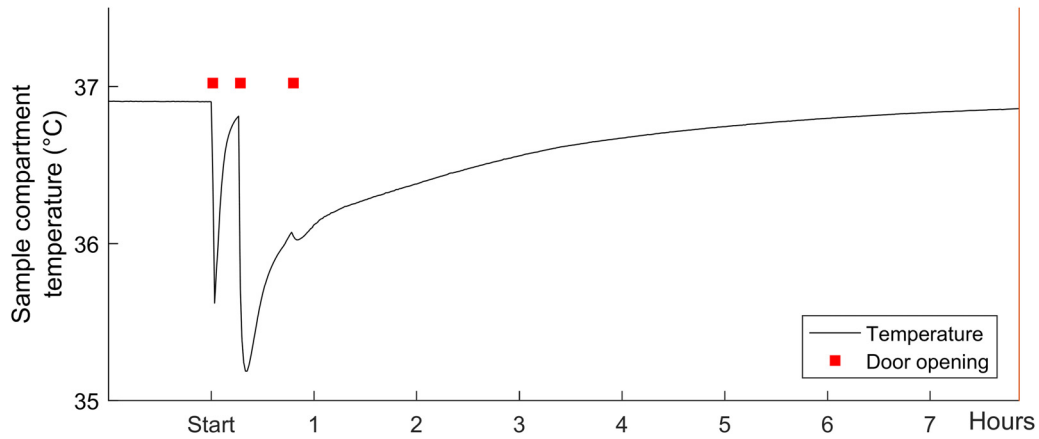
Field Testing

Four prototypes were sent to the Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU) in Laos for field testing in representative environments. Three of the prototypes were deployed in hospitals in Vientiane (17.9°N 102.6°E), Luang Namtha (20.9°N 101.4°E), and Salavan (15.4°N 106.2°E).
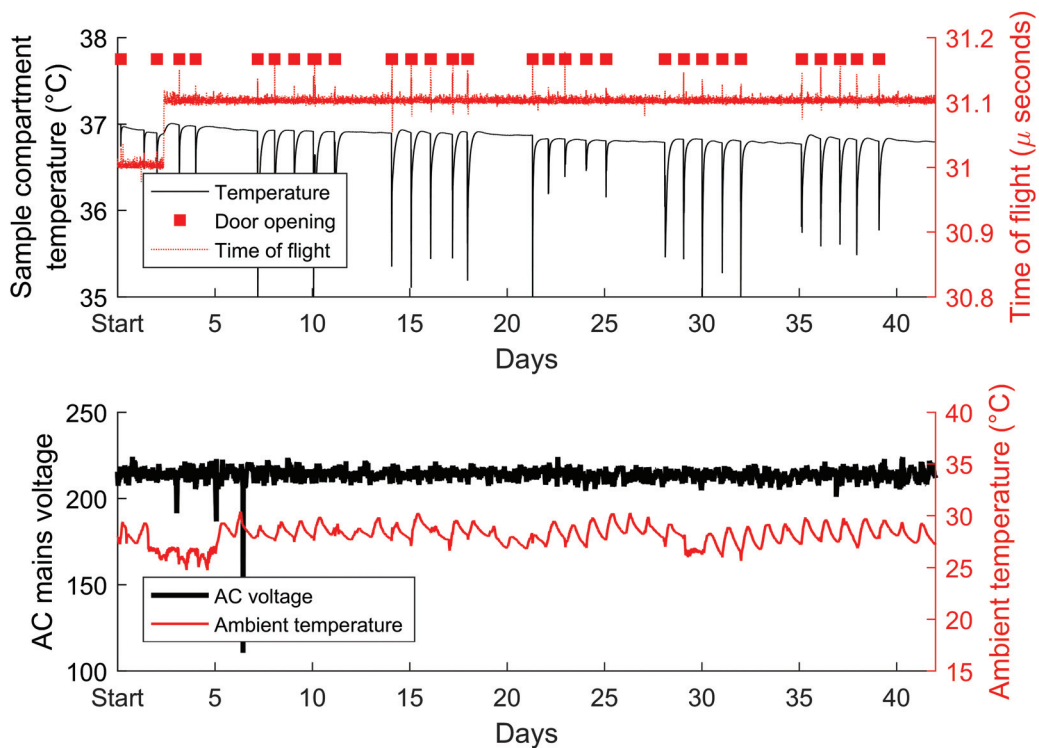
Overall, the prototypes experienced unexpectedly consistent power availability, the longest outage duration being 3.25 h at Salavan. Luang Namtha experienced a maximum outage duration of 0.8 h, and Vientiane did not experience more than 2 s of power outage. The cumulative power outages were 21.8 h over the test duration of 6 weeks. Overall, the power availability remained high for all test locations, at 99.92% in Vientiane, 99.89% in Luang Namtha, and 98.36% in Salavan with an average of less than one daily outage event (0.07, 0.37, and 0.78 outage events per day, respectively).

Fig. 7 shows the time to recovery for door openings as compared against the sample recovery requirements, which were assumed to be of constant slope (e.g., 300 s to recover after a 10 s opening requirement leads to an allowable recovery time of 400 s after a 15 s opening). The line of data points at 60 s corresponds to the data polling time period of once per minute. Recovery temperature is specified as 36 °C as this is the lower limit of the allowable set point $\pm 1\,^{\circ}$C allowable temperature range.

Fig. 8 Example multiple-open short duration sample compartment temperature plot from the field results. Red squares indicate when the door was opened. The sample compartment had not fully recovered to its set point.

Fig. 9 Field test data. The upper graph shows the sample compartment temperature and door openings, the lower graph shows the ambient temperature and power conditions.

Local personnel in all field sites were given a script designed to simulate a typical user interaction with a conventional incubator. In general, two to three door openings and sample vial/bottle swaps per day were performed during weekdays. In addition, due to the once-per-minute recording of data, events under 60 s were assumed to be 60 s—this explains the line of data points formed at 60 s (Fig. 7). Most door openings recovered rapidly, with only four at or above the required ratio of 15 min to recover after 10 s of open time (dotted line) and seven above the preferred ratio of 5 min to recover after 10 s of open time (dot and dashed line) (Fig. 7). These outliers are not strict failure criteria, as the door opening recoveries are highly variable depending on what activity is taking place that requires access to the sample compartment.

Repeated and prolonged door openings will inevitably drop the cavity temperature (Fig. 8). As can be expected, this behavior will continue to increase with additional door openings over short periods, as also will be seen with conventional incubators.

Hospital staff performed ten door openings per week, twice per day, with associated temperature drops in the sample compartment as room temperature sample vials/bottles and Petri dishes were inserted. There were also TOF spikes seen in Fig. 9, but coincident with door openings, we presume that as the door was opened and closed stresses resulted on the PCM container and sample compartment that caused physical movement of the two transducers closer or further from each other. Less regular AC mains voltage fluctuations can be seen, although still close to the nominal value. Note the change of the TOF set point on day 2 from 31.0 to 31.1 μs; although the TOF regulation resumed operation approximately 1 h after the adjustment, the temperature took considerably longer (approximately 6 h) to restabilize.

Conclusion

We demonstrated effective, reliable performance of a prototype microbiological culture incubator during power outages and off nominal voltage and AC frequency variations as well as in very low and very high ambient temperatures, without the use of electrical batteries. The incubator performed well under both simulated power loss events and real-world conditions in hospitals in Laos, maintaining incubation temperatures through heater regulation based on TOF measurements of the PCM. An effective means of ascertaining the physical state of the PCM was developed using ultrasonic transmission, improving control response over a temperature-based system and allowing faster PCM temperature recovery without adversely affecting each sample compartment's temperature. These features will allow a future productized version of this incubator to be operable in low resource settings, increasing the reach and reliability of temperature sensitive or growth detection-based diagnostics in low-income countries. The design should require less frequent maintenance and calibration than traditional incubators while improving accuracy by better adhering to international standards for detection, identification and DST. In the era of AMR, this is a fundamental and basic layer to successfully battle against AMR.

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