Optimizing Data Logger Setup and Use for Refrigerated Vaccine Temperature Monitoring

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Abstract: Accidental freezing of refrigerated vaccine represents a significant public and private healthcare cost. Freeze-damaged vaccines lose their effectiveness, putting public health at risk. U.S. immunization programs have strengthened vaccine storage and handling recommendations to mitigate this danger. However, current publications describing the setup and configuration of vaccine temperature monitoring devices allow for a range of untested interpretations. In this paper, we report on our study of specific temperature monitoring setup variables, including vial size, thermal buffer type, buffer fluid concentration, and placement of the probe-in-vial setup, in an effort to provide definitive guidance on the selection of an optimal temperature monitoring setup and methodology for use in vaccine storage refrigerators.

1. Introduction

Since 2009, the National Institute of Standards and Technology (NIST) has partnered with the Centers for Disease Control and Prevention (CDC) to provide research-based guidelines for better vaccine storage, handling, and temperature monitoring practices in the CDC Vaccines for Children (VFC) program. In order to preserve drug efficacy, stored vaccines must be kept within strict, prescribed temperature limits. Safe and effective vaccine management hinges on the use of temperature monitoring devices capable of accurately tracking vaccine temperature history. Based on previous NIST studies [1–5], the CDC has recommended the use of a digital data logger thermometer probe immersed in a thermal buffering fluid as the preferred device for vaccine temperature monitoring inside VFC storage refrigerators. However, this guidance allows for a range of possible implementations, including different vial sizes and buffer types. For these reasons, we have attempted to evaluate some of these variables in hopes of providing more specific, research-based guidance to vaccine providers. This paper describes our evaluation of different temperature monitoring setup variables to determine an optimal methodology for tracking refrigerated vaccine temperature. Tested variables include: buffer vial size, thermal buffer type, buffer fluid concentration, and placement of the probe-in-vial setup inside two types of widely-used vaccine storage refrigerators. Each setup variable was evaluated in a simulated-use scenario, in which the refrigerator door was opened repeatedly at 5 minute intervals.

2. Background

Beginning in 2012, NIST researchers conducted tests to determine the optimal buffer material, vial size, probe type, and operating conditions for a probe used to monitor refrigerated vaccine temperature. The group evaluated an exhaustive array of these variables in different combinations. However, despite utilizing different mathematical analysis methods, our data did not present clear-cut conclusions capable of satisfying the need for definitive guidance on an optimal vaccine temperature monitoring setup. The complexity of the experimental design and the potential for unforeseen, uncontrolled variables necessitated a reexamination of both the experimental method and measurement objectives. As a result, we have conducted a series of targeted follow-up tests, with the aim of answering some of the questions that developed out of the original measurements. This paper presents the updated results.

In the original 2012 examination of different buffer materials used with a probe for vaccine temperature monitoring, we evaluated glycerin and commercially-available marine/recreational vehicle (RV) antifreeze at 100 % concentration and in a 50 % aqueous solution, alongside glass beads,

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Concentration (% wt)	Ethanol	Propylene Glycol	Glycerin
0 (water)	5.800	5.800	5.80
20	4.648	4.841	5.11
40	3.594	3.962	4.46
60	2.708	3.175	3.87
80	2.029	2.501	3.32
100	1.669	2.002	2.88

Table 1. Thermal conductivities, λ (W/m·K), for different aqueous concentrations of freeze-point depressors, at 10 °C [7, 8].

sand, and air. The marine/RV antifreeze was chosen as a readily-available form of propylene glycol.

During later investigations of different antifreeze preparations, we discovered that a number of antifreeze manufacturers switched from a propylene-glycol based product to an ethanol-based formulation during the past decade. In many cases, the packaging does not clearly specify the chemical composition of the product.

Past CDC and NIST publications recommend the use of propylene glycol as a thermal buffer fluid [1–6], but temperature logger vendors and end-users employ a variety of buffer fluids as well as solid media. For this reason, in our targeted follow-up tests, we have included propylene glycol, glycerin, ethanol, two different, commercially-available antifreeze formulations, as well as solid buffer media, including glass beads, sand, and polytetrafluoroethylene (PTFE).

An effective vaccine temperature monitoring setup must closely replicate the temperature response of stored liquid vaccine. As a result, the chosen thermal buffer material should have a thermal conductivity similar to that of liquid vaccine, which is composed primarily of water. Pure water, however, is not an ideal thermal transfer fluid for this application, since the freezing point of water, 0 °C, is close to the storage temperature range for refrigerated vaccine. A small amount of a freeze-point depressor mixed with water has the property of lowering the freezing point below 0 °C, while exhibiting a thermal conductivity similar to water. At 10 °C, the thermal conductivity of pure water is 5.800 W/m·K. Thermal conductivities of ethanol, propylene glycol, and glycerol in various aqueous solutions are summarized in Table 1.

This study includes a comparison of different aqueous concentrations of the liquid test media, with the objective of determining 1) whether an optimal fluid concentration for this application exists, and 2) whether commercially-available antifreeze formulations perform comparably to thermal buffer solutions mixed by the end user. For example, is the performance of a commercial antifreeze preparation, stated to contain 20 % propylene glycol, equivalent to a 20 % propylene glycol solution mixed on-site?

3. Method

3.A Refrigerator Setup

Sixteen glass sample vials were filled with varying buffer fluid types and concentrations. The sample vials were arranged in a single tray placed in the center of a freezerless, household (domestic) refrigerator. A fine-gauge, calibrated type-T thermocouple was immersed in each vial. Another thermocouple was inserted into a PTFE cylinder cut to match

Type A	
	u_i / °C
Thermocouple Stability at Ice Melting Point	0.01
Thermocouple Accuracy at Ice Melting Point	0.05
Reproducibility of Ice Melting Point	0.001
Total A	0.05
Type B (rectangular distribution)	
Reference Junction Bath Stability	0.002
Measurement System Resolution	0.001
Total B	0.00
Total Standard Uncertainty ($k = 1$)	0.05
Total Expanded Uncertainty ($k = 2$)	0.10

Table 2. Uncertainty budget for the thermocouple measurement system used in this study.

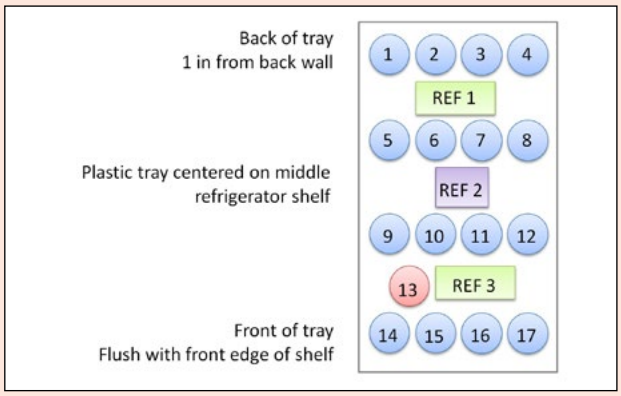

Figure 1. Setup of plastic tray. Sixteen test thermocouples were immersed in buffer-filled vials and one was imbedded in a PTFE block. Three reference thermocouples were inserted into vaccine vials inside packaging issued by the manufacturer.

the dimensions of a 20 mL sample vial. Three more thermocouples were immersed in liquid-containing vaccine vials, which were kept inside closed, manufacturer-issued cardboard packaging, consistent with CDC vaccine storage guidelines. The thermocouples immersed in vaccine liquid supplied the reference temperatures. Because vaccines may be supplied in either single-dose or multi-dose vial formats, we elected to monitor both types of products in this study. Two of the reference thermocouples, REF 1 and REF 3, were immersed in single-dose, 0.5 mL vials. Each of these was inside a box containing 10 single-dose vials. Reference thermocouple, REF 2, was immersed in a multi-dose, 2.5 mL vial, individually packaged in a smaller box. The refrigerator shelves were filled with a moderate vaccine load, and the floor of the unit was packed with water jugs (see Fig. 2a). A diagram of the setup is shown in Fig. 1, and photographs are shown in Figs 2a and 2b.

Figure 2. Figure 2a (left) shows refrigerator setup, with a test tray placed in the center of the refrigerator. Other trays are filled with vaccine boxes to produce a moderate-density load. Figure 2b (right) shows the 20 mL, varying buffer concentration setup tested in trials 1 and 2.

Prior to the refrigerator tests, all thermocouples were calibrated using the ice melting point method outlined in *NIST Technical Note 1411* [9]. This method provides an expanded (k = 2) realization uncertainty of 0.002 °C. The results of the ice melting point tests were used determine a linear offset correction for each thermocouple, allowing us to calibrate each device. The total expanded uncertainty for the thermocouple measurement system (k = 2) is 0.10 °C, as shown in Table 2.

3.B Test Variables

Using the configuration shown above, we tested the following variables:

- Ten types of thermal buffer media
  1. Propylene glycol-based marine/RV antifreeze (PG AF): containing 20 % to 40 % propylene glycol
  2. Ethanol-based marine/RV antifreeze (EtOH AF): containing 20 % ethanol
  3. Propylene glycol USP (PG): Pure, food additive grade, available from pharmacies
  4. Ethanol (EtOH): 190 proof, technical grade
  5. Glycerin/Glycerol (GLYC): CAS# 56-81-5, anhydrous, 99 % to 100 %
  6. Borosilicate glass beads, 5 mm diameter
  7. Borosilicate glass beads, 1 mm diameter
  8. Sand, from craft supply store
  9. PTFE block, machined to match approximate dimensions of 20 mL vials
  10. Air (empty vial)
- Four concentrations of aqueous buffer solutions, liquid media only: 100 %, 50 %, 20 %, and 10 %
- Four sample volumes, using glass sample vials with nominal capacities of 60 mL, 40 mL, 20 mL, and 15 mL

3.C Measurement Pattern

The test variables were patterned across five trials. For the sixth trial, we moved the configuration used in trial 5 to a purpose-built, pharmaceutical grade refrigerator. Table 3 lists the setup configuration for each trial.

Each time a new setup was configured, the refrigerator was allowed to equilibrate overnight with the door closed. Measurement collection was initiated with the door closed to record a baseline, steady-state temperature profile over several hours. This was followed by a door opening trial simulating a high-traffic use pattern. For each trial, we opened the refrigerator door at 5 min intervals for periods of 30 s, repeating the door opening sequence for a total of 1 h. In Trial 1, thermocouple REF 1 did not record data correctly. As a result, we repeated the configuration using an abridged, 30 min door opening sequence (Trial 2), in order to capture the response of REF 1. In all subsequent trials, we performed the full 1 h door opening sequence.

4. Results

4.A Evaluation of Buffer Fluids in Varying Concentrations

The temperature responses of different buffer fluids and concentrations (trials 1 and 2) are shown in Figs. 3a and 3b.

The position of the vial within a single plastic tray appears to have a marked influence on the resulting temperature response. Thermocouples in vials positioned in the front of the tray, closest to the refrigerator door, recorded temperatures more than 1 °C warmer than thermocouples in vials kept in the back of the same tray, closest to the back refrigerator wall – *prior to any door opening*. After the period of door opening, this front-to-back temperature gradient increased to more than 2 °C, as shown in Table 4. The monitored vials positioned in between these front and back planes presented mid-range temperature responses consistent with a front-to-back gradient. Any performance differences between the various buffer liquids and concentrations tested in these trials was overshadowed by the impact of positional temperature gradients.

4.B Evaluation of Buffer Fluids in Varying Sample Volumes

In Trial 3, we examined different vial sizes, ranging from 15 mL to 60 mL. Since we detected no significant performance differences related to buffer fluid concentration in the preceding trials, we eliminated the concentration variable from this trial. For the multi-size vials test, we limited our sample to 20 % aqueous solutions of ethanol, glycerin, and propylene glycol, and undiluted, ethanol-based antifreeze, which is less expensive and more ubiquitous than the propylene-glycol version. The ethanol-based antifreeze formulation is stated to contain 20 % ethanol, so to maintain consistency, we elected to use the other fluids in 20 % concentration. To rule out the possibility of a coincidental, ordering bias affecting our results, we reversed the front-to-back positions of the buffer fluids in this trial (see Table 3).

Despite introducing a range of sample vial sizes and reversing the buffer fluid ordering, the temperature response graph of Trial 3 is strikingly similar to those of the preceding trials. Similar to Trial 1, the front-to-back temperature gradient resulting from the door opening test is in excess of 2 °C (Table 4). Again, successful vaccine temperature monitoring using a probe-in-liquid buffer appears to hinge primarily on the position of the vial. Effects introduced by different buffer fluids, fluid concentrations, and even varying fluid volumes (within the limits of this study), appear to be nearly indistinguishable in comparison to the significant positional temperature gradient exhibited by the storage unit.

Position in Tray	TC #	Trial 1 (20 mL vials)	TC #	Trial 2 (20 mL vials)	TC #	Trial 3 (multi-size vials)	TC #	Trial 4 (20 mL vials)	TC #	Trial 5 (multi-size vials)	TC #	Trial 6 (pharma fridge)
Back	1	EtOH AF 100 %	1	EtOH AF 100 %	1	GLYC 20 %, 60 mL	1	Glass beads 5 mm	1	Glass beads 5 mm, 60 mL	1	Glass beads 5 mm, 60 mL
	2	EtOH AF 50 %	2	EtOH AF 50 %	2	GLYC 20 %, 40 mL	2	Glass beads 1 mm	2	Glass beads 5 mm, 40 mL	2	Glass beads 5 mm, 40 mL
	3	PG AF 100 %	3	PG AF 100 %	3	GLYC 20 %, 20 mL	3	Sand	3	Glass beads 5 mm, 20 mL	3	Glass beads 5 mm, 20 mL
	4	PG AF 50 %	4	PG AF 50 %	4	GLYC 20 %, 15 mL	4	Air	4	Glass beads 5 mm, 15 mL	4	Glass beads 5 mm, 15 mL
					13	Teflon						
Reference Vial			REF 1	REF 1 (0.5 mL)	REF 1	REF 1 (0.5 mL)	REF 1	REF 1 (0.5 mL)	REF 1	REF 1 (0.5 mL)	REF 1	REF 1 (0.5 mL)
Middle	5	EtOH 100 %	5	EtOH 100 %	5	PG 20 %, 60 mL	5	Glass beads 5 mm	5	Sand, 60 mL	5	Sand, 60 mL
	6	EtOH 50 %	6	EtOH 50 %	6	PG 20 %, 40 mL	6	Glass beads 1 mm	6	Sand, 40 mL	6	Sand, 40 mL
	7	EtOH 20 %	7	EtOH 20 %	7	PG 20 %, 20 mL	7	Sand	7	Sand, 20 mL	7	Sand, 20 mL
	8	EtOH 10 %	8	EtOH 10 %	8	PG 20 %, 15 mL	8	Air	8	Sand, 15 mL	8	Sand, 15 mL
Reference Vial	REF 2	REF 2 (5 mL)	REF 2	REF 2 (5 mL)	REF 2	REF 2 (5 mL)	REF 2	REF 2 (5 mL)	REF 2	REF 2 (5 mL)	REF 2	REF 2 (5 mL)
Middle	9	PG 100 %	9	PG 100 %	9	EtOH 20 %, 60 mL	9	Glass beads 5 mm	9	Glass beads 5 mm, 60 mL	9	Glass beads 5 mm, 60 mL
	10	PG 50 %	10	PG 50 %	10	EtOH 20 %, 40 mL	10	Glass beads 1 mm	10	Glass beads 5 mm, 40 mL	10	Glass beads 5 mm, 40 mL
	11	PG 20 %	11	PG 20 %	11	EtOH 20 %, 20 mL	11	Sand	11	Glass beads 5 mm, 20 mL	11	Glass beads 5 mm, 20 mL
	12	PG 10 %	12	PG 10 %	12	EtOH 20 %, 15 mL	12	Air	12	Glass beads 5 mm, 15 mL	12	Glass beads 5 mm, 15 mL
Reference Vial	REF 3	REF 3 (0.5 mL)	REF 3	REF 3 (0.5 mL)	REF 3	REF 3 (0.5 mL)	REF 3	REF 3 (0.5 mL)	REF 3	REF 3 (0.5 mL)	REF 3	REF 3 (0.5 mL)
Front	13	Teflon	13	Teflon								
	14	GLYC 100 %	14	GLYC 100 %	14	EtOH AF 100 %, 60 mL	14	Glass beads 5 mm	14	Glass beads 1 mm, 60 mL	14	Glass beads 1 mm, 60 mL
	15	GLYC 50 %	15	GLYC 50 %	15	EtOH AF 100 %, 60 mL	15	Glass beads 1 mm	15	Glass beads 1 mm, 40 mL	15	Glass beads 1 mm, 40 mL
	16	GLYC 20 %	16	GLYC 20 %	16	EtOH AF 100 %, 20 mL	16	Sand	16	Glass beads 1 mm, 20 mL	16	Glass beads 1 mm, 20 mL
	17	GLYC 10 %	17	GLYC 10 %	17	EtOH AF 100 %, 15 mL	17	Air	17	Glass beads 1 mm, 15 mL	17	Glass beads 1 mm, 15 mL

Table 3. Sample vial volume, contents, and position for each trial.

The PTFE block is also influenced by positional temperature gradients, resulting in a temperature response consistent with the fluid-filled sample vials. The only caveat for its use is the probe-imbedding technique. If the immersion hole is too large, or does not allow proper immersion depth, the thermal buffering properties of the PTFE block will be diminished, and the probe will record something closer to air temperature. In Fig. 3, the PTFE-imbedded thermocouple signal shows more noise during door opening than the liquid-buffered thermocouples. Our tested block (machined in-house) did not exhibit enough noise to significantly impact its performance or discount its validity as a suitable buffer medium. However, commercial manufacturers marketing a PTFE-based setup should be aware of this potential issue. Permanently imbedding a sensor in a PTFE block, or in any buffer-filled vial, makes periodic validation testing more difficult, and the choice of epoxy or adhesive can impact the aggregate thermal conductivity.

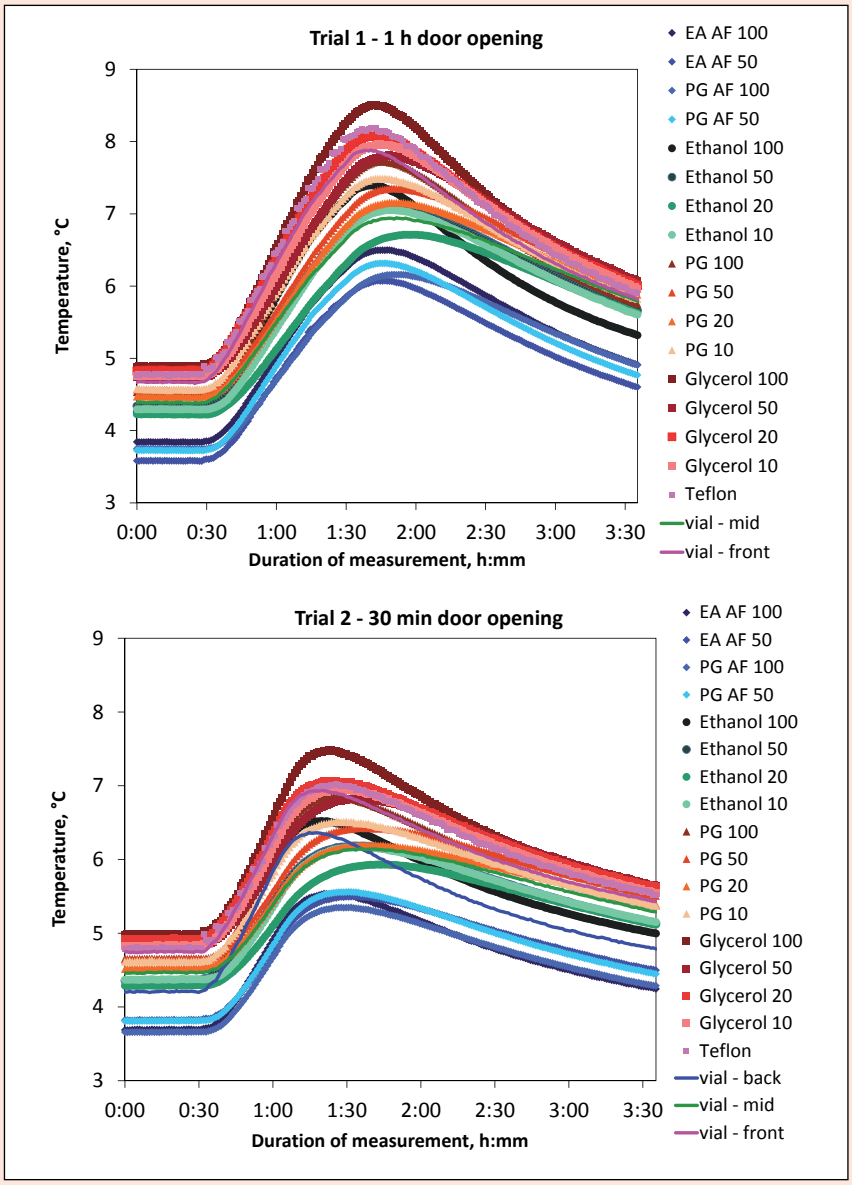

Figure 3. Temperature response of two trials. In each trial, door opening commenced at the 30 min mark as shown in the graphs. The refrigerator was opened for 30 s at 5 min intervals, for a total period of 1 h in Trial 1 (top), and 30 min in Trial 2 (bottom). Symbol color is indicative of item placement in the refrigerator tray, with blues representing the far back of the tray, closest to the back wall of the refrigerator, and reds/pinks representing the front of the tray.

4.C Evaluation of Solid Buffer Media

In Trial 4, we examined non-liquid buffer media alongside empty vials, containing air. Figures 5 and 6 show the results from this trial. Data from filled and un-filled vials is graphed separately, for comparison. All vials tested in this trial were the 20 mL size.

Once again, the position-based temperature gradient is apparent, with a front-to-back temperature difference of 1.6 °C (Table 4). When comparing Figs. 5 and 6, the thermocouples in empty or "air-filled" vials appear to show a faster rate of temperature change during the door opening period than the thermocouples in buffer-filled vials. To compare the air-filled vials and buffer-filled vials in terms of their ability to track liquid vaccine temperature, we calculated the maximum temperature difference between each vial and a nearby reference vaccine at any point during the test (e.g., vial positioned in the front of the tray was compared to the "front" position reference vaccine). On average, buffer-filled vials presented a maximum temperature difference of 0.6 °C, as compared to the temperature of a nearby reference vaccine. For air-filled vials, this average maximum temperature difference was 1.6 °C. These values may be interpreted as the expected "error" of using a particular setup methodology. Based on this, the use of probe placed in an empty, air-filled vial is likely to increase the potential for error by nearly a factor of three, as compared to the use of a probe immersed in buffer media. These results demonstrate that any buffer media, solid or liquid, provides a temperature response that is more consistent with the behavior of liquid vaccine temperature, than is provided by an empty vial.

4.D Evaluation of Solid Buffer Media in Varying Sample Volumes

In Trial 5, we eliminated the air-filled vials, and tested varying sample volumes of solid media. Our results confirmed our conclusions drawn from the liquid media, multi-size vial trial. The largest influence on temperature response was position, not sample volume (see Fig. 7).

4.E Purpose-built, Pharmaceutical-Grade Refrigerator

For the sixth and final trial, we transferred the test vial setup used in Trial 5 to the center shelf a purpose-built, pharmaceutical refrigerator. This refrigerator was again filled with a moderate vaccine load, and we repeated the equilibration and hour-long door opening pattern. Figures 8 and 9 show the results.

In the freezerless refrigerator trials, we observed an average temperature increase of all thermocouples by approximately 3 °C. In the pharmaceutical refrigerator, this effect is greatly diminished. The temperature increase resulting from our door opening procedure was less than 1 °C above the baseline, equilibrium temperature. In addition, positional temperature gradients are much less pronounced. During closed door operation, items placed in the front of the tray were slightly warmer than items in the back of the tray. However, this front-to-back temperature difference was less than 0.4 °C.

One hour of repeated door opening appears to have eliminated the clear front-to-back positional temperature gradient in this particular refrigerator, as items in the back of the tray warmed more quickly than items placed either the middle or the front of

the tray. Even so, the range of thermocouple temperatures measured after the door was opened remained within 0.6 °C (Fig. 9). Because this unit features dozens of fan-forced air vents lining the back walls, along with a sliding glass door, we suspect that the counterintuitive warming response is a result of complex airflow patterns arising during door opening. Further assessment of the pharmaceutical refrigerator in this context is outside the scope of this work.

As shown in Figures 8 and 9, the effect of repeated door opening on this particular unit is small. Unlike the freezerless refrigerator, in which large positional temperature gradients arise during repeated door opening, the pharmaceutical refrigerator maintains a narrow temperature range during both closed door operation and repeated door opening, regardless of the front-to-back position inside the unit.

4.F Summary of Results

To better compare our results across each tested setup, we focused our analysis on two critical temperature points taken from each trial. We designated baseline, steady-state temperature points for each trial using the temperature recorded by each thermocouple exactly 1 h prior to the first door opening action. This snapshot in time provides a good approximation of the thermal environment inside the tray, based on position, during closed-door refrigerator operation. In addition, we selected a "worst-case" temperature point from the temperature recorded by each thermocouple exactly 10 min after the final door opening and closing pattern, as this point typically corresponded to the maximum temperature achieved by the majority of the thermocouples. The "worst case" temperature data for each thermocouple is summarized for all six trials in Table 4.

For each baseline and worst-case point, we averaged sample vial temperatures by location, grouping the vials by the "back," "middle," and "front" positions, as labeled in Table 4. Averaging these results across the five freezerless refrigerator trials gives the mean baseline temperature, $\overline{T}_0$, and the mean worst-case temperature, $\overline{T}_1$, for each position group.

The three reference vaccine vial temperatures were also averaged across all five trials to provide a single baseline reference temperature, $\overline{T}_{ref0}$, and a worst-case reference temperature $\overline{T}_{ref1}$. The mean difference at the baseline temperature point is given by Eq. (1) and the mean difference at the worst-case temperature point is given by Eq. (2):

$$\Delta\overline{T}_0 = \overline{T}_0 - \overline{T}_{ref0}, \quad (1)$$

$$\Delta\overline{T}_1 = \overline{T}_1 - \overline{T}_{ref1}. \quad (2)$$

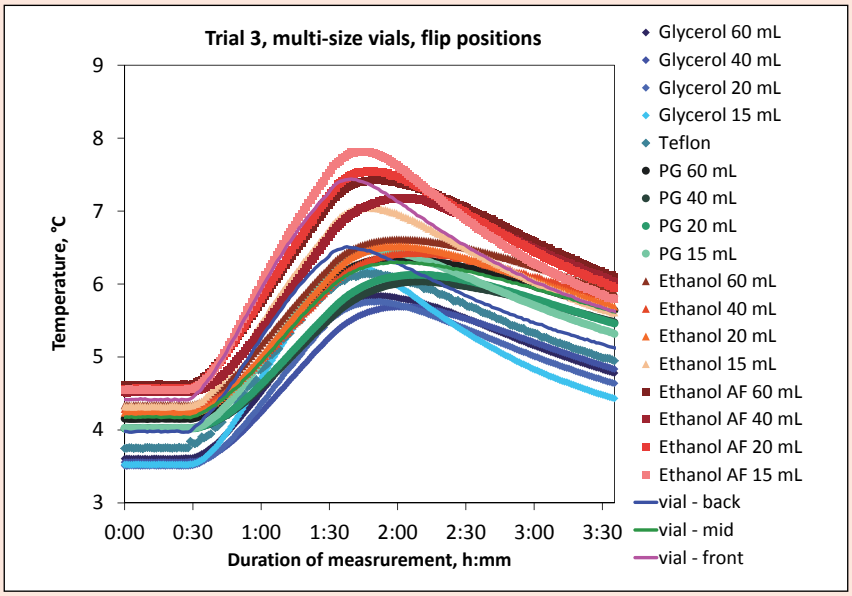

Figure 4. Temperature response of door opening trial. Door was opened for 30 s at 5 min intervals for a period of 1 h, with the first opening occurring at the 2 h mark in the graph above. Sample vials of 60 mL, 40 mL, 20 mL, and 15 mL were used. Symbol color is indicative of item placement in the refrigerator tray, with blues representing the far back of the tray, closest to the back wall of the refrigerator, and reds/pinks representing the front of the tray.

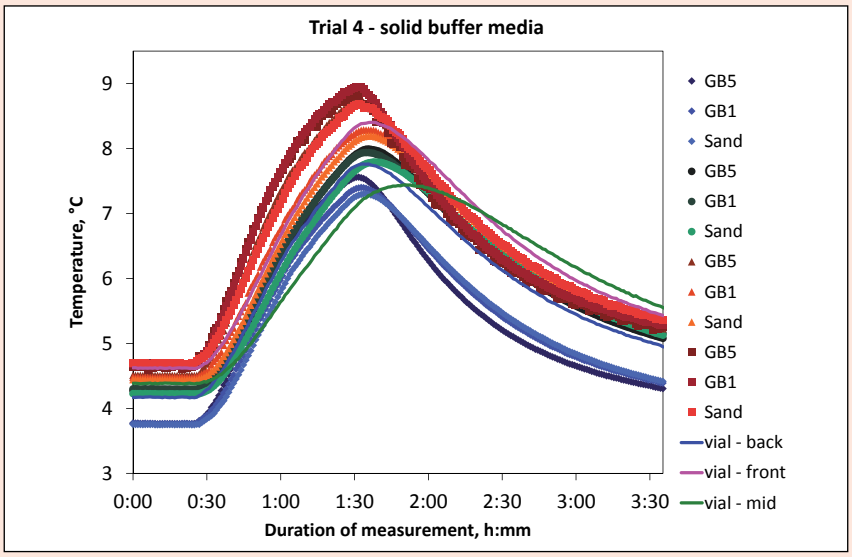

Figure 5. Trial 4 – the door was opened for 30 s at 5 min intervals for a period of 1 h, with the first door opening occurring at the 1 h mark in the graph above. Vials filled with solid media (glass beads, sand) are shown. In the legend, GB5 indicates a vial filled with 5 mm glass beads, and GB1 corresponds to a vial filled with 1 mm glass beads. Symbol color is indicative of item placement in the refrigerator tray, with blues representing the far back of the tray, closest to the back wall of the refrigerator, and reds/pinks representing the front of the tray.

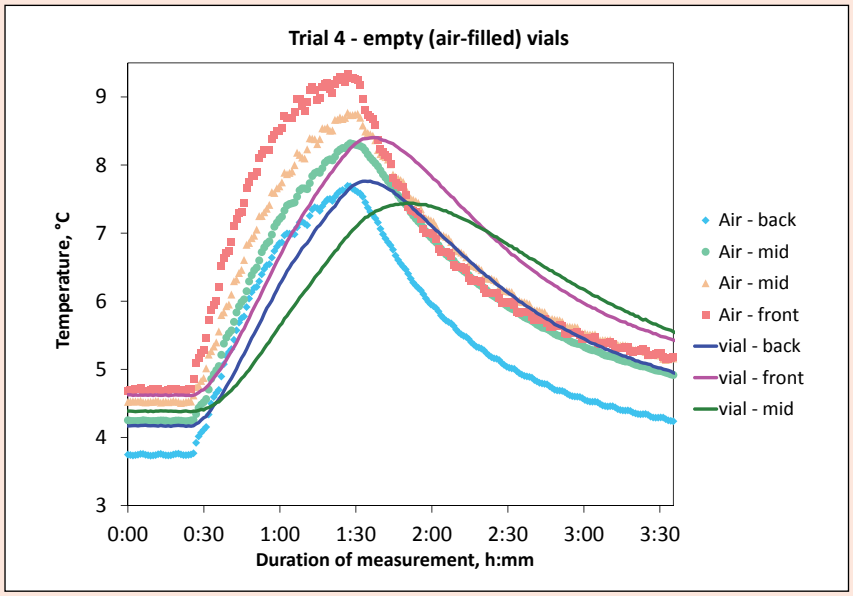

Figure 6. Trial 4 - door was opened for 30 s at 5 min intervals for a period of 1 h, with the first door opening occurring at the 1 h mark in the graph above. Empty, air-filled vials are shown.

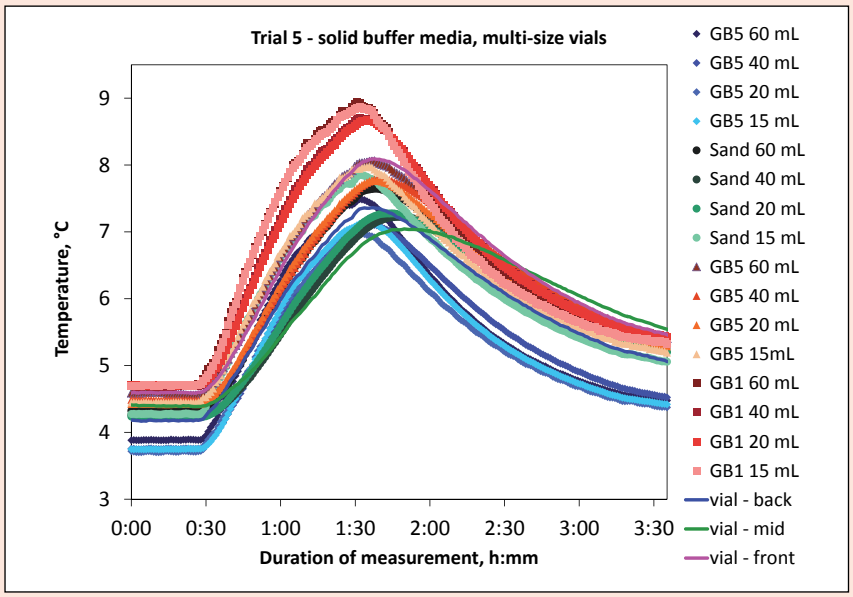

Figure 7. Trial 5 – the door was opened for 30 s at 5 min intervals for a period of 1 h. Solid media were utilized in 60 mL, 40 mL, 20 mL, and 15 mL sample vials.

Table 5 summarizes these values, calculated using the data from all five freezerless refrigerator trials.

During closed-door operation, the average front-to-back temperature gradient in the freezerless refrigerator was 1 °C. After 1 h of repeated door opening, this gradient increased to, on average, 1.5 °C.

A *t*-test confirms the significance of position on thermocouple temperature response. Items placed in the back of the tray register colder temperatures than items in the middle, with $p < 0.01$ to 0.05. Items in the front of the tray register warmer temperatures than items in the middle, with $p < 0.01$.

Table 6 summarizes the pharmaceutical-grade refrigerator trial results. As described above, we designated a baseline, steady-state temperature point 1 h prior to the first door opening, and a worst-case point 10 min after the final door opening/closing, which is given in Table 4.

During closed-door operation, the average front-to-back temperature gradient in the pharmaceutical refrigerator was 0.4 °C. After 1 h of repeated door opening, this gradient decreased to 0.1 °C, as vials placed in the back of the tray warmed more quickly than items placed in the front of the tray.

5. Discussion

The objective of this evaluation was to characterize the performance of different temperature monitoring setup variables to determine an optimal methodology for tracking refrigerated vaccine temperature. We focused our study on a setup used inside a household freezerless refrigerator subjected to frequent door opening, which represents the lowest common denominator in terms of a permissible vaccine storage unit. Despite testing a range of variables, including a variety of liquid and solid buffer media, different aqueous concentrations of liquid buffer media, and various sample vial sizes, the data uniformly shows that the only critical variable in this application is the placement of the buffered temperature monitoring probe.

The freezerless refrigerator exhibits a significant temperature gradient between the back wall and the front door of the unit. During closed-door operation, items stored in the front of a tray were maintained at a temperature approximately 1 °C warmer than items placed in the back of the same tray. Averaging over all probes and trials, this range increased to 1.5 °C after 1 h of high frequency door opening. In some cases, individual thermocouples registered front-to-back temperature differences as large as 2.5 °C after the door opening pattern. In a freezerless refrigerator, any performance differences resulting from variables like buffer media type and vial size are outweighed by the effects of this temperature gradient.

The temperature gradient we observed under the tested conditions was not large enough to seriously threaten vaccine efficacy or exclude the freezerless refrigerator from use as a viable vaccine storage unit. At no point did monitored vaccines or sample vials stray below the lower storage temperature limit of 2 °C. However, some items placed in the front of the tray, including a monitored vaccine vial, exceeded the upper 8 °C limit during one or more door opening trials.

Placing the buffered temperature monitoring probe in the center of a tray along with stored vaccines will help reduce the incidence of high temperature alarms caused by very small excursions (e.g., a data logger recording 8.5 °C following a period of door opening).

Users can mitigate the effects of the freezerless refrigerator's temperature gradient through careful placement of both vaccines and a temperature monitoring probe. The coldest temperatures exist near the back wall of the refrigerator. If for any reason, the refrigerator becomes excessively cold, items closest to this back wall will be the first to experience damaging freezing temperatures. Based on the results of this study, we suggest keeping 7 cm to 10 cm (3 in to 4 in) of space between the back wall and stored vaccines. Selecting smaller trays to fit these dimensions will make it easier for users to avoid accidental placement too close to the back wall. Another option, if space is an issue, is to store freeze-tolerant vaccines in areas known to produce lower temperatures.

Antiquated vaccine storage and handling recommendations may cite the need for airflow around individual vaccine vial boxes. Our current findings do not support this recommendation. Positioning the individual boxes closer together results in a more uniform storage temperature when vaccines are stored in a single tray. However, airflow throughout the refrigerator's interior is critical. Overloading a unit by cramming in too many trays or blocking cold air vents can obstruct critical airflow patterns inside the unit, resulting in increased positional temperature gradients and the formation of potentially damaging pockets of too-cold or too-warm air [1, 2, 6].

Differences in buffer media type and concentration are negligible for this application. We can eliminate position-based biases from our results by considering data from just the centrally-placed probes in the freezerless refrigerator trials, plus all probes in the pharmaceutical-grade refrigerator trials. From this data set, all tested setups successfully tracked liquid vaccine temperature to within 0.5 °C, regardless of buffer media type, liquid media concentration, and sample vial size. Liquid and solid buffer media both function to dampen air temperature fluctuations, resulting in temperature measurements that mimic the conditions of stored vaccines. Empty vials do not provide adequate thermal buffering, and fail to track liquid vaccine temperature during changing temperature conditions.

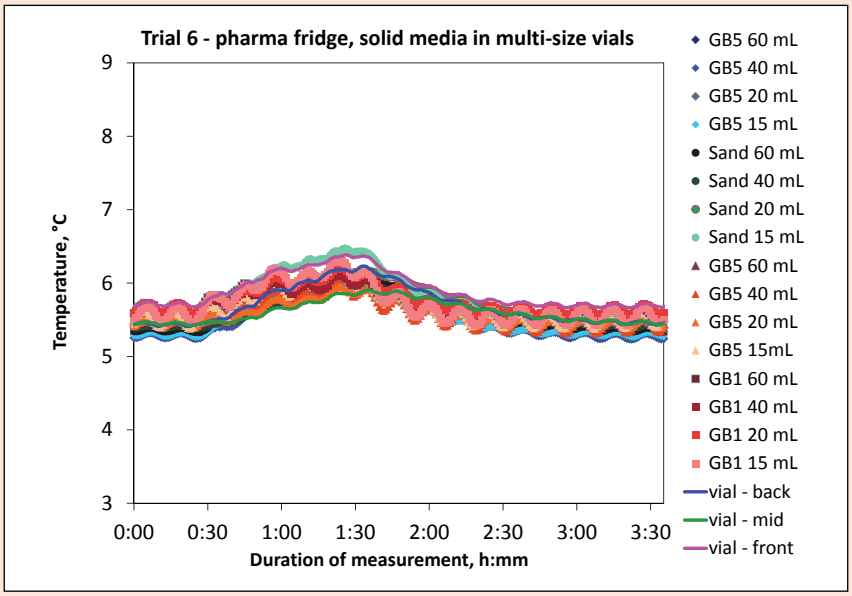

Figure 8. Trial 6 – temperature response of door opening trial for a pharmaceutical refrigerator. The door was opened for 30 s at 5 min intervals for a period of 1 h, with the first door opening occurring at the 1 h mark in the graph above. Sample vials of 60 mL, 40 mL, 20 mL, and 15 mL were filled with solid buffer media. The data are scaled to match the freezerless refrigerator trial graphs.

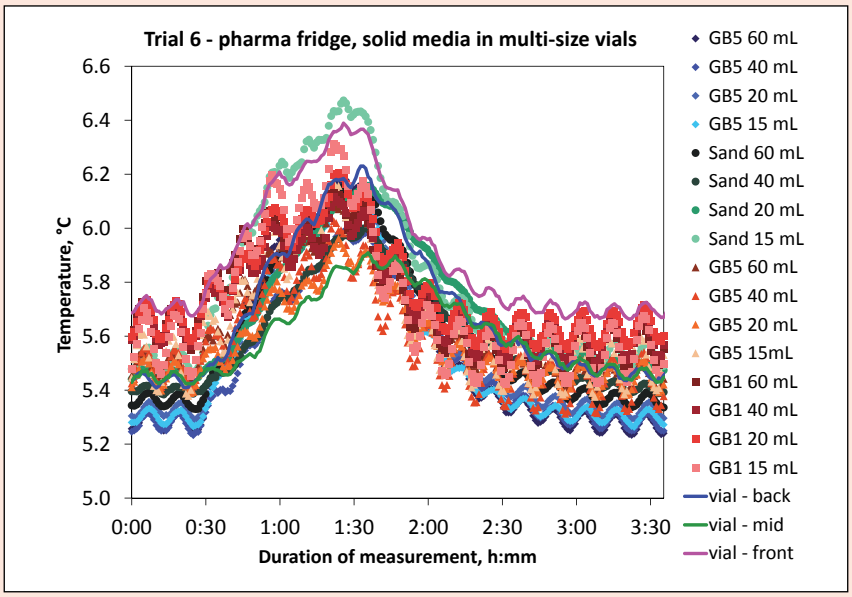

Figure 9. Zoomed-in version of data shown in Fig. 8.

In this study, we tested sample vials with capacities ranging from 15 mL to 60 mL. Any-size sample vial in this range, filled with buffer media, successfully tracks the average temperature of nearby stored vaccine to within 0.5 °C, provided that the probe-plus-sample vial is placed in the center (laterally and front-to-back) of the refrigerator, and that the vaccines are stored appropriately, inside manufacturer-supplied cardboard packaging.

At present, many current (2015), commercially-available data logger probes marketed for cold chain temperature monitoring require an immersion depth of

Position in Tray	Thermocouple #	Domestic, Freezerless Refrigerator Trial 1	Trial 2 (30 min)	Trial 3	Trial 4	Trial 5	Pharmaceutical Refrigerator Trial 6
Back	1	6.5	5.5	5.7	7.4	7.4	6.0
	2	6.0	5.4	5.4	7.3	7.1	5.9
	3	6.1	5.3	5.6	7.3	6.9	6.0
	4	6.3	5.5	6.2	7.2	7.1	6.0
Reference Vial	REF 1 (vaccine)	no data	6.4	6.5	7.7	7.4	6.1
Middle	5	7.4	6.5	6.1	8.0	7.6	6.1
	6	7.0	6.0	5.7	7.9	7.1	5.9
	7	6.6	5.7	5.8	7.8	7.2	6.1
	8	7.0	6.0	6.3	8.0	7.7	6.2
Reference Vial	REF 2 (vaccine)	6.9	6.0	6.1	7.3	6.9	5.9
Middle	9	7.7	6.8	6.4	8.6	8.0	5.9
	10	7.3	6.2	6.1	8.3	7.8	5.7
	11	7.1	6.0	6.2	8.2	7.8	5.8
	12	7.5	6.4	6.9	8.4	7.9	5.8
Reference Vial	REF 3 (vaccine)	7.9	6.9	7.4	8.4	8.1	6.2
Front	14	8.5	7.4	7.3	8.6	8.7	5.8
	15	7.7	6.6	6.8	8.8	8.6	5.9
	16	8.1	7.0	7.4	8.6	8.6	5.9
	17	7.9	6.8	7.7	8.6	8.7	5.8

Table 4. Summary of "worst case" temperature values, recorded 10 min after final door opening, shown for each trial and thermocouple position. Trials 1 through 5 were conducted inside a domestic freezerless refrigerator. Trial 6 was conducted inside a purpose-built, pharmaceutical refrigerator. All temperature values are given in °C.

TC response	Back	Middle	Front
$\bar{T}_0$	3.7	4.4	4.7
$\Delta\bar{T}_0$	−0.7	0.0	0.3
$\bar{T}_1$	6.4	7.1	7.9
$\Delta\bar{T}_1$	−0.7	0.1	0.8

Table 5. Summary of test thermocouple statistics, by position. Values shown are calculated from Eqs. (1) and (2), using thermocouple data from five freezerless refrigerator trials. All values are given in °C.

TC response	Back	Middle	Front
$\bar{T}_0$	5.3	5.5	5.7
$\Delta\bar{T}_0$	−0.2	−0.1	0.1
$\bar{T}_1$	6.0	5.9	5.9
$\Delta\bar{T}_1$	−0.1	−0.1	−0.2

Table 6. Summary of test thermocouple statistics, by position. Values shown are calculated from Eqs. (1) and (2), using thermocouple data from the pharmaceutical refrigerator trial. All values are given in °C.

at least 2.5 cm to minimize stem-conduction errors. Achieving this degree of immersion in a sample vial smaller than 15 mL is likely to be difficult or impossible. For this reason, sample vials smaller than 15 mL are not recommended for use with most standard probes. Multiple commercial vendors offer integrated, probe-in-vial solutions for cold chain temperature monitoring applications. These setups may feature vials smaller than 15 mL, paired with a probe capable of achieving adequate immersion in a very small sample volume. The scope of this study did not include testing of integrated, probe-in-vial products.

Although individual vaccine vials and pre-filled syringes contain volumes as small as 0.5 mL, long-term storage guidelines mandate that vaccines be kept inside an additional layer of manufacturer-supplied cardboard packaging. This packaging adds to the total thermal mass of the stored product, slowing its response to temperature change. For this reason, slightly larger sample volumes (15 mL, 20 mL, 40 mL, 60 mL) provide suitable thermal buffering for the purpose of tracking stored vaccine temperatures. Smaller sample volumes (less than 15 mL) may register thermal excursions while appropriately stored

vaccines are still within prescribed storage limits, resulting in false temperature alarms.

Sample vials larger than 60 mL were not tested in this study. Choosing a sample volume significantly larger than 60 mL is likely to result in excessive thermal buffering, allowing legitimate temperature excursions to go undetected, and putting vaccines at risk.

6. Conclusions

The placement of a data logger probe used for vaccine temperature monitoring is critical to effective device performance. A household, freezerless refrigerator exhibits a significant temperature gradient between the back wall and the refrigerator door. This gradient increases in response to repeated door opening. By contrast, the pharmaceutical refrigerator's temperature control mitigates this effect, eliminating any measurable front to back temperature gradients. Users electing to store vaccines inside a household, freezerless refrigerator must exercise greater caution when placing both vaccines and their temperature monitoring devices inside the unit. Placing the temperature monitoring probe-in-vial setup in the center of a tray on the central refrigerator shelf will prevent the resulting temperature data from being excessively skewed by the anticipated temperature gradients inside the unit.

The choice of buffer media type, liquid media concentration, and sample vial size, within the limits of this study, did not have a significant impact on the setup's ability to track liquid vaccine temperature to within 0.5 °C. Liquid and solid buffer media both function to dampen air temperature fluctuations, resulting in temperature measurements that mimic the conditions of stored vaccines.

7. Acknowledgments

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8. References

[1] M. Chojnacky, W. Miller, D. Ripple, and G. Strouse, "Thermal Analysis of Refrigeration Systems Used for Vaccine Storage," *NIST Interagency or Internal Report (NISTIR) 7656*, 2009.

[2] M. Chojnacky, W. Miller, and G. Strouse, "Thermal Analysis of Refrigeration Systems Used for Vaccine Storage: Report on Pharmaceutical Grade Refrigerator and Household Refrigerator/Freezer," *NIST Interagency or Internal Report (NISTIR) 7753*, 2010.

[3] M. Chojnacky, W. Miller, and G. Strouse, "Data Logger Thermometers for Vaccine Temperature Monitoring," *NIST Interagency or Internal Report (NISTIR) 7899*, 2012.

[4] M. Chojnacky, W. Miller, and G. Strouse, "Thermal Analysis of a Small Pharmaceutical Refrigerator for Vaccine Storage," *NIST Interagency or Internal Report (NISTIR) 7900*, 2012.

[5] M. Chojnacky, W. Miller, and G. Strouse, "Methods for accurate cold-chain temperature monitoring using digital data-logger thermometers," *AIP Conference Proceedings*, vol. 1552, pp. 1014-1019, 2013.

[6] Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases, "Vaccine Storage and Handling Toolkit," May 2014 (http://www.cdc.gov/vaccines/recs/storage/toolkit/storage-handling-toolkit.pdf).

[7] ESDU, "Thermal conductivity of liquids: Mixtures," *ESDU 88004*, Amendment (A), September 2005.

[8] ESDU, "Thermal conductivity of liquids: Glycols, glycerol and their aqueous solutions," *ESDU 99013*, Amendment (B), September 2005.

[9] B. Mangum, "Reproducibility of the Temperature of the Ice Point in Routine Measurements," *NIST Technical Note 1411*, 1995.

[10] ILAC/OIML, "Guidelines for the determination of calibration intervals of measuring instruments," *ILAC G24 / OIML D 10*, 2007.

[11] D. Matthias, J. Robertson, M. Garrison, S. Newland, and C. Nelson, "Freezing temperatures in the vaccine cold chain: A systematic literature review," *Vaccine*, vol. 25, pp. 3980-3986, 2007.