Achieving temperature stability for storage of biological samples in an autodefrost freezer

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OBJECTIVE
To assess the temperature stability of an autodefrost freezer commonly used in veterinary practices, whether the use of a Styrofoam cooler within the freezer provides temperature stability, and the ease of use of a remote monitoring system for the notification of temperature elevations.

ANIMALS
None.

METHODS
Temperature in the freezer and 2 Styrofoam coolers were assessed with remote monitoring thermometers every 15 minutes. Temperature values were monitored from October 11 to December 18, 2023 (for a 68-day period). Data analysis focused on temperatures for the freezer exceeding 0 °C and the elevations in temperatures within the coolers relative to the freezer.

RESULTS
The freezer had an increase in temperature approximately every 16 hours. Over 68 days, the freezer had a temperature greater than 0 °C 27 times, representing 26 separate elevations. The Styrofoam coolers within the freezer never registered a temperature higher than −5 °C. Elevations in temperature within the freezer were larger in magnitude than temperature elevations within the coolers, which showed smaller-magnitude changes in temperature.

CLINICAL RELEVANCE
The temperature stability provided by the Styrofoam cooler would avoid potential freeze-thaw cycles of any stored biological samples. Additionally, the remote temperature monitoring system is easy to install and monitor, providing peace of mind to practice management.

Keywords: freezer, temperature, platelet-rich plasma, biological sample, storage

Bious samples, such as platelet-rich plasma (PRP), are typically frozen at either −80 °C or −20 °C with continuous temperature monitoring of the freezer with alarms in a research setting.1,2 However, in veterinary practice, these samples are usually stored in a multiuse freezer. The practice may also store laboratory testing supplies, ice packs, and other supplies in the same freezer, resulting in the freezer being opened more often than in a typical research setting. Additionally, practices most often purchase refrigerator/freezer combinations with autodefrost cycles. Freezers with autodefrost cycles do require less maintenance by the practice.3 However, at regular intervals, the evaporating coils are heated, melting and evaporating frost to routinely defrost the freezer.3 This causes fluctuation in the temperature of the freezer because the cooling coils are heated.3 Small volumes of samples are more sensitive to these temperature fluctuations. Private practices do not commonly have alarm systems for monitoring of the freezer temperature or for doors left ajar, also raising the possibility of freeze-thaw cycles that may or may not be observed.

PRP preparations are increasing in use in veterinary medicine. PRP has various applications, including musculoskeletal disorders, ophthalmic disorders, and dermatologic disorders.4,5 These biologics primarily act to reduce inflammation through the activity of growth factors, including VEGF, TGF-β, PDGF, platelet-derived epidermal growth factor, fibroblast growth factor, epidermal growth factor, and IGF-1. Multiple methods of collection and preparation have been described.2,4-12
Although these preparations were initially used primarily in academic and large referral centers, this technology is now easily accessible to veterinarians in smaller practices. Given the expense in collecting the samples, excess prepared PRP is commonly stored for additional treatments. Several papers have examined the presence of growth factors after storage.\textsuperscript{6-10} Repeated treatments have shown to be more effective for most conditions.\textsuperscript{5,6,9,11} These samples are commonly less than 5 mL in private practice and can be thawed quickly if the temperature of the freezer rises.

Repeated freeze-thaw cycles may cause early degradation of proteins, including growth factors. Previous work has shown that multiple cytokines and growth factors are degraded with repeated freeze-thaw cycles.\textsuperscript{2} Previous work by Hosnuter et al\textsuperscript{10} described storage for up to 14 days at \(-20^\circ\text{C}\). The study showed that growth factors were present in samples up to the 14th day of storage, though at lower levels than immediately after collection and processing. The study evaluated levels of PDGF, TGF-\(\beta\), epidermal growth factor, IGF-1, VEGF, and P-selectin.\textsuperscript{10}

We sought to evaluate if a freezer in use in private practice could maintain a consistent temperature of \(-20^\circ\text{C}\) for appropriate storage of PRP samples. The primary objective was to track the temperature of the freezer over a period of 68 days, evaluating any elevations in temperature above \(0^\circ\text{C}\), which may result in thawing of the samples. The secondary objective was to assess whether the use of a Styrofoam cooler within the freezer would ameliorate any observed temperature fluctuations to serve as an affordable method in private practice. The third objective was to assess the ease of use of the temperature probe monitoring system and whether this would be practical as a monitoring device in private practice to notify of significant temperature elevations within the freezer that may affect sample integrity.

**Methods**

Temperature monitoring was performed every 15 minutes for 68 days with a remote-monitored temperature probe and transmitter system (SoniShield Duo; Sonicu) with glycol-buffered sensors.\textsuperscript{13} Data was transmitted over wireless internet to a cloud storage system for collection and analysis, with

**Figure 1**—Visual images of the installation of a remote temperature monitoring system. A—The glycol-buffered sensor in the rear of the freezer and the closed 2 freezers. B—Both coolers with the lids removed and the ice blocks on top of the samples and glycol sensors. C—The coolers with the ice blocks removed, exposing the glycol-buffered sensors and samples. D—The transmitters, mounted on the right side of the refrigerator/freezer unit with the flat wires connecting the sensors.
a data point for each of the 3 sensors transmitted every 15 minutes. A top refrigerator/bottom freezer unit (Model #69313; Kenmore) was used.

A glycol sensor was installed in the freezer unit on the left side; this was secured to the wall of the freezer using adhesive mounting brackets and zip ties provided by the manufacturer. Second and third sensors were installed in 2 Styrofoam coolers measuring 9" wide by 12" long by 14" high, with a Styrofoam thickness of each wall and the lid of 1.25". The lids fit snugly on top of each cooler, but no latching system was used. Each cooler contained 1 ice block, measuring 5" wide by 7" long by 0.75" high; the sensors were upright in the corner of the cooler, with the ice block fitting on top of the sensor. Biological samples were next to the sensor and with the ice block also on top of the samples. The flat wires from the sensors were secured along the wall of the freezer using the adhesive mounting brackets and zip ties provided by the manufacturer to exit on the hinge side of the cooler and terminated at the control units mounted to the side of the freezer (Figure 1). Samples were placed in the coolers as needed, including PRP and fresh frozen plasma, and other supplies were accessed from the freezer as needed, generally multiple times daily. Staff could open the freezer as needed throughout testing, reflecting actual activity in a veterinary hospital where ice packs for patient care, sample transit, medications, and biological samples are stored in a multipurpose freezer that is opened multiple times daily by many different staff members.

**Statistical analysis**

The data from the freezer and the data from the coolers are referred to as “freezer,” “cooler 1,” and “cooler 2,” respectively, in the tables and figures, with the average of the latter 2 referred to as “cooler average.” The analyses focused on the temperatures for the coolers when the freezer temperature was ≥ 0°C. Changes from the previous assessment were computed as current−previous to provide information regarding the magnitude of the elevations, and differences between the freezer and cooler average were computed as freezer−average. Summary statistics (mean, SD, minimum, and maximum) are provided for all observations for freezer and cooler average and for the difference of each observation and the immediately preceding observation. Summary statistics are also provided for the values when the freezer temperature was ≥ 0°C. The summary statistics include the 95% CI for the mean difference of the paired comparisons between freezer and cooler average using the t distribution. Normality was tested using the Kolmogorov-Smirnov test for data when the freezer temperature was ≥ 0°C; testing for the normality for all data would not be meaningful due to the large sample size. All analyses were performed using SAS, version 9 (SAS Institute Inc).

**Results**

The remote monitoring software (SoniCloud; Sonicu) was used to export data for 6,525 data points for the freezer and 2 coolers over a 68-day period (Figure 2). Of the 6,525 temperatures recorded for the freezer, 102 were greater than or equal to −5°C, including 27 that were ≥ 0°C. These 27 occurred on 22 separate days (32%), with 17 days having one such elevation and 5 days each having 2. For the latter 5 days, 8 elevations represented separate instances on these days, and the other 2 elevated temperatures were contiguous. The separation between the 22 occurring on different days ranged from 12 hours to 10 days. On the day that
2 sequential readings were ≥ 0 °C, the freezer door was left ajar for approximately 30 minutes; the monitoring system then alerted administrative staff after the second reading above 0 °C, and the freezer was subsequently closed appropriately. No temperature for either cooler was greater than or equal to −5 °C.

Among all temperatures, the means of the temperatures for the freezer and the cooler average were −17.67 °C and −17.32 °C, respectively, with a mean difference of −0.34 °C and a 95% CI of −0.41 °C to −0.28 °C (Table 1). When freezer temperatures were ≥ 0 °C, the means of the temperatures for the freezer and the cooler average were 1.90 °C and −16.25 °C, respectively, with a mean difference of 18.15 °C and a 95% CI of 17.31 °C to 19.00 °C (Table 2). The CIs were computed using the SDs of the paired differences between the freezer and the cooler average, which were assumed to be normally distributed as supported by a P value exceeding .15 using the Kolmogorov-Smirnov test when freezer

### Table 1—Summary statistics for all temperatures.

<table>
<thead>
<tr>
<th></th>
<th>Freezer</th>
<th>Cooler average</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 6,525</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean temperature (°C)</td>
<td>−17.67</td>
<td>−17.32</td>
<td>−0.34</td>
</tr>
<tr>
<td>95% CI</td>
<td>−0.41, −0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>2.73</td>
<td>0.81</td>
<td>2.58</td>
</tr>
<tr>
<td>Minimum (°C)</td>
<td>−20.5</td>
<td>−24.30</td>
<td>−8.20</td>
</tr>
<tr>
<td>Maximum (°C)</td>
<td>7.0</td>
<td>−7.50</td>
<td>23.10</td>
</tr>
<tr>
<td>Difference from previous a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean temperature (°C)</td>
<td>−0.0004</td>
<td>−0.0016</td>
<td>0.0012</td>
</tr>
<tr>
<td>95% CI</td>
<td>−0.054, 0.057</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>2.32</td>
<td>0.14</td>
<td>2.29</td>
</tr>
<tr>
<td>Minimum (°C)</td>
<td>−9.5</td>
<td>−2.90</td>
<td>−9.65</td>
</tr>
<tr>
<td>Maximum (°C)</td>
<td>19.8</td>
<td>3.30</td>
<td>19.15</td>
</tr>
</tbody>
</table>

a N = 6,524 because the first temperature has no previous temperature.

Temperatures in a standard autodefrost freezer and in 2 Styrofoam coolers within the freezer were monitored every 15 minutes over 68 days. This table shows the summary statistics for all temperatures and changes from the previous assessment, showing that the temperature within the coolers had smaller-magnitude changes in temperature throughout the study.

### Table 2—Freezer temperatures ≥ 0 °C.

<table>
<thead>
<tr>
<th></th>
<th>Freezer</th>
<th>Cooler average</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean temperature (°C)</td>
<td>1.90</td>
<td>−16.25</td>
<td>18.15</td>
</tr>
<tr>
<td>95% CI</td>
<td>17.31, 19.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.78</td>
<td>1.61</td>
<td>2.13</td>
</tr>
<tr>
<td>Minimum (°C)</td>
<td>0</td>
<td>−17.70</td>
<td>13.45</td>
</tr>
<tr>
<td>Maximum (°C)</td>
<td>7.0</td>
<td>−10.85</td>
<td>23.10</td>
</tr>
<tr>
<td>Difference from previous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean temperature (°C)</td>
<td>14.76</td>
<td>0.46</td>
<td>14.30</td>
</tr>
<tr>
<td>95% CI</td>
<td>12.54, 16.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>4.44</td>
<td>0.17</td>
<td>4.44</td>
</tr>
<tr>
<td>Minimum (°C)</td>
<td>1.0</td>
<td>0.20</td>
<td>0.50</td>
</tr>
<tr>
<td>Maximum (°C)</td>
<td>19.8</td>
<td>0.80</td>
<td>19.15</td>
</tr>
</tbody>
</table>

Temperatures in a standard autodefrost freezer and in 2 Styrofoam coolers within the freezer were monitored every 15 minutes over 68 days. This table focuses on readings where the temperature in the freezer was ≥ 0 °C and compares them to the average temperature in the coolers at the same time, showing that the average temperature within the coolers did not elevate above 0 °C, which would prevent a freeze-thaw cycle.

Figure 3—Subset of temperature elevations in Figure 1; this line graph shows the longest sustained temperature in the freezer, being over 0 °C for more than 30 minutes. However, the temperature in either cooler never increased above −7 °C. Each circle indicates a single data point for the respective area (freezer, cooler 1, cooler 2).
temperatures were ≥ 0°C. For freezer temperatures ≥ 0°C, the maximum temperatures for the freezer and the cooler average were 7.0°C and −10.85°C, respectively, with the latter occurring when the temperature was 3.1°C for freezer and −13.2°C and −8.5°C for cooler 1 and cooler 2, respectively (Figure 3). The highest temperature reading from either cooler in the 2 hours after the maximum freezer temperature was −14.6°C for cooler 2, which occurred 15 minutes after the elevation in freezer temperature (Figure 4).

Figure 4—Subset of temperature elevations in Figure 1; this line graph shows the highest recorded temperature increase in the freezer, climbing to 7°C. However, the temperature in either cooler never increased above −14°C. Each circle indicates a single data point for the respective area (freezer, cooler 1, cooler 2).

The temperature probes and monitoring system were installed by the primary author in under an hour. She found them easy to install, and the online monitoring system was also simple to set up with customizable alerts for temperature fluctuations outside of the acceptable range. The emergency and rehabilitation staff reported that they were not in the way during their normal course of practice. Because the software system was easy to customize, temperature elevations above the alarm settings resulted in quick notification of these temperature swings as noted above. The remote monitoring system is expandable, and multiple contacts for alert can be added as well.

Discussion

These results show that autodefrost freezers exhibit temperature elevations frequently, which at times are ≥ 0°C. These elevations in temperature above 0°C would result in a freeze-thaw cycle for any biological samples, such as PRP, leading to potential degradation of the growth factors present in PRP. However, by using Styrofoam coolers within the freezer, this variation can be negated. In this study using 2 separate Styrofoam coolers, no such dramatic temperature elevations were seen, with all temperatures being less than −5°C. It is important to recognize that the freezer used in this study was in use in a busy emergency and rehabilitation hospital throughout the length of the study. Staff entered the freezer multiple times daily and removed biological samples and fresh frozen plasma directly from the Styrofoam coolers as needed in the course of their work caring for patients. Most of the times that the freezer was opened, it was closed without a long-lasting effect on the temperature. However, when the freezer door was left open, the freezer temperature stayed over 0°C for more than 30 minutes. Despite this prolonged elevation in temperature, the temperature in both coolers was less than 0°C and would not have resulted in thawing of the sample. This also supported our third objective in that quick notification of administrative staff occurred in a reasonable time to have on-site staff close the freezer that was mistakenly left ajar. Based on the experience of the first author and staff in installation and the reported results, the remote monitoring approach can give practice owners and hospital managers peace of mind that biological samples are appropriately frozen. These results are encouraging for small private practices, who may not have the resources to invest in a manual defrost freezer or the staff time to keep up with the maintenance of these freezers.

These results indicate that the addition of a Styrofoam freezer within an autodefrost freezer is a simple solution to maintaining a steady temperature for biological samples. Additionally, the thermometer system used is an effective additional control for monitoring temperatures for practices that would choose to invest.

While this study is encouraging, we did not measure the growth factors in the samples being stored. Though previous work showed that growth factors are present in PRP after being frozen for up to 6 months at −20°C, no studies have been published evaluating the presence of growth factors after repeated freeze-thaw cycles (more than 1). However, previous work has shown that some biochemical analytes undergo significant decreases after repeated freeze-thaw cycles. A study to measure growth factors at the time of collection, after being frozen for up to 3 to 6 months and after freeze-thaw cycles, would improve our understanding of the risk of freeze-thaw cycles.

This study confirmed that autodefrost freezers do experience significant temperature elevations, including elevations ≥ 0°C. However, the addition of a simple Styrofoam cooler significantly improves the temperature stability of any contained samples, avoiding any temperature elevations greater than or equal to −5°C. Additionally, remote temperature monitoring devices can be used to easily provide peace of mind and alerts of temperature rises in a busy practice environment.

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