A novel low-volume high-density system for sample storage

Stephanie M. Carter, Paul Upton, Cindy Neeley and Joseph Granchelli

Key Words
SampleSeal, Foil Seal, 384 format 0.1mL 2D Tube; Storage miniaturization, Screw Cap Tubes

Abstract
The Thermo Scientific™ SampleSeal™ instrument uses novel technology to create low volume 384 tube high-density sample arrays in which individual 0.1mL Thermo Scientific™ Matrix™ tubes are heat sealed with foil. In this study, tubes sealed with foil were monitored with thermocouples to ensure minimal heat effects on samples during sealing with the SampleSeal instrument. The seal integrity of 0.1mL Matrix tubes in comparison to standard screw cap storage tubes with silicone gaskets was evaluated for both short term and long term sample loss under normal usage, as well as under extreme application conditions. We were able to demonstrate minimal sample loss for the foil sealed tubes after one freeze-thaw cycle in liquid nitrogen* followed by 32 day storage at room temperature. The screw cap tubes resulted in some sample loss due to the evaporation of the sample through the silicone O-ring during room temperature storage. Both tube types were able to maintain seal integrity after storage in liquid nitrogen for eight weeks, as well as after extreme application conditions such as the application of partial vacuum after one freeze-thaw cycle in liquid nitrogen and after ten freeze-thaw cycles at -80°C. In order to ensure that both tube types meet and exceed regulatory requirements for air transportation, a thermal shock/pressure differential test at -40°C and +55°C was successfully performed. In conclusion, the 0.1mL Matrix tubes sealed with the SampleSeal instrument demonstrated either equivalent or improved performance in comparison to standard screw cap tubes with silicone gaskets and is a novel methodology to store low volume samples.

* Tubes should not be submerged directly in liquid nitrogen

Introduction
The Thermo Scientific SampleSeal instrument seals and individualizes 384-format 2D-barcoded storage tubes in a single process. The SampleSeal instrument creates low-volume high-density sample arrays suitable for short and long-term storage across a range of temperatures and is easily pierced with manual or automated pipette tips, supporting compatibility across standalone or integrated storage applications. Sealed 384-format tubes allow laboratories to maximize storage space when compared to lower-density formats. Single-access, 70μL working volume tubes protect sample integrity by eliminating multiple thaw and refreeze cycles needed when working with larger volume samples. The purpose of this study...
was to demonstrate the seal-integrity of 0.1mL Matrix tubes sealed with foil by the SampleSeal instrument in comparison to standard screw cap storage tubes with silicone gaskets for short term and long term sample loss under normal usage, as well as under extreme storage conditions. Both tube types were evaluated by a thermal shock/pressure differential test at -40°C and +55°C as per the requirements for air transportation set by regulatory agents (e.g. US Department of Transportation, International Civil Aviation Organization, and International Air Transport Association).

**Experimental details**

**Materials**
- SampleSeal 384-format Foil Heat Sealing and Cutting Instrument (Cat# 4220-384)
- SampleSeal 25μm Easy-pierce Foil (Cat# 4221-025)
- Thermo Scientific Matrix 0.1mL 384-format 2D Barcoded Tubes (Cat# 3815)
- 0.5mL standard screw cap tubes
- Thermo Scientific Multidrop Combi Reagent Dispenser (Cat# 5840300)
- Thermo Scientific standard bore tubing cartridge (Cat# 24072670)
- RS PTFE Type K thermo couples TM Electronics (Cat# 409-4908)
- USB data logger 8 channel Pico technologies USBTC08 + Picotech picolog software
- Thermo Scientific Matrix 8-Channel Decapper (Cat# 4105MAT)
- OHAUS Precision Standard Balance
- Thermo Scientific Revco ULTIMA Plus -80°C Freezer
- Thermo Scientific Locator 7
- Vacuum Apparatus

**Methods**

Heat Effects of Foil Seal on Samples Sealed with the SampleSeal Instrument

Thermocouple probes were placed in drilled holes on the side of individual 0.1mL Matrix tubes so they would sit just below the meniscus of the sample. Tubes with thermocouple probes were distributed in different areas of the sample rack to evaluate temperature differences due to location bias. Each tube in a full rack of 384 tubes was filled with 70µL of water containing food dye using a Multidrop Combi instrument with a standard bore tubing cartridge. The filled rack was then transferred to the SampleSeal instrument and the data logger was set to log measurements at 100 millisecond intervals from each probe. The log was started and data was collected as tubes were heat sealed with the SampleSeal instrument set to 158°C for 1.6 seconds.

Evaluation of Sample Integrity during Room Temperature Storage following Cryopreservation in Liquid Nitrogen

Samples were randomized into test racks and were visually inspected for defects in seal integrity and/or sample loss. Individual tubes were weighed on an analytical balance to determine the starting weight of the tube. Tubes were stored in liquid nitrogen (LN₂) for 24 hours ± 2 hours and were thawed for 24 hours ± 2 hours before weighing. Each of the 0.5mL screw cap tubes was filled with 350µL of water containing red dye and the 0.1mL foil sealed tubes filled with 70µL of water containing red dye were weighed on an analytical balance for 30 and 32 days, respectively. A visual inspection of tubes was performed at each time point.
Evaluation of Sample Integrity by Leakage Test after Short-term Liquid Nitrogen Storage

Sample racks containing 0.1mL foil sealed tubes filled with 70µL of water containing red dye or the 0.5mL screw cap tubes filled with 350µL water containing red dye were prepared. All tubes were visually inspected for defects in seal integrity and/or sample loss before LN$_2$ storage. Tubes were stored in LN$_2$ for 24 ± 2 hours and were thawed at room temperature for 24 ± 2 hours before weighing. Sample racks were vacuum tested by placing a paper towel on the sample tubes so that leakage of red dye could be visualized. The sample racks were placed in the vacuum chamber upside down using vacuum at 5 inHg for 30 minutes. Tubes that showed evidence of leaking red dye on the paper towel were considered failures.

Evaluation of Sample Integrity after Repeat Freeze-Thaw Cycles

0.1mL foil sealed tubes filled with 70µL of water containing red dye or the 0.5mL screw cap tubes filled with 350µL water containing red dye were prepared and placed at random into test racks. Individual tubes were weighed on an analytical balance to determine the starting weight of the tube and were visually inspected for defects in seal integrity and/or sample loss. Tubes were stored at -80°C and were allowed to thaw between 2 and 7 hours before weighing. Individual tubes were weighed on an analytical balance between each cycle for ten freeze-thaw cycles. A visual inspection of tubes was performed after each freeze-thaw cycle.

Relatively Long Term Liquid Nitrogen Storage

0.1mL foil sealed tubes filled with 70µL of water containing red dye or the 0.5mL screw cap tubes filled with 350µL water containing red dye were prepared and were placed at random into test racks (four racks per tube type were prepared). Individual tubes were weighed on an analytical balance to determine the starting weight of the tube and were visually inspected for defects in seal integrity and/or sample loss before storing in LN$_2$.

One rack was removed from LN$_2$ every 2 weeks over an 8 week time period. Tubes were thawed at room temperature and then weighed to determine sample loss during storage. A visual inspection of tubes was performed after each freeze-thaw cycle.

Thermal Shock and Pressure Differential (Vacuum) Test

Three tubes per tube type containing glycol were randomly selected for the thermal shock and pressure differential (vacuum) testing. Briefly, the samples were placed on their sides on a piece of blotting paper in a -40°C chamber for 2 hours. If after 2 hours no leakage was evident, a 28 inHg vacuum test was performed at -40°C for 30 minutes. Following the 30 minute vacuum test samples were evaluated for leakage. Immediately following thermal shock/pressure differential tests at -40°C, samples were placed on their sides on a piece of blotting paper in the +55°C chamber for 2 hours. If after 2 hours no leakage was evident, a 28 inHg vacuum test was performed at +55°C for 30 minutes. Following the 30 minute vacuum test samples were evaluated for leakage.

In order to pass the test (IATA PI 650), the primary receptacle or the secondary packaging must be capable of withstanding, without leakage, an internal pressure of 95 kPa in the range of -40°C to 55°C (-40°F to 130°F).

Data Analysis

When applicable, the data was organized into summary statistics and the average percent weight loss of each tube type for each test was calculated. Two sample t-tests assuming unequal variances were used to determine the significance of tube performance between foil sealed 0.1mL Matrix tubes and the 0.5mL standard screw cap tubes (p≤0.01).
Results and discussion

Minimal Heat Effects on Samples Sealed with the SampleSeal Instrument

During heat sealing by the SampleSeal instrument, sample temperature was monitored every 100 milliseconds with thermocouples inserted in the 0.1mL Matrix tubes in various locations in the sample rack (Fig. 1). As the ambient temperature slowly rose by about 1°C during the measurements, the small elevation in sample temperature (≤ 2°C) during heat sealing lasted for approximately 400 seconds before returning to ambient temperature with minimal effects to the sample despite tube location.

Evaluation of Sample Integrity during Room Temperature Storage following Cryopreservation in Liquid Nitrogen

Samples were examined by weighing individual tubes between 30 to 32 days at room temperature after thawing from liquid nitrogen cryopreservation (Fig. 2). The screw cap tubes demonstrated gradual sample loss up to 4% over the 30-day room temperature storage due to the evaporation of the sample through the silicone O-ring, whereas minimal sample loss was observed in the foil-sealed tubes by the SampleSeal instrument after 32 days. The foil-sealed 0.1mL Matrix storage tubes significantly outperformed the standard screw cap storage tubes with silicone gaskets during room temperature storage (p= 4.00E-10).

Evaluation of Sample Integrity by Leakage Test after Short Term Liquid Nitrogen Storage

Following liquid nitrogen storage, leak testing was performed using partial vacuum to examine the integrity of the foil seals on Matrix 0.1mL tubes containing water with red dye. Tubes that showed evidence of leaking red dye after the application of vacuum were considered failures. There were no failures observed for the tubes foil-sealed by the SampleSeal instrument (n=20 racks, total of 7680 tubes). There were 8 failures observed in the standard 0.5mL screw cap tubes (n=10 racks, total of 960 tubes) resulting in an approximate 0.8% failure rate (Fig. 3).
Evaluation of Sample Integrity during Repeat Freeze-Thaw Cycles

Closure seal integrity in the storage tubes was verified after ten freeze-thaw cycles at -80°C to simulate extreme application conditions. Both the Matrix 0.1mL tubes sealed by the SampleSeal instrument and the standard 0.5mL screw cap tubes demonstrated less than 1% sample loss after 10 freeze-thaw cycles (Fig. 4).

Evaluation of Sample Integrity during Long Term Liquid Nitrogen Storage

0.1mL Matrix tubes and 0.5mL screw cap tubes were weighed individually every 2 weeks following storage in liquid nitrogen over a total of 8 weeks. The Matrix 0.1mL foil sealed storage tubes remained intact through the 8-week storage in liquid nitrogen. The seal integrity was comparable between the heat sealed tubes and the 0.5mL standard screw cap tubes (p= 0.7841) (Fig. 5).

Thermal Shock and Pressure Differential (Vacuum) Test

In order to ensure that the storage tube types meet and exceed regulatory requirements for air transportation, a thermal shock/pressure differential test at -40°C and +55°C was performed (Table 1). As the primary receptacle for samples, both tube types were capable of withstanding, without leakage, an internal pressure of 95 kPa in the range of -40°C to 55°C (-40°F to 130°F) and are suitable for sample transportation by air and meet the requirements set by regulatory agents (e.g. US Department of Transportation, International Civil Aviation Organization, and International Air Transport Association).

<table>
<thead>
<tr>
<th>Test Sample Description</th>
<th>-40°C Thermal Shock/Pressure Differential Test</th>
<th>+55°C Thermal Shock/Pressure Differential Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thermal Shock Test (2 hours)</td>
<td>28 inHg Pressure Differential Test (30min)</td>
</tr>
<tr>
<td>Matrix 0.1mL tube with foil seal</td>
<td>Pass</td>
<td>Pass</td>
</tr>
<tr>
<td>Standard 0.5mL tube with screw cap/silicone gasket</td>
<td>Pass</td>
<td>Pass</td>
</tr>
</tbody>
</table>

Table 1. Thermal Shock and Pressure Differential (Vacuum) Test
Conclusions

- The SampleSeal instrument uses novel technology to create full sealing and individualization of tubes.
- The SampleSeal instrument creates low-volume high-density sample arrays suitable for short and long-term storage across a range of temperatures.
- The SampleSeal system demonstrates equivalent or improved seal integrity over standard systems, while providing valuable space saving potential.