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

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Introduction

Samples stored in biobanks give researchers and healthcare institutions access to potential datasets of analytes that can be used for both diagnostic and research purposes. The usefulness of these samples in clinical decision-making is highly dependent on their quality and integrity. New technologies that better preserve sample integrity are being developed to meet the requirements of both present and future biobanking needs. Here, we present the Thermo Scientific™ SampleSeal™ instrument, a novel system for sample storage that creates low volume 384 tube high-density sample arrays in which individual 0.1mL Thermo Scientific™ Matrix™ tubes are heat sealed with foil. To evaluate sample quality and seal integrity, 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 tube 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.

Methods

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 (LN_2) for 24 hours ± 2 hours and were thawed for 24 hours ± 2 hours before weighing. Each of the 0.5mL screw cap tubes filled with 350μL of water containing red dye and the 0.1mL foil sealed tubes filled with 70μL of water containing red dye was 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 hours ± 2 hours and were thawed at room temperature for 24 hours ± 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 5inHg 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 randomized 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 randomized 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 28inHg vacuum test was performed at -40ºC 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 +55ºC chamber for 2 hours. If after 2 hours no leakage was evident, a 28inHg vacuum test was performed at +55ºC 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 ($p \leq 0.01$).

Results

Minimal Heat Effects on Samples Sealed with the SampleSeal Instrument

To monitor sample temperature during heat sealing by 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 384-tube sample rack to evaluate temperature differences due to location bias. Sample temperature was monitored every 100 milliseconds during heat sealing. 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 (data not shown).

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

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) (Fig. 2).

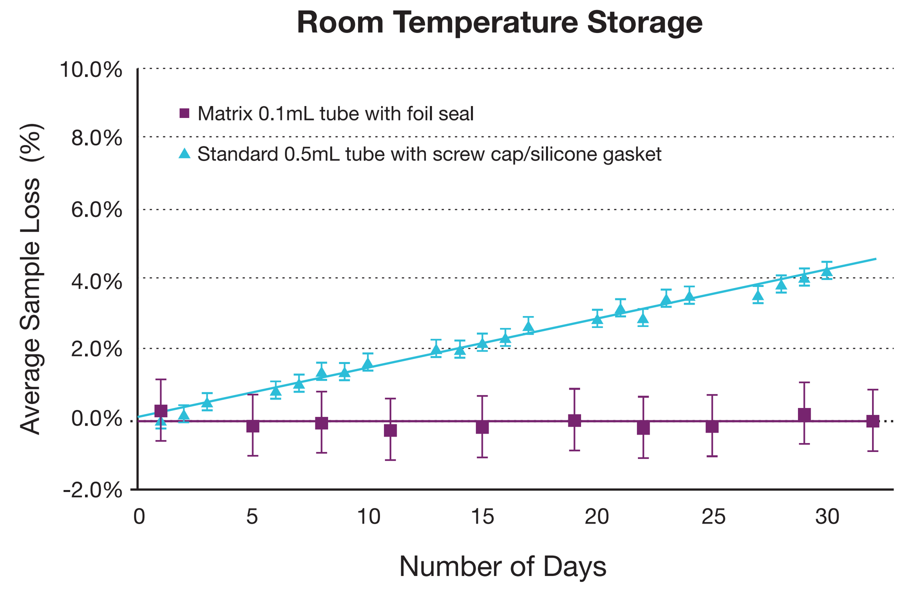

FIGURE 2. Sample integrity during room temperature storage after thawing from liquid nitrogen.

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

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).

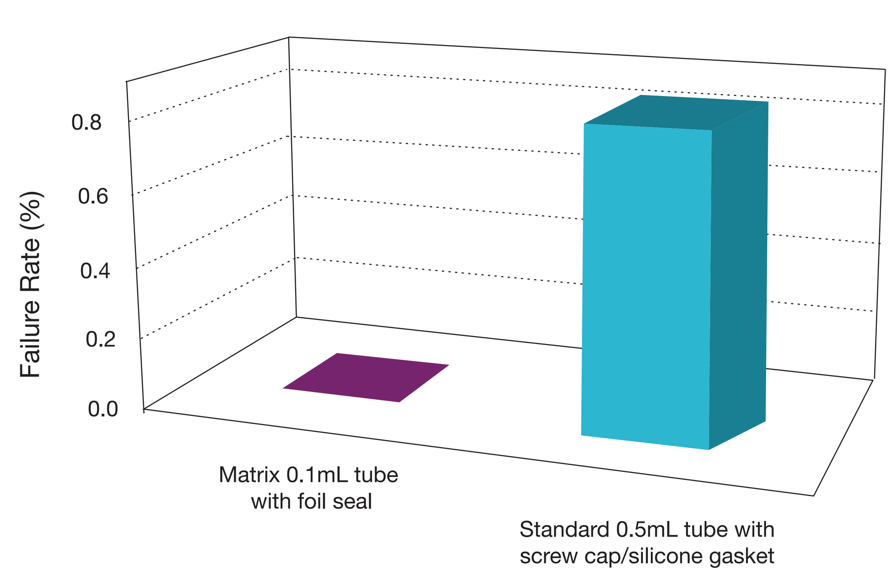

FIGURE 3. Leakage test following liquid nitrogen storage.

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).

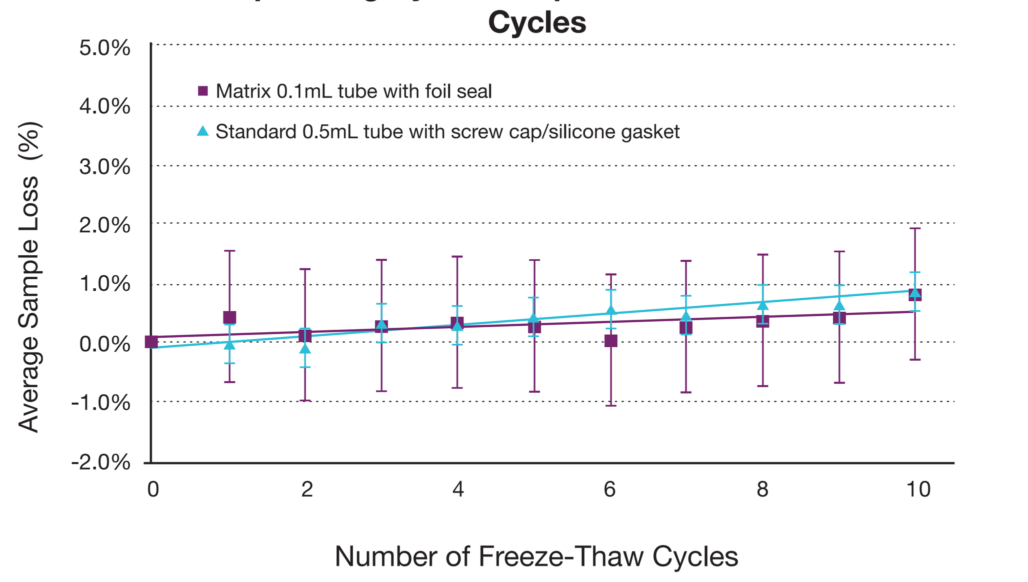

FIGURE 4. Evaluation of closure seal-integrity after 10 freeze and thaw cycles at -80°C.

Evaluation of Sample Integrity during Relatively Long Term Liquid Nitrogen Storage

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).

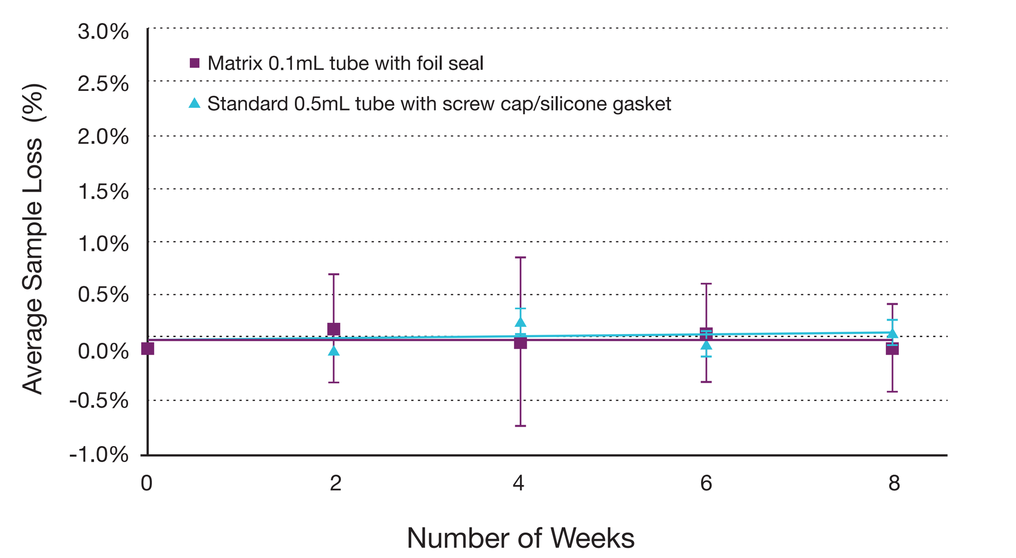

FIGURE 5. Sample Integrity over relatively long term storage in liquid nitrogen.

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 are 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).

	-40°C Thermal Shock/Pressure Differential Test		+55°C Thermal Shock/Pressure Differential Test	
Test Sample Description	Thermal Shock Test (2 hours)	28 In. Hg Pressure Differential Test (30min)	Thermal Shock Test (2 hours)	28 In. Hg Pressure Differential Test (30min)
Matrix 0.1mL tube with foil seal	Pass	Pass	Pass	Pass
Standard 0.5mL tube with screw cap/silicone gasket	Pass	Pass	Pass	Pass

TABLE 1. Thermal Shock and Pressure Differential (Vacuum) Test

Conclusions

- **The SampleSeal instrument uses novel technology to create full sealing and individualization of tubes and better preserves sample integrity to meet the requirements of both present and future biobanking needs.**
- **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 under normal usage, as well as under extreme application conditions.**