APPLICATION NOTE

Container Closure Integrity of Product Requiring Deep Cold Storage at -80°C.
INTRODUCTION
Live viral vaccines and gene and cell-based therapies sometimes require deep cold storage temperatures (-80°C down to cryogenic, -196°C) to maintain the activity and efficacy of the formulations. Studies have shown that these deep cold storage temperatures can introduce risk to the sealing performance of the packaging components. In particular, studies have shown that the low storage temperatures can introduce risk to the container closure integrity (CCI) of vial/rubber stopper combinations traditionally used to fill sterile pharmaceutical product [1]. It is therefore critical that robust development work is done in a holistic framework to choose the appropriate packaging components and to qualify the sealing process such that the risk of CCI issues during deep cold storage and transport is minimized.
EFFECTS OF DEEP COLD STORAGE ON VIAL SEALING

Commonly used rubber butyl stoppers lose their elastic properties during deep cold storage as a result of their glass transition temperature ($T_g$), which lies between -55°C and -70°C. In a range of temperatures around the $T_g$, the rubber stopper becomes brittle. In addition, the primary packaging components, including the glass vial, rubber stopper, and metal crimp, shrink at varying rates due to their different, material dependent, rates of thermal expansion which can lead to gaps at the material interfaces. Figure 1 displays an x-ray tomography image revealing shrinkage of a rubber stopper of roughly 8.5% by volume at cryogenic conditions. Most of this shrinkage occurs at temperatures warmer than the $T_g$. By contrast, the shrinkage by volume of a borosilicate glass vial will be an order of magnitude less. When the rubber stopper loses its elastic properties and if gaps appear between the sealing surfaces due to material shrinkage, there is a risk that seal integrity could be lost.

If CCI is lost during -80°C storage, non-sterile, cold, dense gas from the storage environment (i.e. air from a -80°C freezer or carbon dioxide from dry ice) can leak into the stored vial. When the leaking vial is taken out of cold storage and warms up to a temperature above the $T_g$, the stopper regains its elastic properties, the packaging components expand to their original forms, and the sample can reseal, potentially trapping the cold dense gas from the storage environment inside. This temporary leak could risk the stability as well as sterility of the pharmaceutical product and would not be detected by traditional CCI test methods.

The work done so far investigating CCI of stoppered vials at -80°C has identified the following critical areas for ensuring CCI at deep cold storage temperatures:

- The choice of appropriate packaging component combinations taking careful consideration of worst-case fits and stack tolerance.
- Qualification of the capping & crimping process to produce robust consistent stopper compression for sealing.

![Figure 1. An x-ray tomography image showing shrinkage of a rubber stopper at cryogenic conditions. The blue line shows the form of the stopper at room temperature.](image-url)
In order to quantify the risk of -80°C storage to the CCI of stoppered vials, CCI test methods are required that can generate robust science-based data during development studies. Over the past few years, a significant amount of data has been generated for deep cold storage product using CCI test methods based on headspace gas analysis. Most of this work has been performed in development to support the choice of appropriate packaging components and to qualify the sealing process. There are also examples of 100% CCI testing of GMP product [2].

Laser-based headspace analysis as a technique for CCI testing has several advantages including:

- The measurement is non-destructive meaning samples that have been analyzed for CCI can be kept for other purposes.
- The measurement is rapid; a measurement time of a few seconds allows for data to be generated in a straightforward manner on hundreds (or thousands) of vials to gain statistical insight into the sealing performance as a function of various parameters.
- Product vials can immediately be measured upon removal from the cold storage environment; no thawing is required.
Using headspace analysis for CCI testing leverages the fact that a leaking vial will exchange gas with the surrounding storage environment. When the vials are initially stored at -80°C from room temperature, a partial vacuum within the headspace develops due to the decrease in temperature. If a leak is present, the atmosphere of the storage environment will flow into the vial headspace. When the leaking vial is brought out of cold storage, the vial can reseal itself trapping cold dense gas inside. As the vial continues to warm up, the trapped gas expands creating an overpressure in the vial. This type of leakage during deep cold storage can drastically change the initial vial headspace conditions resulting in various measurable conditions depending on the storage environment and the defined headspace CCI test method. The graphs in Figure 2 demonstrate how headspace measurements identify three vials that have lost CCI while being stored on dry ice. A temporary loss of CCI while on dry ice resulted in drastic changes of the vial headspace composition. An ingress of cold dense CO₂ gas from the storage environment into the leaking vials replaced the initial vial air headspace and, as the vial warmed up and resealed, caused an overpressure in the vial.

**Figure 2.** Headspace measurement results of media vials stored on dry ice. The measurements identified three vials that had leaked during the deep cold storage, revealing elevated CO₂ levels, depleted oxygen levels, and an overpressure.
RESIDUAL SEAL FORCE FOR QUALIFYING CAPPING AND CRIMPING TO PRODUCE GOOD SEALING AT -80°C

To seal a stoppered vial, an applied stress (sealing force) deforms or compresses the elastomeric stopper against the container sealing surface. This induces a corresponding strain in the stopper, creating a contact stress at the vial/stopper interface. The strain is “locked-in” by applying and crimping an aluminum ferrule over the stopper. This locked-in compression, or stored internal energy, is known as the Residual Seal Force (RSF). RSF is the stress a compressed rubber stopper flange continues to exert on the vial’s sealing surface after crimping the aluminum seal and is a measure of vial seal tightness. By correlating RSF measurements to stopper compression and CCI testing during packaging development, they may be used as a predictor of the risk to container closure seal integrity. In particular, studies have shown RSF measurements to be a suitable qualitative test method to evaluate seal quality in deep cold storage applications [3]. Figure 3 summarizes the results of a packaging development study correlating RSF to the rubber stopper compression achieved by varying the capping and crimping settings.

As shown in Figure 3, RSF testing can be used to characterize the seal quality of capped vials through a correlation with stopper compression. These values can be used to establish the optimal cap sealing process parameters for various types of capping equipment, which can then facilitate the comparison and consistency of seal quality of sealed vials manufactured on different equipment in different facilities [4]. The data in Figure 4 demonstrates how RSF testing enabled the characterization, optimization, and eventual qualification of a capping and crimping line. Initial RSF testing showed the capping and crimping line produced vials with relatively low RSF values (~35 N) and a large vial-to-vial variability. Optimizing the capping and crimping parameters improved the line performance such that vials were being produced with higher RSF values in a more consistent range of values.

As mentioned earlier, studies have shown that robust capping and crimping can be critical for product vials to maintain CCI during deep cold storage. In addition to conducting RSF correlation studies with stopper compression, studies can be done to investigate the
Studies have shown that deep cold storage at temperatures around -80°C can introduce risk to the CCI of stoppered vials. Effects of the low temperature on the different primary packaging materials can cause a temporary loss of CCI during the deep cold storage. Identification of appropriate primary packaging components and the qualification of a robust capping and crimping process can lower the risk to CCI. A holistic science-based approach involves executing packaging and process development studies to generate robust data demonstrating the consistent production of vials that maintain good CCI during deep cold storage and transport.

**SUMMARY**

Studies have shown that deep cold storage at temperatures around -80°C can introduce risk to the CCI of stoppered vials. Effects of the low temperature on the different primary packaging materials can cause a temporary loss of CCI during the deep cold storage. Identification of appropriate primary packaging components and the qualification of a robust capping and crimping process can lower the risk to CCI. A holistic science-based approach involves executing packaging and process development studies to generate robust data demonstrating the consistent production of vials that maintain good CCI during deep cold storage and transport.

**Figure 4.** Results showing how RSF testing can enable the optimization of capping and crimping parameters to consistently produce tightly sealed vials.

**Figure 5.** RSF vs CCI correlation data showing that low RSF values correlate to increased risk of CCI issues.

correlation between RSF and CCI. Figure 5 shows the results of a study performed on vaccine product vials that were stored at -80°C. The results of non-destructive CCI testing using headspace analysis were correlated to RSF measurements made on the same product vials. The red data points represent product vials that were rejected as having lost CCI during cold storage according to a leak limit defined as part of a headspace CCI test method, while green data points represent product vials accepted as having maintained good CCI. The results in Figure 5 show that low RSF values correlated to an increased risk for losing CCI. As part of a holistic approach for lowering the risk to CCI during deep cold storage, the manufacturer initiated a program in which the capping and crimping lines where optimized and qualified using RSF measurements, and finished product was CCI tested using headspace analysis as part of in-process control activities.
REFERENCES


2. Presentation ‘Ensuring container closure integrity of a gene therapy cancer vaccine needing deep cold storage’, Josine Wilmer, 2019 PDA Parenteral Packaging Conference, Venice, Italy

3. Presentation ‘Correlating Vial Seal Tightness to Container Closure Integrity at Various Storage Temperatures’, Derek Duncan and Roger Asselta, 2015 PDA Parenteral Packaging Conference, Frankfurt, Germany