Ensuring Container Closure Integrity of a Gene Therapy Cancer Vaccine Needing Deep Cold Storage\textsuperscript{1}. 

\textsuperscript{1}This case study is based on a presentation given at the 2019 PDA Parenteral Packaging Conference, Venice, Italy.
Introduction:

The following case study describes how a gene therapy manufacturer handled a possible Container Closure Integrity (CCI) issue due to deep cold storage at -80°C. The product was in clinical trial when the manufacturer discovered potential breaches in CCI. The identified issue occurred when a product vial from a stability study was punctured with a syringe and apparent overpressure in the vial moved the syringe plunger upwards. Referral to previous work involving a live viral vaccine stored at -80°C lead to investigation of CCI [1]. Scheduled clinical trial was halted and product batches were put under quarantine. Discussions with the national regulator were started and a root cause analysis was initiated with the objective to characterize the problem and propose corrective actions. The regulator would approve restart of the trial once suitable corrective actions were implemented.

Study Objective:

The objectives for this study are summarized as follows:
1) Investigate if a fundamental CCI issue exists with the specific vial-stopper combination at deep cold storage.
2) Demonstrate the feasibility of using Headspace CO₂ Analysis as a basis for CCI testing.
3) Perform Residual Seal Force (RSF) testing to gain insight into the sealing process quality and capping/crimping process.

An extensive amount of development studies were performed, including method development for CCI testing at cold temperatures. In this particular case, the manufacturer required all products to be stored in a GMP cold storage environment and product samples were not allowed to thaw during CCI testing. Critical CCI testing requirements were that the test be non-destructive and that the sample excursion time from deep cold storage should remain below 60 seconds.
Headspace Carbon Dioxide Analysis:

Several product batches were made available for headspace analysis as part of initial testing: Batch 1 (engineering batch), Batch 2 (GMP batch used in clinical trial), Batch 3 and 4 (GMP batches prepared for use in clinical trial). In total, 986 product samples stored at -80°C were selected and transferred to dry ice for more than 120 hours. In addition, positive control samples were added to the -80°C storage and were transferred to dry ice together with the product samples. The positive controls consisted of empty stoppered vials with a 10 micron capillary inserted in the robber stopper. After storage on dry ice, headspace CO₂ analysis was performed. A leaking vial was identified by the detection of CO₂ ingress into the container. For each measurement, samples were transferred from dry ice for less than 60 seconds.

Figure 1 gives an overview of headspace CO₂ levels in all batches. Batch 4 (bottom graph), the most recently manufactured batch, had only slightly elevated headspace CO₂ levels when compared to the other three batches (top graph).
Residual Seal Force Testing:

RSF testing was performed as part of a root cause analysis to investigate the potential correlation of sealing quality (capping & crimping) to the CCI results determined by using headspace analysis. The RSF measurement is associated with the stopper compression force, and can therefore be used to quantitatively characterize seal quality. Figure 2 compares RSF measurements of product samples in the different batches, Batches 1 and 2 had lower RSF values than Batches 3 and 4 suggesting that an inconsistent capping and crimping process, resulting in low RSF values, correlates to an increased risk to CCI during deep cold storage.

The red data points in Figure 2 belong to product samples that had been identified as leaking and were rejected by the CCI test. The green data points belong to vials that did not leak. These results show a clear trend: leaking vials tend to have low RSF values suggesting that vials which are more loosely crimped have a higher risk to lose CCI at deep cold storage temperatures.

*Figure 2: Residual Seal Force measurements of Batches 1-4. Red data points indicate rejected (leaking vials), green data points indicate accepted (non-leaking) vials.*
Corrective Actions:

By performing both headspace analysis and RSF testing, it was concluded that these product batches were suffering from (temporary) leaking vials due to the -80°C storage as well as variation in the capping and crimping process. Two options were defined for potential corrective actions:

Option 1 – Adjust the storage temperature conditions to a warmer temperature. In this case, the manufacturer already had stability data for storage at -20°C for up to one year.

Option 2 – Adjust and/or improve the packaging components.

Additional CCI studies performed with the packaging components at -20°C revealed no breaches in CCI at this temperature. It was therefore decided to increase the storage temperature to -20°C and focus on generating more stability data. Furthermore, additional RSF studies were performed to further optimize and qualify the capping and crimping process.

In addition to these corrective actions, an additional issue needed to be addressed. Product vials were still temporarily exposed to deep cold temperatures due to a snap freezing step after capping and crimping in the production process (see Figure 3). Lighthouse supported the manufacturer with performing additional CCI studies which demonstrated that any vials losing CCI in the snap freezing step could be detected with a headspace CO₂ ingress measurement. The manufacturer therefore implemented an in-process control test performing 100% CCI testing after the quick-freezing step and before storage at -20°C.

Figure 3: Overview of the final production process, including an In-Process Control CCI test using Headspace CO₂ Analysis (red) after the dry ice snap freezing step.
Case Study Conclusions:

A gene therapy manufacturer identified a potential CCI issue in product vials needing deep cold storage at -80°C. A CCI test method was developed that enabled non-destructive CCI testing of product vials at these cold temperatures. The capability to non-destructively test clinical product in storage enabled troubleshooting studies. These studies confirmed product vials could temporarily lose CCI during deep cold storage. The vials resealed at room temperature resulting in an overpressure in the stoppered vial observed during administration. A root cause investigation showed that the risk of losing CCI correlated to low stopper compression and the quality of the capping and crimping process as measured by RSF. The corrective actions included changing the product storage temperature to -20°C, improving the capping and crimping process based on RSF characterization, and implementing a 100% IPC CCI test. The corrective actions were approved by the regulator allowing the manufacturer to rapidly restart clinical trials (Figure 4).

![Figure 4: Case study timeline.](image)

Reference


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