WHITE PAPER

mRNA therapies on the rise

Challenges for primary packaging and how to test for best performance

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mRNA, key indications, future applications and market overview

Covid-19 provided the breakthrough for messenger RNA (mRNA) technology platforms, and prophylactic vaccines are now the key indications set to dominate this market for years to come.

Vaccines for respiratory infections caused by viruses include the well-known Influenza Virus, but also the Respiratory Syncytial Virus (RSV) and, last but not least, Corona Viruses like SARS-CoV-2 but also other versions as SARS-CoV-1 which emerged in 2002.
Except for the loss of taste and smell, the symptoms are often similar and, in addition, dual infections are more common than believed.

The future application of mRNA technology in prophylactic vaccines is likely to be a combined vaccine protecting against the flu and SARS viruses or even including protection against RSV as per the Nature article "mRNA vaccines and treatments: beyond COVID-19"[1].

Future applications may also include vaccinations against HIV / AIDS. Studies have shown meaningful potential. However, the genetic variability of HIV is very high. Nevertheless, the National Institutes of Health advised on March 14th 2022 the launch of a Phase 1 clinical trial evaluating three experimental HIV vaccines based on a mRNA platform[2].

Another future application is cancer. Most of the current developments are for personalized treatments or focused on single cancers as published by Miao L. et al in their paper "mRNA vaccine for cancer immunotherapy" [3].

The mRNA pipeline with 171 drug candidates in other therapeutic areas including Influenza, HIV/AIDS, RVS and Cancer, as shown in Figure 01, is promising and it can be expected that the market will be back to Covid-19 pandemic level in about ten years. As a consequence, the demand for deep-freeze primary packaging solutions will remain and even increase.

The key route of administration is intramuscular (IM) as shown in Figure 02. Contrary to Covid-19 pandemic vaccines, the preferred primary packaging solution is expected to, at least partially, transition from multi-dose vials to single-dose syringes.

Especially for prophylactic vaccines this can also be explained by the fact that most flu vaccines are filled in ready-to-fill (RTF) glass syringes.

For the Covid-19 vaccines, Time to Market was the crucial factor for deciding on multi-dose vials. When it comes to endemic diseases, the focus is on Time, Ease of Use and Waste Reduction.

Taking Pfizer BionTech Cormirnaty as an example, the theoretical dead volume per vial, calculated from the Professional Information[6], is 0.24 mL taking into consideration the dead volume per plastic syringe used for administration of 0.035 mL.

Using a 1mL short glass syringe with a 25G 5/8" needle, as standard for flu vaccines, the dead volume is around 0.01 mL [7].

Considering the six doses which theoretically can be drawn from a vial, the transition to syringes represents a significant saving of more than 20% in drug product required.

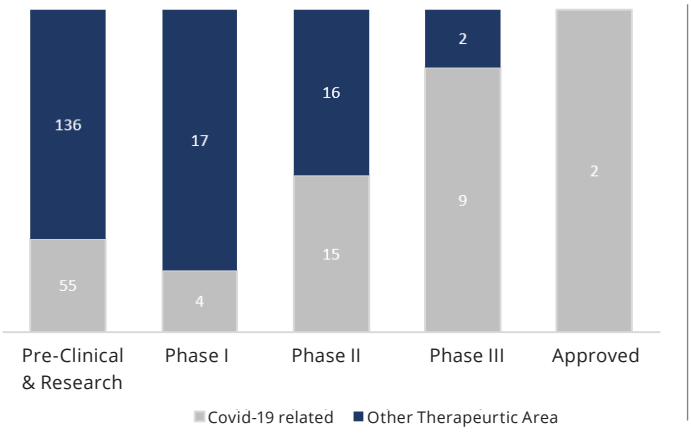


Figure 01:
Pipeline of mRNA drugs[5]

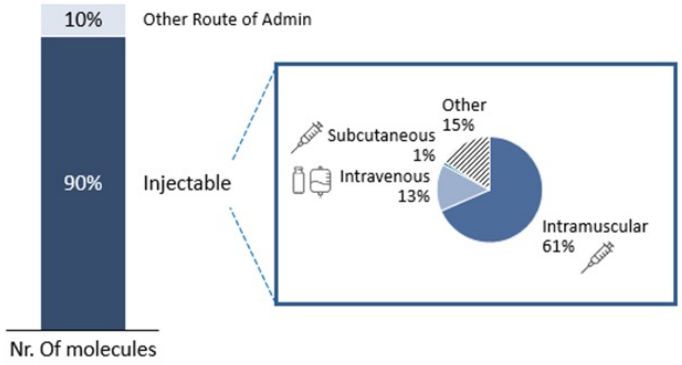
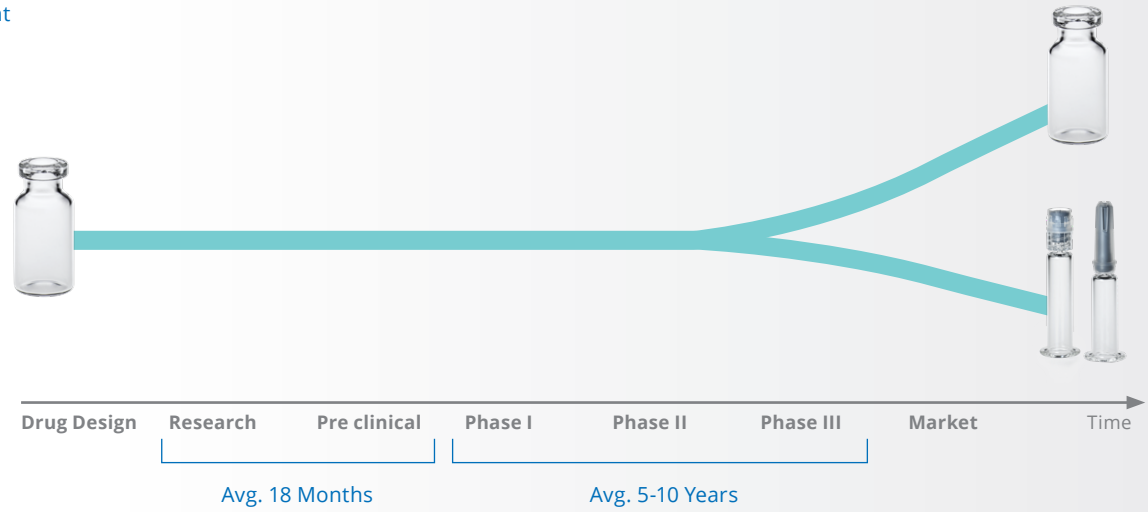


Figure 02:
Key route of administration of mRNA drugs in pipeline[5]

References
1. Nature Publishing Group. (n.d.). mRNA vaccines and treatments: beyond COVID-19. Nature news. Retrieved June 30, 2022, from https://www.nature.com/articles/d42473-022-00132-y - 2. U.S. Department of Health and Human Services. (2022, March 14). NIH launches clinical trial of three mRNA HIV vaccines. National Institutes of Health. Retrieved June 30, 2022, from https://www.nih.gov/news-events/news-releases/nih-launches-clinical-trial-three-mrna-hiv-vaccines - 3. Miao, L., Zhang, Y., & Huang, L. (2021, February 25). MRNA vaccine for cancer immunotherapy - molecular cancer. BioMed Central. Retrieved June 30, 2022, from https://doi.org/10.1186/s12943-021-01335-5 - 4. Data from Globaldata, March 2022, Nr. of Molecules - 5. Data from Globaldata, March 2022, Key Route of Administration of mRNA Drugs in Pipeline - 6. Pfizer. (n.d.). Professional Information Comirnaty covid-19 vaccine Inj Konz. compendium.ch. Retrieved June 30, 2022, from https://compendium.ch/product/1462602-comirnaty-covid-19-vaccine-inj-konz/mpro# - 7. Stevanato Group, Internal SG Study Report, "Evaluation of performance requirements of prefilled syringes according to ISO 11040- 8:2016 paragraphs 6 and 7.5", May 2021

Figure 03:
Roadmap of vaccine development



Challenges

The advantages of mRNA, such as easiness to adapt to new viruses, scalability, safe immune response and not involving infectious elements, outnumber the challenges.

mRNA vaccine and therapeutics scale-up requires new manufacturing techniques and is based on multiple technology platforms. Plasmids (pDNA) are the key raw material and the mRNA molecule is produced following a complex enzymatic in vitro transcription (IVT) process. After purification and encapsulation, deep-freeze cold storage is required.

Current developments include the optimization of the mRNA molecule to allow for proper drug delivery and, over time, deep-freeze storage conditions may come up to more standard levels. In addition, there will be other potential forms of delivery, such as lyophilized drug products.

As shown in Figure 03 above, the roadmap will potentially lead to the use of prefilled syringes adding additional challenges in meeting the Container Closure Integrity (CCI) requirements in deep freeze conditions.

CCI is one of the biggest challenges to overcome as syringes are far more complex than vials. The impact of deep-freezing temperatures on CCI and also on the functionality of the syringes are key elements to be verified.

Stevanato Group developed specific testing methods to evaluate CCI of prefilled syringes at -20°C and -70°C to assess the suitability of EZ-fill® glass syringes for applications requiring such storage conditions as standard test methods for CCI cannot be used under deep-freeze conditions.

Tests and solutions to overcome the challenges

For CCI two techniques were used, the mass extraction (ME) method (Test 1.1) and head space analysis (Test 1.2). In both tests, adapted for testing under deep-freeze conditions, empty and water-filled samples were tested.

In addition, break-loose and gliding force (Test 2), as well as burst testing (Test 3) were performed, along with an unscrew and opening test of the Integrated Tip Cap (ITC) closure (Test 4).

An additional aspect, plunger movement, was also tested – and motion can be observed for empty syringes but not for filled syringes, likely due to the difference in compressible air volume.

This leads to the recommendation to either adopt an airless filling process or, if not possible, to keep the amount of air between drug solution and stopper to a minimum.

These key questions were also discussed by Marco Povolo and Alan Xu of Stevanato Group in their paper “Influence of Freezing Storage Condition on Glass Syringe Performance – Two Methods Investigating Container Closure Integrity of 1ml Syringes” presented at the PDA Annual Meeting 2022 in Dallas Texas[8].

References:

8. Marco Povolo, Alan Xu, Stevanato Group, PDA Annual Meeting 2022 Presentation, Dallas Texas “Influence of Freezing Storage Condition on Glass Syringe Performance – Two Methods Investigating Container Closure Integrity of 1ml Syringes”

1. Container Closure Integrity Testing

Two methods were used for testing CCI. First the Mass Extraction (ME) method in which test samples are maintained at -20° C. This test was developed with Pfeiffer Vacuum, Inc. Secondly a Laser Headspace (LH) analysis on samples after a -70°C freeze/thaw cycle.

1.1 Mass Extraction

The ME method is carried out under vacuum. During the test, the sample is placed in a vacuum chamber and the chamber is evacuated. The leakage rate of the test unit is determined by the flow (leak) from the sample (test unit) to the vacuum reservoir. The test was performed at a temperature of -20°C with empty and water-filled samples, including samples with a 5µ leak.

The ME method is able to differentiate between positive and negative controls for empty syringes (Figure 04). Empty syringes show a worst-case scenario compared to a frozen liquid which could occlude the positive control holes. In addition, air is in contact with all surfaces that may leak.

TEST METHOD: Mass Extraction
Positive controls
1ml ITC syringes

RESULTS: Vacuum decay flow (µg/min)

SAMPLE PREPARATION: Positive control samples (with 5µ leak artifacts)
Non-defective syringes "Test Samples" (without artifacts)

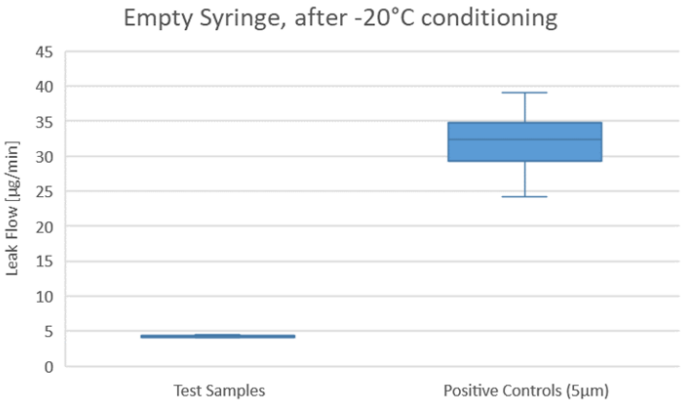


Figure 04: Modified Mass Extraction (at -20°C)

1.2 Headspace Analyis

Laser Headspace Analysis is performed on samples after being conditioned at -70° C in a CO_2 rich environment measuring the ingress of CO_2 into water-filled syringes, including positive test control samples. No detection of CO_2 has been detected in all tested samples.

Certain CO_2 ingress was detected in the filled positive controls (Figure 06a), likely due to the smaller headspace and potential dissolution of CO_2 in the water. Total test time must be taken into consideration to ensure positive controls still have CO_2 after measuring test samples.

TEST METHOD: Headspace gas analysis (CO_2)
1ml ITC positive control samples
1ml ITC syringes – 30 samples for each test condition

RESULTS: CO_2 pressure (mbar)
7 days at -70°C in rich CO_2 environment.

SAMPLE PREPARATION: Sample: water-filled

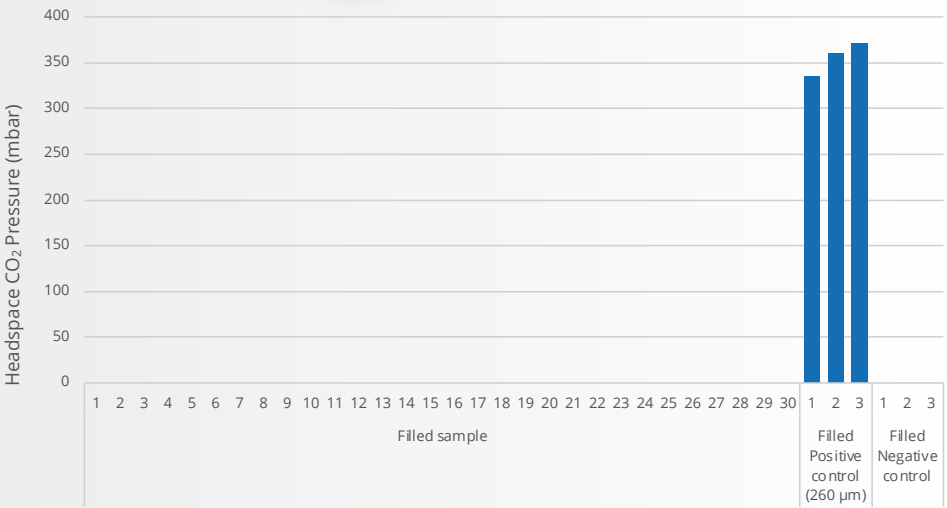


Figure 06a: CCIT result water-filled syringes

2. Break-Loose and Gliding Test

The goal of this test was to measure the influence of deep freezing on 1 ml ITC syringes with regard to the gliding force of empty syringe barrels and to assess the quality and consistency of silicone oil lubrication within the inner syringe barrel. The tests were performed using a load frame to determine the Break-Loose force and Gliding Force on samples conditioned for seven days at the respective temperature.

In accordance to ISO 11040-4:2015, Break-Loose and Gliding Force tests were carried out on filled EZ-fill® ITC glass syringes after being stored at the respective temperature for seven days. They are showing excellent Break Loose Forces even slightly lower at -20° C and -40°C respectively (Figure 08a) and a Gliding Force performance with comparable results before and after freezing storage (Figure 08b).

TEST METHOD:	ISO 11040-4:2015 Annex E Syringes 1mL ITC - 30 samples per temperature range
RESULTS:	Break-Loose Force Mean Gliding Force
SAMPLE PREPARATION:	Sample 1: 7 days at 20°C Sample 2: 7 days at -20°C Sample 3: 7 days at -40°C

Figure 08a:
Break-Loose

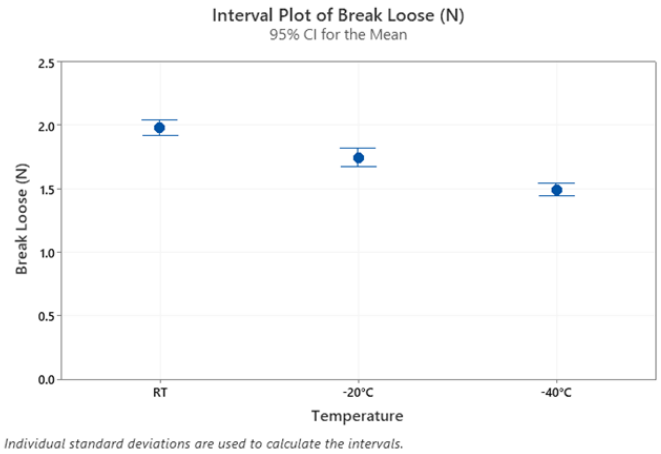
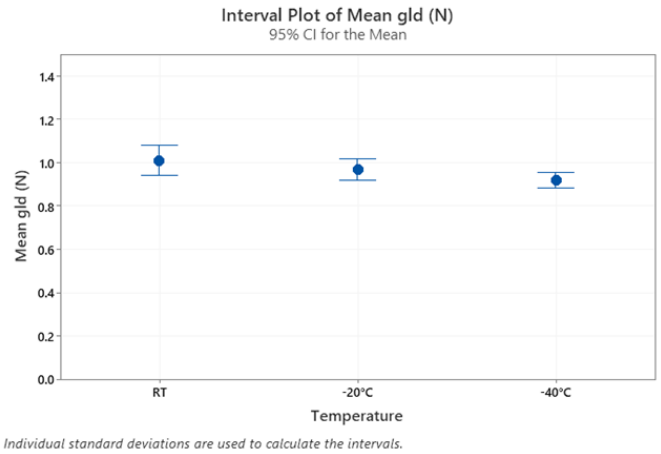


Figure 08b: Mean
Gliding Force



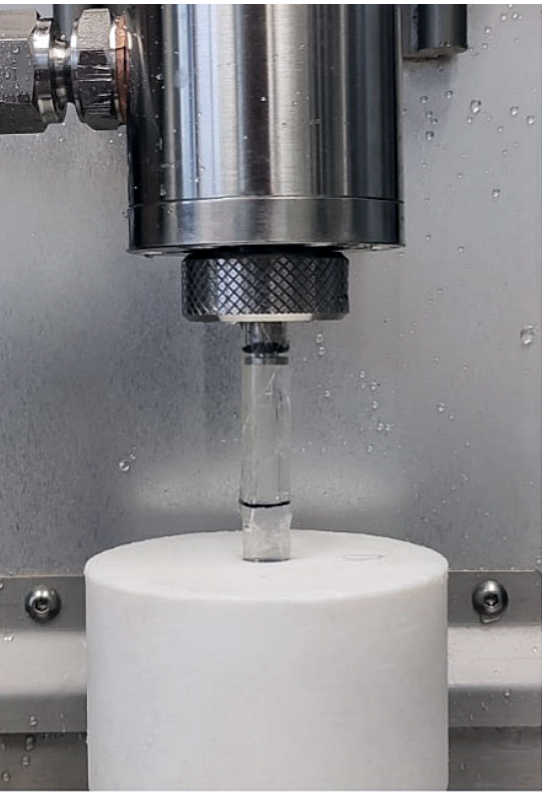


Figure 10:
Destructive Burst test

3. Mechanical Burst Test

Burst Test evaluation was made to understand the influence of -40°C storage temperature on the mechanical performance (Maximum Burst Resistance) of 1 ml ITC syringes. The burst test is performed using a special fixture developed by Stevanato Group. As shown in Figure 10, the test is destructive.

The Burst test was performed on glass ITC syringes according to ISO 7458:2004. Results did not show a statistically relevant difference in glass barrel resistance between -40°C and room temperature storage conditions.

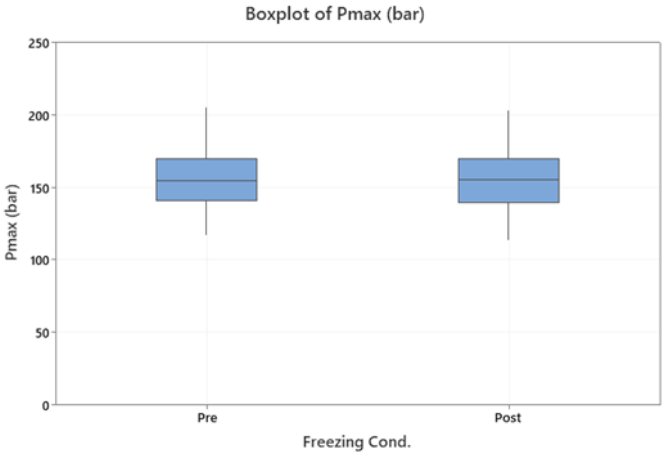
The test results show freezing storage did not impact mechanical resistance (Burst Test) of the glass syringes at all.

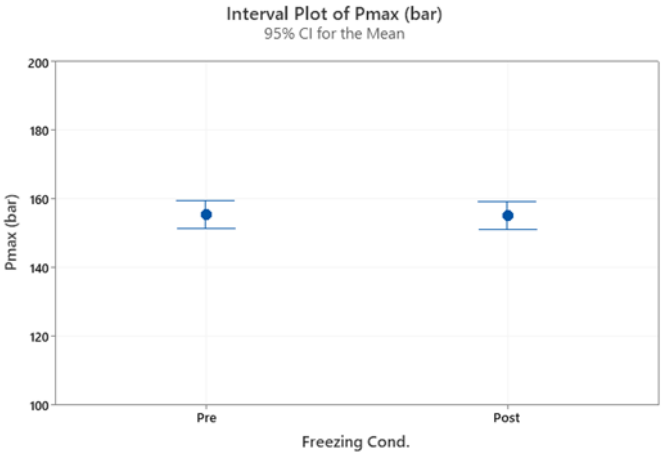
TEST METHOD: ISO 7458:2004, Method B
Syringes 1mL ITC - 100 samples per temperature range

RESULTS: Maximum internal pressure resistance (bar)

SAMPLE PREPARATION: Sample 1: Pre - 7 days at +20°C
Sample 2: Post - filled and stored 7 days at -40°C
Figure 08: Mechanical Test Scheme

Figure 09:
Boxplot and Interval Plot of the maximum internal pressure resistance





4. ITC Unscrewing and Opening

A test was performed to assess the influence of a freezing cycle on the torque and opening force of the rigid tip cap ITC of a sterilized sub-assembled syringe ready for filling. The test is carried out in a load frame using a fixture in which the test sample is placed, with a load capable of measuring a torque.

Test results show freezing storage did not impact the Opening Force and had a slight influence on torque values.

TEST METHOD: ISO 11040-4:2015 Annex G5
Syringes 1mL ITC - 50 samples per category
Unscrew of 100° at 500°/min, opening 400 mm/min

RESULTS: Unscrewing Torque: maximum value of torque required to unscrew the closure
Opening Force: maximum value of force during closure opening

SAMPLE PREPARATION: Category 001: Baseline
Category 002: 3x cycle -50°C
Category 003: 5x cycle -50°C

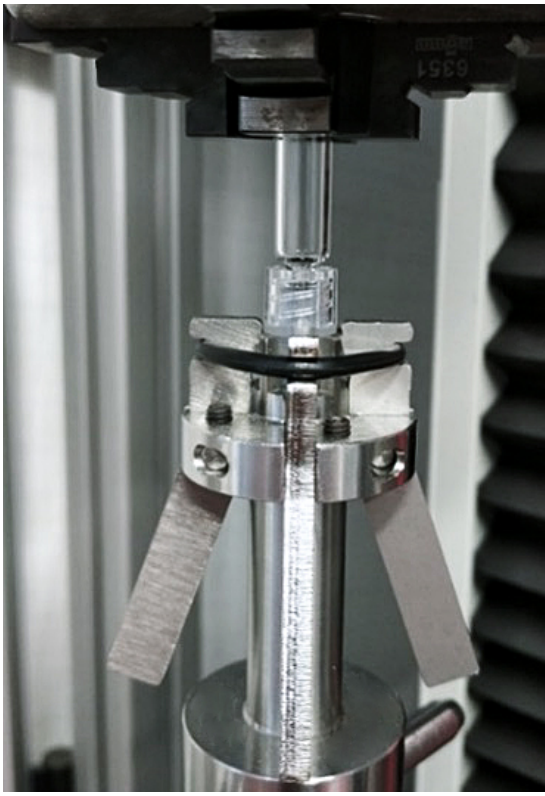
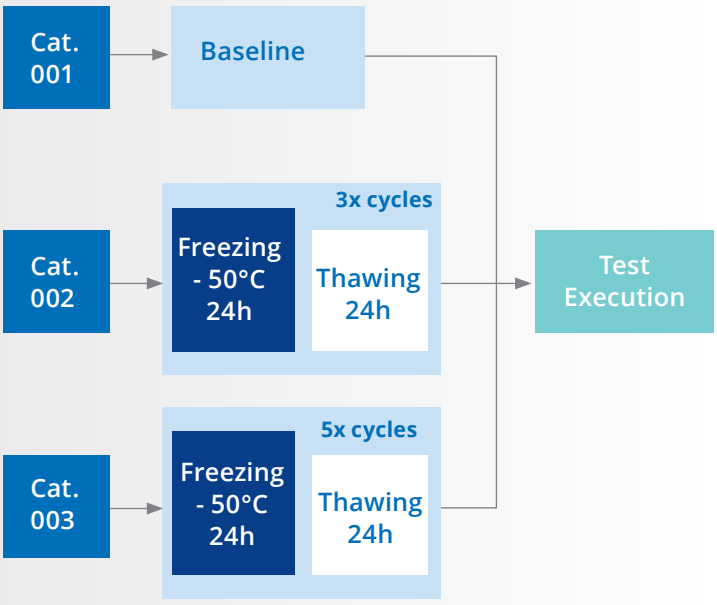


Figure 11: Unscrewing & Opening tool

Figure 12: Test Scheme



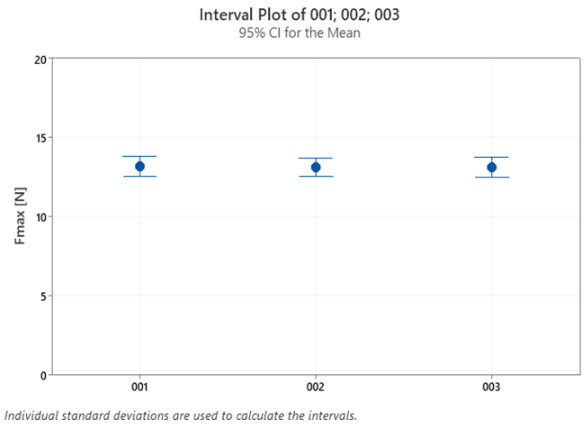


Figure 13a: ITC opening results

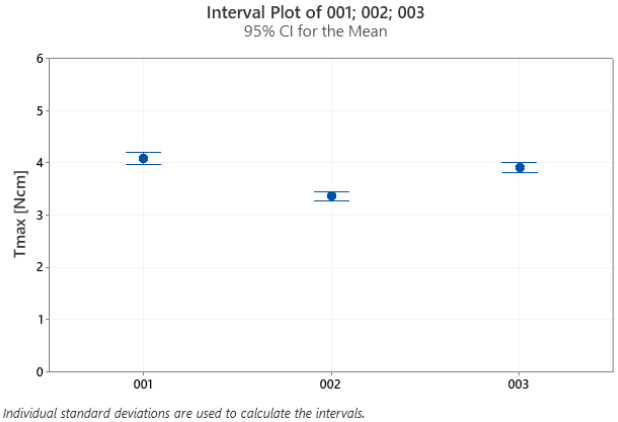


Figure 13b: ITC unscrewing results

Conclusions

Glass Syringes are a viable solution for drug products requiring low-temperature storage

The sterility of 1ml ITC syringes is not affected by storage at -70°C since no impact on CCI has been detected.

Freezing storage showed no or only a slight, non-critical influence on functional or mechanical performance of 1ml ITC syringes. In particular, no impact on the glass was detected, a fact proven during the pandemic by the vials supplied for billions of Covid-19 vaccine doses.

Overall, Glass PFSs can be considered a good solution for Lifecycle Management of vaccine applications requiring low storage temperatures (e.g., mRNA Vaccines) and the extensive testing performed confirmed the suitability of Stevanato Group's EZ-fill® ITC glass syringes as a perfect solution for drugs requiring deep-freeze storage.

Customer-specific solutions can be created from these elements, including an assessment to assure full conformity to critical quality attributes

Stevanato Group offers a comprehensive range of syringes and components. With experience and a long-term commitment through continuous capacity increases and quality improvements, Stevanato Group confirms its position as one of the leaders in this industry.

Glass Syringes are available in bulk as well as ready-to-fill with a variety of add-on components like stoppers, plunger rods, finger flanges and with proven application with auto-injectors and safety systems

Syringe format and components may have an impact on the Critical to Quality Attributes. A dedicated assessment should be performed for each new combination. Analytical services are an essential resource enabling pharma companies to choose the right drug containment solution for their needs. Stevanato Group Technology Excellence Centers in Italy[9] and Boston[10], MA provide the required scientific expertise to support pharma companies on their journey to a successful implementation of this promising technology.

References:

9. Stevanato Group, EMEA Technology Excellence Center, Stevanato Group, via Molinella 17, 35017 Piombino Dese (PD) Italy - 10. Stevanato Group, US Technology Excellence Center, Stevanato Group, 451 D St Suite 704 Boston, MA 02210 USA

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Silvia Gallina is currently part of the **Product Management team** for the **syringe platform** at Stevanato Group.

After her master's degree in Pharmacy from the University of Padova (Italy), she built-up her experience within a pharmaceutical company working in the Medical Information and Pharmacovigilance departments.

She joined Stevanato Group in 2018 as Technical and Quality support Specialist. She manages relations with Key Accounts from different market areas, building a deep knowledge of pharmaceutical industry needs and expectations.

Marco Povolo

Marco Povolo is currently a member of the **Technology Excellence Center team** at Stevanato Group.

He obtained a master's degree in Mechanical Engineering and a Ph.D. in Mechanics and Advanced Engineering Sciences at the University of Bologna (Italy).

He joined Stevanato Group as Senior Research Analyst in SG analytical services laboratories after years of experience in the research field and publications.

He specializes in the characterization of the mechanical, physical, and functional performances of container closure systems and drug delivery devices.

About Stevanato Group

Founded in 1949, Stevanato Group is a leading global provider of drug containment, drug delivery and diagnostic solutions to the pharmaceutical, biotechnology and life sciences industries. The Group delivers an integrated, end-to-end portfolio of products, processes and services that address customer needs across the entire drug lifecycle at each of the development, clinical and commercial stages. Stevanato Group's core capabilities in scientific research and development, its commitment to technical innovation and its engineering excellence are central to its ability to offer value-added solutions to clients.

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