WHITE PAPER

mRNA therapies on the rise

Challenges for primary packaging and how to test for best performance

Stevanato Group

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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, the Respiratory Syncytial Virus (RSV) and, last but not least, Corona Viruses like SARS-CoV-2. 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.

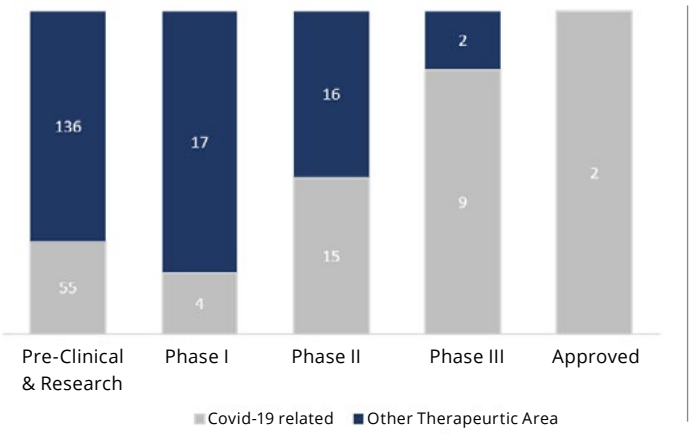


Figure 01:
Pipeline of mRNA drugs[4]

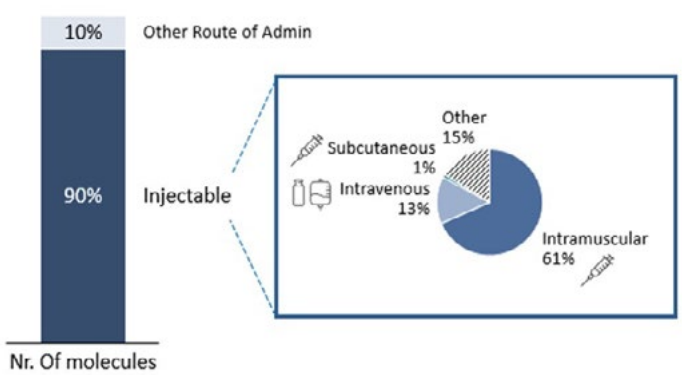


Figure 02:
Key route of administration of mRNA drugs in pipeline[5]

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 -

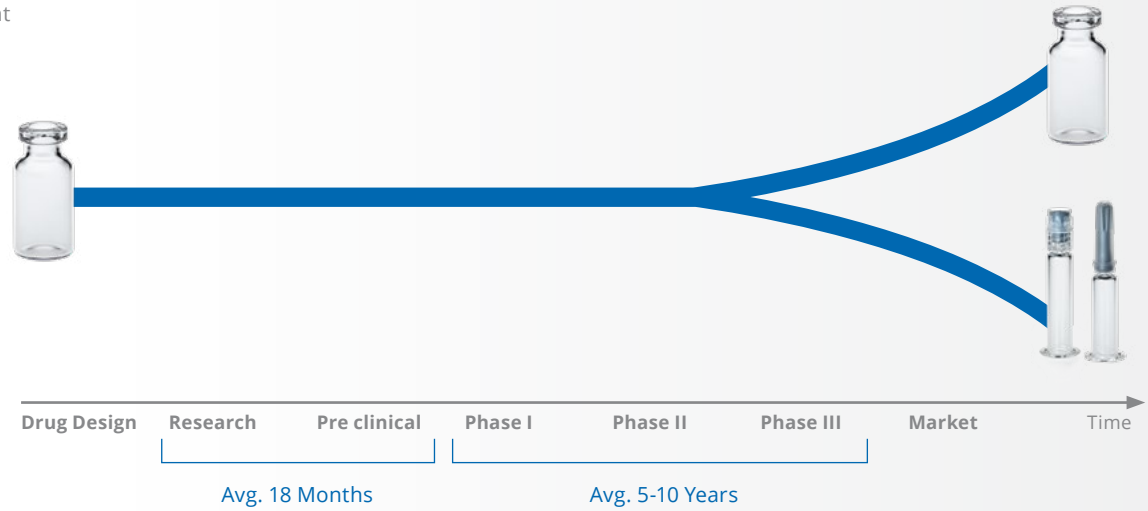
4. Data from Globaldata, March 2022, Nr. of Molecules

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 protocols 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). Both tests were adapted for testing deep-freeze conditions.

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).

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

Additionally, plunger movement has also been evaluated. Displacement was observed which may occur depending on the balance between pressure (air contraction and liquid expansion when freezing), plunger break-loose force and physico-chemical properties of the components and liquid fill. Excessive plunger movement may result in loss of CCI or sterility, so it is recommended to carefully evaluate the headspace related to the previously mentioned factors based on the intended storage and shipping conditions.

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 normally carried out under vacuum at room temperature. During this test, the sample was placed in a vacuum chamber in a freezer and the chamber is evacuated of air.

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 1 ml standard (STD) ITC syringes, including positive control syringes with a 5 µm leak.

The ME method is able to differentiate between positive controls and the tested samples which showed a lower leak flow rate than the 5 µm positive controls. (Figure 04).

TEST METHOD:	Mass Extraction (Air flow rate, µg/min)
SAMPLE PREPARATION:	Positive control: 1 ml STD ITC syringes with 5 µm leak artifact. Sample: 1 ml STD ITC syringe
RESULTS:	Test samples showed lower flow rate compared to 5 µm positive controls

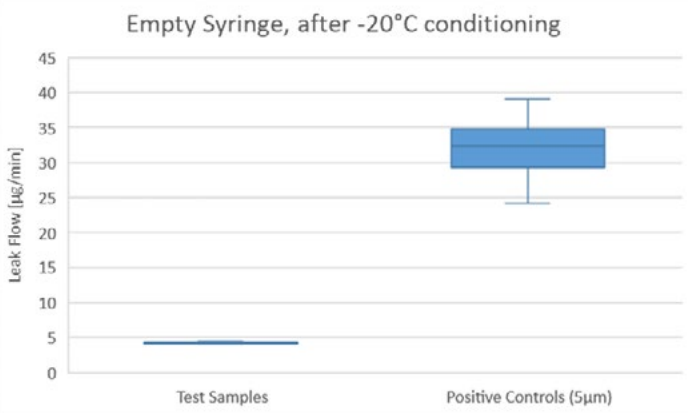


Figure 04:
Modified Mass Extraction (at -20°C)

1.2 Headspace Analysis

Laser Headspace Analysis was performed on samples after being conditioned at -70° C in a CO_2 -rich environment measuring the ingress of CO_2 into 1 ml long ITC syringes, including positive test control samples.

No increase of CO_2 was detected in the test samples while CO_2 increased as expected in the positive controls.

Total test time was taken into consideration to ensure positive controls still have CO_2 after measuring test samples.

TEST METHOD: Headspace gas analysis (CO_2 pressure, mbar)

SAMPLE PREPARATION: Positive control: 1 ml Long ITC syringes, with 260 µm leak artifact
Negative control: 1 ml Long ITC syringes, stored at room temperature in air environment.
Sample: 1 ml Long ITC syringes, stored 7 days at -70°C in CO_2 rich environment

RESULTS: No CO_2 increase detected in test samples

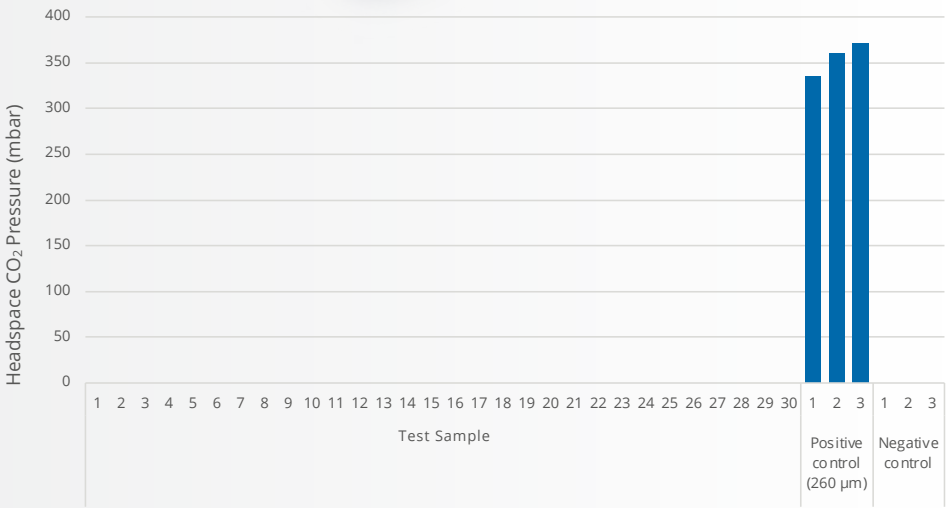


Figure 5: CCIT result for syringes

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 06a) and a Gliding Force performance with comparable results before and after freezing storage (Figure 06b).

TEST METHOD:	Break-Loose, Mean Gliding Force per ISO 11040-4:2015 Annex E
SAMPLE PREPARATION:	1 ml STD ITC Syringes - 30 samples per temperature Set 1: 7 days at 20°C Set 2: 7 days at -20°C Set 3: 7 days at -40°C
RESULTS:	Break-Loose Force comparable or lower after freezing Mean Gliding Force comparable after freezing

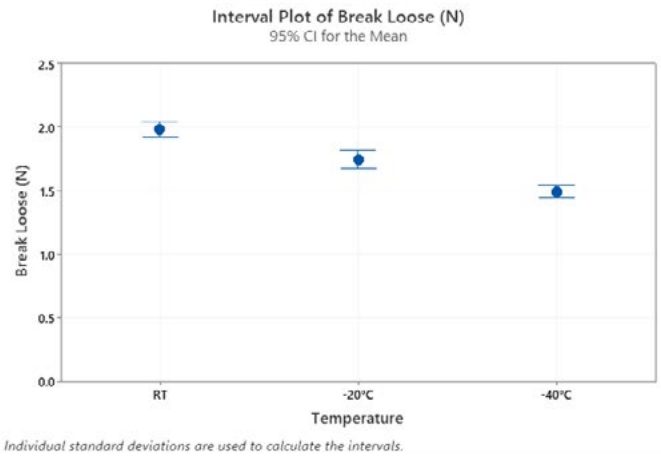


Figure 06a: Break-Loose

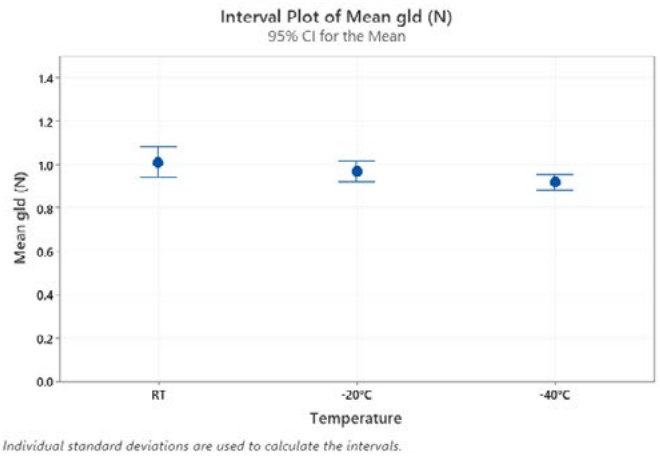


Figure 06b: Mean Gliding Force

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. 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:	Maximum internal pressure resistance per ISO 7458:2004, Method B Syringes
SAMPLE PREPARATION:	1.25 mL ITC Syringes - 100 samples per temperature range Set 1 “Pre”: 7 days at +20°C Set 2 “Post”: - filled and stored 7 days at -40°C
RESULTS:	Pressure resistance comparable after freezing

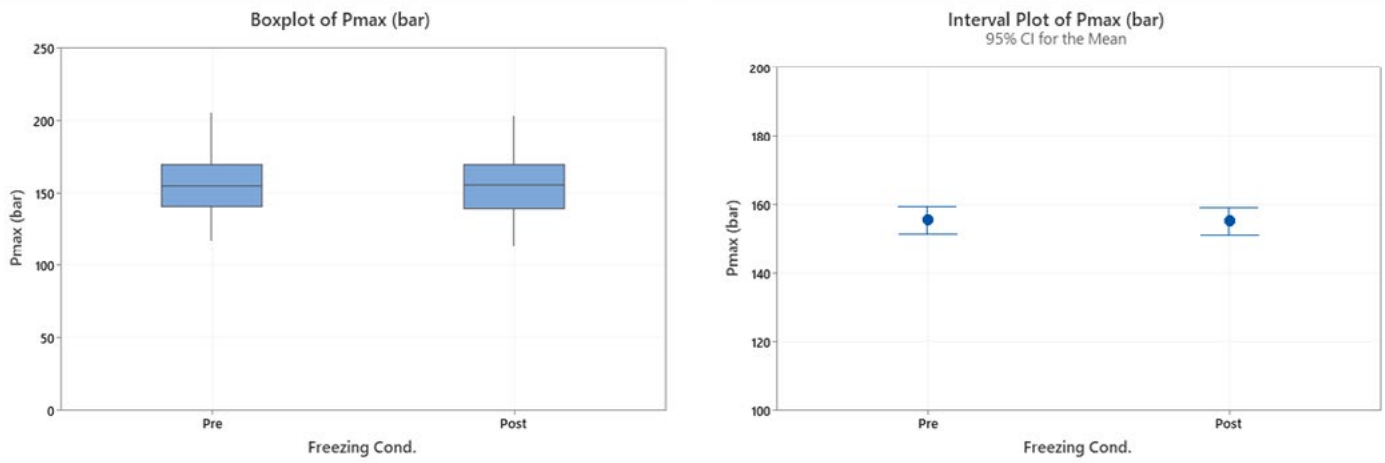


Figure 07: Boxplot and Interval Plot of the maximum internal pressure resistance

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: Unscrewing Torque per ISO 11040-4:2015 Annex G5
Opening Force: maximum value during closure opening

SAMPLE PREPARATION: 1 ml Long ITC Syringes - 50 samples per category
Category 001: Baseline
Category 002: 3x cycle -50°C
Category 003: 5x cycle -50°C

RESULTS: Unscrewing Torque: comparable or slight influence after freezing
Opening Force: comparable after freezing

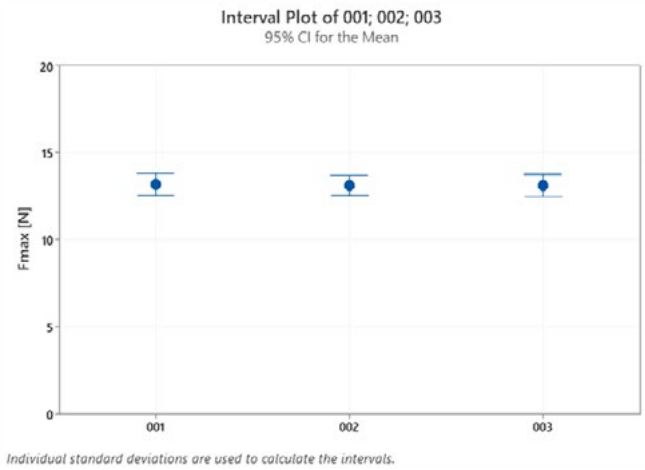


Figure 8a: ITC opening results

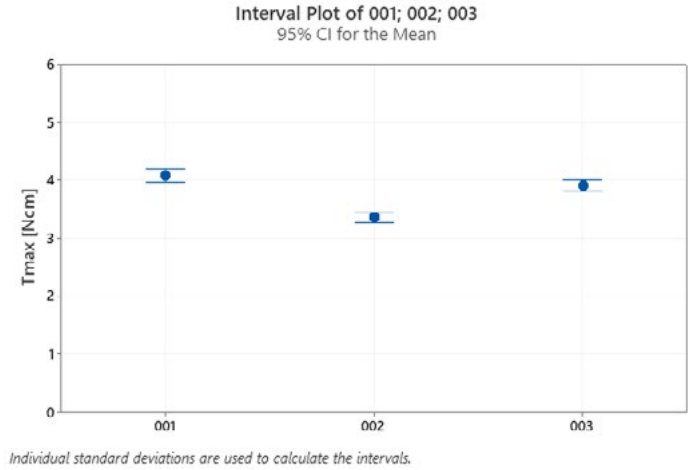


Figure 8b: ITC unscrewing results

Conclusions

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

CCI is one of the most parameters evaluated for drug sterility and stability. Based on the testing here, ITC syringes consistently outperformed their positive controls down to -70°C.

Additionally, our tests after freezing storage at -40°C or multiple freeze/thaw cycles at -50°C showed no critical influence on functional or mechanical performance to the tested ITC syringes. In particular, no impact on the glass burst resistance 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 viable 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 compatibility 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 joined Stevanato Group as a Technical Account Manager in 2018 before she became a member of the Product Management team for the syringe platform.

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 has managed relations with Key Accounts from different market areas, building a deep knowledge of pharmaceutical industry needs and expectations.

Marco Povolo

Marco Povolo joined Stevanato Group's EMEA TEC as a Senior Research Analyst after years of experience in the research field and publications.

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 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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