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## 3.2.P.2.5 Microbiological Attributes

mRNA-1273 Drug Product is manufactured by a conventional aseptic process using sterilizing filtration. Prefiltration bioburden is monitored as part of the manufacturing process. The microbiological quality attributes are monitored by testing for sterility and endotoxins at release. Sterility is also monitored annually as part of the stability testing program. The microbiological suitability of the selected primary container closure system has been demonstrated through container closure integrity (CCI) testing studies.

### 3.2.P.2.5.1 Container Closure Integrity Qualification

Container closure integrity (CCI) qualification of the container closure is described below.

CCI studies were performed to define the range of capper crimp settings for sealing the 10R vial at Catalent, Bloomington, IN site on the Flexi-Fill line (System 2324-03), that result in an integral seal for the mRNA-1273 Drug Product stored at -20°C (-15°C to -25°C) and ensures that all microbiological quality attributes are met through product expiry and use (USP <1207>). The data also verifies the suitability of the container closure system to form an integral seal under the long-term storage condition.

A non-product-specific helium-leak method available at West Analytical Laboratories (West Pharmaceutical Services Inc., Exton, PA), with a test temperature of -20°C, was used in this study. The CCI of the 10R vial container closure system at different capper settings was demonstrated by comparing the observed helium-leak rate to the USP <1207> defined maximum allowable leakage limit (MALL). To demonstrate integrity of the closure, the MALL must be less than $6 \times 10^{-6}$ mbar L/s (at 25°C; measured by helium mass spectrometry in the vacuum mode). The corresponding limit at -20°C would be less than or equal to $5.1 \times 10^{-6}$ mbar L/s. This leakage rate equates to the presence of an orifice of nominal diameter of between 0.1 and 0.3 µm. At this leakage rate, the probability of microbial ingress has been determined to be < 0.10 (equivalent to 10%) (Kirsch et al., 1997). Selecting this conservative MALL ensures a low risk of microbial ingress and liquid leakage and can eliminate the need to perform additional microbial ingress or liquid challenge studies as a function of leak size (USP <1207>).

Five (5) different capper crimp settings were used to generate a set of test vials during the filling of the engineering batch at Catalent. The capper crimp pressure settings were evaluated using the center point, two settings of higher compression, and two settings of lower compression to bracket and thus create an operating range from low to high crimp pressure (1.0 to 3.0 bar).

The results demosntrate that all the tested capper crimp setting parameters are suitable to obtain integral vials at -20°C storage condition with leak rates well below the maximum allowable leak level (MALL) of $5.1\times 10^{-6}$ mbar L/s. Leak rate results are provided in Table 1. The center point of the capper setting is intended to be used in routine manufacture.

**Table 1: Helium Leak Rate Data for 10R Glass Vials from ENG20037 Capper Setting Study at -20°C**

| Sample ID (per West CoA) | Sample Description | Average Leak rate measured mbar L/s | Maximum Leak rate measured in study mbar L/s | MALL (USP<1207>) [a] mbar L/s |
|---|---|---|---|---|
| S20013534 | Lot#: VL-085JUN20, Nominal Crimp Pressure:1.2 Min Crimp Pressure:1.0, Max Crimp Pressure: 1.4 | 8.4E-9 ± 3.6E-9 (n = 32) | 2.0E-8 | ≤ 5.1E-6 |
| S20013535 | Lot#: VL-085JUN20, Nominal Crimp Pressure:1.6, Min Crimp Pressure:1.4, Max Crimp Pressure: 1.8 | 8.8E-9 ± 2.9E-9 (n = 12) | 1.2E-8 | ≤ 5.1E-6 |
| S20013536 | Lot#: VL-085JUN20, Nominal Crimp Pressure:2.0, Min Crimp Pressure:1.8, Max Crimp Pressure:2.2 | 9.6E-9 ± 1.8E-9 (n = 12) | 1.3E-8 | ≤ 5.1E-6 |
| S20013537 | Lot#: VL-085JUN20, Nominal Crimp Pressure:2.4, Min Crimp Pressure:2.2, Max Crimp Pressure: 2.6 | 7.3E-9 ± 1.9E-9 (n = 12) | 1.0E-8 | ≤ 5.1E-6 |
| S20013538 | Lot#: VL-085JUN20, Nominal Crimp Pressure:2.8, Min Crimp Pressure:2.6, Max Crimp Pressure: 3.0 | 14.4E-9 ± 7.0E-9 (n = 32) | 4.4E-8 | ≤ 5.1E-6 |

Abbreviation: MALL = maximum allowable leakage limits
a Recalculated from $6 \times 10^{-6}$ mbar L/s at 25°C

## 3.2.P.2.5.2 Container Closure Integrity Test Method Development and Validation

A product-specific helium-leak method is being developed and qualified at West Analytical Laboratories (West Pharmaceutical Services Inc., Exton, PA) for the container closure integrity testing of the mRNA-1273 Drug Product at the intended long-term storage condition of -20°C (-15°C to -25°C). This method will be used for performing CCI testing in lieu of sterility testing on stability for the mRNA-1273 Drug Product in an ongoing manner.

### 3.2.P.2.5.3 Microbiological Growth Promotion Characteristics to Assess Suitability as an Unpreserved Multiple-Dose Product

mRNA-1273 Drug Product does not include a preservative. The lipid nanoparticle-based product is incompatible with common preservatives as shown in Section 3.2.P.2.2.1.4. It is however presented as a multiple-dose product. The ability of the product to be used as an unpreserved multiple-dose product was evaluated in a microbial challenge hold study that examines the ability of the product to support or hinder microbial growth.

A microbial challenge hold time study, also known as growth promotion study was performed to support the proposed "Beyond Use Time" of 6 hours from initial needle puncture/vial entry for the mRNA-1273 Drug Product. The study involved inoculating low levels of selected microorganisms (specified in USP <51>, as well as an additional typical skin flora, *S. epidermidis*) and evaluating the product's ability to promote or hinder growth of the microorganisms over a timeframe corresponding to the proposed "Beyond Use Time" of the entered vial. The low level inoculum levels are representative of contamination that may occur in an in-use situation when multiple doses are withdrawn from the same vial (Metcalfe, 2009). The study used a concentration bracketing approach (0.10 and 0.5 mg/mL) to cover the Phase 3 and Emergency Use Authorization (EUA)/commercial product concentration (0.20 mg/mL CX-024414 mRNA). The data is summarized in Table 2 and Table 3. The results show that growth of the inoculated microorganism is hindered for up to 24 hours at room temperature [= "No Increase" as defined in USP <51>; (Metcalfe, 2009)]. It can be concluded that the mRNA-1273 Drug Product solution hinders the growth of common potential contaminant microorganisms well over the proposed "Beyond Use Time" of 6 hours at room temperature conditions.

**Table 2: Growth in Number of Microorganisms when Inoculated into mRNA-1273 Drug Product Solution (0.5 mg/mL CX-024414 mRNA) and held at 20°C - 25°C**

| Organism (ATCC) | Inoculated Level (Log CFU/mL) | Log Change from Inoculum | | | |
|---|---|---|---|---|---|
| | | 0 h | 8 h | 12 h | 24 h |
| *S. aureus* (6538) | 1.5 | 0.1 | 0.2 | 0.2 | 0.2 |
| *P. aeruginosa* (9027) | 1.2 | 0.2 | 0.2 | -0.1 | 0.1 |
| *E. coli* (8739) | 1.7 | 0.0 | 0.0 | -0.1 | 0.4 |
| *S. epidermidis* (12228) | 1.3 | 0.0 | -0.3 | -0.7 | -1.3 |
| *C. albicans* (10231) | 1.3 | 0.1 | 0.0 | 0.2 | 0.2 |
| *A. brasiliensis* (16404) | 1.3 | 0.0 | 0.0 | 0.0 | 0.0 |

Abbreviations: ATCC = American Type Culture Collection; CFU = colony-forming unit(s); DP = drug product

**Table 3: Growth in Number of Microorganisms when Inoculated into mRNA-1273 Drug Product Solution (0.10 mg/mL CX-024414 mRNA) and held at 20°C - 25°C**

| Organism (ATCC) | Inoculated Level (Log CFU/mL) | Log Change from Inoculum | | | |
|---|---|---|---|---|---|
| | | 0 h | 8 h | 12 h | 24 h |
| *S. aureus* (6538) | 1.5 | 0.2 | 0.1 | 0.1 | 0.1 |
| *P. aeruginosa* (9027) | 1.2 | 0.1 | 0.2 | -0.1 | 0.1 |
| *E. coli* (8739) | 1.7 | 0.0 | 0.0 | 0.1 | 0.4 |
| *S. epidermidis* (12228) | 1.3 | 0.0 | -0.1 | -0.3 | -0.8 |
| *C. albicans* (10231) | 1.3 | 0.2 | 0.1 | 0.2 | 0.1 |
| *A. brasiliensis* (16404) | 1.3 | 0.0 | 0.0 | 0.1 | 0.0 |

Abbreviations: ATCC = American Type Culture Collection; CFU = colony-forming unit(s); DP = drug product