SINGLE USE SUPPORT.

PIONEERING BIOPHARMA

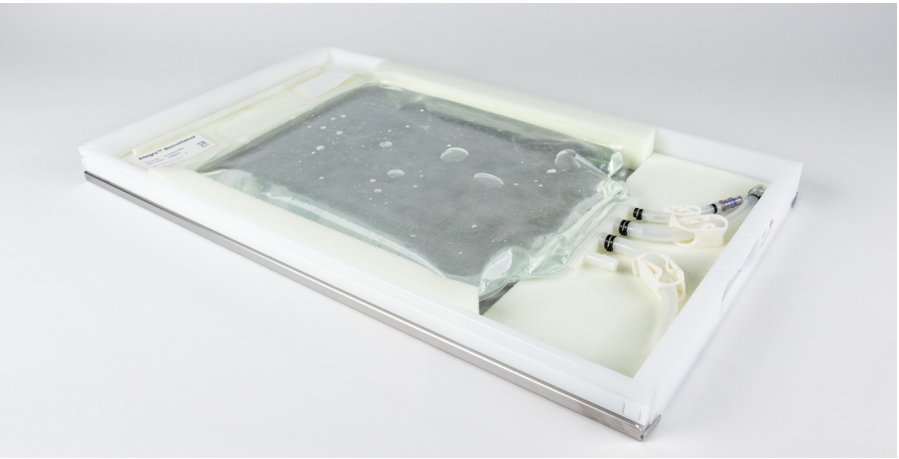
# CASE STUDY

## Bioburden Testing of RoSS®

# 1 | The Situation

RoSS® is the safety standard for any single use bag. “Robust Storage and Shipping” is an innovation from Single Use Support providing a protection for single use bags to avoid product loss of highly valuable biopharmaceuticals. It can be applied in different areas of manufacturing in Biopharma, be it cell & gene therapies or liquid transfer of large bulk drug substances, such as blockbuster vaccine production.

Despite its short time on the market, RoSS shells are of high demand. The scalable protective system supporting single-use technologies is well-established in numerous Big Pharma companies and is in use worldwide proving its effectiveness in real-life every day.



Picture: RoSS shell in use clean

# 2 | The Problem

RoSS shells contain 3D foams to protect and immobilize the filled and frozen bags while transporting.

Usage of foams is uncommon in the biopharmaceutical industry resulting in concerns that may arise in this regard.

- **Do RoSS shells expose particles when in use in cleanroom environments?**
- **Do foams of RoSS pose a microbial contamination source in general or by absorbing condensate during thawing?**

In the following these questions will be fact-checked with conducted studies.

# 3 | The Results

## 3.1 | Study Particles Exposed from RoSS in general

The goal of the study was to investigate whether the introduction and handling of RoSS shows any negative impact on the particle count within an ISO 5 environment. Single-use bags have been filled with water and were placed inside RoSS shells in assembly environment at Single Use Support and subjected to a standard freeze – transport – thaw process.

After thawing, RoSS were subjected to material lock-in processes from ISO 8 up to ISO 5 (cleaning by IPA wipe and opening).

Particle emission was measured in an ISO 5 environment, whereby different scenarios were considered during routine and rough handling, standard (1.2m) and worst-case height (probe below shell).

### Particle count in ISO 5 cleanroom during RoSS handling

| RoSS sample ID# | 0,3 µm/m³ | 0,5 µm/m³ | 1,0 µm/m | 5,0 µm/m | cleanroom class ISO 14644 | Comment |
|---|---|---|---|---|---|---|
| 7 | 0 | 0 | 0 | 0 | 5 | Standard measurement |
| 8 | 212 | 71 | 35 | 0 | 5 | Standard measurement |
| 9 | 0 | 0 | 0 | 0 | 5 | Standard measurement |
| 10 | 318 | 35 | 0 | 0 | 5 | Standard measurement |
| 11 | 1377 | 388 | 0 | 0 | 5 | rough handling during measurement |
| 12 | 530 | 247 | 35 | 0 | 5 | Standard measurement |
| 7w | 247 | 35 | 0 | 0 | 5 | worst-case measurement height |
| 8w | 106 | 35 | 0 | 0 | 5 | worst-case measurement height |
| 10w | 212 | 106 | 71 | 35 | 5 | worst-case measurement height |
| 12w | 318 | 141 | 141 | 0 | 5 | worst-case measurement height |

| Class | Max. particle amount (particle/m³ air) | | | |
|---|---|---|---|---|
| | 0,3 µm | 0,5 µm | 1 µm | 5 µm |
| ISO 5 | 10.200 | 3.520 | 832 | - |

**The introduction of RoSS into an ISO 5 environment and its routine or even rough handling did not cause any violations of the particle count limits.**

## 3.2 | Study Contamination Exposed from 3D Foam

The goal of this study was to investigate if the foam absorbs condensate during thawing and thereby could promote microbial growth. Additionally, the surface bioburden of the foam was analyzed.

Single-use bags filled with water were placed inside RoSS shells in assembly (CNC) environment at Single Use Support and subjected to a standard freeze and thaw process.

After thawing, RoSS were subjected to material lock-in processes from warehouse area to CNC (surface cleaning by IPA wipe). Microbial sampling was performed (contact plates), and the mass difference between foam mass before freezing and after thawing was determined.

### Mass differences of the tubing foams:

| RoSS sample ID# | mass tubing foam before freezing [g] | mass tubing foam after thawing [g] | Difference [g] |
|---|---|---|---|
| 1 | 138 | 336 | 192[1)] |
| 2 | 131 | 133 | 2 |
| 3 | 129 | 133 | 0 |
| 4 | 126 | 129 | 0 |
| 5 | 131 | 130 | 1 |
| 6 | 129 | 127 | -2 |
| 1) the pinch clamp of the tubing was leaky (water leaked out of the bag) | | | |

**The RoSS foam does not show absorbing behavior during thawing and is therefore not likely to contribute to a microbial growth-promoting environment. The results indicate a very low microbial load on the RoSS foam.**

# 4 | The Conclusion

| Samplepoint | Bioburden [cfu/25cm2] |
|---|---|
| upper tubing foam RoSS 1 | 0 |
| bottom tubing foam RoSS 1 | 15 [1)] |
| Side Foam RoSS 1 | 0 |
| Back Foam RoSS 1 | 0 |
| upper tubing foam RoSS 2 | 0 |
| bottom tubing foam RoSS 2 | 0 |
| Side Foam RoSS 2 | 1 |
| Back Foam RoSS 2 | 0 |
| upper tubing foam RoSS 3 | 0 |
| bottom tubing foam RoSS 3 | 0 |
| Side Foam RoSS 3 | 0 |
| Back Foam RoSS 3 | 0 |
| upper tubing foam RoSS 4 | 0 |
| bottom tubing foam RoSS 4 | 0 |
| Side Foam RoSS 4 | 2 |
| Back Foam RoSS 4 | 0 |
| upper tubing foam RoSS 5 | 0 |
| bottom tubing foam RoSS 5 | 0 |
| Side Foam RoSS 5 | 0 |
| Back Foam RoSS 5 | 47 [2)] |
| upper tubing foam RoSS 6 | 0 |
| bottom tubing foam RoSS 6 | 0 |
| Side Foam RoSS 6 | 0 |
| Back Foam RoSS 6 | 0 |
| BW upper tubing foam | 1 |
| BW bottom tubing foam | 0 |
| BW Tubing Side Foam | 0 |

**1) Contamination may be related to the leaky pinch clamp**
**2) The side and tubing foams of the same shell show no microbiological growth. It cannot be excluded that the contact plate may have been contaminated during sampling (possible handling error).**

**The results indicate a very low microbial load on the RoSS foam.**

The RoSS shell does not have impact on bioburden in cleanroom environment. Conducted studies have proven that:

- **The introduction of RoSS into an ISO 5 environment and its routine or even rough handling did not cause any violations of the particle count limits.**
- **The RoSS foam does not show absorbing behavior during thawing and is the refore not likely to contribute to a microbial growth-promoting environment.**