Depyrogenation Study on Primary Packaging

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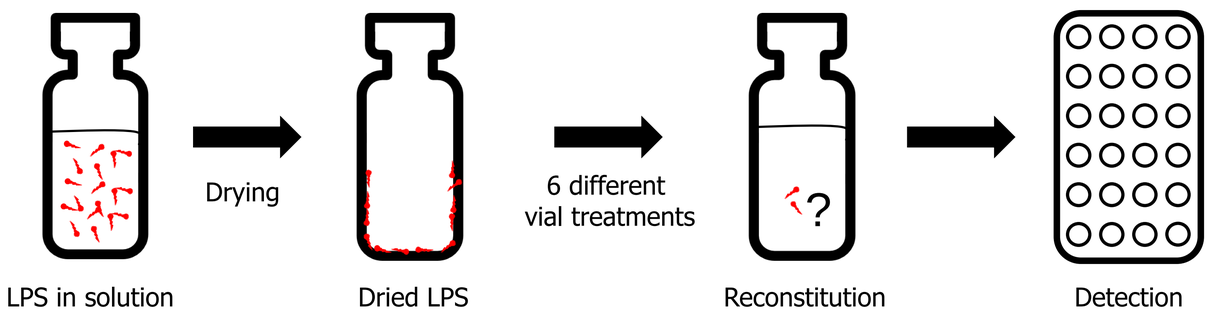
Abstract

Endotoxin (LPS), if present in the bloodstream, is already in small doses a severe health risk. Thus depyrogenation of primary packing is important to ensure patient safety. Dry-heat depyrogenation is the primary method used for endotoxin inactivation. A depyrogenation process should demonstrate at least 99.9 % (3-log) endotoxin reduction. Common processes used in the pharmaceutical industry to prepare vials before filling with parenteralia often include sonication, washing and heating. Sonication and washing are reducing the number of particles in the glass vials, but are these vial treatments able to reduce the contamination with endotoxin as well or is heating absolutely necessary? Very few data is available about endotoxin reduction in vials at different glass depyrogenation treatments.

In this study a systematic approach was used to test 6 different combinations of vial treatments and their influence on endotoxin contamination of 5 different glass vials (n = 9-10).

Method and Materials

Workflow

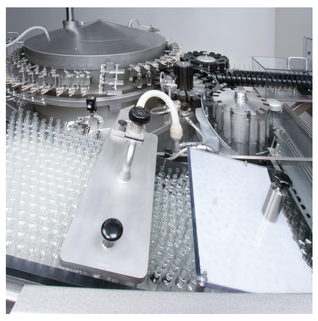


- Drying: 1000 EU/vial, over night
- Vial Treatments: 6 different methods (0, a-e), negative controls (NC) without addition of endotoxin and undried LPS (R) was used as controls (each n = 9-10)
- Reconstitution: in 0.2 % SDS for 2.5 h including vortexing
- Sample dilution in LRW: 1:100, 1:1000
- Detection: rFC Method (Endozyme II)

Overview – 6 different vial treatments

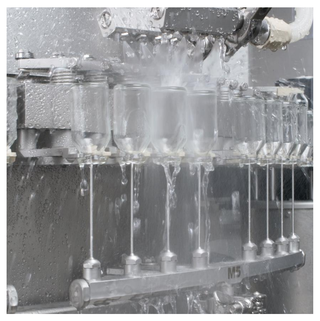
0	a	b	c	d	e
		Sonication		Sonication	
	Washing	Washing	Washing	Washing	
			Heating	Heating	Heating

Sonication



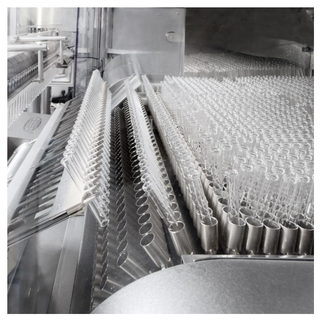
Sonicator
1 min 70 °C
240 W

Washing



Cleaning machine
3 x wasching
water temperature 80 °C

Heating



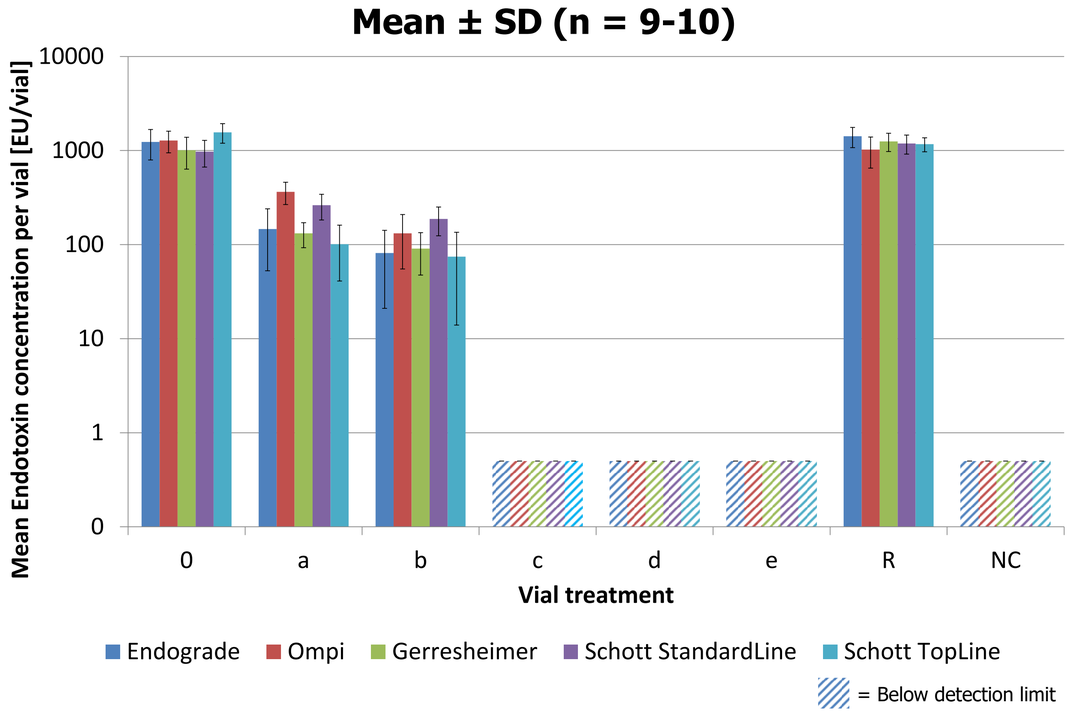
Heat tunnel
15 min
320 °C

5 different glass vials used for the study

- Endograde Glass Test Tube (Hyglos)
- ISO 2R Clear Glass Vial (Nuova Ompi)
- ISO 2R Flacon (Gerresheimer Chalon)
- ISO 2R Fiolax clear StandardLine (Schott)
- ISO 2R Fiolax clear TopLine (Schott)

Results

Endotoxin concentration after vial treatment
Mean ± SD (n = 9-10)



Log reduction of endotoxin per vial

Vial treatment	Endograde	Ompi	Gerres-heimer	Schott (SL)	Schott (TL)
a	0.93	0.55	0.88	0.57	1.19
b	1.18	0.99	1.05	0.72	1.32
c	> 3.39	> 3.41	> 3.30	> 3.9	> 3.49
d	> 3.39	> 3.41	> 3.30	> 3.29	> 3.49
e	> 3.39	> 3.41	> 3.30	> 3.29	> 3.49
NC	> 3.39	> 3.41	> 3.30	> 3.29	> 3.49

- LPS concentration is not influenced by drying method (0 vs. R)
- 0.57-log to 1.32-log LPS reduction for treatment a and b
- > 3-log reduction for treatments c, d and e (equal NC)

Conclusion

- Endotoxin in different glass vials behaves comparable with and without treatments
- For reconstitution of endotoxin a dedicated sample preparation (0.2 % SDS) was applied
- Washing and sonicating is not sufficient to reduce endotoxin (reduction ≤ 1.32-log)

⟶ **A 3-log endotoxin reduction was only possible after heat treatment**

References

- *Tech Report #3 Validation of Dry Heat Processes Used for Sterilization and Depyrogenation, PDA*
- *USP <1211> Dry-Heat Sterilization/Depyrogenation*