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Title:
Report for Microbiological Tightness
Validation for the Membrane of the
OneMum and all Ardo Pumpset
ARDO MEDICAL AG

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Report for Microbiological Tightness Validation for the Membrane of the OneMum and all Ardo Pumpset

1.0. Document Approval

Historic	Title	Name	Date	Approval Signature
Written by	Laboratory MEDISTRI SA	G. Farron	19 th July 2011	
Approved by	General Manager MEDISTRI SA	S. Nilforoushan	19 th July 2011	
Approved by	Quality responsible at ARDO MEDICAL AG	R. Speck	19 th July 2011	

2.0. Revision Table

Revision	Date	Page(s) concerned	Specification of revision
0	18 th July 2011	---	Creation of basis document
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3.0. General Information

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ID of product tested		Membrane of OneMum PumpSet
Description of testing		The aim of the present test was to validate the tightness of the PumpSet membrane for microbiological contamination. The membrane must not allow potential microbiological contamination to pass from and contaminate the product compartments separated by the membrane, i.e. from the milk bottle to the tube and vice versa, respectively. The tightness was tested during simulated use of the device.

4.0. Rationale

The OneMum PumpSet has been designed to collect breast milk into a milk bottle. The suction process is guaranteed by an electrically operated pump connected to the top cover via a plastic tube (refer to picture, p. 3 of the present report).

The internal side of the milk bottle is separated from the plastic tube by a membrane (refer to picture, p. 3). The membrane must act as a sterility barrier in order to guarantee that no microbiological contamination potentially present on one side of the membrane contaminates the other side.

For this reason, we tested the effectiveness of the membrane to act as a sterile barrier using an artificial microbiological contamination. The testing procedure was done under conditions of simulated product use.

5.0. Procedure

The test set-up was approved by ARDO Medical AG by the signature of the protocol 0652-220611.

The microbiological evaluation of membrane tightness during simulated use was performed with artificial contamination, using a fresh culture of *B. subtilis*. This referenced bacterium was chosen because it is easily recognizable and produces resistance structures which weren't affected by the suction process.

The following points were investigated:

1. The ability of microbes to pass through the membrane barrier from the lower side to the upper side.
2. The ability of microbes to contaminate a liquid by passing through the membrane barrier from the upper part.

The supposed not contaminated (lower, upper) parts were investigated by bioburden tests, according to Pharmacopée Européenne, chap 2.6.12, United States Pharmacopeia 7.0 : 32 (61) and SN EN ISO 11737-1.

5.1. Methods

1. Organisms. *B. subtilis* was chosen. Reasons:

Referenced and traceable (documentation)
Easily recognizable (morphology)
Easily culturable (enrichment, testing) and recoverable
Resistant to physical stress by spore forming (against vacuum)



2. Growth conditions (media, temperature, time) and specification

Enrichment culture incubation: Inoculation of *B. subtilis* in TSB, incubation 30-35°C, 3 days
Bioburden incubation: Only for Bacteria: TSA, 30-35°C, 5 days

3. Devices : 10 sterile OneMum pump set from Ardo Medical AG
 1 pump

4. Bioburden testing:
Total aerobic microbial count TAMC (bioburden, membrane filtration)

The enrichment culture permitted to perform simulated use. The membrane tightness was tested as follow :

- a. Tightness from up to down : 2 ml of enrichment culture were poured on the upper part of the membrane (as a dense contamination spot). Breast simulation : a sterile pierced glove (at the end of a finger) was filled with 150 ml of sterile peptoned solution. For simulated use : the glove was placed on the funnel, pump on (worst case : highest cycle, highest vacuum). This procedure optimally simulated a normal use. After use, bioburden was performed by membrane filtration of the peptoned solution.

Number of tests : 5

- b. Tightness from down to up : A sterile pierced glove (at the end of a finger) was filled with 150 ml of enrichment culture. For simulated use : the glove was placed on the funnel, pump on (worst case : highest cycle, highest vacuum). This procedure optimally simulated a normal use and filled the OneMum bottle. After use, bioburden was performed on the upper part of the membrane by pouring 5 ml of peptoned solution (homogenisation for optimal recovery). 145 ml of peptoned solution was added before membrane filtration (of 145+5 ml of peptoned solution).

Number of tests: 5

5. Controls

Population control : triplicates. 1ml of each enrichment culture was homogenized with 9 ml of TSB; five suspension/dilution were performed and 0.2 ml/dilution were poured on TSA. Bacteria were enumerated after 48 hours of incubation at 30-35°C.

Evaluation of bacterial inhibition by gloves: After 10 minutes of contact with the inner part of three gloves, 1ml of each enrichment culture was homogenized with 9 ml of TSB; five suspension/dilution were performed and 0.2 ml/dilution were poured on TSA. Bacteria were enumerated after 48 hours of incubation at 30-35°C.

Sterility of the filtration ramps and media before use (=negative control) and after use

6. Acceptance criteria

Enrichment culture (cfu/ml) : minimum of 10^4 /ml

Bioburden : minimal log reduction of 10^{-3} , but a maximum of 50 cfu

Summary: The tightness (microbiological barrier) of the **protective membrane** of the OneMum pump set during use was evaluated.



A: The contaminant weren't able to pass from a contaminated liquid in the bottle to the upper part of the membrane barrier



B: The contaminant weren't able to pass through the membrane from a contaminated spot on the upper part to a non-contaminated liquid in the bottle

**USE OF A WORST CASE
MODEL: *B. subtilis***

**HIGH RESISTANCE
HIGH CONTAMINATION LEVEL**



A : Contamination. 150 ml of enrichment culture were poured into the bottle through a pierced glove

B: Contamination. 2 ml of enrichment culture were poured into the upper part of the membrane

Simulated use with the pump switched on for 5-10 minutes; the main opening was obturated with a pierced glove and a manual rotation simulated the mother movements to favour the contamination opportunities



**WORST CASE USE:
HIGHEST VACUUM
HIGHEST CYCLES**



Note: the vacuum produced an important suction which lead the liquid to wet the lower part of the membrane

BIOBURDEN

6.0 Results

6.1. Controls

6.1.1. ufc /ml in the enrichment culture

Sample ID	<i>Bacillus subtilis</i> contamination (cfu / ml)
1	1.470x10 ⁹
2	1.560x10 ⁹
3	1.440x10 ⁹
Average	1.490x10 ⁹

6.1.2. Evaluation of bacterial inhibition by gloves. (enrichment culture on the internal part of the gloves for 10 minutes)

Sample ID	<i>Bacillus subtilis</i> contamination (cfu / ml)
1	1.405x10 ⁹
2	1.520x10 ⁹
3	1.345x10 ⁹
Average	1.423x10 ⁹

Evaluation of controls: 95.5% of the contamination was transferred from the gloves to the OneMum pump set. An average of 1.423 billion of bacteria/ml of medium was calculated as contamination:

The minimum of 10⁴ living bacteria/ml was reached and successfully transferred

6.2. Bioburden testing

6.2.1 Results of the contamination of the upper part of the membrane after use (when the liquid is contaminated)

Sample ID	Bioburden: nb of <i>B.subtilis</i> counted	Reduction (log)	Negative control (ramp and media)
1	0	10 ⁻⁹	Before: Negative After: Negative
2	0	10 ⁻⁹	
3	0	10 ⁻⁹	
4	0	10 ⁻⁹	
5	0	10 ⁻⁹	



Note: The contaminated liquid inoculated into the bottle by simulated use didn't show microbiological evidence for transfer in the upper part of the membrane, as shown in picture A (from left to right: 1-5). The negative controls (B: From left to right before and after) remained negative during the tests (the ramps were carefully rinsed with sterile peptoned water between filtration).

6.2.2 Results of the contamination of the liquid in the bottle after use (when the upper part of the membrane is contaminated)

Sample ID	Bioburden: nb of <i>B.subtilis</i> counted	Reduction (log)	Negative control (ramp and media)
1	0	10 ⁻⁹	Before: Negative After: Negative
2	0	10 ⁻⁹	
3	0	10 ⁻⁹	
4	0	10 ⁻⁹	
5	0	10 ⁻⁹	



Note: The uncontaminated liquid inoculated into the bottle by simulated use didn't show microbiological evidence for contamination, as shown in picture C (from left to right: 1-5). The negative controls (D. From left to right: before and after tests) remained negative during the tests (the ramps were carefully rinsed with sterile peptoned water between filtration).

7.0. Interpretation and discussion

The bioburden tests were performed according to Pharmacopée Européenne, chap 2.6.12, United States Pharmacopeia 7.0 : 32 (61) and SN EN ISO 11737-1.

The assembly instructions of the OneMum pump set given by Ardo Medical AG were followed.

Worst case of simulated use of the pump and contamination level revealed that the membrane didn't show evidence of bacterial transfer in our conditions, during tests and incubation conditions.

The acceptance criterion for enrichment culture: minimum of 10⁴ cfu/ml; 10⁹cfu/ml was reached.

The acceptance criteria for bioburden: minimal log reduction of 10⁻³, but a maximum of 50 cfu; log reduction 10⁻⁹, 0 cfu were reached.

- Considering the conditions of use, the predefined acceptance criteria and thank to its tightness, the membrane seem to be an appropriate element to prevent contamination transfers during use.