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Verification of the sporicidal efficacy
of the product Puristerl340[®] at 37°C
in the Fresenius 5008 dialysis machine
with ONLINE *plus* + Bicarbonate and 1 x Acid CDS

1. Objective

Verify the degree of efficacy of a disinfection procedure using the product Puristeril® 340 at 37°C in the Fresenius 5008 dialysis machine with ONLINE *plus* + Bicarbonate and 1 x Acid CDS .

2. Method

The disinfecting effect was tested after artificial contamination of the machine. A \log_{10} reduction factor of at minimum 5,0 should be reached at the various check points of the fluid-carrying system.

2.1 Test organism

As test organisms we used spores of *Bacillus subtilis*, variato *globigii*, which were suspended in ethanol at a concentration of $3 \times 10^{10}/\text{ml}$.

We used these test organisms since the vegetative organisms which are regularly employed in disinfectant tests (such as *E. coli*, *Pseudomonas aeruginosa*, *Candida albicans* etc.) are not suitable for the evaluation of highly-efficient procedures . The most resistant, vegetative (non- sporeforming) test organism is *Streptococcus faecium*.

A \log_{10} reduction factor of 8 for this test organism corresponds to approximately a value of a \log_{10} -reduction factor of 5 for *B. subtilis* spores. For tests under practice conditions a number of organisms of 10^9 (final dilution in the machine) would have to be used. This would constitute an unrealistic high load of biological material .

2.2 Artificial contamination of the dialysis machine

Before each test run 1 ml of the test organism suspension was introduced into the fluid-carrying system via the rinsing chamber , resulting in a final concentration of the test organisms between 10^7 and $10^8/\text{ml}$ of dialysing fluid. In order to achieve a

uniform distribution of the test organisms the On-Line *plus* filter was bypassed. Then the artificially contaminated dialysing fluid was recirculated for 10 minutes .

Thereafter the control samples **before** disinfection were taken at measuring point 3 (see table 1) , since a sufficient distribution throughout has been reached in numerous other tests with the same machine .

2.3 Test runs

After the artificial contamination the filter was activated again and the disinfection cycle started.

After completion of the disinfection cycle the control samples **after** disinfection were taken at the measuring points 1, 2 and 4-7 (see table 1).

A total of 5 independent test runs were carried out . The tests were performed at our Institute on from December 2, until December 6, 2002.

2.4 Evaluation

The CFU numbers were, where needed, determined by means of dilution series in sterile Casein-Peptide- broth.

As comparative figure for the achieved disinfecting effect the log-reduction factor (RF) was calculated:

$$\log_{10} \frac{\text{Number of organisms before disinfection}}{\text{Number of organisms after disinfection}}$$

3. Results

The challenge before disinfection amounted in all tests to 10^6 CFU/ml.

The achieved reduction factors are listed in table 2. The figures are arithmetical mean values from double determinations.

4. Rating

With the product Puristeril® 340 at 37°C the required \log_{10} reduction factors of 5 or more were by far exceeded at all measuring points .

Due to the demonstrated high sporicidal effect the tested procedure is as effective as other procedures which are approved for decontamination, for example in Hepatitis.

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Table 1 pertaining to evaluation dated December 20, 2002

Verification of the sporicidal efficacy of the product Puristeril340® at 37°C in the Fresenius 5008 dialysis machine with ONLINE *plus* + Bicarbonate and 1 x Acid CDS

Measuring points	L o c a t i o n
1	Upstream of P02 feeding pump
2	Upstream of V 29s, air separator valve
3	Upstream of conductivity/temperature measuring cell (only positive control)
4	Dialysing fluid supply line
5	Downstream of pump P06
6	Between V28 and V19
7	Downstream of balancing chamber P03

Table 2 pertaining to evaluation dated December 20, 2002

Verification of the sporicidal efficacy of the product Puristeril340® at 37°C in the Fresenius 5008 dialysis machine with ONLINE *plus* + Bicarbonate and 1 x Acid CDS

Achieved reduction factors					
Measuring point	Test run				
	1	2	3	4	5
1	>6,20	>6,18	>6,25	>6,34	>6,13
2	>6,20	>6,18	>6,25	>6,34	>6,13
4	>6,20	>6,18	>6,25	>6,34	>6,13
5	>6,20	>6,18	>6,25	>6,34	>6,13
6	>6,20	>6,18	>6,25	>6,34	>6,13
7	5,72	5,88	5,95	6,16	>6,13