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Validation of filter sterilisation in autoclaves

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Abstract

Filter cartridges for sterilising filtration are often sterilised by autoclavation. Although this process should be fully validated, not all process parameters are always considered. In this paper, the influence of positioning and packaging on the $F_0^{121°C}$-values at the coldest spot of the filter was investigated in the Sealkleen® and Kleenpak® housings with hydrophilic and hydrophobic filters (Pall Process Filtration Ltd., Portsmouth, UK). A temperature mapping of the assembled filters was performed and it was shown that the coldest spot was located at the sterile side at the top of the filter. The vertical position with the non-sterile side down showed the highest $F_0^{121°C}$-values at the coldest spot. The use of biological indicators confirmed this finding. Different packaging methods with medical paper had no significant influence on $F_0^{121°C}$-values for the Kleenpak® filter. The hydrophilic Sealkleen® showed significantly lower $F_0^{121°C}$-values in the open filter compared to the filter closed at the sterile side, while the hydrophobic Sealkleen® showed significantly lower $F_0^{121°C}$-values for the filter in a bag compared to the open filter and the sterile side-closed filter.

Keywords: Sterilisation; Validation; Filter cartridges; Autoclavation

1. Introduction

Membrane filters used for sterilising filtration are commonly sterilised in autoclaves or by in-line steaming. Although in line sterilisation avoids the contamination risk during aseptic installation, autoclavation of catridge filters is still frequently used. Few data are published about the autoclavation of filter cartridges. Kovary et al. (1983) reported on the Steam-In-Place (S.I.P.) sterilisation of disc filter housings and membranes. They indicated that the design of the filter housing was very important to obtain a sterile filter. Also, thermocouple data and microbiologically evaluated filter membranes provided the assurance required for qualification and validation of the S.I.P. sterilisation procedure. Myers and Chrai (1982) described the S.I.P. of cartridge filters in-line with a receiving tank. Filter configuration seemed to be important to optimize the condensate drainage and the removal of entrapped air. Concerning the autoclavation of filters, Steere and Meltzer (1993) mentioned that the filter itself is usually oriented with its outlet down so that condensed liquid will not collect in its core.

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In our study, the effect of positioning and packaging on $F_0^{121°C}$-values at the coldest spot in the filter cartridge was investigated.

2. Materials and methods

2.1. Materials

Two types of filter assemblies were used, the Sealkleen® and the Kleenpak®, both from Pall Process Filtration Ltd. (Portsmouth, England). For each of these assemblies, two types of membranes were tested: hydrophilic filters, N66 Posidyne (SLK7002NFZP) for the Sealkleen® and Ultipor N66 (KA3NFP1) for the Kleenpak®, and hydrophobic filters, Emflon II (SLK7002V002PV) for the Sealkleen® and (KA3V002PV1) for the Kleenpak®. In those cases where the housings were packed, medical paper (SPS, Coulommiers, France) was used. Temperature was monitored using thermocouples (type T, Thermoelectric, Balen, Belgium) and a datalogger (Type DTL 1214, Thermoelectric, Balen, Belgium). The integrity of the autoclaved filters was tested with an integrity test equipment (Palltronic FFE04, Pall Process Filtration Ltd., Portsmouth, England). Biological indicators (spore strips, 106 B. Stearothermophillus) were from Raven Bio-Lab inc. (Omaha, USA).

2.2. Methods

Throughout the study, the autoclave cycles were as follows: initial single vacuum, followed by steam injection. When the temperature of 121°C was reached at the drain of the autoclave, a sterilisation time of 20 min was applied, followed by a drying (vacuum) time of 20 min. In order to have a minimal influence of the preheating phase, data were rejected if the preheating time of the autoclave exceeded 6 min. Every filter was cooled to room temperature before the next test.

The aim of the first part of the study was to detect for the coldest spot in the filter housing. Therefore, a temperature mapping was performed using six thermocouples for the Sealkleen® and four for the Kleenpak® and a datalogger. Thermocouples were fixed to the filter housings with the use of a stainless steel connector and fittings (Fig. 1a and b). The mapping was performed with the filter housing in four different positions in the autoclave. These four positions are shown in Fig. 2. Two horizontal positions were tested, one with the vent and drain upwards (H_{up}) and one with the vent and drain downwards (H_{down}). Two vertical positions were also tested, the upright position (V_{up}) and the upsidedown position (V_{down}). The filters were not packed and vent and drain valves were open while autoclaving. During the autoclaving cycle, the temperature was recorded every 10 s, and these data were processed in LOTUS 123. This experiment was performed on the Sealkleen® and the Kleenpak® filterassemblies, both with the hydrophilic and hydrophobic filters. For the Sealkleen®, the temperature mapping was performed six times on the same hydrophilic filter (intravariability) and on six different hydrophilic and six different hydrophobic filters (intervariabil-

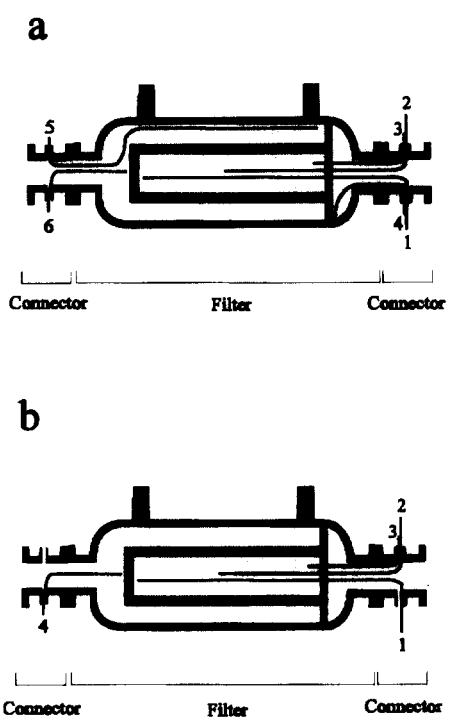

Fig. 1. (a) Sealkleen® housing with six thermocouples fixed with the two connectors. (b) Kleenpak® housing with four thermocouples fixed with the two connectors.

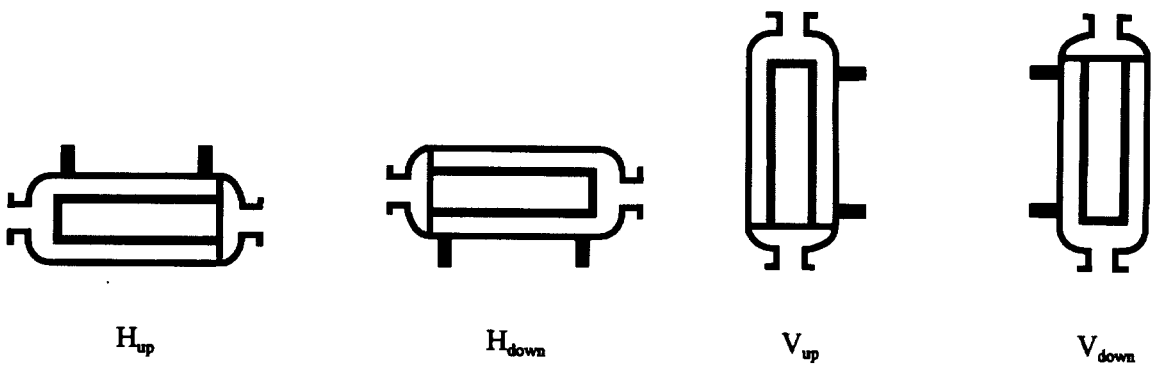

Fig. 2. The four different positions of the filter in the autoclave. H_{up}: filter in horizontal position with vent and drain upwards; H_{down}: filter in horizontal position with vent and drain downwards; V_{up}: filter in vertical position with non-sterile side upwards; V_{down} filter in vertical position with non-sterile side downwards.

ity). For the Kleenpak®, the temperature mapping was performed once on a hydrophilic and once on a hydrophobic filter for the four different positions. Using one thermocouple at the coldest spot, six hydrophilic and hydrophobic filters were tested in the four different positions. The data are expressed as mean $F_0^{121°C}$-values ($\pm$ S.D.) ($F_0^{121°C} = \Sigma 10^{(T-121)/Z}.\Delta t$ where $Z = 10$). Statistical evaluation was done using the Wilcoxon signed rank test [Siegel and Castellan, 1988] with a significancy level of $P < 0.05$ (two-tailed).

Because the stainless steel connectors used to fix the thermocouples can produce lower $F_0^{121°C}$-values, the influence of the connector diameter was investigated in the Sealkleen® housing. Two diameters were tested, 2.5 cm and 3.5 cm, while the length of the connector was 6 cm. The diameter of the inlet and of the outlet of the Sealkleen® housing is 3.2 cm and 3.5 cm, respectively. This test was performed three times on one hydrophilic Sealkleen® filter in the four different positions.

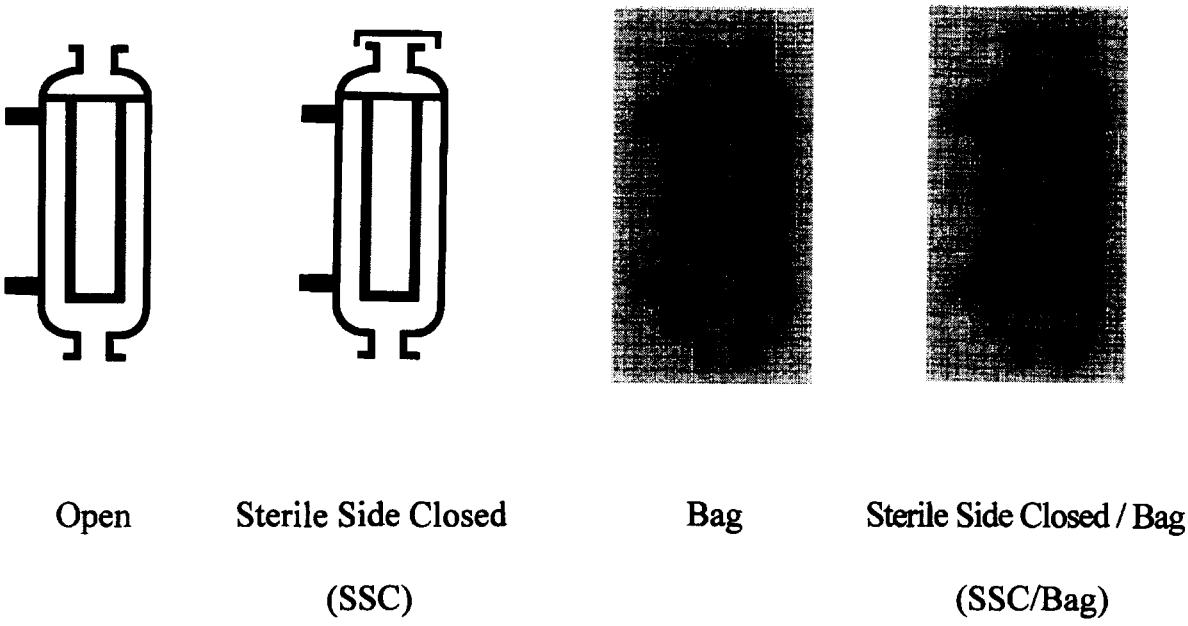

Fig. 3. The different packaging methods tested in the V_{down} position Open: the filter is not packed; SSC: the sterile side of the filter is closed with medical paper; Bag: the filter is put in a medical paper bag and the sterile side is not closed; SSC/bag: the filter is put in a medical paper bag and the sterile side is closed with medical paper.

Table 1
Mapping and positioning of the Sealkleen® housing with six thermocouples. The results are presented as mean $F_0^{121°C}$-values in s (± S.D.)

	Position	Thermocouple no. 1	2	3	4	5	6
A	H_{up}	789.7 ± 99.9[a,b]	817.0 ± 84.4	990.8 ± 117.4	1169.2 ± 157.2	1216.9 ± 62.1	1780.3 ± 28.1
	H_{down}	1079.8 ± 55.4	1093.0 ± 47.8	1189.8 ± 55.7	1415.0 ± 92.1	1905.8 ± 20.7	1801.8 ± 16.8
	V_{up}	601.8 ± 123.9[b]	633.3 ± 117.0	757.3 ± 133.8	1190.6 ± 227.0	1279.8 ± 166.9	1149.1 ± 134.8
	V_{down}	1148.5 ± 107.8	1298.6 ± 152.7	1586.5 ± 197.8	1785.6 ± 182.7	1911.4 ± 106.0	1788.3 ± 27.9
B	H_{up}	1016.6 ± 65.4[a,b]	1008.9 ± 42.7	1104.2 ± 45.4	1174.5 ± 113.1	1425.8 ± 62.5	1745.7 ± 68.8
	H_{down}	1223.4 ± 103.5	1243.3 ± 111.7	1355.2 ± 125.5	1528.5 ± 153.2	1883.9 ± 94.0	1356.0 ± 82.8
	V_{up}	894.0 ± 288.3	981.6 ± 403.8	1115.8 ± 423.4	1365.5 ± 367.7	1596.2 ± 222.3	1356.0 ± 304.8
	V_{down}	1237.3 ± 127.0	1381.1 ± 156.2	1676.9 ± 155.2	1820.0 ± 130.4	1932.3 ± 91.0	1844.4 ± 87.2

A: six different hydrophilic filters; B: Six different hydrophobic filters.
[a]Significantly lower than H_{down}; [b]significantly lower than V_{down}.

Data are expressed as mean $F_0^{121°C}$-values (± S.D.). Statistical evaluation was done using the data obtained at the coldest spot in the filter housing with the Wilcoxon signed rank test.

When filters are autoclaved, the sterile side of the housing should be protected against contamination after sterilisation. In this study, medical paper (SPS, Coulommiers, France) was used. A comparison was made between four different situations: the filter assembly totally open (open), the sterile side of the filter housing closed with paper (SSC), the filter housing packed in a paper bag (Bag), the sterile side closed with paper and the filter housing packed in a paper bag (SSC/Bag) (Fig. 3). For the Sealkleen® assembly, one hydrophilic and one hydrophobic filter cartridge was tested six times in the V_{down} position. The Kleenpak® assembly was tested for six different hydrophilic and six different hydrophobic filters in the V_{down} position. Data are expressed as $F_0^{121°C}$-values. Statistical evaluation was done on the data obtained at the coldest spot in the filter housing using the Wilcoxon signed rank test (two-tailed, $P < 0.05$).

In the last part of the study, spore strips were used (*B. stearothermophillus* with D-value = 10^6, Raven Bio-Lab Inc., Omaha, USA) to show the correlation between the calculated $F_0^{121°C}$-values

Table 2
Mapping and positioning of the Kleenpak® housing with four thermocouples. The results are presented as mean $F_0^{121°C}$-values in s (± s.d. for $n = 6$)

	Position	Thermocouple no 1		2	3	4
		$n = 1$	$n = 6$	$n = 1$	$n = 1$	$n = 1$
A	H_{up}	1439.6	1566.1 ± 69.6	1505.7	1803.6	1487.5
	H_{down}	1447.0	1630.4 ± 54.5	1528.2	1865.5	1568.2
	V_{up}	1431.5	1327.3 ± 121.5[a,b,c]	1522.1	1754.2	1819.5
	V_{down}	1286.0	1717.4 ± 54.8	1662.9	1850.7	1499.4
B	H_{up}	1484.9	1387.5 ± 87.9	1580.8	1654.8	1551.9
	H_{down}	1562.8	1459.1 ± 58.5	1636.7	1531.3	1611.9
	V_{up}	1570.6	1046.6 ± 125.1[a,b,c]	1656.9	1644.0	1669.8
	V_{down}	1608.0	1480.0 ± 28.8	1765.4	1817.2	1799.6

A: hydrophilic filter; B: hydrophobic filter.
[a]significantly lower than H_{up}; [b]significantly lower than H_{down}; [c]significantly lower than V_{down}.

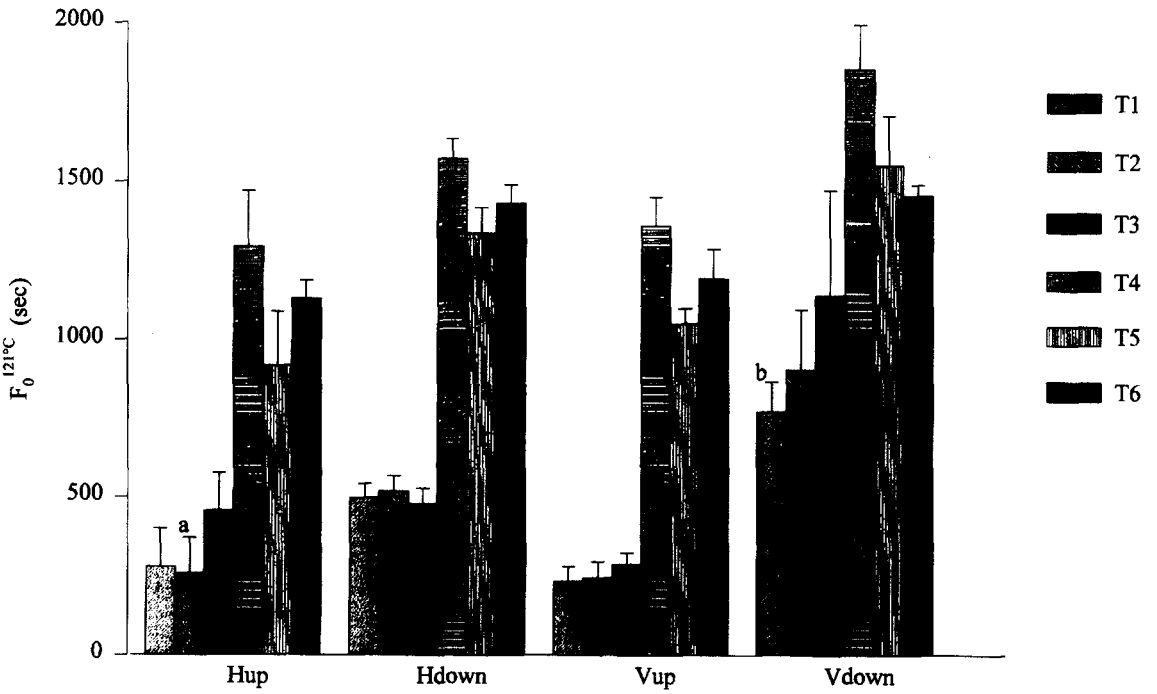

Fig. 4. Mapping of one hydrophilic Sealkleen® with six therrnocouples (n = 6). [a]significant lower than T1; [b]significant lower than T2 (error bars = ± S.D.).

and the number of residual viable cells (colony forming units, CFU) on the spore strips. Four hydrophilic Kleenpak® filters were autoclaved at the same time each in a different position (Fig. 2). The sterile side was closed with medical paper. In order to be able to discriminate between the different filter positions, the sterilisation phase of the autoclaving cycle was set at 6 min. The spore strip was attached with autoclave tape to the thermocouple placed at the coldest spot. This experiment was performed twelve times. After sterilisation, the strip was extracted with peptone water, appropriate dilutions up to 10^{-4} were made with peptone and incubated at 56° for 3 days. The number of colony forming units (CFU) of *B. stearothermophilus* were counted on tryptic soy agar (TSA).

3. Results and discussion

The results of the temperature mapping of the filterhousings, are shown in Table 1 for the Sealkleen® and Table 2 for the Kleenpak®. Fig. 4 shows the results for the temperature mapping of the hydrophobic Sealkleen® filter. The data revealed that thermocouple no. 1 (T1) detected the coldest spot in both the Sealkleen® and the Kleenpak®, although this did not always reach a significance level of 0.05 between the three thermocouples at the sterile side in the H_{up} and H_{down} positions in the Sealkleen® housing. A plausible reason for the cold spot location was an air entrapment causing an inadequate steam penetration. Although, it has to be emphasized that the testing was done with a single vacuum cycle. Therefore, the retention of air within the filter core and the resultant temperature gradient is exacerbated. In a typical production autoclave cycle, with multiple vacuum applications, this difference is likely to be reduced. Taking the

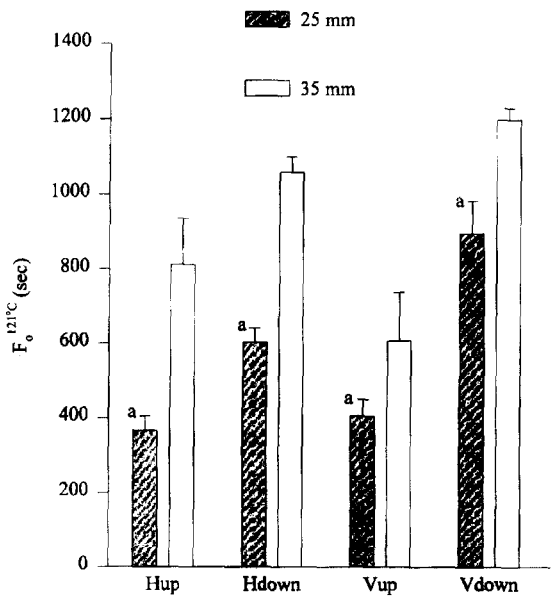

Fig. 5. Influence of connector diameter on $F_0^{121°C}$-values at the coldest spot in the hydrophilic Sealkleen® (n = 3). [a]significantly lower than 35 mm (P = 0.125) (error bars = ± S.D.).

position of thermocouple no.5 in the Sealkleen®, much higher $F_0^{121°C}$-values were obtained than expected. This can be due to the fact that the drain valve, which was always open during autoclaving and positioned near the bottom of the housing (Fig. 1a) allowed the steam to flow through. Another reason could be the contact of the thermocouple with the metal housing. As thermocouple no.1 was detected as the coldest spot, the $F_0^{121°C}$-values for that thermocouple were further used as comparable parameter.

Table 3
Influence of the packaging method on $F_0^{121°C}$-values (s) for the Sealkleen® in V_{down} position (mean ± S.D., n = 6)

	Hydrophilic	Hydrophobic
Open	913.8 ± 146.4[a]	1229.9 ± 320.3
SSC	1096.8 ± 151.0	1258.3 ± 154.4
Bag	1175.6 ± 319.6	1010.7 ± 126.4[a,b]
SSC/BAG	1116.2 ± 285.4	918.0 ± 65.4

[a]significantly lower than SSC; [b]significantly lower than Open.

Table 4
Influence of the packaging method on $F_0^{121°C}$-values (s) for the Kleenpak® in V_{down} position (mean ± S.D., n = 6)

	Hydrophilic	Hydrophobic
Open	1717.4 ± 54.8	1606.9 ± 28.8
SSC	1594.0 ± 83.7	1480.0 ± 45.2
Bag	1620.3 ± 165	1492.1 ± 230.0
SSC/Bag	1474.4 ± 138.5	1510.6 ± 55.5

The study on the positioning of the filter housing revealed that the V_{up} position was the worst position showing the lowest $F_0^{121°C}$-values and that V_{down} was the optimal position with the highest $F_0^{121°C}$-values (Table 1) at thermocouple no.1. The hydrophilic Sealkleen® filter tested six times in the V_{down} position gave a significantly higher $F_0^{121°C}$-value than the three other positions. The $F_0^{121°C}$-value of the V_{up} position was significantly lower than the H_{down} and V_{down} position, respectively. For the six different hydrophilic Sealkleen® filters, the $F_0^{121°C}$-value for the V_{down} position was significantly higher than the $F_0^{121°C}$-values for the V_{up} and the H_{up} position, respectively. The $F_0^{121°C}$-values for the V_{up} position were significantly lower than all the three other positions. The V_{up} $F_0^{121°C}$-values for the six hydrophobic Sealkleen® filters were significantly lower than in the H_{up} position. The $F_0^{121°C}$-values for the V_{down} position were significantly higher than in the H_{up} position.

These results were confirmed for the Kleenpak® capsules. Because the Kleenpak® capsules are made of a plastic body, the heating time is much shorter in comparison with the metal Sealkleen® filter housing and differences in $F_0^{121°C}$-values were much smaller. The $F_0^{121°C}$-values for the V_{up} position in the hydrophilic filter were significantly lower than those for the three other positions, while $F_0^{121°C}$-values for the V_{down} position were significantly higher than in the V_{up} and the H_{up} positions, respectively. This indicates the ideal position for in-line steaming is the worst position for autoclaving. This is probably because air is entrapped in the filter when placed in the V_{up} position. Rotation of the filter might also improve the evacuation of condensate from the metal housing in the Sealkleen® filter.

As the thermocouples were fixed using a connector, the influence of the diameter of the connector was investigated on the $F_0^{121°C}$-values for thermocouples no.1 with the filter in the four

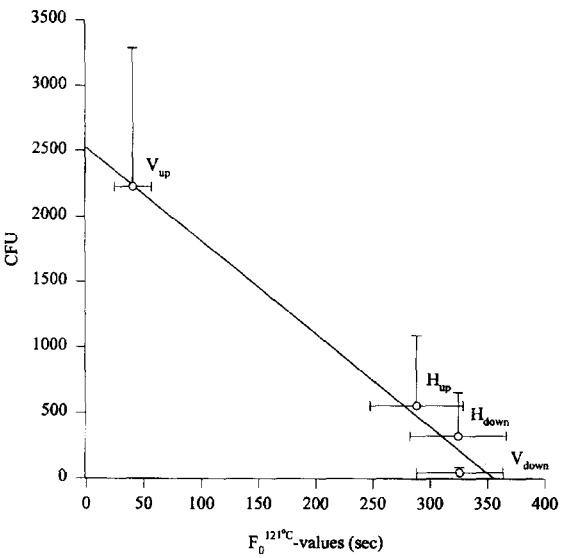

Fig. 6. CFU vs. $F_0^{121°C}$-values for the hydrophilic Kleenpak® assembly (error bars = ± S.D.).

different positions. On both the in- and the outlet of the filter a connector was attached and every test was repeated three times. The results (Fig. 5) showed that the $F_0^{121°C}$-values produced at the coldest spot were significantly lower for the smaller connector ($P = 0.125, n = 3$). This indicated that connecting tubes to the filter housing should not be modified without a previous validation step. Young et al. (1994) described that steam sterilisation of deadlegs was mostly influenced by tube diameter and less by length.

After sterilisation, the filter should be protected against recontamination. Several methods are currently used in industry, as placing the filter housing in a bag, or closing the sterile side of the housing with steam permeable paper or a combination of both techniques. Several materials are available for these purposes, like medical paper, Tyvec®, etc.

In this study, a comparison was made between the different methods available using a non-packed filter housing as a control. Only the optimal V_{down} position was tested. Table 3 shows the results for the Sealkleen® filterhousing. In the case of the hydrophilic filter, the open (non-packed) filter showed a significantly lower $F_0^{121°C}$-value than the filter with the sterile side closed. There was no significant difference between the other packagings. The $F_0^{121°C}$-values for the hydrophobic filter positioned in the bag were significantly lower than the open filter and the filter with the sterile side closed, respectively. For the Kleenpak® filter housing, no difference was observed in $F_0^{121°C}$-values between the hydrophobic and the hydrophilic filter (Table 4).

In order to show the relevance of the calculated $F_0^{121°C}$-values to allow a discrimination between the different situations, spore strips were applied. The filter used for this study was the hydrophilic Kleenpak® filter. The correlation between the mean CFU of *B. stearothermophilus* and the mean $F_0^{121°C}$-values was (r^2) 0.985 (Fig. 6). The CFU values for the V_{up} positioned filter were significantly higher than all three other positions while the CFU values for the V_{down} position was significantly lower than for the three other positions. The $F_0^{121°C}$-values were significantly lower for the V_{up} position compared to all other positions. This curve confirmed that the V_{down} position seemed to be the position of choice. The sterilisation procedure of the filters considered in this study should be fully validated in the V_{up} position.

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