



Technical Report

180612.3WUS

In-Use Evaluation of Pall Kleenpak™ KA2IN4 Disposable Filter Capsule used with the Follett® Symphony Plus™ Water and Ice Dispenser

1 Introduction

The Pall Kleenpak KA2IN4 Disposable Filter Capsule is designed and validated to reduce the presence of waterborne pathogens within influent supply water and is composed of a double layer of 0.2 µm sterilizing grade Supor® media, incorporating a graded pre-filtration membrane.

KA2IN4 has been validated according to the industrial standard liquid microbial challenge tests for 0.2 µm sterilizing grade filters using *Brevundimonas diminuta*^{1,2}. The Filter Capsule can operate at a continuous water pressure up to 75 psi at up to 185 °F without affecting its integrity and, its chemical compatibility has been demonstrated for a wide range of chemical compounds commonly used in water treatment².

Performance of KA2IN4 has been investigated in conjunction with the Symphony Plus™ Water and Ice Dispenser (Follett LLC, PA, USA) under real life conditions simulated in a laboratory setting. Following initial evaluation of the bioburden of the delivered dispenser, a KA2IN4 Filter Capsule was fitted upstream of the Symphony Plus dispenser and the microbiological quality of the dispensed ice tested for the presence of *Pseudomonas aeruginosa*, *Pseudomonas* spp., *Escherichia coli*, coliforms and total aerobic count (TAC) at 71.6 °F and at 98.6 °F over a 6 month period (189 days).

In this evaluation a comparison of the microbial load of the ice was assessed in the presence and absence of a KA2IN4 Filter Capsule each over a 6 month period.

2 Material and methods

Crossover evaluation was carried out over a 12 month period under real life conditions simulated in a laboratory setting. During this period, the quality of the ice dispensed by the Symphony Plus was microbiologically

tested to determine the presence of bacterial contamination and identify potential establishment of biofilms.

Prior to the evaluation, and upon its completion, the general quality of the influent water was assessed by Silt Density Index (SDI) Test³. This test provides an indication of the level of particulate and colloidal materials in the water. In addition, the microbiological quality of the influent water was tested for *Pseudomonas aeruginosa*, *Pseudomonas* spp., *Escherichia coli*, coliforms and Total Aerobic Count (TAC) at 71.6 °F and at 98.6 °F.

To simulate real-life usage conditions during the evaluation, the Symphony Plus dispenser was programmed to automatically dispense ice and water for 6 seconds every 6 minutes over a 16 hour period each day. The ice was dispensed into a chute and the water straight into the drip tray. Samples for testing were collected directly from the dispense shoot into sterile containers, containing sodium thiosulfate pentahydrate, which acts as a chlorine neutralizer.

During the first 6 months of the evaluation (October-March), the KA2IN4 Filter Capsule was installed upstream of the water inlet of the Symphony Plus dispenser machine. 0.26 gal (1 L) of ice was aseptically collected and left to thaw at room temperature. Aliquots of the thawed ice (3.38 oz / 100 mL) were tested for *Pseudomonas aeruginosa*, *Pseudomonas* spp., *Escherichia coli* and coliforms, respectively. Aliquots (0.2 tbsp / 1 mL) were analysed for TAC at 71.6 °F and at 98.6 °F. Ice samples were collected and analysed on a weekly basis up to day 28. Following this period, the analysis was carried out biweekly up to day 189 inclusive.

At the end of the 6 months, the KA2IN4 Filter Capsule was disconnected from the Symphony Plus dispenser and its integrity assessed by Liquid Microbiological Challenge Test with *Brevundimonas diminuta*¹ and non-destructive Forward Flow Integrity Test⁴.

The Symphony Plus was then descaled and sanitized as described in the “optimal cleaning practice” report⁵. A further 6 months of samples (April - September) were collected and analysed against the same panel of bacteria and at the same frequency as the filtered ice samples.

Additionally, swab samples were taken at defined points on the Symphony Plus dispenser at the beginning and end of each 6 month period to identify potential establishment of biofilms. The swabs were then analysed for total aerobic counts at 71.6 °F and at 98.6 °F.

3 Results

3.1 Silt Density Index (SDI)

SDI was determined for the incoming water using 0.45 µm filter discs. The estimated SDI values was 3.2 in the analysis carried out pre-evaluation and 3.5 for the post-evaluation (Table 1). Both values indicate a low level of > 0.4 µm particulate in the influent water.

Table 1.
SDI tests carried out pre- and post- evaluation.

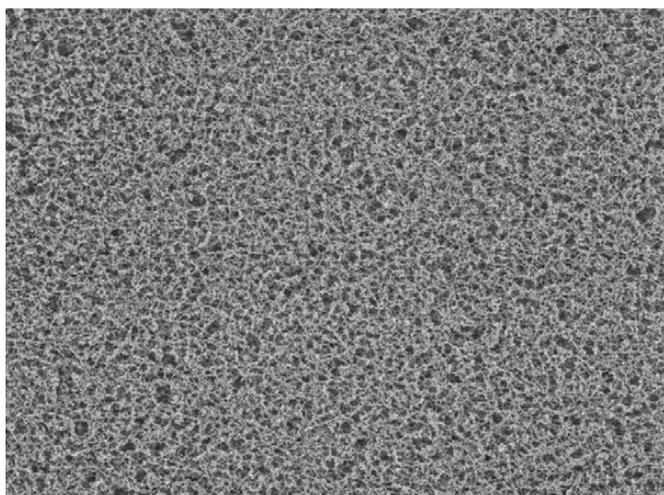
Sample	Temperature	SDI Value
Pre-evaluation	13.5 °C	3.2
Post-evaluation	11 °C	3.5

3.2 Scanning electronic microscope (SEM) and Energy-Dispersive Spectroscopy (EDS) analysis

The membrane used to estimate the SDI value post-evaluation, was analysed by SEM/EDS, which provides more detailed information on particulate matter retained on the membrane. The results from the SEM and EDS analysis are shown in Figure 1 and Table 2, respectively.

Figure 1.

A) SEM of the control SDI membrane (x 1000).



B) SEM of the post-evaluation SDI membrane (x 1000)

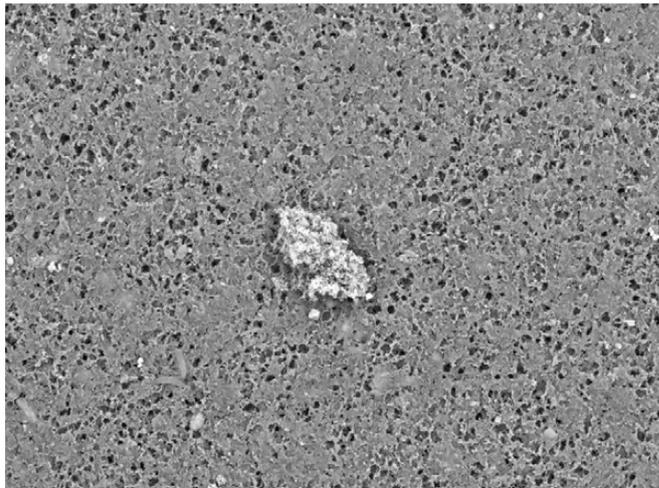


Table 2.
EDS results: detection of < 1% not included. *Gold (from SEM coating process).

Elements	Weight (%)	
	Control Membrane	Post-Evaluation SDI Membrane
Oxygen	56.7	50.7
Carbon	36.2	24.2
*Gold	7.1	15.4
Aluminium	-	4.5
Calcium	-	1.9
Iron	-	1.7
Silicon	-	1.6

3.3 Microbiological analysis

3.3.1 Influent Water

Prior to the evaluation, 0.26 gal (1 L) of water was aseptically collected from the mains water system to test for the presence of bacteria. Aliquots (0.2 tbsp, 1 mL) of the collected water were tested for the total aerobic count (TAC).

Aliquots of 3.38 oz (100 mL) were used to investigate the presence of *Pseudomonas* spp., *E.coli* and coliforms. The results of the microbiological analysis of the influent water are shown in Table 3.

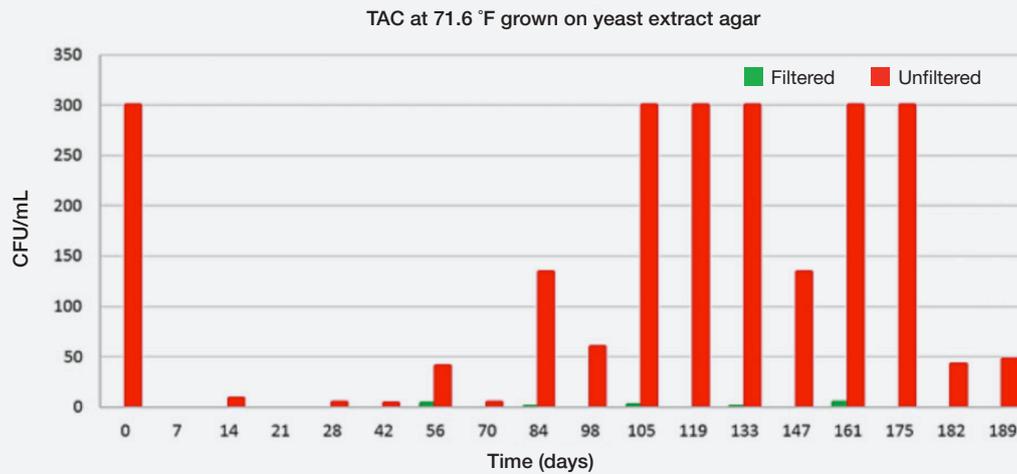
Table 3.

Influent water microbiological analysis. The samples were collected immediately and after 1 minute flushing.

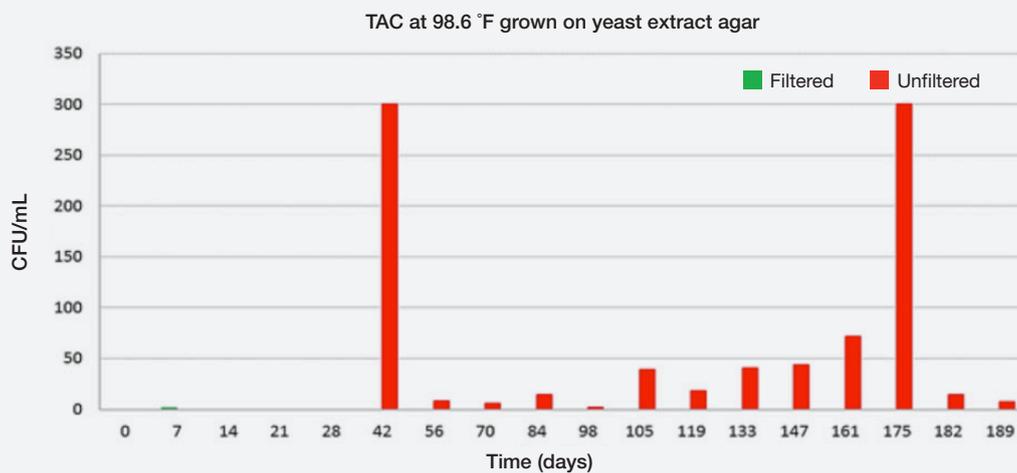
Sampling time	Total Aerobic Count (TAC)		<i>Pseudomonas</i> spp. (CFU/mL)	<i>Pseudomonas aeruginosa</i> (CFU/mL)	<i>Escherichia coli</i> (CFU/mL)	Coliforms (CFU/mL)
	71.6 °F (CFU/mL)	98.6 °F (CFU/mL)				
Immediate	>300	>300	0.64	0	0	0
Post - 1 min flush	>300	7	0.49	0	0	0

Figure 2.

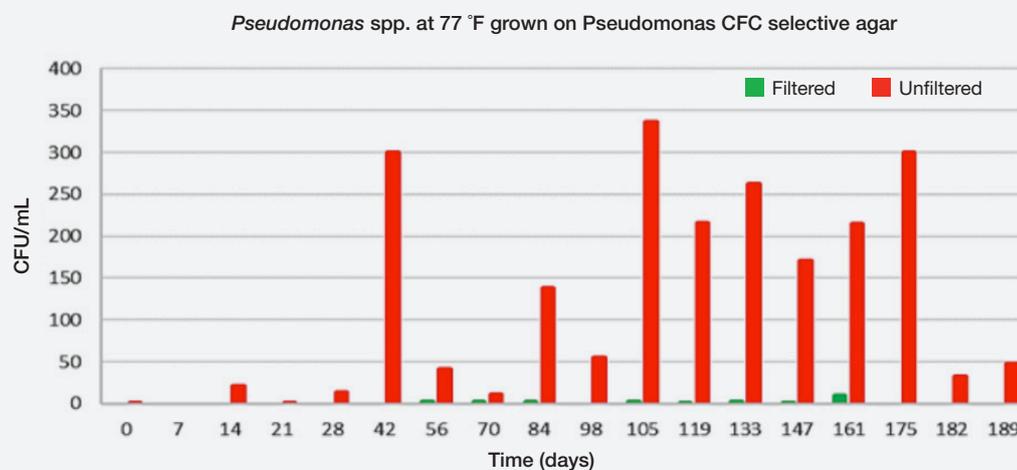
A) Total aerobic count (TAC) incubated at 71.6 °F from filtered and unfiltered ice samples.



B) Total aerobic count (TAC) incubated at 98.6 °F from filtered and unfiltered ice samples.



C) *Pseudomonas* spp. counts from filtered and unfiltered ice samples.



Note: between 175 and 189 days, the filtered ice samples were not collected due to a failure of the ice dispenser valve.

3.3.2 Ice samples

Ice (0.26 gallons/ 1 L) was collected from the Symphony Plus dispenser and tested for TAC, *Pseudomonas aeruginosa*, *Pseudomonas* spp., *Escherichia coli* and coliforms. The samples were taken at regular periods and analysed over the 12 months.

The results of the microbiological analyses, carried out on the ice samples collected during the filtered and unfiltered period, are summarised in Figure 2. Full data are shown in Appendix 1.

Pseudomonas fluorescens, *Pseudomonas putida*, *Pseudomonas stutzeri* and *Pseudomonas mendocina*, were identified among the *Pseudomonas* species in both filtered and unfiltered ice samples. *Pseudomonas* species were characterised using the RapID NF Plus system (Thermo Fisher Scientific).

Pseudomonas aeruginosa, *E.coli* and coliforms were not detected at any time during either the filtered or unfiltered periods of the evaluation.

3.3.3 Microbial Challenge Testing

The KA2IN4 Filter Capsule was tested after the 6 month evaluation period to assure robustness and assess bacterial retention capability using *Brevundimonas diminuta*. A summary of the bacterial challenge is shown in Table 4.

Table 4.

Liquid bacterial Microbial Challenge Test Results for the KA2IN4 Filter Capsule used in the 6 month evaluation vs. new KA2IN4 Filter Capsule (Control).

	Filter Capsule Number	Total Challenge	Challenge per Effective Filter Area (CFU/cm)	Recovery	Titre Reduction
6 month used KA2IN4	IX37710070	1.78E10	2.39E07	0	>1.78E10
Control	IX37710011	1.78E10	2.39E07	0	>1.78E10

3.3.4 Forward Flow Integrity Test

The filter passed the Forward Flow Integrity Test with limits correlated to bacterial retention following the 6 month evaluation period.

Table 5.

Forward Flow Integrity Test results following the 6 month in-use evaluation period.

Filter number	Integrity Test	Remarks
IX37710070	Pass	None

3.3.5 Swabs

Tables 6 and 7 show the results of the microbiological analysis of the swab samples taken at day 0 and day 189 of each 6 month test period, respectively. The swabs were used to sample 8 pre-determined surfaces within the Symphony Plus during the filtered and unfiltered period. Prior to starting the evaluation and after removing the KA2IN4 filter capsule, the in-use system was sanitized following the “optimal cleaning practice” report⁵.

Due to a valve failure on the water reservoir, there had been no movement of water through the reservoir from day 175 to day 189 whilst the replacement valve was ordered. During these two weeks, it was not possible to collect ice samples.

A schematic representation of the swab sample points is shown in Figure 3 (see page 7).

Table 6.

Total aerobic count (TAC) of swab samples collected at day 0 of the filtered and unfiltered (post-cleaning) period.

Sample	Location	Day 0		Day 0 (post-cleaning)	
		Filtered		Unfiltered	
		CFU/mL @ 71.6 °F	CFU/mL @ 98.6 °F	CFU/mL @ 71.6 °F	CFU/mL @ 98.6 °F
1	Ice spout	0	1	89	0
2	Water spout	0	0	0	0
3	Drip tray	0	0	>300	0
4	Ice hopper ledge	0	0	0	0
5	Ice mixing blade	0	0	6	0
6	Ice hose	0	0	29	0
7	Water reservoir	0	1	72	0
8	Sanitiser cup	0	0	0	0

Table 7.

Total aerobic count (TAC) of swab samples collected at day 189 of the filtered and unfiltered period.

Sample	Location	Day 189		Day 189	
		Filtered		Unfiltered	
		CFU/mL @ 71.6 °F	CFU/mL @ 98.6 °F	CFU/mL @ 71.6 °F	CFU/mL @ 98.6 °F
1	Ice spout	3	0	>300	>300
2	Water spout	0	0	0	1
3	Drip tray	>300	>300	>300	>300
4	Ice hopper ledge	0	0	18	3
5	Ice mixing blade	0	0	11	3
6	Ice hose	0	0	>300	>300
7	Water reservoir	>300	>300	2	0
8	Sanitiser cup	0	1	0	0

Figure 3.
Symphony Plus Water and Ice Dispenser Swab Sample Points.



4 Discussion

This evaluation has demonstrated that the Pall Kleenpak KA2IN4 Disposable Filter Capsule can improve the microbiological quality of ice dispensed by the Symphony Plus for up to six calendar months in a laboratory setting.

4.1 Influent water

The microbiological analysis of the influent water showed no presence of *Pseudomonas aeruginosa*. However, significant bacterial contamination was detected within the mains water system as indicated by the high number of aerobic colonies present in both, immediate and post-flush water samples (Table 3).

On the contrary, both SDI measurements taken pre- and post- evaluation, showed a relatively low particulate and colloidal loading in the influent water, as also confirmed by the SEM/EDS analysis. The low particle concentration detected in the water enables long filter lifetime without an installed upstream pre-filter system.

The flow rate of the water dispensed by the Symphony Plus was not affected by the presence of the Filter Capsule as shown in Table 8.

Table 8.

Symphony Plus flow rate during the evaluation period.

Filter Capsule Presence	Minimum Dispensed Water Flow	Maximum Flow Dispensed Water Flow
Filtered	0.41 gal/min (1.55 L/min)	0.88 gal/min (3.35 L/min)
Unfiltered	0.40 gal/min (1.51 L/min)	0.89 gal/min (3.37 L/min)

4.2 Evaluation period

During the 6 month unfiltered period, significant bacterial growth was observed on the TAC plates incubated at both 71.6 °F and 98.6 °F. These values ranged from 0 to over 300 CFU/mL. As shown in Figure 2, the bacterial growth for the unfiltered samples varied throughout the testing period with a substantial increase observed after day 42. These fluctuations were likely associated to environmental changes in the influent water (i.e. temperature, carbon source, pH, etc.), or to the establishment of biofilm within the ice and water dispenser.

In contrast, during the filtered period a minimal growth was observed on the TAC plates of the ice samples. The colony count ranged from 0 to 5 CFU/mL on plates incubated at 71.6 °F, and from 0 to 1 CFU/mL on the plates incubated at 98.6 °F.

Pseudomonas spp. were low in the filtered ice samples, with an average value of 2 CFU/mL per plate (range: from 0 to 9.9 CFU/mL). Conversely, more than 94 % of *Pseudomonas* positive samples were found in the unfiltered ice samples.

Overall four different species of *Pseudomonas* were identified from the ice samples. These included: *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas stutzeri* and *Pseudomonas mendocina*. These species may represent a potential risk for immunocompromised patients⁶⁻⁹, and any contamination found in a healthcare setting should be assessed by the Water Safety Team¹⁰.

4.3 Filter integrity

Results from the Microbiological Challenge and the Forward Flow Integrity Test demonstrated that the KA2IN4 Disposable Filter Capsule maintained its integrity through the fatigue of 30,240 ON/OFF cycles and more of 2,600 gallons (10,000 L) of water filtered during the study period.

4.4 Swab samples

The swab data showed minimal evidence of bacterial contamination at day 0 of the filtered phase when compared to the unfiltered period (Table 6 and 7). It should be noted that the unfiltered evaluation was carried out after the filtered study and it is likely that the sanitization process is unable to effectively remove all microorganisms within the dispenser either before the Filter Capsule was installed or after its removal.

At the end of the 6 month filtered period, positive swab samples were found only in sample 3 (drip tray) and sample 7 (water reservoir). It should also be noted that the water reservoir valve failed between day 175 and day 189 during the filtered study. In contrast, at the end of the unfiltered period there was bacteria detected at every swab point indicating biofilm establishment.

The installation of the Pall Kleenpak KA2IN4 Disposable Filter Capsule upstream of the Follett Symphony Plus minimized the microbiological contamination in the dispensed ice and also reduced the growth of bacteria within the dispenser.

5 Conclusion

This evaluation has demonstrated that the Pall Kleenpak KA2IN4 Disposable Filter Capsule can improve the microbiological quality of the ice dispensed by the Symphony Plus for up to six calendar months.

Appendix 1

Table 9.

Baseline microbial analysis of ice samples pre- and post- sanitisation prior to starting the evaluation.

Evaluation Period	Days	Total Aerobic Count (TAC)		<i>Pseudomonas</i> spp. (CFU/mL)	<i>Pseudomonas aeruginosa</i> (CFU/mL)	<i>Escherichia coli</i> (CFU/mL)	Coliforms (CFU/mL)
		71.6 °F (CFU/mL)	98.6 °F (CFU/mL)				
Baseline	Pre sanitisation	>300	>300	0	0	0	0
	Post sanitisation	0	0	0	0	0	0
	1 week post sanitisation	0	2	0	0	0	0

Table 10.

Test microbial analysis of ice samples from filtered water (with Pall Kleenpak KA2IN4).

Evaluation Period	Days	Total Aerobic Count (TAC)		<i>Pseudomonas</i> spp. (CFU/mL)	<i>Pseudomonas aeruginosa</i> (CFU/mL)	<i>Escherichia coli</i> (CFU/mL)	Coliforms (CFU/mL)
		71.6 °F (CFU/mL)	98.6 °F (CFU/mL)				
Filtered	0	0	0	0	0	0	0
	7	0	1	0	0	0	0
	14	0	0	0	0	0	0
	21	0	0	0	0	0	0
	28	0	0	0	0	0	0
	42	0	0	0	0	0	0
	56	4	0	3	0	0	0
	70	0	0	3	0	0	0
	84	1	0	3	0	0	0
	98	0	0	0	0	0	0
	105	2	0	3	0	0	0
	119	0	0	0.02	0	0	0
	133	1	0	3	0	0	0
	147	0	0	0.01	0	0	0
	161	5	0	9.9	0	0	0
	175(*)	N/a	N/a	N/a	N/a	N/a	N/a
	182(*)	N/a	N/a	N/a	N/a	N/a	N/a
	189	N/a	N/a	N/a	N/a	N/a	N/a

(*) Ice samples were not collected due to a failure of the ice valve.

Table 11.

Control microbial analysis of ice samples from unfiltered water.

Evaluation Period	Days	Total Aerobic Count (TAC)		<i>Pseudomonas</i> spp. (CFU/mL)	<i>Pseudomonas aeruginosa</i> (CFU/mL)	<i>Escherichia coli</i> (CFU/mL)	Coliforms (CFU/mL)
		71.6 °F (CFU/mL)	98.6 °F (CFU/mL)				
Unfiltered	0	>300	0	0.1	0	0	0
	7	0	0	0	0	0	0
	14	9	0	21	0	0	0
	21	0	0	0.3	0	0	0
	28	5	0	13.5	0	0	0
	42	4	>300	>300	0	0	0
	56	41	8	41	0	0	0
	70	5	6	11	0	0	0
	84	134	14	138	0	0	0
	98	60	2	55	0	0	0
	105	>300	39	337	0	0	0
	119	>300	18	216	0	0	0
	133	>300	41	262	0	0	0
	147	134	44	171	0	0	0
	161	>300	72	215	0	0	0
	175	>300	>300	>300	0	0	0
	182	43	14	33	0	0	0
	189	48	7	47	0	0	0
	189 post sanitization	176	0	181	0	0	0

6 References

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