

Bacteriostatic Evaluation of Paper Impregnated with Aquastat® X11 Over a Four-Week Period

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OVERVIEW

Paper sheet containing Aquastat® X11 antimicrobial (lot#5822) manufactured by K2 Concepts, Inc was tested against standard (untreated) paper sheet for its ability to prohibit bacteria and fungi growth in paper over a 4- week period.

Samples (2 x 3 inches) were cut from the sheet of Aquastat® X11 impregnated paper as well as a control paper (untreated), weighed and placed standing on end in 200 mL beakers. 10 mL of a bacteria/fungi suspension, prepared from E coli, Flavobacterium (Chryseobacterium-indologenes) and a fungi (approximately 1000 CFU/mL) was added to beakers. The beakers were sealed with parafilm for 24 hours. The following day the parafilm was replaced with a petri dish to allow ventilation. Additional stock of bacteria/fungi suspension (1000CFU/mL) was added twice weekly to the beakers to replenish the stock (see figure 1).

SAMPLING PROCEDURE

One strip of approx. 1 cm width of each paper was cut and then divided into 4 pieces, top to bottom. The top and bottom of each paper sample was placed in a sterile tube with 2 mL of sterile CaCl2 solution and then sonicated for 10 minutes. A sample of 0.5 mL of the resulting solution (suspension) was added to a R2A agar plate and incubated for three days. Samples were taken and analyzed weekly.

RESULTS

Week No	Paper section	Sample 1	Sample 2
		No antimicrobial	Aquastat® X11
1	top	+++ (F)	0
	bottom	+++ (B, F)	0
2	top	0	0 - 1
	bottom	++++ (B, F)	0
3	top	+++++ (B, F)	0
	bottom	+++++ (B, F)	0
4	top	++++ (B, F)	± B
	bottom	++ (B, F)	± B, ± F

Legend
B: Bacteria
F: Fungi
(++++) Heavy growth (TNTC)
(++) Moderate growth
(+) Slight growth
(±) 1-2 colonies

SPECIAL NOTE

After week 3 of the testing traces of bacteria colonies began to appear on all the R2A agar plates after 7 days of incubation. This bacteria species was isolated and identified by staining and found to be a gram positive spore forming Bacillus sp. Spore forming bacteria are well known for their ability to hibernate or become dormant while in a harsh or inhospitable environment. However, when these spores are transferred into a nutrient rich environment such as that found on the R2A agar plate, the spores become active and colonize. Due to the extended 7-day incubation period these Bacillus spores as found on the paper were thus not active while on the paper, but when relocated to the R2A agar became active. In spite of the presence of these spores, the Aquastat® X11 treated paper sample continued to control bacterial and fungal growth through the entire test period. Photo of Bacillus species is shown in Figure 1.

CONCLUSION

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Disclaimer:
 All results are based on independent study conducted by the authors named above. K2 Concepts, Inc maintains no involvement in these findings.



FIGURE 1

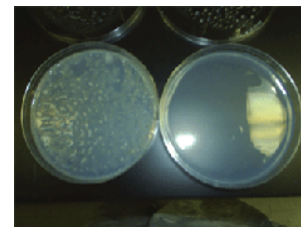


FIGURE 2
 Bacteria/Fungi found on control sample vs. sample containing Aquastat® X11

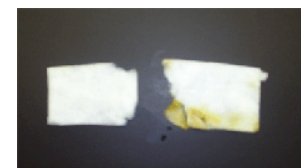


FIGURE 3
 Treated (left), un-treated (right)

Results from the R2A agar plates indicate that the Aquastat® X11 impregnated paper controlled bacterial and fungal growth even after being contaminated by guest spore forming bacteria (Bacillus) while the untreated paper supported significant bacteria and fungal growth (see Figure 2). Additionally, distinct visible differences in the treated vs. untreated paper samples were observed. Pictures of the treated vs. untreated sample are shown in Figure 4.

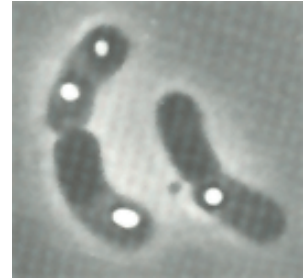


FIGURE 4
Photo of Bacillus sp.