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Biomaterials Research Report
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Pathocept Aerosol and Surface Disinfection

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Purpose – The purposes of this study were to:

1. evaluate the capability of Pathocept disinfectant to kill aerosolized salivary bacteria.
2. investigate the ability of sprayed disinfectant to kill representative aerosolized bacteria.
3. demonstrate the ability of Pathocept disinfectant to kill vegetative bacteria on "cleaned" environmental surfaces.

Materials and Methods – The experimental ***Pathocept*** disinfectant was received from *Pathocept Corporation.* Freshly collected saliva was used as the organic debris challenge for aerosol and environmental surface experiments. Individual test bacteria were: *Staphylococcus aureus* ATCC #25923, *Pseudomonas aeruginosa* ATCC #27853, and *Escherichia coli* ATCC # 25922. Cultures of each were grown aerobically in tryptic soy broth at 37C for 24 hours prior to study. Five replications of each test were performed. The chamber used for the aerosol portion of the study was prepared by cutting two 2.5 inch portals on either end of a 37 x 51 inch plastic container (Fig.1).

Phase I – Prepared suspensions of whole saliva, *S. aureus, P. aeruginosa,* or *E. coli* were sprayed simultaneously through the two portals for 5 seconds. The disinfecting capabilities of aerosolized *Pathocept* were assayed by spraying disinfectant into the chamber 1 minute after previous contamination with aerosolized bacteria or saliva (Fig. 2). One minute after treatment with ***Pathocept,*** the lids of 5 tryptic soy agar plates were removed and the plates placed at the bottom of the chamber as aseptically as possible in order to collect viable airborne bacteria (Fig 3). Test tryptic soy agar plates exposed for 5 minutes to both microbial contaminants and the disinfectant were covered and incubated aerobically at 37C for 24 hours before observation. Disinfectant aerosol controls were run using commercial *Lysol II Spray.* Negative controls were also included by assessing the presence of remaining microbial contamination in chamber air after spraying with distilled water only. Control seeded agar plates were subsequently covered and incubated aerobically as described above. It should be noted that the interior walls of the enclosed chamber were rigorously cleaned and disinfected between each test run to ensure no carry-over of contaminant bacteria.

Figure 1: Enclosed aerosol-testing chamber

Figure 2: Disinfectant introduced into chamber following aerosolized bacteria contamination

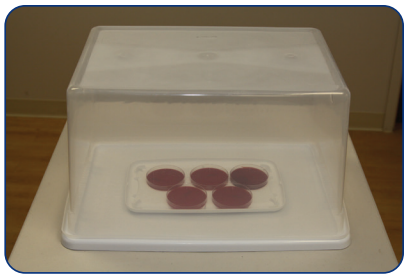

Figure 3: Collection of airborne bacteria onto culture media

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Phase II – Tryptic soy broth cultures of the three test bacteria were initially diluted 1:10 in broth. A 0.2 mL aliquot of each was applied and spread over the surfaces of five previously cleaned 2 x 2 inches counter tiles. The wetted surfaces were allowed to dry completely before disinfectant testing. The coated tiles were then treated with ***Pathocept*** or distilled water (control) by holding the disinfectant container 6-12" from the tile surface and spraying until the entire tile surface was moist. The surfaces remained wet for the recommended intermediate-level disinfection interval (10 minutes), and then wiped to remove excess liquid. Control coated tiles were sprayed with distilled water and wiped after three minutes to remove remaining liquid. The presence of viable bacteria remaining on the surfaces after wiping was assayed by replica plating the tiles on tryptic soy agar plates containing 5% sheep blood and incubated at 37C for 24 hrs.

Results –

Phase I – Data are presented as colony-forming units (CFU) recovered in Table 1. Tryptic soy agar plates that were exposed to microbial contaminants of whole saliva, *S. aureus, P. aeruginosa,* or *E. coli* in conjunction with the ***Pathocept*** disinfectant yielded no or minimal colony growth after 24 hours incubation at 37 C (Fig. 4). Samples assayed with the above microbial contaminants and *Lysol II Spray,* however, yielded residual colony growth in three of the four test systems (Fig. 5). Of the bacteria tested with this latter aerosol disinfectant, the most growth was noted for the *S. aureus* cultures, where colonies were too numerous to count (TNTC). The negative distilled water spray control data for all test bacteria and organic debris resulted in extensive microbial growth (Fig. 6).

Table 1. Efficacy of aerosolized disinfectants on microbial challenges [CFU (range)].

	Pathocept	Lysol	Distilled Water
Whole Saliva	0 (0)	251 (234-268)	TNTC*
E. coli	3.5 (1-7)	6 (3-11)	TNTC
S. aureus	7 (3-11)	TNTC	TNTC
P. aeruginosa	0 (0)	0 (0)	TNTC

*Too Numerous To Count

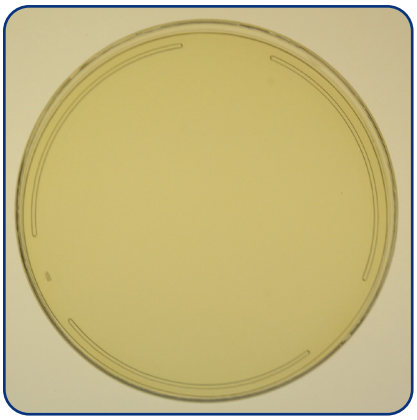

Figure 4: Aerosolized saliva treated with ***Pathocept*** disinfectant

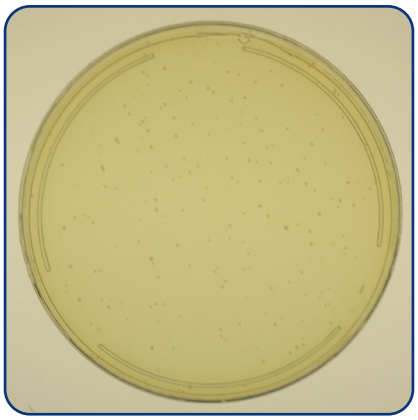

Figure 5: Aerosolized saliva treated with ***Lysol Spray II*** disinfectant

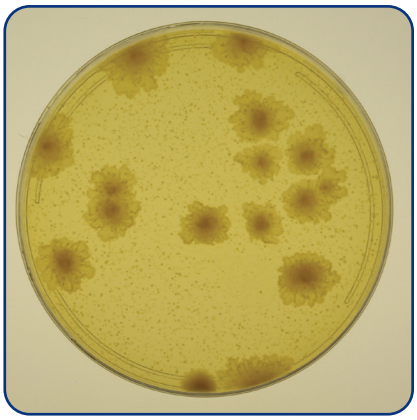

Figure 6: Aerosolized saliva treated with distilled water serving as the control

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Phase II – After 24 hours incubation, replica plate cultures for all three test bacteria disinfected with surface spraying of ***Pathocept*** showed few microbial colonies (Fig 7). In contrast, control tiles that were sprayed and wiped with only distilled water demonstrated substantially more bacterial growth (Table 2; Fig 8). Bacteria replica plate controls yielded confluent growth (Fig 9).

Table 2. Surface disinfection of bacterial-coated environmental surfaces [CFU (range)].

	Pathocept	Distilled Water
E. coli	12 (1-24)	TNTC
S. aureus	428 (225-650)	TNTC
P. aeruginosa	22 (5-40)	TNTC

*Too Numerous To Count

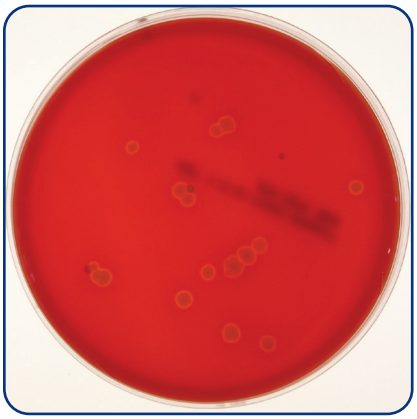

Figure 7: E. coli-contaminated surface treated with ***Pathocept***

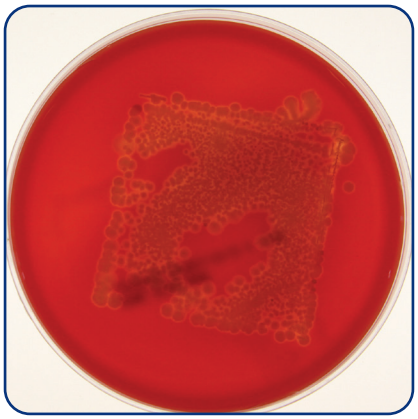

Figure 8: E. coli-contaminated surface treated with distilled water

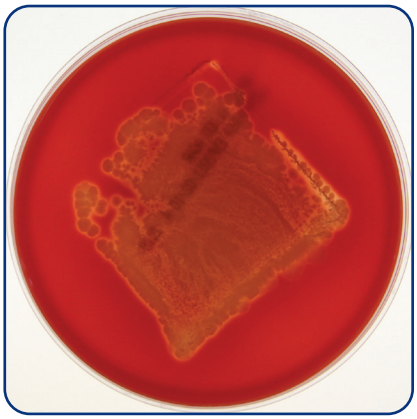

Figure 9: E. coli contaminated surface control replica plate

Conclusions – ***Pathocept*** was found to be effective as an aerosolized disinfecting agent against salivary bacteria and representative individual bacterial species. Environmental surface disinfection studies also indicated that ***Pathocept*** was successful in killing contaminant organisms.

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