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***In Vitro* Assessment of Antimicrobial Protection in Extension Lines of Central Venous Catheters Treated with Chlorhexidine after 7 Days of Use**

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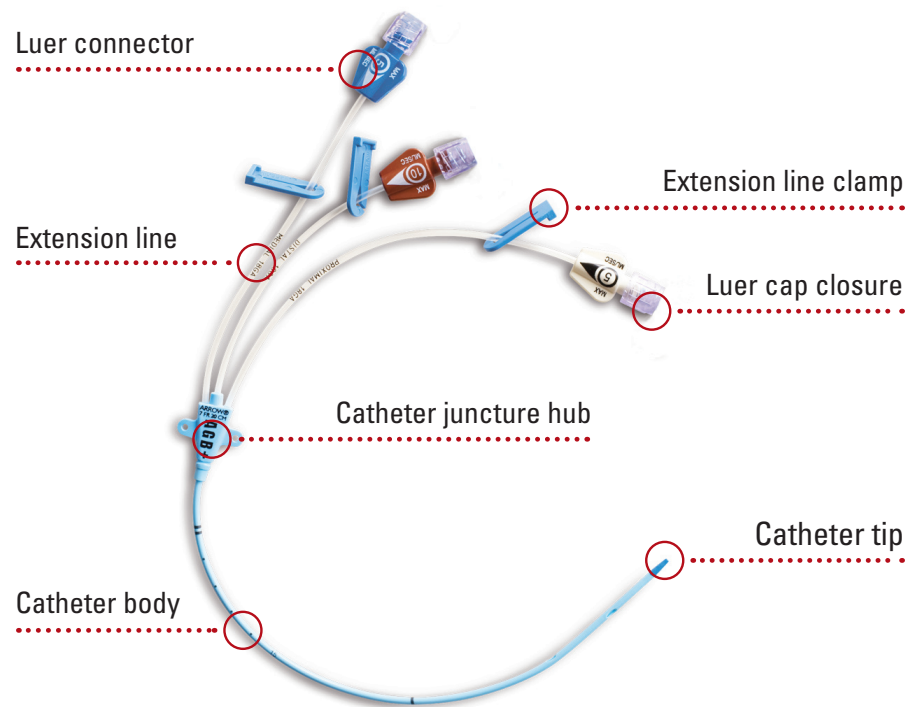
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In Vitro Assessment of Antimicrobial Protection in Extension Lines of Central Venous Catheters Treated with Chlorhexidine after 7 Days of Use

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Background: The luers and extension lines of venous access catheters are known to be entry points for luminal colonization by microorganisms.¹ Various clinical studies indicate that the longer catheters are in place, the more likely luminal pathways will become contaminated.² Microorganisms can contaminate the catheter hub and lumen at the time of catheter insertion, or later, during catheter manipulation. Sources of pathogens colonizing catheter luers and lumens of extension lines include contaminated infusates and flora from the patient, care providers, or the local environment.³ Contaminated catheter luers lead to microbial adherence and biofilm formation in the lumens of extension lines, which can become a reservoir of pathogens that later shed and contribute to bacteremias. As illustrated in the photograph below, there is more luminal surface area available for colonization in the extension lines of a 7 French triple lumen central venous catheter than in the catheter body that indwells within veins. Bacteremias with negative catheter-tip cultures have been reported.⁴



Objective: A prior study of commercially available antimicrobial CVCs⁵ showed that only the chlorhexidine treated CVC was able to prevent colonization of extension lines following a 24-hour microbial challenge. In this study we tested the ability of chlorhexidine treated extension lines to inhibit colonization via two different microbial challenge pathways following 7 days of simulated extension line use.

Methods: ARROWg⁺ard Blue PLUS[®] CVCs and untreated control CVCs were removed from their packages and the extension lines aseptically cut at the catheter hubs. The test articles consisted of the luers and full-length extension lines with open ends. To simulate use, each day they were flushed with phosphate buffered saline (PBS) and then locked for 24 hours at 37°C allowing antimicrobial agent extraction. To preclude leakage from the extension lines following locking, a hemostat was used to clamp the line at the open end, and the sliding clamps provided with the catheter were used to clamp the extension line at the luer end.

To simulate challenge by a contaminated infusate, following the 7th day of locking, the extension lines were flushed and then inoculated with challenge organisms (10³ CFU/ml): *S. aureus* (ATCC 33591), *C. albicans* (ATCC 10231), *S. epidermidis* (ATCC 35983), *E. faecalis* (ATCC 51229) and *P. aeruginosa* (ATCC 27853). After incubation for 24 hours at 37°C, the lines were flushed, the clamped ends were cut off, the remainders were sliced lengthwise to expose the luminal surfaces and then were sonicated in DE neutralizing broth. Dilution plating utilized DE neutralizing agar.

To simulate challenge by contaminated closures, the extension lines were also filled with broth and then closed with a screw-on cap that had been pre-colonized for 48 hours with the same challenge organisms. After 24 hours incubation with the contaminated caps, the same recovery and plating method used on the extension lines in the contaminated infusate challenge was utilized here.

Results: Recovered organisms from the simulated contaminated infusate challenges are presented in the table below:

Organism	Untreated Extension Line (cfu/mL)	Chlorhexidine Treated Extension Line (cfu/mL)
<i>Staphylococcus aureus</i>	3.50E+04	0.00E+00
<i>Staphylococcus epidermidis</i>	3.50E+05	0.00E+00
<i>Enterococcus faecalis</i>	8.40E+03	0.00E+00
<i>Pseudomonas aeruginosa</i>	6.00E+06	0.00E+00
<i>Candida albicans</i>	2.80E+03	0.00E+00

Recovered organisms from the simulated contaminated cap challenges are presented in the table below:

Organism	Untreated Extension Line (cfu/mL)	Chlorhexidine Treated Extension Line (cfu/mL)
<i>Staphylococcus aureus</i>	1.20E+04	0.00E+00
<i>Staphylococcus epidermidis</i>	4.80E+05	0.00E+00
<i>Enterococcus faecalis</i>	4.00E+02	0.00E+00
<i>Pseudomonas aeruginosa</i>	6.40E+03	0.00E+00
<i>Candida albicans</i>	2.40E+04	0.00E+00

Conclusions:

- Following 24 hours of incubation, both the contaminated caps as well as the contaminated infusates were able to colonize extension lines.
- Significant populations of pathogens were able to colonize untreated extension lines.
- After 7 days of simulated use, the chlorhexidine treatment was effective in preventing colonization of extension lines both via contaminated luers as well as via contaminated infusates.

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