Effect of a Novel Catheter Locking Solution for the Prevention of Hemodialysis Catheter-Related Infection

By

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A Thesis submitted to the Faculty of Graduate Studies of The University of Manitoba
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Abstract

Catheter-related infections (CRIs) are a growing problem in the hemodialysis population. There is therefore an increased need for an effective catheter locking solution for CRI prevention. Current antibiotic locking solutions can prevent infection, but microbial resistance may be a significant limitation. Our solution containing ethanol and trisodium citrate (TSC) should eliminate this concern while providing anticoagulation in the catheter between treatments. In this *in vitro* study, we hypothesized that our novel ethanol/TSC solution would kill bacteria in culture and prevent biofilm formation by organisms that cause hemodialysis CRI. Using a two part experimental procedure, testing a suspension of bacteria in culture and testing biofilm growth using the Calgary Biofilm Device, we confirmed that our ethanol/TSC catheter locking solution kills organisms in culture within 1 hour and prevents biofilm formation. These results are very promising and further clinical investigations are warranted in the hemodialysis population.

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1. Background

1.1 Epidemiology

The prevalence of end-stage renal disease (ESRD) has increased dramatically in North America over the last decade. For example, in Canada, from 1990 to 2004, there was a 158% increase in the number of patients with ESRD (Figure 1). [1, 2] Similarly, in the United States from 1990 to 2004, there was a 151% increase in the prevalent number of ESRD patients. [3] It is thought that this increase may be due to the growing number of people with diabetes in the population. [4]

For management of ESRD, patients can undergo one of three different treatments. The patient may receive a kidney transplant, may undergo peritoneal dialysis or may need to be treated using hemodialysis. In Canada, from 1990 to 2004, there were significant decreases in the percentage of ESRD patients with a functional kidney transplant, as well as those undergoing peritoneal dialysis as a treatment (Figure 2). [1, 2, 5] A dramatic increase in the use of hemodialysis as a treatment has resulted. Hemodialysis use in Canada has increased from approximately 32% in 1990 to almost 50% in 2004 (Figure 2). [1, 2, 5] Over 15,000 Canadians with ESRD currently utilize hemodialysis for treatment of ESRD, making hemodialysis the most prevalent treatment for ESRD in Canada. [2] Similarly, in the United States, the prevalence of hemodialysis increased from 62% in 1990 to 66% in 2003. [6]

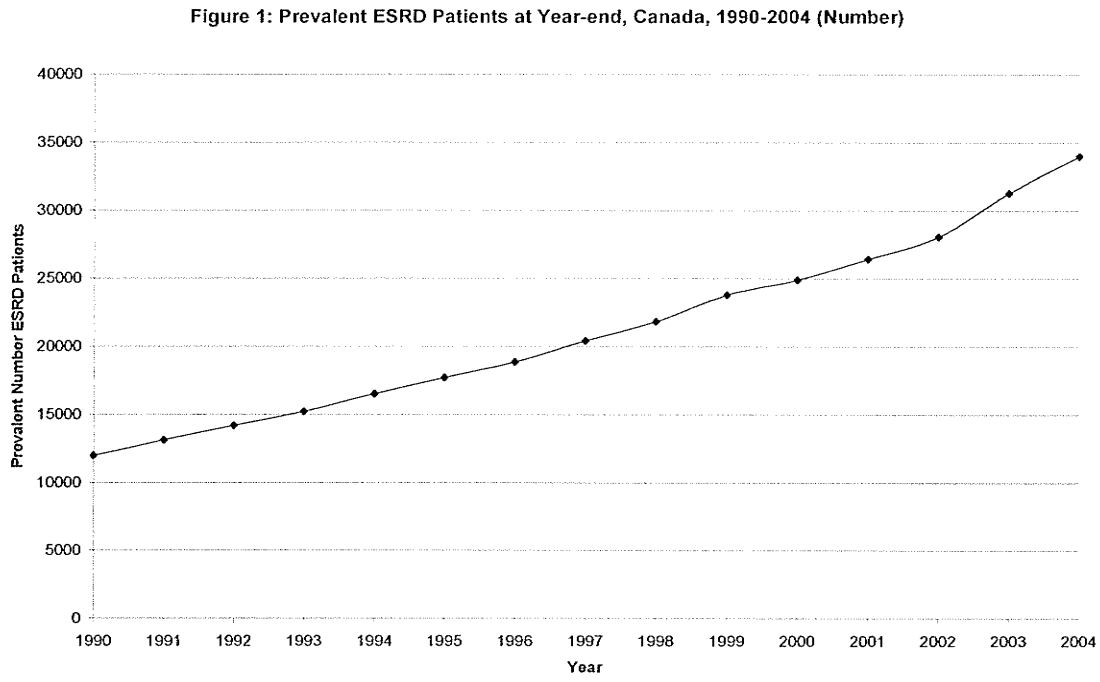

Figure 1. Prevalent End-Stage Renal Disease Patients at Year-end in Canada, 1990-2004 (Number) [5]

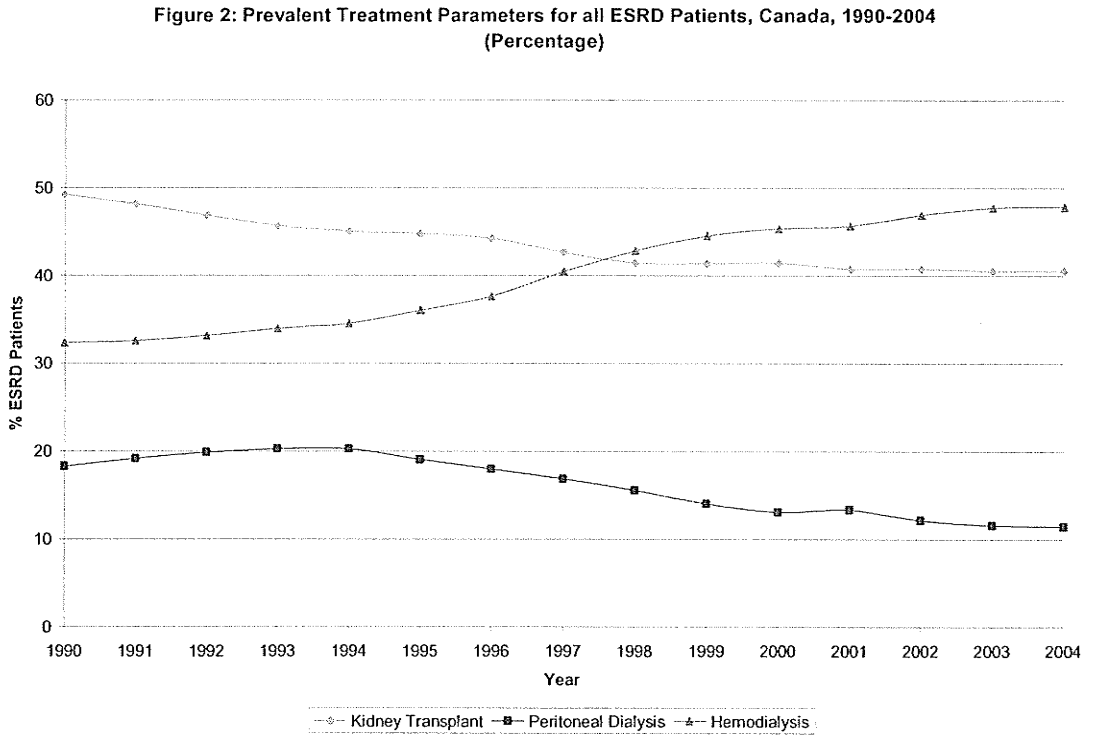

Figure 2. Prevalent Treatment Parameters for all End-Stage Renal Disease Patients in Canada, 1990-2004 (Percentage) [5]

Hemodialysis involves pumping the patient's blood out of the body and through a dialyser. The blood undergoes diffusion of toxins, electrolytes and water against a solution of electrolytes across a semi-permeable membrane, and then returns to the patient. The procedure is primarily carried out three times a week, for four hours at a time, in hemodialysis centres. In order to provide hemodialysis as treatment for ESRD, access to blood vessels is required. The three main vascular access types used in hemodialysis are arteriovenous (AV) fistulas, polytetrafluoroethylene (PTFE) grafts and cuffed, tunneled catheters. A common type of AV fistula is created by surgically joining the brachial artery and the cephalic vein, creating a region of high pressure in which a needle can be inserted for vascular access. They are the ideal and preferred method of access due to low thrombosis and infection rates. While the prevalence of AV fistulas has remained relatively constant from 1991 to 2000, a very slight decrease has been seen recently (Figure 3). [1, 5] PTFE graft formation involves linking the brachial artery and the cephalic vein using a synthetic material such as PTFE (Gortex ®). Its use has also declined over the same time period (Figure 3). [1, 5] Concurrently, permanent catheter use has increased dramatically. As shown in Figure 3, roughly 5% of vascular accesses in 1991 were with permanent catheters. That percentage quickly grew over the next decade and reached nearly 28% in 2000. [1, 5] More recent literature suggests that the percentage of permanent catheter use for vascular access in hemodialysis is now approximately 32%.[7, 8] This increase in the use of catheters clearly demonstrates that while they are not the most ideal method of vascular access, they have become essential in the management of hemodialysis patients.

Figure 3: Prevalence of Arteriovenous Access Types Used in Hemodialysis, Canada, 1991-2000 (Percentage)

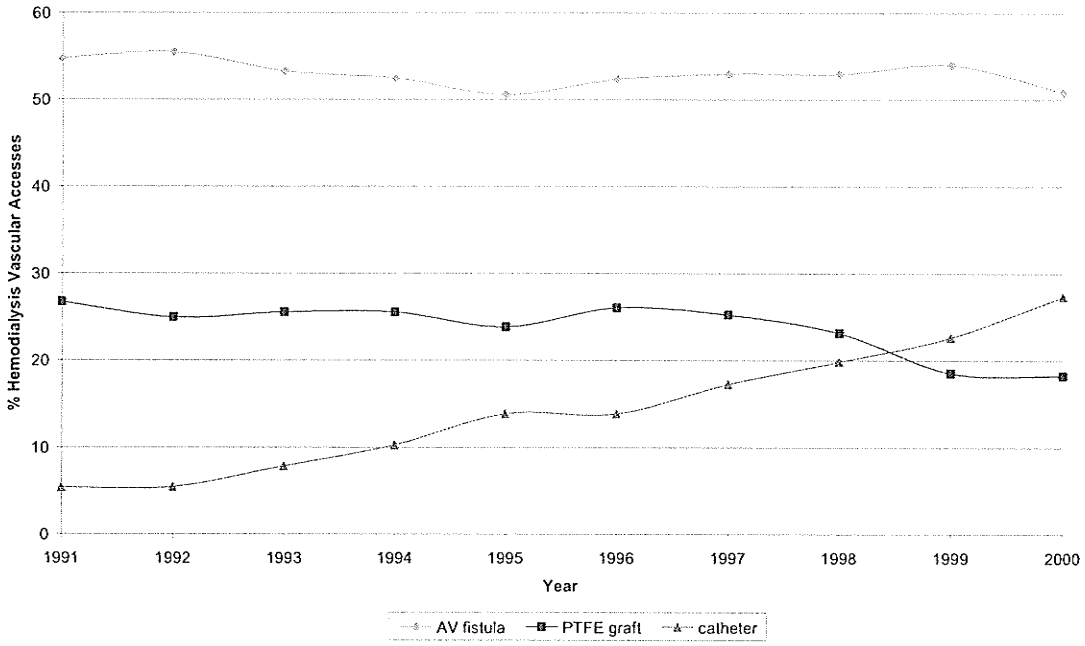

Figure 3. Prevalent Arteriovenous Access Types Used in Hemodialysis in Canada, 1991-2004 (Percentage) [5]

The high prevalence of cuffed, tunneled catheter use can be mainly attributed to the fact that they are suitable for immediate use following insertion. This is especially true in acute situations where the patient needs immediate hemodialysis and there is not enough time for an AV fistula to mature. As well, catheters can be used in nearly any patient, even in patients with fragile vessels in which development of an AV fistula would be very problematic. [9] One more advantage to the use of catheters is the elimination of the morbidity associated with accessing the blood via an AV fistula. Vascular access by way of a fistula or a graft involves puncturing the patient's skin using two large needles. This procedure is repeated three times a week and can be very painful and unpleasant for the patient, despite the use of topical anesthetics.

1.2 Hemodialysis Catheter-Related Infections

While the effectiveness of catheters cannot go unnoticed, many complications can arise from their use. These include infection, thrombosis, complications in catheter placement, inadequate catheter flow, increased cost and increased risk of death, which is most likely the result of poorer baseline clinical characteristics in patients using catheters compared to patients with fistulas. [9-11] Catheter-related infections (CRIs) are the most severe complication, and are a major cause of morbidity and mortality among hemodialysis patients using catheters for vascular access. [9, 12, 13] CRIs are commonly classified as one of three main types; exit site infection, tunnel infection (only applies to permanent catheters), or intraluminal infections causing bacteremia. [9]

The frequency of catheter-related bacteremia detailed in several large hemodialysis studies ranged between 2.5 and 6.5 cases per 1,000 catheter days. [12, 14-25] This translates into between roughly 4,400 and 11,400 hemodialysis catheter-related bacteremias each year in Canada. Literature suggests that approximately 10% of patients with CRI are diagnosed with severe sepsis or a metastatic complication such as endocarditis, osteomyelitis, epidural abscess or septic arthritis. [12-14, 16, 17, 20, 23, 26]

Several catheter factors affect rates of CRI. They include catheter type, material and placement. Numerous studies have shown higher incidence of infection in patients with temporary catheters than among patients with cuffed, tunneled catheters. [16, 27-29] Non-tunneled catheters are reported to have bacteremia rates varying from 3.8–6.5 per 1,000 catheter days while rates of catheter-related bacteremia for permanent cuffed, tunneled catheters range from 2.5 to 5.5 per 1,000 catheter days (Table 1). [12, 14-25]

Table 1. Rates of Infection in Hemodialysis Catheters

Catheter type		Infection Rate (episodes/1,000 catheter days)
Untunneled (temporary) catheter		**3.8-6.5** [19, 22, 30]
	Femoral	4.5-7.6
	Internal Jugular	5.6
	Subclavian	0.7-2.7
Tunneled (permanent) catheter		**2.5-5.5** [12, 14-18, 20, 21, 23, 30, 31]

The rate of CRI is affected by catheter material as well. The majority of catheters used have been composed of silicone, polyurethane, polyvinyl chloride, polypropylene and Teflon. [32] Several studies have shown that catheters made of polyvinyl chloride, polyethylene and Teflon are more susceptible to the adherence of microorganisms as compared to catheters made of silicone elastomer, or polyurethane. [33-36] The differences in the risk of infection between the varying types of catheters may be due to differences in number of surface irregularities that enhance some microbial adherence [34, 37-39] or due to differences in rates of thrombogenicity. [34, 39, 40] Consequently, catheters in use today are generally made of polyurethane or a new "second generation polyurethane" known as carbothane.

Catheter placement has also been shown to influence the rate of CRI. The risk of infection is highest for catheters placed in the femoral vein and lower for catheters placed in the internal jugular vein, while catheters placed in the subclavian vein appear to have the smallest risk of bacterial infection (Table 1). [27, 30, 34, 41-43] Currently however, catheters are not placed in the subclavian vein because of difficulties with venous stenosis (a non-infectious complication). [19]

Infections can be caused by a single organism or by more than one organism at one time. In the studies reported in Table 2, Gram-positive microbes were isolated in 67% to 86% of CRIs and Gram-negative microbes were isolated in 34% to 49%. [14, 16, 17, 20, 21, 23] Monomicrobial infections account for between 79% and 89% of all cases of CRIs while polymicrobial infections account for 5% to 21%. [14, 16, 17, 21, 23] The most common Gram-positive pathogens are *Staphylococcus aureus* and *Staphylococcus epidermidis.* Other coagulase-negative staphylococci and enterococci can cause CRIs as well. A variety of Gram-negative bacteria have been isolated, including *Pseudomonas* sp., *Klebsiella* sp. and *Enterobacter* sp. [14-17, 20, 23, 24] In addition to bacteria, fungi such as *Candida* sp. have been isolated in a small number of CRIs. [14-17, 20, 23, 24] One study by Mokrzycki et al. showed that HIV+ patients were five times more likely to have a CRI caused by a Gram-negative organism, and seven times more likely to have a fungal infection as compared to control patients. [21]

Microorganisms can cause a CRI by colonizing the catheter either extraluminally or intraluminally. Extraluminal and intraluminal infections are distinguished by the site of initial adherence of the microorganism. Extraluminal catheter infections can be caused by migration of the infecting organism from the insertion site at the skin to the catheter tip, although permanent catheters have cuffs that minimize the frequency of infections caused in this manner. [24, 44] Extraluminal infections may also be caused by hematogenous organisms from distant sources of infection that adhere to the external surface of the catheter. [44, 45] Intraluminal infections are caused by contamination of the catheter hub by

Table 2. Common Organisms Isolated in Catheter Related Infections [a]

	Marr et al. (1997)[16] (n=62)[b]	Beathard (1999)[14] (n=123)	Saad (1999)[17] (n=86)	Krishnasami et al. (2002)[20] (n=98)[c]	Poole et al. (2004)[23] (n=83)	Mokrzycki et al. (2000)[21] (n=109)[d]
Gram positive bacteria (%)	73	86	67	78	83	72
Coagulase negative Staphylococci	18		40			
Staphylococcus epidermidis		37			35	11
Staphylococcus aureus		30		49		53
Methicillin resistant *S. aureus*	27		5	21	6	
Methicillin sensitive *S. aureus*	18		17	18	12	
Enterococcus sp.	8	12	20	23	8	1
Gram negative bacteria (%)	37	34	45	49	34	9
Enterobacter sp.	5	9	16	12	7	1
Klebsiella sp.	5	3	12	14	2	
Serratia sp.		2	1	6	1	
Escherichia sp.	6	1	13	5	4	2
Pseudomonas sp.	5	7	13		5	1
Citrobacter sp.	2	4	4	1		
Acinetobacter sp.	6	4	11	3		2
Fungi (%)	2			9		4
Candida sp.	2			9		3
Polymicrobial infection (%)	11	16	21		16	5

n, number of catheter related infections (CRIs)

Only studies reporting more than 50 episodes of CRI have been included.

[a] Definition of catheter related infections vary between studies from only bacteremia to bacteremia plus exit site infection.

[b] Percentages of gram positive and negative isolates include both monomicrobial and polymicrobial infections.

[c] Percentages given in this study are per total number of isolates, not per number of CRIs.

[d] HIV infection is not a significant risk factor for CRI but is associated with a higher prevalence of Gram-negative and fungal species.

skin flora or during handling of the catheter by patients or dialysis personnel. [24, 44, 45] Because catheter hubs are frequently manipulated, intraluminal contamination occur more often compared with extraluminal contamination in long-term use catheters. [46, 47]

Microorganisms colonizing the internal or external surface of the catheter can form a biofilm. A biofilm is an aggregate of microbes that forms when free-floating or planktonic cells adhere to a foreign surface such as a catheter. [36] Bacteria multiply and construct a hydrated polysaccharide network on the outer surface of the cell (exopolysaccharide matrix) known as a glycocalyx which facilitates microbial adherence and formation of a sessile microbial colony. [36] The glycocalyx is composed of roughly 99% water as well as fibrous polysaccharides stabilized by cross-linking proteins known as lectins. [48-50] Mature biofilms attract additional diverse microorganisms to attach and the entire microcolony grows, enclosed in and protected by the thick glycocalyx matrix.[36] Infected central venous catheters provide model conditions for the production and growth of biofilms, including an ideal growth temperature and a rapidly flowing fluid environment to provide nutrition to the microcolony. [36] Several microorganisms causing CRIs have also been found to form biofilms under desirable conditions. [51] These include strains of *S. aureus, S. epidermidis, P. aeruginosa* and *E. coli* as well as *Candida* sp. [36] When an organism forms a biofilm on the surface of the catheter, the microorganisms in the colony are sheltered from all host defenses and antibiotics, making the biofilm very difficult to eliminate. [36] Currently, the treatment for catheters infected with biofilm is catheter removal or exchange over a guidewire. [13, 16, 36]

1.3 Management of Catheter-Related Infections

1.3a Systemic Antibiotics

According to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative Guidelines for Vascular Access, a patient with catheter-related bacteremia should immediately be treated with systemic antibiotics appropriate for the organisms suspected, regardless of whether the patient is displaying symptoms of bacteremia. [52] As numerous studies have shown, catheter-related bacteremia can be caused by a variety of organisms including both Gram-negative and Gram-positive organisms (Table 2). [14, 16, 17, 20, 21, 23] Therefore, until culture results are available to determine the organism causing bacteremia, optimal initial systemic antibiotic treatment should include antibiotics for both Gram-positive and Gram-negative organisms. [12] Locally, this treatment commonly involves the use of cefazolin and gentamicin. However, elimination of catheter-related bacteremia by systemic antibiotics alone has been shown in two studies to have very low rates of success (Table 3). [16, 17] A prospective, non-randomized observational study done by Saad resulted in 36.7% success when systemic antibiotics alone were used to treat catheter-related bacteremia (i.e. without catheter removal). [17] In a similar study carried out by Marr et al, 32% success using systemic antibiotics alone was observed. [16] The definition of success in the Marr et al. study was more stringent than that in the Saad study which may account for the slightly lower rate of success observed. However, overall, the rate of clinical cure using systemic antibiotics alone is consistently low, ranging from 22% to 37%.[12, 16, 17, 53, 54]

Table 3. Catheter Salvage Using Systemic Antibiotics Alone

Authors/ year	Design	Inclusion Criteria	Dose	Protocol	Catheters (Type): Lumen Volumes	N (pts) N (caths)	Definition of Success	% success	Most common organisms
Marr et al. (1997)[16]	prospective non-randomized observational study	patients with end-stage renal disease (ESRD) undergoing hemodialysis with dual-lumen cuffed catheters with suspected catheter-related bacteremia who were not hospitalized and did not develop clinical signs of sepsis, tunnel infection or persistent fever	vancomycin (loading dose, 20 mg/kg of body weight) and gentamicin (loading dose, 2 mg/kg). Short-course therapy: ≤ 2 weeks; long-course therapy: >2 weeks	assess the efficacy of catheter salvage using systemic antibiotics alone	permanent, tunneled-cuffed catheters	41 patients 62 catheters	catheter that was in place at the time of the episode of catheter related bacteremia was still in place at the end of 3 months or if the catheter was removed for a reason other than persistence of recurrent bacteremia caused by the same organism	32%	Gram negative bacteria: 37%; MRSA: 27%; MSSA: 18%; Coagulase negative Staphylococci: 18%
Saad (1999)[17]	prospective non-randomized observational study	patients with ESRD undergoing hemodialysis with dual-lumen cuffed catheters with catheter related bacteremia confirmed by positive blood cultures	initial coverage in most cases consisted of vancomycin combined with either gentamicin or ceftazidime. Antibiotics were continued for 21 days unless other indications warranted a longer course	compare efficacy of catheter salvage using systemic antibiotics alone vs. catheter exchange over a guidewire	included Soft-Cell, Circle-C, PermCath. Permanent, tunneled-cuffed catheters	52 patients 86 catheters	if the patient remained asymptomatic and the fever stayed away for more than 30 days after completion of antibiotic therapy	36.7%	coagulase-negative Staphylococci: 40%; MSSA: 17%; *Enterococcus:* 20%; Gram negative organisms: 45%

1.3b Catheter Guidewire Exchange

As it is known that the main source of catheter-related bacteremia is a biofilm which forms on the surface of the catheter, it is reasonable to speculate that bacteremia persists with systemic antibiotic treatment alone without catheter removal due to biofilm stability in the presence of low concentrations of antibiotics. [12, 55] It is therefore necessary to eliminate the biofilm that has formed on the catheter in order to cure the infection. To accomplish this, the infected catheter can be removed, and a new catheter placed in a new location in the body. Drawbacks to this technique include the need for short-term vascular access, more surgical procedures required by the patient and a decreasing number of access sites available for new permanent catheters over time.

Catheter exchange over a guidewire has proven to be an effective strategy for treatment of CRI, allowing for access site salvage and eliminating the need for short-term vascular access. Several studies have shown success rates varying from 80% to 88% using catheter exchange over a guidewire to treat CRI (Table 4). [12, 14, 17, 26, 56-58] Exchange over a guidewire appears to be a very successful method of treating catheter-related bacteremia as it removes the source of infection completely. However, it still requires the patient to undergo additional surgical procedures.

Table 4. Catheter Exchange Over Guidewire

Authors/ year	Design	Inclusion Criteria	Dose	Protocol	Catheters (Type): Lumen Volumes	N (pts) N (caths)	Definition of Success	% success	Most common organisms
Saad (1999)[17]	prospective non-randomized observational study	patients with end-stage renal disease (ESRD) undergoing hemodialysis with dual-lumen cuffed catheters with catheter related bacteremia confirmed by positive blood cultures	initial coverage in most cases consisted of vancomycin combined with either gentamicin or ceftazidime. Antibiotics were continued for 21 days unless other indications warranted a longer course	compare efficacy of catheter salvage using a) systemic antibiotics alone vs. b) catheter exchange over a guidewire	included Soft-Cell, Circle-C, PermCath. Permanent, tunneled-cuffed catheters	52 patients 86 catheters	if the patient remained asymptomatic and the fever stayed away for more than 30 days after completion of antibiotic therapy	a) 36.7%; b) 81.4%	coagulase-negative Staphylococci: 40%; MSSA: 17%; *Enterococcus:* 20%; Gram-negative organisms: 45%
Shaffer (1995)[57]	non-randomized, observational study	patients with ESRD undergoing hemodialysis with dual-lumen cuffed catheters who were symptomatic at the time of the study	systemic antibiotics were continued for 1 to 2 weeks following guidewire exchange	all episodes of catheter related sepsis were treated with catheter exchange over a guidewire	long-term, tunneled, dual-lumen silicone central venous dialysis catheters, Quinton PermCath	10 patients 13 catheters	negative catheter tip culture	76.9%	coagulase-negative Staphylococci, *Serratia marcescens, Enterococcus, S.aureus*
Beathard (1999)[14]	prospective, comparative, observational study, non-randomized	patients with ESRD undergoing hemodialysis with dual-lumen cuffed catheters with catheter-related bacteremia	exchange over guidewire plus three weeks systemic antibiotic therapy	a) catheter exchange over a guidewire vs. b) exchange over guidewire plus creation of new tunneled site vs. c) catheter removal and delayed replacement	permanent, tunneled, cuffed catheters	95 patients 123 catheters	culture based cure-negative blood cultures at least 1 week after therapy complete plus 45 day-symptom free interval; clinically based cure-culture not obtained and cure based only on 45 day-symptom free interval	a) 87.8%; b) 75%; c) 86.5%	*S.epi:* 37%; *S.aureus:* 30%; *Enterococcus:* 12%; Gram-negative microbes; 34%
Robinson et al. (1998)[56]	prospective non-randomized observational study	patients with ESRD who have bacteremia without an identifiable source except the catheter and have defervescence with intravenous antibiotics within 48 hours and no sign of tunnel tract infection	exchange over guidewire plus three weeks systemic antibiotic therapy (vancomycin and gentamicin)	evaluate efficacy of catheter exchange over guidewire and systemic antibiotic therapy in elimination of catheter related bacteremia	PermCath, tunneled, cuffed, permanent catheters	21 patients 23 catheters	no bacteremia within 90 days after exchange	83.0%	*S.aureus*, Coagulase negative Staphylococci, *Enterococcus*

1.3c Antibiotic Lock Technique for Catheter-Related Infection Treatment

Depending on the clinical presentation of the infection, various antibiotic locks have been studied in an attempt to salvage the catheter without removal or exchange. [59] When successful, the antibiotic lock technique eliminates the need for surgical procedures as it allows for access site as well as catheter salvage. The technique involves the insertion of a solution of antibiotic plus anticoagulant into the catheter lumen at the catheter hub between dialysis sessions. In theory, the presence of concentrated antibiotic in the catheter lumen over a prolonged period of time could lead to the eradication of the microorganisms causing the infection. While it currently remains unclear which antibiotic-anticoagulant lock would be most effective, a number of recent investigations have evaluated the efficacy of various lock solutions in the treatment of hemodialysis CRI (Table 5). [20, 23, 60-62] Several studies demonstrate success rates of between 60% and 100% (Table 5). [20, 23, 60] A recent study carried out by Poole et al. in 2004 prospectively analyzed the likelihood of achieving a clinical cure using the antibiotic lock protocol and determined whether the bacterial pathogen had any effect on the rate of success. [23] The lock was composed of either vancomycin (2.5 mg/mL) and heparin (2500 U/mL), cefazolin (5 mg/mL) and heparin (2500 U/mL), ceftazidime (5 mg/mL) and heparin (2500 U/mL), or vancomycin (2.5 mg/mL) with ceftazidime (5 mg/mL) and heparin (2500 U/mL). Success in this study was defined as catheter salvage with resolution of symptoms within 48 hours of the start of the protocol in addition to negative surveillance cultures 1 week after completing the systemic antibiotic regimen. The protocol was successful in 70% of cases. The authors also found that rate of success varied depending on the pathogen; 75% success with *S. epidermidis* infections, 40% for *S. aureus*

infections and 87% for Gram-negative infections. Overall, the protocol appeared to be successful with the best outcomes observed with Gram-negative and *S. epidermidis* infections.

Table 5. Antibiotic Lock for Treatment of Catheter-Related Infection

Authors/year	Design	Inclusion Criteria	Dose	Protocol	Catheters (type): Lumen Volumes	N (pts) N (caths)	Definition of Success	% Success	Most common organisms
Poole et al. (2004)[23]	historically controlled interventional study, prospectively analyzed the likelihood of clinical cure with antibiotic lock protocol	patients with end-stage renal disease (ESRD) who undergo hemodialysis with tunneled dialysis catheters, who had suspected catheter-related bacteremia whose blood cultures did not grow *Enterococcus*	loading dose of vancomycin (20 mg/kg) and ceftazidime (1g) followed by maintenance doses of 500 mg for vancomycin and 1g for ceftazidime. Antibiotic lock: vancomycin (2.5 mg/mL)/heparin (2500 U/mL); cefazolin (5 mg/mL)/heparin (2500 U/mL); ceftazidime (5 mg/mL)/heparin (2500 U/mL); vancomycin (2.5 mg/mL)/ceftazidime (5 mg/mL)/heparin (2500 U/mL)	evaluate whether likelihood of a cure depends on type of pathogen	permanent, tunneled, cuffed catheter	83 catheters	catheter salvage with resolution of symptoms within 48 hours of initiation of the antibiotic lock protocol and negative surveillance cultures 1 week after completing the antibiotic regimen	70% (overall success); 87% (Gram-negative); 75% (*S.epi*); 40% (*S.aureus*)	MRSE: 29%; *S.aureus:* 15%; *Enterococcus:* 7%; Gram-negative rods: 17%
Krishnasami et al. (2002)[20]	non-randomized, observational, prospective study which analyzes efficacy of antibiotic lock and systemic antibiotics	patients with ESRD who undergo hemodialysis with tunneled dialysis catheters, who had suspected catheter-related bacteremia who did not have persistent fever 48 hours after start of antibiotic therapy	loading dose of vancomycin (20 mg/kg) and gentamicin (1.5 mg/kg) followed by maintenance doses of antibiotics for the following 3 weeks. Maintenance dose of vancomycin (500 mg) and gentamicin (1mg/kg) but not exceeding 100 mg Antibiotic lock: vancomycin (2.5mg/mL)/gentamicin (1 mg/mL)/heparin (2500 U/mL); vancomycin (2.5mg/mL)/heparin (2500 U/mL); gentamicin (1 mg/mL)/heparin (2500 U/mL); cefazolin (5 mg/mL)/heparin (2500 U/mL); cefazolin (5 mg/mL)/gentamicin (1 mg/mL)/heparin (2500 U/mL)	evaluate efficacy of antibiotic lock and systemic antibiotics	permanent, tunneled, cuffed catheter	98 catheters	resolution of fever and negative surveillance cultures 1 week after completion of protocol	65%	MRSE: 19%; *Enterococcus:* 12%; Gram-negative rods: 34%; *Klebsiella:* 12%
Capdevila et al. (1993)[60]	prospective, non-randomized, observational study to determine likelihood of clinical cure without catheter removal using antibiotic lock protocol	patients with ESRD who undergo hemodialysis with tunneled dialysis catheters who had confirmed catheter-related sepsis with no exit site infection, no yeast infection and whose bacteremia persisted 48-72 hours after start of antibiotic therapy	intravenous vancomycin or ciprofloxacin. Lock of 100 µg/mL of vancomycin or ciprofloxacilin. Antibiotic given for 15 days	determine likelihood of clinical cure without catheter removal using antibiotic lock protocol	PermCath, Quinton, double lumen in internal jugular vein	11 patients 13 catheters	no positive blood cultures after 6 weeks of diagnosis of catheter-related bacteremia	100%	*S. epi, P. aeruginosa* (together made up 77% of microbes)

1.4 Antibiotic Lock Technique for Catheter-Related Infection Prevention

Treating CRIs is a fundamental aspect of enhancing the wellbeing of hemodialysis patients utilizing central venous catheters. However, rather than treating CRIs after they occur, it would be ideal to prevent them from occurring at the outset.

Several studies have evaluated the effectiveness of various antibiotics in combination with an anticoagulant for locking into the catheter in order to prevent CRIs in the hemodialysis population (Table 6). [18, 63-69] A double-blind randomized study conducted by Dogra et al. compared a 5000 U/ml heparin lock solution with a gentamicin/citrate lock solution (40 mg/ml gentamicin and 3.13% citrate) (Table 6). [18] Eighty-three tunneled, cuffed catheters in 112 hemodialysis patients were randomly allocated to either the control or treatment group. Infection-free catheter survival, defined as the number of days from catheter insertion to diagnosis of infection, was compared. The results of the study showed infection-free catheter survival of 282 days in the treatment group, and only 181 days in the control group (P=0.002). In addition, the authors reported a significant difference in infection rates per 100 catheter days; 0.03 infections/100 catheter days in the treatment group compared with 0.42 infections/100 catheter days in control group. As summarized in Table 6, comparable studies have shown similar results. While these studies clearly indicate a reduction in the infection rate with the use of a prophylactic antibiotic lock, there still exists the main concern with the use of antibiotics in a locked solution over time; the development of resistant organisms. With the antibiotic lock in place over a prolonged period of time, there will be leakage of antibiotic into the bloodstream at the catheter tip. Over time, the body may be exposed to repeated low concentrations of antibiotics, allowing organisms to develop resistance.

Thus, further investigations have evaluated non-antibiotic locking solutions for the prevention of infections.

One prospective, randomized control trial carried out by Betjes et al. evaluated the efficacy of a taurolidine locking solution (Table 6). [65] Taurolidine is a broad spectrum antimicrobial agent derived from the amino acid taurine. It is effective against bacteria and fungi, and there is no evidence of bacterial resistance to taurolidine. [11, 62, 70] The ability of the taurolidine catheter locking solution (1.35% taurolidine and 4% TSC) to prevent CRIs was compared with a locking solution of heparin alone (5,000 U/mL). Seventy-six catheters in 58 hemodialysis patients were randomly allocated to either the control or treatment group. Sepsis-free catheter survival, defined as number of days from catheter insertion to diagnosis of catheter-related sepsis, was compared. The results of the study showed 100% sepsis-free catheter survival in the treatment group, compared with approximately 85% in the control group (P=0.047). The study also evaluated bacterial-colonization free survival, defined as the number of days from catheter insertion to positive bacterial blood culture. The frequency of positive blood cultures 30 days after catheter placement was 7% for the citrate–taurolidine group and 9% for the heparin group.

Another study performed by Allon in 2003 also evaluated a taurolidine-citrate catheter locking solution (1.35% taurolidine and 4% TSC) compared with a heparin lock (5,000 U/mL) for the prevention of CRIs (Table 6). [63] After 90 days, bacteremia-free survival was 94% in patients who received the taurolidine-citrate lock and 47% in control patients whose catheters were locked with heparin alone (P<0.001). However, Allon also discovered that catheter patency was lower among patients who received the taurolidine

lock (32%) compared with control patients (76%) (P<0.001). Thus, a decrease in catheter patency appears to be as significant disadvantage of using taurolidine as a catheter locking solution. [63]

A multi-center, double-blind, randomized control trial done in 2005 by Weijmer et al. evaluated the efficacy of 30% TSC alone as a catheter locking solution compared to heparin. [69] Two hundred and ninety-one hemodialysis patients were randomized to receive either 30% TSC or 5000 U/mL unfractionated heparin as a locking solution for prevention of catheter complications including catheter-related bacteremia and thrombosis. Ninety-eight catheters were permanent, tunneled cuffed catheters, and 193 were temporary, non-tunneled catheters. Forty-six percent of catheters in the heparin group had to be removed because of complications compared to 28% in the TSC group. Rates of catheter-related bacteremia in both groups also differed significantly. For the heparin group, the rate of catheter-related bacteremia was 4.1 episodes per 1,000 catheter days, while for the TSC group, the rate was only 1.1 per 1,000 catheter days (P<0.001). The authors therefore concluded that 30% TSC reduces catheter-related bacteremia and other catheter complications for both permanent and temporary catheters without the occurrence of any serious adverse effects. It has been previously suggested that a maximum TSC concentration of 30% can be safely used clinically as a catheter locking solution. [31, 69, 71] However, elevated concentrations of serum citrate can lead to life-threatening conditions such as cardiac dysrythmia, seizures and bleeding. [72, 73] One hemodialysis patient died of cardiac arrest as a result of the injection of a 47.6% concentration of TSC into a permanent catheter. [73] Concentrations as high as 30% leave little room for error, and thus, 30% TSC is not approved for use in Canada or the United

States. Four percent TSC is approved for use in Canada (Health Canada Device License: 385425-1) [74], and in the United States, the FDA issued a warning against the use of concentrations of citrate higher than 4%. [70]

Table 6. Antibiotic Lock for Prevention of Catheter-Related Infection

Authors/year	Design	Inclusion Criteria	Dose	Protocol	Catheters (type): Lumen Volumes	N (pts) N (caths)	Definition of Success	% Success	Most common organisms
Bleyer et al. (2005)[66]	prospective, double-blind, randomized controlled trial	patients older than 18 yrs with end-stage renal disease (ESRD) who received a new catheter for hemodialysis, who did not have infection at time of insertion, are antibiotic free	either a) heparin or b) minocycline-EDTA (3 mg/mL minocycline and 30 mg/mL EDTA)	to asses efficacy of lock with minocycline and EDTA compared with heparin alone	permanent, tunneled, or temporary, non-tunneled catheter	57 catheters	decreased catheter colonization	a) 64.3% vs. b) 9.1%	*Staphylococcus* sp.
Kim et al. (2006)[67]	prospective, double-blind, randomized controlled trial	ESRD patients requiring a temporary catheter for hemodialysis while waiting for placement and maturation of an arteriovenous fistula or graft, who did not already have an infection or were undergoing antibiotic therapy	either a) non-antibiotic (heparin (1,000 U/mL)) or b) antibiotic lock (gentamicin and heparin (5 mg/mL gentamicin and 1,000 U/mL heparin) or cefazolin and heparin (10 mg/mL cefazolin and 1,000 U/mL heparin))	to asses efficacy of antibiotic lock compared with non-antibiotic lock (heparin alone)	temporary, non-tunneled catheter	120 catheters	decreased bacteremia rate and decreased catheter colonization	a) 3.12/1000 catheter days and 11.7% vs. b) 0.44/1000 catheter days and 1.7%	*S.epi, S.aureus*
McIntyre et al. (2004)[68]	prospective, randomized controlled trial	patients with ESRD who require a catheter for hemodialysis, who did not have recent infection, are not on immunosuppressant medications, have no evidence of CRI and are antibiotic free for at least 28 days before catheter insertion	either a) heparin (5,000 U/mL) or b) gentamicin and heparin (5 mg/mL gentamicin and 5,000 U/mL heparin)	to asses efficacy of lock with gentamicin and heparin compared with heparin alone	permanent, tunneled, cuffed catheter. Kirnal KSC split Ash catheter	50 catheters	bacteremia-free survival	a) 4 infections/1000 catheter days vs. b) 0.3 infections/1000 catheter days	*S.epi, S.aureus, E.coli*
Dogra et al. (2002)[18]	double-blind, randomized controlled trial	patients with ESRD who undergo hemodialysis with tunneled dialysis catheters	either a) heparin (5,000 U/mL) or b) gentamicin and citrate (2 mL of 40 mg/mL gentamicin and 1 mL 3.13% tri-sodium citrate)	to asses efficacy of lock with gentamicin and citrate compared with heparin lock	permanent, tunneled, cuffed catheter. Mahurkar Permcath	83 patients 112 catheters	infection-free catheter survival defined as number of days from catheter insertion to diagnosis of infection	a) 181 days (0.42 infections/100 catheter days) vs. b) 282 days (0.03 infections/100 catheter days)	*S.epi, S.aureus*, MRSA, *Enterobacter*
Betjes et al. (2004)[65]	prospective, randomized controlled trial	patients with ESRD who require a catheter for starting or continuing hemodialysis, who are not in ICU unit or on antibiotics	either a) heparin (5,000 U/mL) or b) citrate-taurolidine (1.35% taurolidine and 4% sodium citrate)	to asses efficacy of lock with taurolidine and citrate compared with heparin lock	permanent, tunneled (Tesio Cath and Ash Split Cath), or temporary, non-tunneled catheter (Medcomp.)	58 patients 76 catheters	sepsis-free survival defined as number of days from catheter insertion to diagnosis of catheter-related sepsis	a) ~85% vs. b) 100%	*S.epi, S.aureus*
Allon (2003)[13]	prospective, non-randomized, comparative study	patients with ESRD, undergoing dialysis with tunneled dialysis catheters, who did not have positive blood cultures or antibiotic therapy within 2 weeks of enrollment, evidence of exit-site infection....etc	either a) heparin (5,000 U/mL) or b) citrate-taurolidine (1.35% taurolidine and 4% sodium citrate)	to asses efficacy of lock with taurolidine and citrate compared with heparin lock	permanent, tunneled, cuffed catheter	20 catheters	bacteremia-free survival at 90 days	a) 47% vs. b) 94%	*S.epi, S.aureus*
Sodemann et al. (2001)[64]	pilot study to evaluate efficacy of Dialock in combination with new heparin free antimicrobial lock solution	patients with ESRD who undergo hemodialysis with tunneled dialysis catheters at least 18 years of age with vessel exhaustion and/or congestive heart failure, not pregnant, able to give informed consent	3.0 mL of lock consisting of taurolidine (0.034g) and citrate (0.4 mmol) inserted into each lumen of Dialock system	evaluate efficacy of Dialock in combination with new heparin free antimicrobial lock solution	Dialock system	70 patients	no infection occurs within 30 days of implantation	60%	N/A
Weijmer et al. (2005)[60]	double-blind, randomized controlled trial	patients with ESRD, older than 18 years, not admitted to ICU, require catheter for hemodialysis, newly inserted catheter, who do not have heparin-induced thrombocytopenia or thrombosis, sepsis, or infection requiring antibiotics	either a) 30% TSC or b) 5,000 U/mL unfractionated heparin	to asses efficacy of lock with 30% citrate compared with heparin lock	permanent, tunneled, or temporary, non-tunneled catheter	291 patients 301 catheters	decreased infection rate	a) 1.1 infections/1000 catheter days vs. b) 4.1 infections/1000 catheter days	*S.epi, S.aureus*

EDTA=ethylenediamine tetraacetic acid

1.5 Novel Ethanol/Trisodium Citrate Lock for Infection Prevention

Our locking solution composed of 30% ethanol and 4% TSC surpasses other locking solutions that have been proposed thus far. The main potential advantage of our novel locking solution compared with other antibiotic locks (e.g. gentamicin) would be avoidance of the development of resistant organisms with chronic use, while still preventing catheter-related bacteremia, and preventing thrombosis. Also, the combination of 30% ethanol and 4% TSC is relatively inexpensive and easy to prepare. Furthermore, it contains a greatly reduced concentration of TSC to allow for an additional margin of safety while providing an appropriate anticoagulant effect. [75-77]

Four percent TSC is used as the anticoagulant in our catheter lock solution. [72] Traditionally, between 1,000 and 10,000 IU/mL of heparin have been used as an anticoagulant, however, using heparin as an anticoagulant put patients at risk of several complications including bleeding complications. [69, 72] In addition, heparin immediately precipitates when combined with ethanol. [78] Thus, a lock comprised of ethanol and heparin would not be clinically practical. Four percent TSC was first used as an anticoagulant in 1914, and since then, has been used in several extracorporeal blood circuits including dialysis. [72] It prevents clotting by chelating serum ionized calcium which is required for clotting. Recent studies verify that TSC is as effective, or more effective, than heparin for thrombosis prevention. [76, 77]

Ethanol acts as the antimicrobial component of our catheter lock solution. It kills bacteria by targeting the cell membrane and causing membrane leakage. [79] The cell membrane of Gram-positive cells is found under a thick peptidoglycan layer while the cell membrane of Gram-negative cells is located under a thin peptidoglycan layer found

under an outer phospholipid membrane. Peptidoglycan is composed of polysaccharide chains cross linked by small peptides. Ethanol dehydrates the peptidoglycan layer, thereby "poking holes" in the structure, and is able to reach the cell membrane. In the case of Gram-negative cells, ethanol must first dissolve the outer phopholipid membrane in order to expose the thin peptidoglycan layer. Bacterial cell membranes are composed of a phospholipid bilayer and membrane protein molecules. It is thought that ethanol disturbs the membrane by inserting in between the phospholipid tails making the membrane less fluid. [79, 80] Ethanol can also denature membrane spanning proteins in the cell membrane and cause cell leakage. Ethanol is able to make its way into the cell and denature proteins required by the cell to carry out normal processes required for its existence.

Ethanol is commonly used as an antiseptic and there has been no known acquired resistance to ethanol to date. As discussed, ethanol works by denaturing membrane proteins resulting in cell membrane leakage, thereby making it less likely to result in the development of resistant organisms. Since the mechanism of cell elimination by ethanol is unlike that of antibiotics, resistance to ethanol should not develop, for example, by specific cellular changes in binding proteins, or production of neutralizing enzymes.

In order to develop the 30% ethanol/4% TSC lock for clinical use, it was previously reported that 30% is the highest concentration of ethanol compatible with 4% TSC *in vitro*, therefore compatibility complications will not arise. [78] Moreover, the 30% ethanol/4% TSC solution does not appear to significantly affect the integrity of hemodialysis catheters made of carbothane, and promising long term confirmatory studies are presently near completion. [81]

Based on these observations, it was reasonable to continue developing the 30% ethanol/4% TSC lock solution for clinical use, but as a preliminary step, it was necessary to assess its efficacy by testing the solution on both planktonic organisms, and assess its ability to prevent biofilm formation.

2. Research Goal and Hypotheses

Research Goal

To develop a novel catheter locking solution to prevent CRIs in hemodialysis patients.

Hypotheses

1) The 30% ethanol/4% TSC solution will successfully kill bacteria that commonly cause hemodialysis CRIs *in vitro* within 48 hours.

2) The 30% ethanol/4% TSC solution will successfully prevent the formation of biofilm *in vitro* by organisms that commonly cause hemodialysis CRIs.

3. Methods

The study was planned with two main parts to evaluate the effectiveness of the locking solution for the prevention of CRI. Because we were unsure of the killing effect of 30 % ethanol over 48 hours (the time between dialysis sessions), Part 1 was carried out to test the effectiveness of the solution on both Gram-positive and Gram-negative organisms growing in culture. Part 2 then tested the solution's ability to prevent biofilm formation using a Calgary Biofilm Device (CBD) (Figure 4) [82, 83]

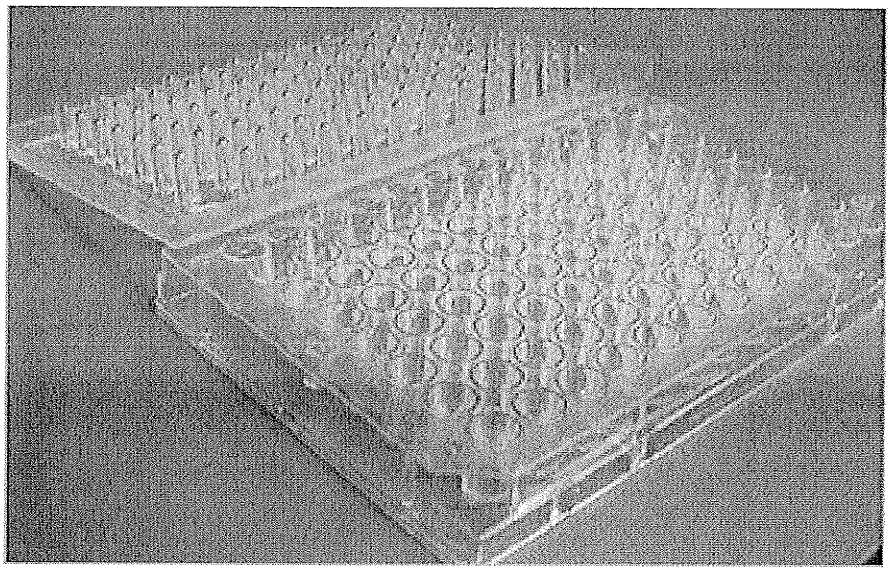

Figure 4. The Calgary Biofilm Device

3.1 Part I Methods

3.1a Collection of Clinical Isolates

Clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) (n=4), methicillin-sensitive *S. aureus* (MSSA) (n=8), methicillin-resistant *Staphylococcus epidermidis* (MRSE) (n=8), *Pseudomonas aeruginosa* (n=4) and *Escherichia coli* (n=4) were used. The number of isolates tested was based on an approximation of the distribution of organisms in our population and the number of isolates available locally. Isolates were identified using a microbiology laboratory database (Microscan®, Dade Diagnostics Corp., Mississauga, Ontario) at the St. Boniface Hospital, Winnipeg, Manitoba, Canada. All isolates, except MRSA, were obtained from blood cultures of hemodialysis patients with catheter-related bacteremia between the years 2000 and 2005. Because MRSA bacteremias in Manitoba dialysis centres are rare, MRSA isolates were obtained from urine, sputum or blood cultures of patients who were not hemodialysis patients.

All isolates were collected from the St. Boniface Hospital Clinical Microbiology lab in frozen vials. A small sample was taken and plated onto tryptic soy agar (TSA) plates and incubated at 37°C overnight. A sample from that plate was taken and re-plated onto TSA to isolate colonies. The plates were incubated at 37°C overnight. Isolated colonies were taken from those plates and were again plated on TSA to isolate colonies, and plates were incubated at 37°C overnight. This step is done to revive the organisms after they are taken out of the freezer. Finally, approximately 3 to 4 isolated colonies from the third plating were put into Eppendorf tubes containing 1 mL sterile skim milk.

These stock cultures were labeled and placed into the freezer for long-term storage and future use.

Immediately prior to beginning the experiment, a 0.5 McFarland ($1.5x10^8$ cfu/mL) was prepared. A small sample from a frozen stock culture was plated onto a TSA plate and subcultured for isolated colonies. A small number of colonies were then suspended in 3 mL of sterile normal saline. Turbidity of the suspension was determined using VITEK® Densicheck™ (bioMérieux, France).

3.1b Composition of the Control and Lock Solutions

The 30% ethanol/4% TSC lock solution consisted of 3.8 mL ethanol stock, 1 mL TSC stock and 5.1 mL MHB. The control solution consisted of 5.1 mL Mueller-Hinton broth (MHB) and 4.8 mL normal saline. These proportions in the control solution were used to match the "broth" versus "non-broth" proportions of the lock solution.

A 78.9% sterile ethanol stock solution was used (Stock: dehydrated ethyl alcohol for injection 7.89 g/10 mL, DIN 00394394, Sandoz Canada Inc., QC, Canada). A 40% TSC stock solution was used, and was prepared by dissolving 4.0 g of TSC powder (Sodium citrate tribasic: dehydrate powder, Sigma-Aldrich Co., St. Louis, MO, USA) in 10 mL distilled water. The pH was adjusted to between 6.4 and 7.5 using 1M citric acid which was prepared by dissolving 21.014 g of citric acid monohydrate powder (MW=210.14 g/mol) into 100 mL distilled water. The solution of TSC was filtered through a 0.45 μm filter using a sterile syringe and sterile technique.

3.1c Part I Study Design

Part I of the experimental procedures involved testing the 30% ethanol/4% TSC solution against cultures of each isolate. An outline of the study design is shown in Figure 5. Bacterial suspensions using a 0.5 McFarland were prepared for each isolate. Control and lock solutions were inoculated with 100 µL of bacterial suspension to yield approximately $1x10^6$ cfu/mL. Solutions were incubated at 37°C for a total of 48 hours. Samples were collected at t=0 hours (before exposure to the ethanol/TSC lock), t=1 hours (one hour after exposure to the ethanol/TSC lock), t=24 hours and t=48 hours.

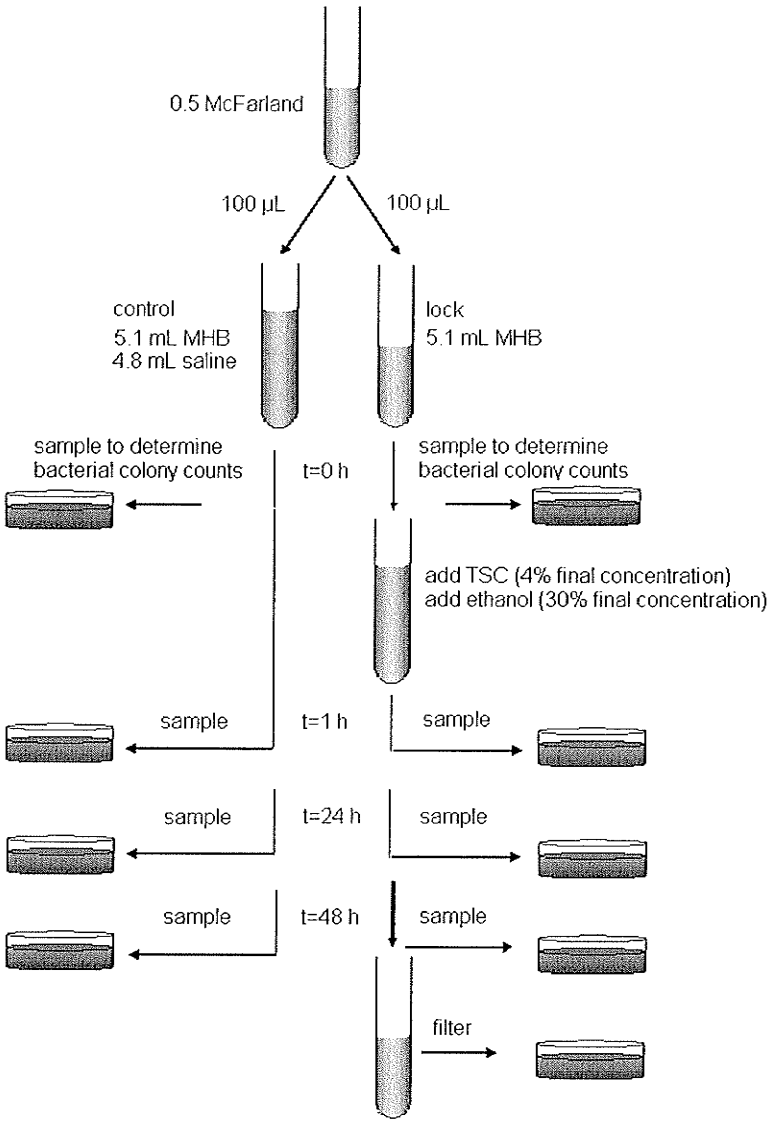

Figure 5. Summary of Part I Study Design

The purpose of the t=0 hour test was to confirm the initial presence of viable organisms in both the control and lock solutions. Samples of the lock solution were taken before the addition of the 30% ethanol/4% TSC solution. Therefore, the lock solution at t=0 hours consisted only of 5.1 mL MHB while the control solution consisted of 4.8 mL normal saline and 5.1 mL of MHB (9.9 mL total). At t=0 hours, 100 µL of the 0.5 McFarland was put into each solution, bringing the total volume of the control solution to

the desired 10 mL. The volume of the lock solution at t=0 hours was only 5.2 mL after the addition of 100 µL of the organism; only 52% of the control solution volume. Since the volume of the lock solution was approximately half that of the control solution, counts for the lock solution were approximately double the counts in the control solution at the same dilution. To correct for this factor in the determination of the number of colony forming units in the lock solution at t=0 hours, the following adjustment was carried out:

$$\text{cfu/mL in lock solution} = \frac{\text{\# colonies} \times \frac{5.2}{10}}{\text{dilution plated} \times \text{volume plated}} \quad [84]$$

After samples of both the control and lock solutions were taken for the t=0 hour test, the 30% ethanol/4% TSC solution (3.8 mL of 78.9% sterile ethanol and 1 mL 40% TSC) was added to the remaining 5.1 mL lock solution (after removal of 100 µL sample for t=0 hours), to bring the final volume of the lock solution to 9.9 mL.

At t=0, 1, 24 and 48 hours, samples of the control and lock solutions were serially diluted in normal saline, and 10 µL aliquots were plated onto ¼ TSA plates. For the lock solutions at t=1, 24 and 48 hours, undiluted samples of 100 µL were also plated onto half of the TSA plate. This step was carried out to ensure that any viable organisms in the suspension would be detected. All plates were incubated for 24 hours at 37°C and viable colonies between 10 and 100 were counted. The lower limit of detection using the colony-count method was $1x10^2$ cfu/mL. All experiments were conducted in duplicate for all isolates and on separate days.

During sampling in the experiment, ethanol may theoretically have been absorbed into the agar and may have potentially prevented the growth of the organism. Thus, at t=48 hours the remaining volume of the lock solution (approximately 9 mL) was filtered through a 0.45 μm Nalgene Analytical Test Filter Funnel, Series 145 (Nalge Nunc International, Rochester, New York). The filter was then rinsed with 15 mL sterile water to remove any residual alcohol. The filter was removed using sterile forceps and placed onto solid TSA plates which were incubated at 37°C for 24 hours. Any observation of colonies on the filter would indicate presence of viable organisms in the lock solution.

3.1d Part I Data Analysis

The data provided is the mean cfu/mL (+/- standard deviation) calculated from a total of 16 samples (when n=8) or 8 samples (when n=4) for each organism; n isolates per organism, done in duplicate on 2 different days. Since the study has a parallel-design, in which two groups, lock and control, were tested during the same period of time, data analysis would include the student's t-test for normally distributed data (parametric data), and the Mann-Whitney U test for non-parametric data where appropriate. [85]

3.2 Part I Results

Results of all tests for Part I are shown in Figure 6. Results indicate that all control and lock solutions had between 5.1×10^5 and 1.7×10^6 cfu/mL in the solution at t=0 hours. Thus, organisms were present in both the lock and control solutions at the start of the study, and therefore, both cultures had a similar capacity to grow. At t=1 hour, a slight increase in colony counts for the control group was observed. Conversely, in the

lock solution, 0 colonies were observed in all duplicates of all isolates at t=1 hour. Recall, the limit of detection using this method is $1x10^2$ cfu/mL. In the control group, organisms continued to grow as expected and reached approximately $4.3x10^8$ cfu/mL by t=24 hours. Again, the lock solution showed no viable organisms at t=24 hours. Figure 6 indicates that the control organisms grew as expected over the 48 hour time period. The lock solution continued to show no viable organisms at t=48 hours. In addition, no growth on the filters was observed for any isolates after filtering the lock solution at t=48 hours.

Since there was no detectable growth in the lock solution and florid growth in the controls, it was clear that there was a substantial difference between the groups. In addition, there was no detectable growth in the lock solution to perform statistical analysis.

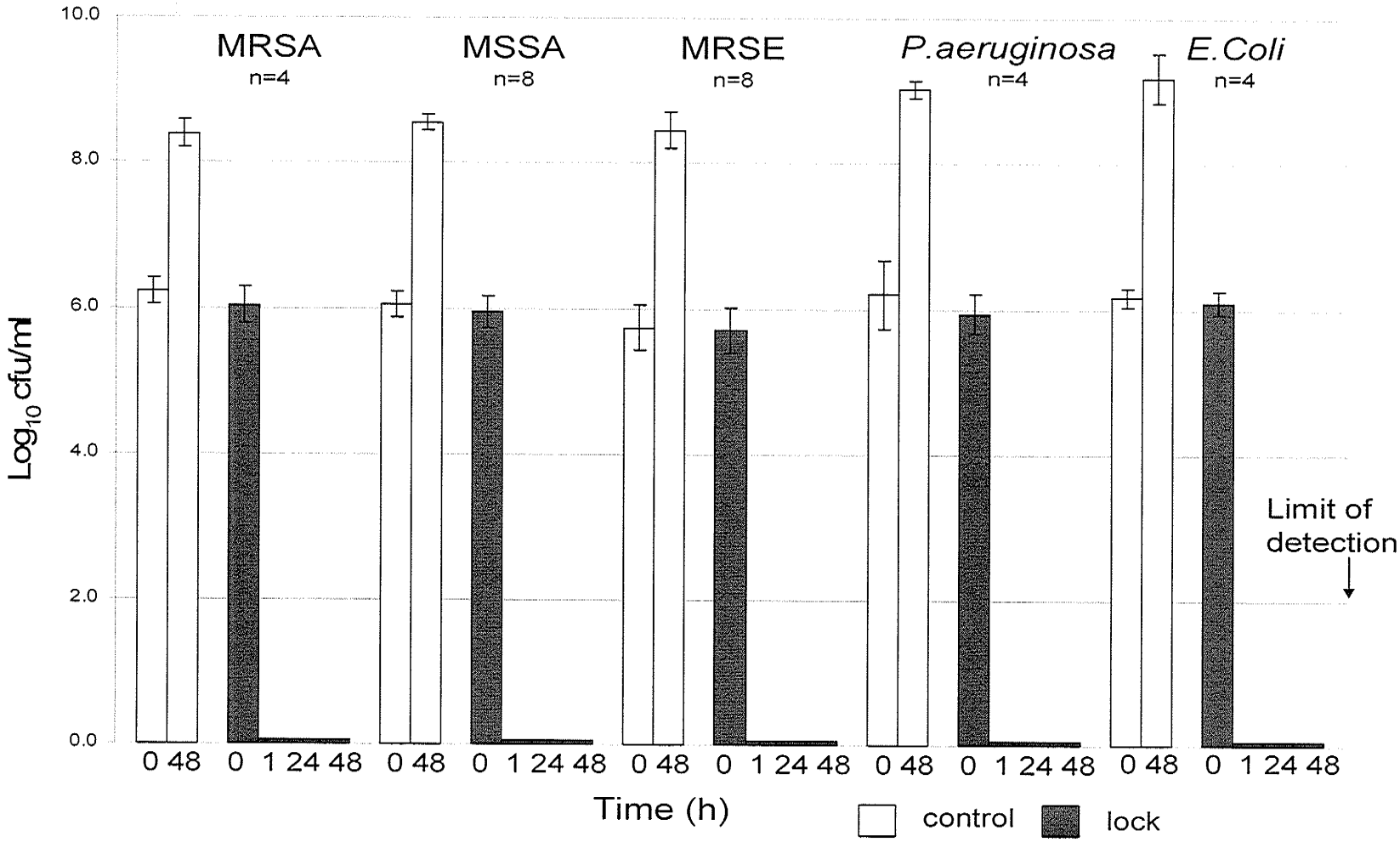

Figure 6. Effect of Control and Lock Solutions on Bacterial Growth. n=number of isolates. Values are means +/- SD.

3.3 Part I Discussion

The results of Part I demonstrated that the 30% ethanol/4% TSC solution was able to quickly eradicate the organisms growing in culture. The antimicrobial effect of our locking solution is most likely a result of the effect of ethanol, since a 4% citrate concentration has minimal antibacterial effect. [31] All organisms tested, both Gram-positive (including MRSA), and Gram-negative, were consistently killed within 1 hour of exposure to the locking solution. The results obtained in this experiment are valuable because we were unsure of the killing capabilities of a 30% ethanol concentration over this time period. As 70% ethanol is widely used as an antiseptic, it is known that concentrations of ethanol as high as 70% are bactericidal. [86] Our results now show that 30% is also very effective as a lock solution *in vitro*. This outcome is ideal since the variety of organisms tested in this study are frequently found in clinical cases of hemodialysis CRIs. If used clinically, the lock should be able to quickly kill common organisms that enter into the catheter lumen.

However, these results only demonstrate that the locking solution is effective *in vitro*. The *in vivo* situation may differ. In patients, the catheter is placed in the blood vessel, and there is some mixing of blood at the catheter tip. The presence of blood in the catheter, even in slight amounts, could potentially alter the efficacy of the locking solution. In an effort to more closely mimic the *in vivo* situation, we decided to include a small volume of blood or albumin in with the lock solution to observe whether it would affect the killing action of the solution. Unfortunately, the albumin precipitated out of the solution upon contact with the ethanol, and the mixing of blood with the lock solution led to coagulation of the blood. Both outcomes made it impossible to proceed, and so, we

opted to omit the addition of both the blood and the albumin from the experimental procedure. Clinically, there may be leakage of ethanol out of the catheter between dialysis sessions. As the tip of the catheter is in the right atrium in the presence of high rate of blood flow, any leakage of ethanol should be quickly diluted and should not be of concern. In the event that some blood "back titrates" in the tip of the catheter as ethanol leaks out, there could be very small amounts of protein in blood that denatures right at the tip of the catheter. This is of questionable clinical significance, nevertheless, rates of catheter thrombosis at the catheter tip should be monitored in early clinical trials.

Another limitation of this experiment is that it does not address the potential for the 30% ethanol/4% TSC to prevent biofilm formation on the catheter. Thus, after demonstrating that the lock solution could effectively kill organisms in culture, the next step was to determine whether the lock solution could potentially prevent infections *in vivo* by first demonstrating its ability to prevent the formation of biofilm *in vitro*.

3.4 Part II Methods

3.4a Collection of Clinical Isolates

All 28 isolates used in Part I were not tested in Part II of the experiment. This is because not all 28 isolates formed biofilm. Biofilm formers were determined by running a preliminary trial in which each of the 28 isolates tested in Part I was tested using the Calgary Biofilm Device (Figure 4). [82, 83] The Calgary Biofilm Device (CBD) is an established technique for growing biofilm, developed by researchers at the University of Calgary. [82] It is comprised of 2 components. The bottom component is a basic 96-well plate, and the top component is a lid with 96 identical pegs, each of which is situated

directly above a well of the 96-well plate. The device is to be placed on a rocker such that the medium in the wells flows rapidly along the pegs to enhance biofilm formation. The flow of medium in each of the wells provides a consistent shear force to each of the 96 pegs, producing up to 96 equivalent biofilms. The CBD procedure used in this study is based on the protocol described by the CBD manufacturer (MBEC^{TM} Bioproducts Inc.).

The CBD was initially used in order to determine which of the 28 isolates were biofilm formers. Twenty-five µL of a 0.5 McFarland culture was put into 2.475 mL MHB, and 250 µL aliquots of that culture were placed into two wells of the CBD. The CBD was placed on a rocker (Orbital Shaker, Cole-Parmer) (90 rpm) at room temperature for a total of 72 hours. The biofilm were fed with 250 µL MHB every 24 hours in order to provide the best possible conditions for biofilm growth. After 72 hours, the pegs were rinsed by filling wells of a 96-well plate with 250 µL normal saline, placing the pegs in the well, and rocking the plate at 90 rpm for 1 minute. The rinse step was carried out to remove from the biofilm surface any residual organisms that were not part of the established biofilm. This is to ensure that only cells that were part of the biofilm would be counted. The pegs were then broken off the CBD using a sterile hemostat, and placed into 0.5 mL normal saline. The pegs in saline were placed in the sonicator (Ultrasonic Cleaner, Aquasonic Model 250T, VWR Scientific International) for 5 minutes to disrupt the biofilm, removing the organisms from the peg. The resulting solution was subsequently sampled and serially diluted, and 10 µL aliquots were plated on ¼ of a TSA plate. Plates were incubated for 24 hours at 37°C and viable colonies between 10 and 100 were counted to determine bacterial titer on each peg. Ceri et al. indicate that after 24 hours incubation at 35°C, biofilms of *E. coli* and *P. aeruginosa* should reach densities of

approximately $1x10^7$ cfu/mL, and *S. aureus* biofilms should reach $1x10^5$ cfu/mL. [82] Based on this data, and taking into consideration the fact that the biofilms were initially being grown at room temperature instead of 35°C, we decided to include all isolates that grew biofilm with densities of at least 1×10^6 cfu/mL. The organisms that were shown to have the ability to produce biofilm were used for testing against our locking solution. Of the 28 initial isolates, only 10 had densities of at least $1x10^6$ cfu/mL after 72 hours, and were tested in Part II of the experiment; MRSA (n=2), MSSA (n=2), MRSE (n=2), *P. aeruginosa* (n=2) and *E. coli* (n=2).

3.4b Composition of the Control and Lock Solutions

Two-hundred and fifty µL of the lock solution was placed into each well of the CBD. A lock solution was made consisting of 0.95 mL ethanol stock, 0.25 mL TSC stock, 1.275 mL MHB and 25 µL of the 0.5 McFarland culture. The control solution consisted of 1.275 mL MHB, 1.2 mL normal saline and 25 µL of the 0.5 McFarland culture. These proportions in the control solution were used to match the "broth" versus "non-broth" proportions of the lock solution. As the biofilms had to be fed every 24 hours to ensure presence of sufficient growth medium, a fresh 'feeding' solution was prepared every 24 hours for both the control and lock biofilms. The lock solution was composed of 3.8 mL ethanol stock, 1 mL TSC stock, 5.1 mL MHB and 100 µL normal saline. The control solution was composed of 5.1 mL MHB and 4.9 mL normal saline. All solutions were made with identical stock solutions used in Part I.

3.4c Part II Study Design

Part II of the experimental procedure involved testing the effectiveness of the 30% ethanol/4% TSC solution on the prevention of biofilm formation by each isolate, using the CBD. An outline of the study design is shown in Figure 7. Bacterial suspensions using a 0.5 McFarland were prepared for each isolate. Control and lock solutions (2.475 mL each) were inoculated with 25 µL of bacterial suspension to yield a 2.5 mL culture containing approximately $1x10^6$ cfu/mL. Two-hundred and fifty µL of solution was placed into each appropriate well of the CBD plate. Plates were placed on a rocker in a 37°C incubator, and allowed to incubate for a total of 72 hours. Every 24 hours, 250 µL of feeding solution was placed into the appropriate wells. After 72 hours, the pegs were rinsed by filling wells of a 96-well plate with 250 µL normal saline, placing the pegs in the well, and rocking the plate at 90 rpm for 1 minute to remove any residual organisms from the surface of the biofilm. The pegs were then broken off the CBD using a sterile hemostat, and placed into 0.5 mL normal saline. The pegs in saline were sonicated for 5 minutes to disrupt the biofilm on the peg. The resulting solution was subsequently sampled and serially diluted, and 10 µL aliquots were plated on ¼ of a TSA plate. Plates were incubated for 24 hours at 37°C and viable colonies between 10 and 100 were counted to determine bacterial titer on each peg. The lower limit of detection using the colony-count method was $1x10^2$ cfu/mL. Biofilms were grown on duplicate pegs for all isolates, and on separate days.

To determine whether the growth of cells in the presence of the lock solution was simply inhibited and could resume after removal of the lock solution, or if the cells were in fact killed by the lock solution, we took 100 µL of the solution remaining after

sonication of the peg and put it into 9.9 mL MHB. The suspension was incubated at 37°C for 48 hours. The solution was subsequently serially diluted and 10 µL aliquots were plated on ¼ of a TSA plate. Plates were incubated for 24 hours at 37°C and viable colonies between 10 and 100 were counted.

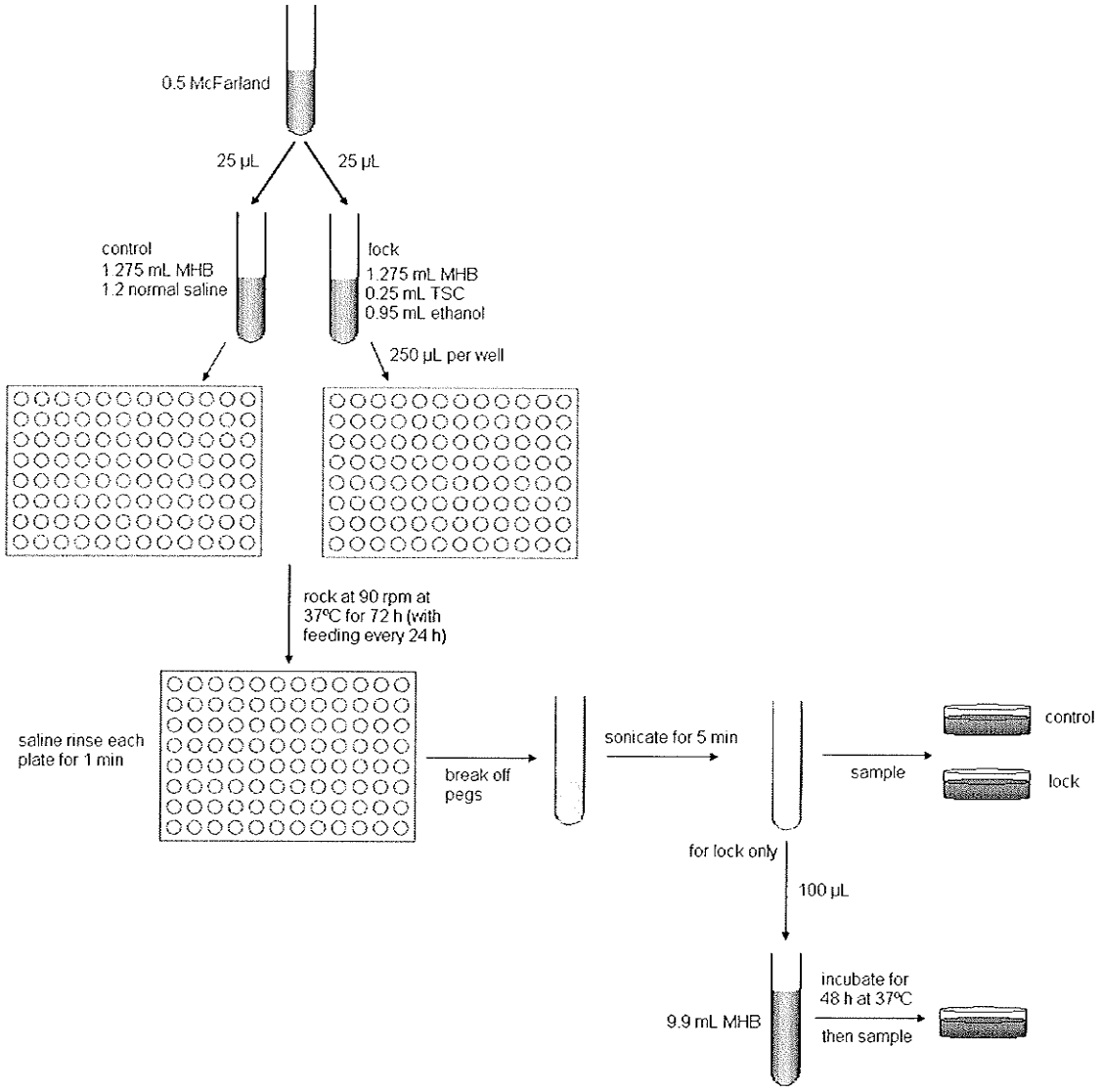

Figure 7. Summary of Part II Study Design

3.4d Part II Data Analysis

The data provided is the mean cfu/mL (+/- standard deviation) calculated from a total of 8 samples for each organism; 2 isolates per organism, 2 pegs per isolate, and done in duplicate on 2 different days. Since the study is designed as a parallel-design study, in which two groups, lock and control, were tested during the same period of time, data analysis would include the student's t-test for normally distributed data (parametric data), and the Mann-Whitney U test for non-parametric data where appropriate. [85]

3.5 Part II Results

Results of all tests for Part II are shown in Figure 8. Standard deviations of means are also given. The results indicate that all control biofilm had, on average, between $6x10^6$ and $7.4x10^7$ cfu/mL, confirming that the organisms had the ability to grow in the absence of the lock solution. The biofilm growing in the presence of the lock solution did not produce detectable growth after 72 hours incubation. Furthermore, the supplementary test performed after 72 hours gave no positive results indicating that organisms were in fact killed.

Since there was no detectable growth in the lock solution and florid growth in the controls, it was clear that there was a substantial difference between the groups. In addition, there was no detectable growth in the lock solution to perform statistical analysis.

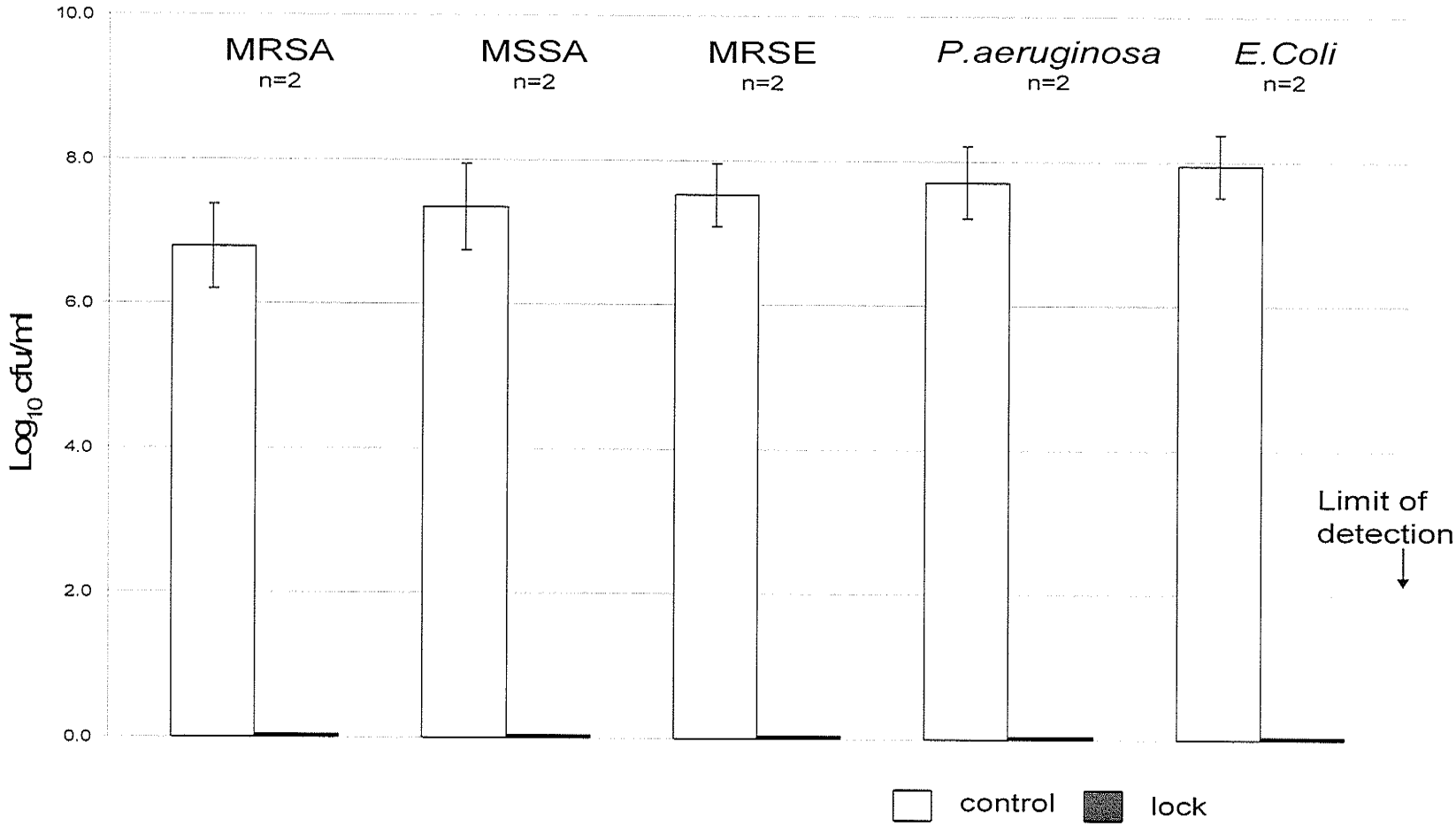

Figure 8. Effect of Control and Lock Solutions on Biofilm Growth After 72 h Incubation.
n=number of isolates. Values are means +/- SD.

3.6 Part II Discussion

The results of Part II demonstrated that the 30% ethanol/4% TSC solution prevents biofilm formation *in vitro*. This is an exceptional outcome, as it indicates that the 30% ethanol/4% TSC solution, if locked in the catheter from the point of catheter insertion into the patient, could potentially prevent biofilm formation in the catheter lumen. Both Gram-positive and Gram-negative organisms tested did not grow biofilm in the presence of the lock solution. Additionally, the supplementary test performed on the biofilm pegs growing in the lock solution after 72 hours confirmed that the organisms were killed, not merely inhibited, by the lock solution.

Results from Part II however, only show that the locking solution can prevent biofilm formation *in vitro*. In the blood vessel, the catheter surface is consistently exposed to components of blood such as platelets, fibrin and proteins during the dialysis procedure. Fibrin may facilitate the development of biofilm in the catheter lumen by allowing organisms to attach to the catheter surface, producing exopolysaccharide which may in turn interact with other blood components, further shielding the cells of the biofilm. This experiment did not address this issue. However, the rapid kill of organisms by ethanol would inhibit bacterial adherence to the catheter surface, effectively eliminating the production of the exopolysaccharide matrix by the cells, thereby preventing subsequent biofilm formation altogether.

A second limitation of this experiment is that it was carried out on only 10 isolates. Part I tested 28 isolates, but as discussed, it was not possible to test all 28 isolates in Part II due to the fact that they did not all form satisfactory biofilm. This is a limitation as it could be possible that other strains would not respond to ethanol in the

same way. However, based on these results, and the ability to prevent biofilm *in vitro*, our results are rather convincing.

Another limitation of the study is that the experiments were carried out using the CBD as a surface for biofilm growth rather than catheters. A comparable test could have been carried out using catheters, however, the cost of each catheter is roughly $250 to $300, and to test multiple organisms in duplicate would have become cost prohibitive. While it is quite unlikely that the results would have differed if the test had been carried out using catheters, the catheter polymer is different from the CBD peg and may affect the ability of organisms to adhere to it.

Finally, this test did not show data regarding treatment of CRI by eradication of the biofilm. While it would be valuable to show that the 30% ethanol/4% TSC solution eliminates biofilm from the catheter surface, it is more beneficial to show that infections could be prevented by inhibiting the formation of biofilm from the outset.

4. Conclusion of Part I and Part II

At the completion of both Parts I and II of the experimental procedure, both of the proposed hypotheses had been tested and confirmed. The ethanol/TSC catheter locking solution kills bacteria in culture and also prevents biofilm formation *in vitro* in the organisms tested.

5. Future Development

This *in vitro* study established that the 30% ethanol/4% TSC solution successfully kills bacteria that commonly cause hemodialysis CRIs. Furthermore, it successfully prevents the *in vitro* biofilm formation by organisms implicated in hemodialysis CRIs. The results of this study are very promising, and they support that this novel locking solution could potentially be used as an effective prevention for hemodialysis CRIs.

Our ethanol/TSC locking solution should eliminate the concern of antibiotic resistance developing due to the use of long-term antibiotic locks for prevention of CRI. In addition, it is inexpensive and easy to prepare, stable under standard conditions, and it does not affect the integrity of the catheter over time. [78, 81] Practically, we anticipate that there should be minimal safety concerns with the use of ethanol in a locked solution. However, before any definitive conclusions regarding safety can be made, clinical trials involving large sample sizes need to be carried out.

Possible clinical concerns pertaining to safety are mainly attributed to the presence of ethanol in the blood. Ethanol could leak out of the catheter into the blood stream and enter into the circulation. [87] While this may occur, the volume of ethanol that would enter the blood stream would be very small, and would be diluted quickly by the blood, posing a minimal concern.

Generally, in the development of new clinical entities, studies are usually performed in animals before carrying out a clinical trial in human subjects. An animal study testing an ethanol lock protocol in a sheep model has already been completed. Chambers et al. conducted a double-blinded, block-randomized trial in a sheep model in 2007. [88] At day 0, 12 Hickman central venous catheters were inserted into the internal

jugular vein of 12 three-year-old female Coopworth sheep. All catheters were inoculated with 1.0 mL of a 0.5 McFarland *S. epidermidis* culture at day 0 which was locked *in situ* for 72 hours. At day 3, the culture was aspirated and the catheter was flushed with normal saline. The sheep were randomly assigned to receive either 1 mL of ethanol/water (70:30 v/v) or heparinised saline (100 units/mL) as the first treatment, which was locked in the catheter lumen for 3 hours. The fluid was then aspirated out of the catheter, and the catheter was flushed with saline once again, and locked with 1 mL heparinised saline (100 units/mL) until day 5. At that point, the catheters were removed and samples were taken for culturing. After at least 8 catheter-free days, a new catheter was inserted into the contralateral internal jugular vein, and the 5 day process was repeated with the sheep receiving the alternate treatment. The primary purpose of the study was to determine the efficacy of a single, brief treatment with ethanol in sterilizing the infected catheters. Out of the 11 catheters which completed the study (one catheter was removed due to blockage while undergoing the treatment phase of study), 9 were successfully sterilized using the ethanol lock protocol, while none were sterilized using the heparinised saline lock ($P<0.01$). The authors point out that the blockage observed in one catheter during the treatment phase of the study could not be due to the ethanol because the blockage occurred before it was locked with ethanol (i.e. between day 0 and day 3). Furthermore, the study does not refer to any adverse side effects experienced by the sheep throughout the trail despite using 70% ethanol in the catheter.

Table 7. Ethanol Lock Technique

Authors/ year	Design	Inclusion Criteria	Dose	Protocol	Catheters (Type): Lumen Volumes	N (pts) N (caths)	Definition of Success	% success	Most common organisms
Metcalf et al. (2003)[90]	prospective prevention	case study patient with TPN catheter	3 mL 70% ethanol for 2 hours, later left *in situ* between each infusion PLUS intravenous amoxicillin	70% ethanol for 2 hours, later left *in situ* between each infusion then used for prevention for 3 years	long term Hickman catheter for TPN	1 patient 1 catheter	infection prevention	no infections for 3 years	*E. coli*
Dannenberg et al. (2003)[89]	retrospective, non-randomized	pediatric oncology patients who presented with positive blood cultures and clinical signs of infection	a) 2.3 mL 74% ethanol for 20-24 hours, over 3 days, PLUS intravenous antibiotics b) appropriate systemic antibiotic	ethanol lock carried out 24 times (18 pts), systemic antibiotics alone used 15 times (13 pts)	Broviac catheters	28 patients 39 catheters	no infectious relapse of any kind within 4 weeks of treatment	a) 67% vs. b) 47%	*S. epi*
Chambers et al. (2007)[88]	double-blinded, block-randomized trial conducted in sheep model	3-year-old female Coopworth sheep	a) 1 mL of ethanol/water (70:30, v/v) or b) 1 mL heparinised saline (100 units/mL)	solution instilled through access port and locked in the catheter for 3 hours	Hickman central venous catheters	11 sheep 22 catheters	catheter sterilization	a) 9/11 sterilized catheters vs. b) 0/11 sterilized catheters	catheters inoculated with *S. epi*
Onland et al. (2006)[91]	retrospective review	older than 6 months, silicone catheter with patent lumen prior to treatment, not allergic to ethanol, persistent positive blood cultures or multiple CRIs	70% ethanol; 0.8 mL (single-lumen), 1.2 mL (double-lumen), 1.4 mL (Port-A-Cath)	single-lumen IVD: ethanol dwell for 12-24 hours, then removed and flush lumen with saline. Repeat for 5 days. Double-lumen IVD: alternate lumens over 10 days, ethanol dwell in each lumen for 24 hours then flush	silicone catheters. Single-lumen tunneled Broviac, double-lumen tunneled Hickman and Port-A-Cath	40 patients 42 catheters	a) clearance of infection and retention of IVD b) decreased incidence of recurrence	a) 100% b) 12%	coagulase-negative staphylococci

IVD= intravascular device
TPN=total parenteral nutrition

In addition to the animal study done by Chambers et al., an ethanol lock protocol has been successfully used in patients in non-hemodialysis populations, but has yet to be attempted in hemodialysis patients requiring catheters for vascular access. Several recent studies have demonstrated its efficacy in patients requiring catheters for parenteral nutrition and in the oncology population (Table 7). [89-91] One case study carried out by Metcalf et al. involved a patient who required a catheter for total parenteral nutrition (TPN) (Table 7). [90] In a 7 year period, the patient had 22 episodes of fever and bacteremia which was considered catheter related. On one occasion, the patient had an *E. coli* bacteremia identified by isolation from blood cultures from both central and peripheral sites. He received systemic antibiotics specific to the infecting organism and he was administered an ethanol lock. Three mL of 70% ethanol were inserted into the catheter lumen after each infusion of TPN. At first, the ethanol remained in the lumen for 2 hours and was then flushed through at the request of the patient (70% ethanol lock pushed into the patient instead of being removed). Afterward, the patient chose to instead leave the lock in between each TPN infusion. Since that episode of bacteremia in 2000, the patient had no additional episodes for at least 3 years. This study suggests that the ethanol lock technique may be effective in prevention of catheter-related bacteremia. It also demonstrates that the ethanol lock may be safe as the patient did not develop any undesirable side effects when the lock was flushed through over a 3 year duration. The authors point out that a safer option would be to remove the lock before using the catheter. A limitation is that this study was carried out on only one individual, and may not be generalizable to a larger hemodialysis population.

A larger analysis carried out by Dannenberg et al. retrospectively reviewed charts of pediatric oncology patients with catheters who had acquired a bloodstream infection. [89] The authors evaluated the effectiveness and safety of the ethanol lock technique in comparison to systemic antibiotics alone for the management of bacteremia or sepsis. In this study, the authors defined bacteremia as "a combination of positive blood culture and fever or a rise of infectious laboratory findings", and sepsis as "having a positive blood culture and at least five of the eight following symptoms: fever/hypothermia, chills, tachycardia/bradycardia, tachypnea, hypotonia, prolonged capillary refill time, oliguria or altered mental status". A total of 28 patients had 39 infections which were divided into two treatment groups. The first group was treated with systemic antibiotics alone while the other group received the same systemic antibiotic treatment in combination with the ethanol lock. The procedure involved filling the catheter lumen with 2.3 mL of 74% ethanol for 20-24 hours with subsequent flushing of the solution through in order to prevent clotting in the catheter. Twenty-four infections (3 sepses, 21 bacteremias) were treated using the ethanol lock technique while 15 (3 sepses, 12 bacteremias) were treated with systemic antibiotics alone. Sixty-seven percent of episodes treated by the ethanol lock technique resulted in no new infection for the patient within 4 weeks compared to only 47% of episodes treated using systemic antibiotics alone. In addition, none of the catheters in patients who had undergone the ethanol lock protocol had to be removed because of a persistent infection. It was also demonstrated that the ethanol flush did not result in any severe side effects in the pediatric patients; only mild side effects such as headaches, dizziness and nausea were reported. This study shows promising evidence that an ethanol lock technique is effective as a treatment, however, the ethanol lock

technique was compared to systemic antibiotics and as discussed previously, systemic antibiotics alone as a treatment is not optimal. Furthermore, the authors state that the ethanol lock technique appears to be safe. While they have demonstrated that the ethanol flush in children showed no major side effects, the study is still too small to make any definitive conclusions regarding safety.

Another study carried out in children by Onland et al. retrospectively reported the efficacy of an ethanol lock technique for intraluminal disinfection and catheter salvage among children requiring long-term central venous catheters between June 1, 2004 and June 22, 2005. [91] During the study period, an infection rate of 6.3 infections/1,000 catheter days was reported. The ethanol lock protocol for treatment of infection consisted of instilling 70% ethanol locks in single-lumen catheters for 12 to 24 hours after which it was removed, and the catheter was flushed with an isotonic sodium chloride solution. The technique was repeated for 5 consecutive days during which the patient was also receiving IV antibiotics. A similar procedure was carried out in patients with double-lumen catheters except the ethanol lock was left to dwell for 24 hours in one lumen, while the other lumen was used for antibiotic infusions. The procedure was repeated alternating lumens over a 10 day period. The study also intended to document any adverse side effects of the ethanol treatment. The authors defined treatment success as "resolution of fever within 24 hours, no recurrence of positive blood cultures with the same organism, and retention of the intravenous vascular device" and defined treatment failure as "recurrence within 30 days with the same pathogen or removal of the IVD because of a persistent infection." In total, 51 treatments in 40 patients (42 catheters) were included in the review. All 51 treatments were successful as defined by the authors,

and there was also a low rate of recurrence (12% recurrence of same pathogen within 30 days). In addition, no adverse side effects were observed throughout the treatment. However, a prospective, randomized controlled trial with a much larger sample size as well as post-marketing surveillance is required to conclude that the ethanol lock technique is a safe and successful treatment strategy.

Our application of the ethanol lock protocol differs from those in the previous studies described in several ways. First, they report instilling ethanol concentrations of at least 70% into the catheter lumen. In addition, there is no indication of the presence of an anticoagulant combined with the ethanol lock. Our solution consists of a much lower concentration of ethanol (30%) in combination with TSC (4%) as the anticoagulant. A decreased concentration of ethanol and TSC allows for an increased margin of safety and decreased odds of observing any adverse side effects. Metcalf et al. and Dannenberg et al. describe flushing the ethanol lock into the patient. [89, 90] We propose, whenever possible, that the ethanol lock be removed before dialyzing to avoid any possibility of harming the patient. The majority of these studies were carried out over a short period of time, while if our lock were to be used clinically, it would be instilled 3 times a week for up to several months. Finally, as opposed to treating infections using an ethanol lock as described in studies above, we hope to use our ethanol/TSC locking solution for prevention of infection. Clinically, our solution would be locked into the catheter lumen from the time of insertion of the catheter into the patient, and we anticipate that, if used regularly, it would successfully prevent CRIs.

While there have yet to be any major side effects reported with the use of ethanol locks, if our novel 30% ethanol/4% TSC lock were to be used in patients, adverse effects

would need to be monitored. The primary concern would be the effect of ethanol on the blood near the catheter tip, therefore, rates of catheter thrombosis at the catheter tip should be closely monitored in early clinical stages.

6. Conclusion

The outcome of our research is extremely promising. The study hypotheses were tested and the results prove that our novel ethanol/TSC catheter locking solution quickly kills bacteria *in vitro,* and it successfully prevents the *in vitro* formation of biofilm by organisms that commonly cause hemodialysis CRIs. In addition to research carried out in animals, human studies in non-hemodialysis patients also show that an ethanol lock can be effectively used *in vivo* for the management of CRIs. Our *in vitro* data, in combination with *in vivo* data from non-hemodialysis patients, suggest that our ethanol/TSC lock could be used, with minimal risk, in a small pilot clinical study in the hemodialysis population.

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