THE DEVELOPMENT AND VALIDATION OF A STANDARD *IN VITRO* METHOD TO EVALUATE THE EFFICACY OF SURFACE MODIFIED URINARY CATHETERS

by

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ABSTRACT

Urinary catheters are a critical medical device in modern medicine, used in almost every healthcare setting worldwide. Catheter associated urinary tract infections (CAUTI) account for 37% of all healthcare associated infections. Many surface modifications, such as antimicrobial coatings, have been proposed but none have resulted in a significant decrease in CAUTI. A variety of test methods exist to evaluate the efficacy of surface modified urinary catheters, but there is no validated *in vitro* standard method. This thesis reports on a standard test method which aims to replicate the two routes by which infection may occur, intraluminally and extraluminally, through two quantitative, *in vitro* models. The first, the Intraluminal Catheter Model (ICM), was devised to evaluate the efficacy of surface modifications to inhibit biofilm growth on the catheter lumen. The ICM was subjected to a rigorous statistical evaluation of its ruggedness. Specifically, the amount the log density and log reduction changed with small adjustments to key operational factors. Five operational factors were varied - inoculum concertation, flow of media through the catheter, pH of the artificial urine media, temperature of the incubator, and biofilm removal technique. The results of the analysis highlighted that biofilm growth is sensitive to changes in pH, which indicates that the growth media must to optimized to increase the method's ruggedness. The analysis also demonstrated that sonication was more efficient than scraping as a means to harvest biofilm from the catheter surface. With further optimization of the procedure, the ICM has potential to become a useful tool to evaluate the efficacy of surface modified urinary catheters. Three extraluminal models were tested but did not meet the statistical requirements necessary for standardization. Extraluminal infections account for 66% of all CAUTIs, therefore, the *in vitro* evaluation of a surface modification's ability to inhibit migration of bacteria along the extraluminal surface of a catheter is critical to fully understand how a modification will perform in the clinical setting. The development of a standardized *in vitro* method which reflects the physiological conditions of CAUTI will help FDA regulators to compare efficacies across products enabling them to recognize effective treatments for patients.

CHAPTER ONE

INTRODUCTION

Epidemiology

Urinary catheters are one of the most commonly utilized medical devices worldwide. They are used in virtually every healthcare setting, improving patient care by relieving urinary retention, reducing risk for injury following traumatic surgery, and allowing for accurate urine output readings (e.g. hemodynamics, electrolyte balance). The global market for urinary catheters is expected to grow to $5.51 billion by 2024[1], largely due to the increasing elderly, obese and diabetic populations. The most common indwelling urinary catheter is the Foley catheter, which consists of a tube that is inserted through the urethra and held in the bladder by an inflatable balloon (Figure 1). While urinary catheters provide invaluable aid to patients, they are not without complications, the most notable being catheter associated urinary tract infections (CAUTI). The CDC defines CAUTI as a positive urine culture ($\geq 10^5$ colony forming unit (CFU) per milliliter (mL)) in concordance with at least one common symptom (e.g. fever, urinary urgency and frequency) in a patient who has had a catheter in place for longer than two days [1]. Catheter associated infections account for 37% of all hospital acquired infections (HAI) and 70% of all nosocomial urinary tract infections (UTI) in the U.S. [2, 3].

[1] https://www.grandviewresearch.com/industry-analysis/urinary-catheters-market. Accessed June 19, 2018.

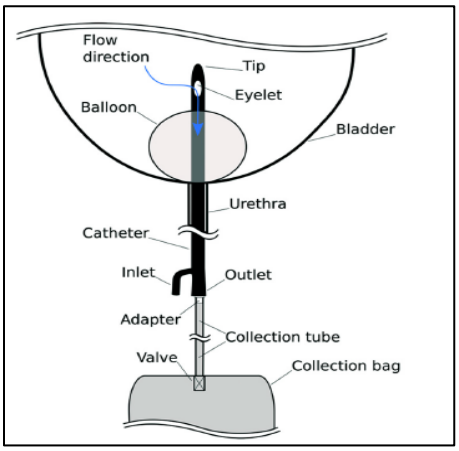

Figure 1. Diagram of a 2-way, Foley catheter inserted through urethra into the bladder. The blue arrow represents the flow of urine through the catheter into the external collection bag. (Reprinted from reference [4].)

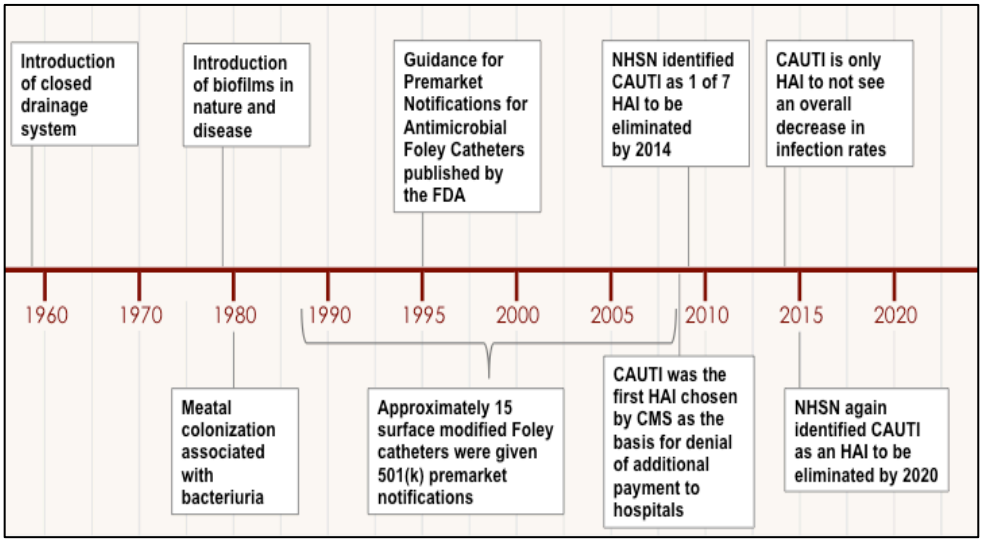

Figure 2. Timeline showing the evolution of urinary catheters. The modern Foley catheter was invented by Dr. Frederic Foley in 1937 [5]. However, the use of tubes, reeds, straws, etc. to relieve urinary retention has been recorded since the time of the early Egyptians, approximately 1500 B.C. [6, 7].

The last major advancement in reducing CAUTI rates was with the introduction of the closed drainage system in the late 1950's (Figure 2). This improvement resulted in a 50% reduction of incidence of infection [8, 9]. Continued advancements were made to aseptic catheterization techniques in the decades following, but infection rates didn't fall

below 30% [10]. Garibaldi et. al. hypothesized that with the use of aseptic closed drainage systems, the main point of entry for bacteria into the bladder became the periurethral mucous sheath outside the catheter [11]. While this hypothesis was eventually proved correct, researchers were still missing a piece of the puzzle. In the late 1970's Dr. William (Bill) Costerton introduced the missing piece with his hypothesis of how bacteria stick to surfaces. He suggested bacteria colonize a surface by producing a glycocalyx of fibers which adhere to a surface and other aggregates of cells forming complex colonies, or biofilms [12]. This discovery lead to many investigative studies throughout the 1980's all inquiring what role biofilms play in medical infections [13, 14]. By the end of the decade, researchers had found that bacteria form biofilm on almost any surface, from rocks in streams to intrauterine devices (IUDs) [15]. Since, it has been shown that virtually all implantable medical devices are susceptible to biofilm colonization and infection [16-20].

Following this advancement, the FDA published Premarket Notifications for Antimicrobial Foley catheters in 1995 to help industry develop new technologies [21]. 510(k) approvals were given to 15 surface modified catheters between 1987 and 2008[2]. However, even with the introduction of these new technologies, CAUTI still accounted for approximately 30% of all nosocomial infections [22]. Due to the significant economic and labor-intensive burden of these infections, the Center for Medicaid and Medicare (CMM) identified CAUTI as the first hospital associated infection that was the basis for denial of government aid to hospitals in 2008 [23, 24]. In response, the following year the

[2] https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm. Accessed June 19, 2018.

CDC published the "National Action Plan to Prevent Health Care-Associated Infections: Road Map to Elimination (HAI Action Plan)" [25]. The plan identified the top 7 HAIs reported in the U.S., including CAUTI, and set incidence rate reduction goals for each. CAUTI was the only HAI to lack a significant decrease in incidence rate between 2009 and 2014 despite advancements in the understanding of infection pathogenesis and surface modification technology [22].

Pathogenesis

To properly assess whether a surface modified catheter will perform as designed a robust understanding of the pathogenesis of CAUTI is necessary. Clinically urinary tract infections (UTI) are classified as either uncomplicated or complicated. Uncomplicated UTIs occur in otherwise healthy individuals with no urinary tract abnormalities [26]. Typically, the body's innate defense mechanisms, such as micturition, mucosal secretions and urine itself, make it more challenging for an infection to start [27-30]. However, there are several risk factors which may make a person more susceptible to uncomplicated UTIs, including sexual activity, the female gender, and diabetes [31-33]. Complicated UTIs are a result of factors which compromise the body's natural defenses, such as urinary retention from neurological diseases or the presence of a foreign object [34, 35]. Upon insertion of a foreign object, such as a urinary catheter, most of the host defenses are disrupted, and a patient is significantly more susceptible to infection [36, 37]. The CDC advises that each day an indwelling urinary catheter remains *in situ* a patient has a 3-7% increased risk for infection [1, 38]. Many hospital patients are inherently immunodeficient and introduction of a catheter further compromises a

patient's natural defenses. Allowing them to be easily colonized by cross-transmission through the hands of health-care professionals or even by their own perineal flora [13, 39, 40]. Once a urinary catheter is in place pathogens may migrate to the bladder one of two ways, through the catheter lumen or extraluminally along the periurethral space [41, 42]. Intraluminal contamination accounts for approximately 33% of CAUTIs and is most commonly associated with a break in the closed sterile system or contamination of the collection bag urine [42, 43]. In this case, bacteriuria has been shown to occur within 48 hours [44]. If a strict sterile collection system is kept, the extraluminal route becomes more important, accounting for 66% of CAUTIs [42, 45]. Extraluminal contamination may occur early if the tip of the catheter is contaminated upon insertion, which then smears the bacteria up the urethra into the bladder, or later by bacteria ascending the periurethral space outside the catheter [39, 46]. If late contamination occurs bacteriuria may take as long as 168 hours to become apparent [44]. CAUTIs may be caused by a broad spectrum of microorganisms, including both gram negative positive bacteria and certain fungi. The most common causative agents include Uropathogenic *Escherichia coli* (UPEC), *Candida albicans*, *Enterococcus* sp., and *Pseudomonas aeruginosa* (Table 1) [3].

Table 1. Distribution and rank order of pathogens frequently reported with CAUTIs across the United States for two reporting periods. Reporting criteria changed between the two periods.

	January 2006-October 2007		January 2011-December 2014	
Pathogen	Percent of Pathogenic Isolates	Rank[a]	Percent of Pathogenic Isolates	Rank[a]
Coagulase-negative *staphylococci*	2.5	7	2.4	13
Staphyloccoccus aureus	2.2	8	1.6	14
Enterococcus species		3		
E. faecalis	3.6		7.0	5
E. faecium	6.0		2.7	11
Other *Enterococococcus* or NOS	5.3		4.1	7
Candida species		2		
C. albicans	14.5		11.7	2
Candida glabrata			2.7	12
Other *Candida* spp. or NOS	6.5		3.4	10
Escherichia coli	21.4	1	23.9	1
Pseudomonas aeruginosa	10.0	4	10.3	3
Klebsiella spp.			10.1	4
K. pneumoniae	7.7	5		
K. oxytoca	0.9	10		
Enterobacter spp.	4.1	6	3.7	9
Acinetobacter baumannii	1.2	9		
Yeast NOS			6.1	6
Proteus spp.			4.0	8
Other	14.1		6.4	
Total	100		100	

[a] The 10 (2006-2007) and 14 (2011-2014) most common pathogens are listed and ranked according to how frequently they were reported to the CDC's National Health Safety Network (NHSN) [3, 47]. The rankings were established based on all pathogens reported.

For a uropathogen to initiate infection it must first attach to either the uroepithelial tissue or the catheter surface [48, 49]. There is limited knowledge on the specific adhesions pathogens use to adhere to catheter surfaces, however researchers have made inferences based on the knowledge of pathogenesis during uncomplicated UTIs.

For example, it is hypothesized that UPEC employs type 1 fimbriae to adhere to catheter surfaces as it has been observed using fimbriae to attach to and colonize host tissue in uncompromised urinary tracts [37, 40]. In addition to initiating infection, preliminary *in vitro* studies have shown the expression of type 1 and type 3 fimbriae by *Klebsiella pnuemoniae* promotes biofilm formation on the surface of urinary catheters [50]. The formation of biofilm is a common survival tactic employed by uropathogens, allowing them to persist and cause recurrent infections [51, 52]. The biofilm structure shields organisms from the stresses of the harsh environment such as antimicrobial treatment, host immune responses, and urine itself, described in detail elsewhere [52-57]. A classic example of these protective communities formed during CAUTI is the crystalline biofilms produced by *Proteus* sp. *Proteus mirabillis*, along with other uropathogens, produces urease, which hydrolyses urea to produce carbon dioxide and ammonia [52, 58]. This increases the pH of the local environment, subsequently generating calcium crystals and magnesium ammonium phosphate (struvite) precipitates [40, 51]. These stones become entangled with the bacteria (Figure 3a) and continue to build on one another ultimately resulting in complete blockage of the catheter lumen (Figure 3b) [59].

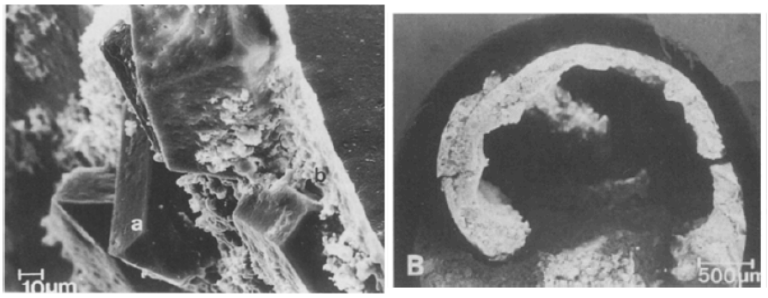

Figure 3. Freeze-dried preparations of *ex vivo* catheters colonized by pure cultures of *Proteus mirabillis* showing large crystals (a) and a cross section of the blockage in lumen (b). (Reprinted from reference [59])

The longer a catheter remains in place, the greater the possibility these organisms will form biofilms and the greater the risk for adverse events (i.e. catheter obstruction, kidney infection, etc.). The possibility of CAUTI is so likely that prevention guidelines go as far as to advise health-care professions to avoid the use of indwelling urinary catheter when at all possible [24], although this is not a feasible option for many patients. Clinicians are also limited to which antibiotics they may prescribe due to the increasing frequency of antibiotic resistance among pathogens, leaving the health-care industry desperate for realistic treatment options [3, 60]. It would be a considerable benefit for patients to have a technology available which significantly delayed or completely inhibited the bacterial colonization of urinary catheters.

Selecting a Test Method

Over the last three decades researchers have modified the surface of urinary catheters to reduce bacterial attachment, trying everything from impregnating the catheters with antibiotics to printing micropatterns on the surface [61-63]. One of the few modified catheters to make it to market and remain there was a hydrogel-silver coated catheter. Initial *in vitro* testing demonstrated hydrogel-silver coated latex catheters

significantly reduced the adhesion of *E.coli* and *P. aeruginosa* when compared to uncoated catheters [64]. This study ultimately led to the product's approval for clinical use in the late 90's. Since the approval, there have been multiple clinical trials involving hydrogel-silver coated catheters which have contradicting conclusions. A few of the studies reported favorable results for the antimicrobial catheters, others reported no difference at all, and some found a difference but concluded the difference was not enough for there to be an economic benefit in using the coated catheters [65-68]. So, what happened, why don't the clinical results correlate with *in vitro* results? A more critical review of *in vitro* methods used by Gabriel et al. demonstrates the methods do not reflect the conditions existing during an infection, and the data was over extrapolated, resulting in an inaccurate prediction of clinical performance.

The objective of this study was to develop a quantitative *in vitro* method which would assist FDA regulators in evaluating the efficacy of surface modified urinary catheters. To achieve this goal, an extensive review of all methods commonly used in the literature was completed and critical parameters necessary to simulate the physiological environment were identified.

When thoughtfully engineered, an *in vitro* method can be an important tool in predicting the clinical efficacy of a product. This section aims to examine the most frequently used laboratory methods in anti-biofilm urinary catheter testing and evaluate their usefulness based on [69]:

- **Relevance**: A method is said to be relevant to a real-world scenario if given the same inputs the laboratory outcome is predictive of the real-world outcome.

- **Repeatability**: Independent repeats of the same experiment in the same lab produce nearly the same response.
- **Responsiveness**: A method should be sensitive enough that is can detect important changes in parameters of interest.

In clinical terms, to reduce infection a surface modified catheter must delay the time it takes for bacteria to reach a concentration of 10^5 CFU/mL in the urine. When choosing a method to evaluate the efficacy of a new catheter, the outputs provided must be able to provide insight to how well it might be able to meet this criterion. Comprehensive lists of *in vitro* methods most commonly reported in the literature to model intraluminal and extraluminal infections are compared and contrasted in Tables 3 and 4, respectively. The intraluminal route of infection differs significantly from the extraluminal route. During intraluminal infection the catheter is exposed to the flow of urine over the surface for extended periods, compared to extraluminal infections where there is a lack of flow but there is a complex relationship between the dynamic uroepithelium and catheter. Therefore, one model cannot accurately capture the complexity of both infections. Ideally, two models will be employed to evaluate the *in vitro* efficacy of the catheter to best predict *in vivo* performance.

Table 2. *In vitro* models to simulate intraluminal colonization by uropathogens.

Method	Description	Pros	Cons	Ability to Reflect CAUTI	Possible Claim	Enumeration Technique	References
Flow Through	Inoculation of proximal end of catheter with test organism. Entire system is incubated at 37 °C. AUM is delivered at rate within laminar flow regimen. Effluent and catheter samples may be collected at multiple time points.	Simulates *in vivo* environment. Produces thick biofilm. Can run for clinically relevant timespan. Simple model set-up.	Only evaluates ability to delay intraluminal colonization. Uses large volumes of media.	Simulates bacterial access to the intraluminal surface. Mimics the spreading of an infection from one location.	Efficacy of surface to delay bacterial adherence and migration on intraluminal surface of catheter in presence of urine flow.	Viable colony counts from sampled urine and catheter segments. Non-invasive optical biomass sensor. SEM and TEM.	[81, 82]
False Bladder	False bladder (i.e. glass vessel) with AUM maintained at 37 °C. This allows a reservoir (~30 mL) of urine to collect in the bladder below the level of the catheter eyehole. As the volume of supplied urine increases, the overflow drains through the catheter to the collection bag. Residual urine in the model is inoculated directly. Samples collected from bladder, effluent flow, and catheter segments.	Simulates aspects of *in vivo* environment. Able to relate bacteriuria to intraluminal growth.	Does not simulate constant challenge of bacteria migrating up extraluminal surface. Complex design decreases reproducibility across studies. Uses large amount of media.	Partially simulates "early" extraluminal inoculation.	Ability for tip to "sterilize" bladder for time frame. Ability to inhibit bacteria from gaining access to intraluminal surface. Efficacy of surface to delay bacterial adherence and migration on intraluminal surface of catheter in presence of urine flow.	Viable colony counts from sampled urine and catheter segments. SEM and TEM.	[46, 83-86]

Modified Drip Flow Reactor (mDFR)	Device with four separate chambers with sealing lids. Catheter segments are connected to influent and effluent ports within the chambers. The device is connected to batch culture, which is pumped through at 1mL/min for 2 hours, after that time sterile nutrient broth is pumped though lumen of catheters at 0.5ml/min for specified timeframe. The catheters are then segmented, and biofilm is harvested for enumeration.	Simulates constant low shear flow of urine over catheter surface. Modified version of standardized method. Produces thick biofilm.	Uses large amount of media. Shortened length of catheter could affect ability to reflect *in vivo* biofilm.	Simulating bacterial access to the intraluminal surface.	Efficacy of surface to delay bacterial adherence and migration on intraluminal surface of catheter in presence of urine flow.	Viable colony count from catheter segments. SEM	[62, 80, 87, 88]
Flow Cell	Inoculum pumped across cell for 1 hour. Following inoculation period media is flowed through cell for given time period. Biofilm imaged using confocal microscopy.	Simulates constant low shear flow of urine over catheter surface. Allows real time microscopic investigation. Uses little media.	Expensive and expertise needed. Shortened length of catheter could affect ability to reflect *in vivo* biofilm.	Morphology of biofilm seen in flow cell does not typically represent that seen in infection [89].	Efficacy of surface to delay bacterial adherence and migration on intraluminal surface of catheter in presence of urine flow.	Bioluminescence for monitoring biofilm accumulation.	[80, 90-92]
Modified Robin Device (MRD)	Ports sit linearly along rectangular channel. Plugs may be inserted into each port. Batch or continuous culture fluid is fed through the device, submerging the substrata. Biofilm density is sampled by removing plugs from the side of cell.	Simulates constant low shear flow of urine over catheter surface.	Simple disks of catheter material do not represent complex device.	Simulating bacterial access to the intraluminal surface.	Efficacy of surface to delay bacterial adherence and migration on intraluminal surface of catheter in presence of urine flow.	Viable colony counts.	[61, 93-95]

Table 3. Common *in vitro* methods used to model extraluminal migration of bacteria during CAUTI and evaluate the efficacy of surface modified urinary catheters.

Method	Description	Pros	Cons	Ability to Reflect CAUTI	Possible Claim	Enumeration Technique	References
Anatomically Correct	Growth media drips into catheterized bladder. Pools in bladder then drip around catheter down urethra (i.e. glass tube). Inoculum introduced around false meatus. Migration of bacteria tracked along the length of catheter and into the bladder.	Able to run for extended timeframe. Captures complexity of device.	Complex design decreases reproducibility across studies. Urethra-catheter interface not well represented with glass tube. Uses large amounts of media	Attempted to simulate the physiological environment that the extraluminal is exposed to *in vivo*. Film of urine around extraluminal surface not seen clinically.	Delay of bacteriuria and bacterial migration along extraluminal surface.	Viable colony counts from sampled urine and catheter segments.	[46, 70]
False Urethra	In a capped tube, agar is poured around a sealed segment of catheter creating an agar column. The top of this column has 3mL of space which act as the 'bladder' and at the bottom a portion of the catheter is exposed representing the 'meatus'. The meatus is inoculated with bacterium and bladder is sampled until bacterium is detected.	Simplified model of anatomical structure. Surrounds the catheter with nutrient media, creating pseudo-tissue environment. Little media required.	Significant issues with agar track decrease reproducibility across studies. Bacteria find other routes to false bladder other than up catheter therefore rate of migration is not accurate.	Attempted to simulate the physiological environment that the extraluminal is exposed to *in vivo*.	Delay of bacteriuria and bacterial migration along extraluminal surface.	Viable colony counts from sampled urine. Roll plate method-semi quantitatively determine bacterial growth on surface.	[71]
Modified Motility Assay	PDMS template with surface modification is inoculated with a uropathogen then placed face down on growth media, which	Allows real time microscopic investigation.	Expensive and expertise needed.	Reflects the interstitial space between catheter and tissue.	Delay of bacterial migration along extraluminal surface.	Low magnification phase-contrast microscopy	[63, 96]

	has been poured on top of a microscope slide. System is incubated then imaged using phase contrast microscopy.	Uses little media. Test for non-eluting technologies.	Cannot directly predict long term efficacy Does not contain final device in test as required by FDA.[3]		Inhibition of bacterial expansion on catheter surface.	imaging and image analysis.	
Minimum Biofilm Eradication Concentration Device (MBEC)	System consists of a conventional 96- well bottom plate and a top plate which has been modified so that there are 96 conical-shaped ‘‘pegs’’ with flattened tips protruding from the lid. Pegs can be dip coated with selected surface modification. During incubation, biofilms grow on the surface of the pegs and the nutrients for biofilm growth are replenished daily by aseptically transferring the lid to a new bottom plate containing fresh sterile medium.	Commercially available device. Uses little media. Pegs can be coated in any material allowing for the screening of numerous treatments.	Does not differentiate between matrix, living and dead cells attached to the surface. Does not contain final device in test as required by FDA. [4]	Does not reflect infection environment. Does not reflect the catheter tissue interface seen *in vivo*.	Measure efficacy of antibiofilm technology to prevent biofilm adherence. Measures total attached biomass.	Bioluminescence for monitoring biofilm accumulation. Crystal violet stain. Optical density measurements. Viable cell counts. SEM	[80, 97-99]
Plate Migration Assay	1 cm channels are cut in nutrient agar plates. One side of channel is inoculated with uropathogen, 1 cm segment of catheter is placed in channel connecting the two sides. Plates are incubated and monitored for bacterial migration across catheter bridge.	Simple rapid screening. Little media needed. Test for non-eluting technologies.	Overly simplistic. No relevant nutrients present.	Does not reflect infection environment. Does not reflect the catheter tissue interface seen *in vivo*.	Delay of bacterial migration along extraluminal surface.	Presence/Absence	[100-102]

[3] https://www.fda.gov/MedicalDevices/ucm080884.htm. Accessed August 13, 2018.
[4] https://www.fda.gov/MedicalDevices/ucm080884.htm. Accessed August 13, 2018.

Inherently *in vitro* methods are unable to completely replace *in vivo* methods because it is impossible to model the complex host-pathogen relationship. However, there are several factors which are typically neglected in an effort to simplify testing that may easily be included in laboratory tests to improve clinical correlation. Running tests for a relevant time frame is one critical parameter which is often dismissed in laboratory testing. Some methods, such as the liquid broth test, are typically only run for a period of hours when clinically an antimicrobial would be expected to delay infection over the course of several days. It is not reasonable to claim a surface modification will be effective when it was only exposed to a challenge for a few hours, especially in reference to intraluminal infections, where urine will flow over the surface for much longer. Simply increasing the experimental time or testing the device until failure will help make the data collected from an *in vitro* model more relevant.

Table 4. Antibiotic Susceptibility Assays.

Method	Description	Pros	Cons	Ability to Reflect CAUTI	Possible Claim	Enumeration Technique	References
Zone of Inhibition	0.5-cm segments of catheter are embedded vertically in trypticase soy agar seeded on the surface with 10^8 CFU/mL of the test organism. The plates are incubated at 37°C for 24 hours. The zones of inhibition recorded. Catheters transferred daily to fresh plates until no ZOI occurred.	Rapid Cheap Simple Reproducible	Only dependent on chemistry's ability to diffuse into the agar. Not useable for non-eluting surface modifications.	Does not reflect environment seen during infection.	Eluting chemistry. Antibiotic susceptibility to clinically relevant pathogens.	Zone of inhibition around the catheter segments, excluding the diameters of the catheters are measured.	[61, 83-86]
Minimum Inhibitory Concentration (MIC)	Tubes containing increasing concentrations of antibiotic are inoculated with specific concentration of bacterium and incubated at 37°C for 24 hours.	Rapid Cheap Simple Reproducible	No surface tested.	Does not test a surface's ability to prevent colonization or migration of bacteria. Does not reflect environment seen during infection.	Antibiotic susceptibility to clinically relevant pathogens.	MIC defined as lowest concentration of antibiotic at which there is no visible bacterial growth.	[75, 87-89]
Liquid Broth Test	Surface modified catheter segments are incubated at 37°C for specified timespan in nutrient broth inoculated with specific concentration of bacterium. After incubation, segments are removed and washed. The segments are then placed in PBS and sonicated. Samples are serially diluted and plated for viable CFU counts.	Surface is in constant contact with relevant media. Simple Rapid Cheap Reproducible	Typically, not done for relevant time frame. Static growth conditions.	Simulates constant contact with urine. Does not reflect environment seen during infection.	Eluting Chemistry. Inhibition of growth of free floating bacteria. Prevents microbial adhesion under static conditions. Antibiotic susceptibility to clinically relevant pathogens.	Viable colony counts of attached bacteria Confocal laser scanning microscopy. SEM Count of free floating viable bacteria.	[64, 79, 86, 89-91]

Growth media is another notable factor that is often assumed to have little influence on the results. *In vitro* models are frequently conducted using a minimal, well-defined growth media (e.g. tryptic soy broth, lysogeny broth). It has been shown repeatably, though, that bacteria respond differently to varying growth media, especially urine [54, 109, 110]. Due to its low pH and high osmolality urine is naturally antimicrobial and creates an especially stressful environment for bacteria, ultimately resulting in altered metabolic pathways, virulence, and motility [29, 111, 112]. Not only does urine influence how bacteria grow and survive, it has also been shown to increase the MICs of several common antibiotics and decreases their overall efficacy [77]. It is therefore reasonable to assume that if urine is not used, results do not accurately predict how a catheter will perform in clinical settings.

Since urine has been identified as a necessary parameter for an *in vitro* model to be considered relevant, the decision becomes whether to use human urine or artificial urine media (AUM). When considering the importance of repeatability and reproducibility AUM represents a good balance between reasonableness and relevance. The use of human urine does have some benefits, for example it would contain natural constituents that are difficult to include in synthetic urine (i.e. hormones, iron chelators, and pyrophosphates). However, human urine is dynamic in composition, changing dramatically depending on age, gender, and health status [103, 113, 114]. A single commercial supplier offers over 15 different populations to choose from: Normal, Caffeine Free, Nicotine Free, Pregnancy First, Second or Third Trimester, Pre-menopausal women (on or off hormone modalities, including birth control), Post-

menopausal women (on or off hormone replacement therapies), Drug Free (Please specify drug), Lipemic, Fasted, or *Race/Ethnicity* (Black, Caucasian, Asian, Hispanic). In addition to wide chemical variation, volume limits are also a concern, with commercially available human urine costing up to $260/L[5]. Table 5 summarizes options for growth media available to researchers and benefits and drawbacks associated with each. More options do exist, as many have been developed depending on researchers' goals and specific applications. For example, Nowatzki et al. created their own AUM to better simulate the effects of salicytic acid release [80]. These recipes were developed with no intention for universal use and most have chemical components well out of physiological range. A significant improvement would be the development of a validated AUM recipe which closely resembles "normal human urine" and supports a broad range of uropathogens.

[5]https://www.innov-research.com/product/normal-human-urine?c=1 Accessed June 29th, 2018.

Table 5. Comparison of varying growth mediums used for *in vitro* models simulating CAUTIs.

Growth Medium	Description	Pro	Con	Ability to Reflect	References
Human Urine	Pooled urine, typically from 1-3 donors. Normalized by adjusting dilution, concentration, and pH according to specific gravity and osmolality.	Shown to support more abundant biofilm growth than artificial recipes [88]. Support growth of broad range of uropathogens. Contains natural components synthetic recipes may not be able to incorporate.	Expensive. Variation between individuals is challenging for standardizing studies. Large amounts of urine for models makes use impractical.	Human urine containing natural constitutes.	[75, 88, 103]
Artificial Urine Media (AUM) within physiological ranges	Synthetic human urine with osmolality, pH and composition as close to physiological ranges as possible.	Able to standardize across studies. Concentrations of components within physiological range. Support growth of broad range of uropathogens.	Compositions depend on clinical urine composition reports researchers' reference. Excludes some natural constituents of human urine (hormones, iron chelators).	Provides conditions similar to that of "normal human urine".	[75, 81, 88, 104-106]
Artificial Urine Media (AUM) for crystal aggregation	Elevated concentrations of specific solutes to encourage stone formation.	Able to simulate infection for specific population/disease.	Certain chemical components out of physiological range. Designed for specific infection, not planned for universal use. Does not support growth of all uropathogens. Potentially cytotoxic.	Accurately represents environment in patients prone to developing kidney stones. Not relevant for studies of general population.	[54, 106-108]
Nutrient Broth	Minimal, well-defined growth media.	Simple Reproducible	Lacks all natural constitutes of human urine.	Does not reflect environment seen during infection.	[54, 64, 75, 77, 78]

Broths commonly used include: Tryptic Soy broth (TSB), Lysogeny broth (LB), or Mueller-Hinton broth.	Supports growth of broad range of uropathogens.	Metabolic activities of pathogens may be altered from those typically seen in infection. MICs of antibiotics in nutrient broth are significantly lower than those observed in urine.	When used to determine bacterial susceptibility may not reflect the ability of bacteria in urine to resist the antibacterial action.

The objective of this research was to develop two quantitative, *in vitro* models which accurately reflect the conditions of the different routes of which CAUTI may occur. The development of standardized, laboratory methods will assist FDA regulators to compare efficacies across products and to bring more effective treatments to patients.

Chapter 2 establishes and statistically assesses a laboratory test method to evaluate the efficacy of surface modifications of the intraluminal surface of Foley catheters against UPEC biofilm. This chapter also discusses operational factors which remain to be optimized. Prospective studies, including optimization of the AUM recipe and the development of a model to test extraluminal surface modifications, are summarized in Chapter 3.

Literature Cited

1. Control, C.f.D., *Urinary Tract Infection (Catheter-Associated Urinary Tract Infection [CAUTI] and Non-Catheter-Associated Urinary Tract Infection [UTI] and Other Urinary System Infection [USI] Events.* 2018(Device-associated Module-UTI).

2. Lo, E., et al., *Strategies to prevent catheter-associated urinary tract infections in acute care hospitals: 2014 update.* Infect Control Hosp Epidemiol, 2014. **35 Suppl 2**: p. S32-47.

3. Weiner, L.M., et al., *Antimicrobial-Resistant Pathogens Associated With Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011-2014.* Infect Control Hosp Epidemiol, 2016. **37**(11): p. 1288-1301.

4. Marzo, A., et al., *An engineering approach towards a more discrete and efficient urinary drainage system.* Proc Inst Mech Eng H, 2018: p. 954411918790286.

5. Foley, F.E.B., *A Hemostatic Bag Catheter.* The Journal of Urology, 1937. **38**(1): p. 134-139.

6. Feneley, R.C., I.B. Hopley, and P.N. Wells, *Urinary catheters: history, current status, adverse events and research agenda.* J Med Eng Technol, 2015. **39**(8): p. 459-70.

7. Hanafy, H.M., S.M. Saad, and M.M. Al-Ghorab, *Ancient Egyptian medicine: contribution to urology.* Urology, 1974. **4**(1): p. 114-20.

8. Miller, A., et al., *Postoperative infection in urology.* Lancet, 1958. **2**(7047): p. 608-12.

9. Gillespie, W.A., et al., *The diagnosis, epidemiology and control of urinary infection in urology and gynaecology.* J Clin Pathol, 1960. **13**: p. 187-94.

10. Garibaldi, R.A., et al., *Factors predisposing to bacteriuria during indwelling urethral catheterization.* N Engl J Med, 1974. **291**(5): p. 215-9.

11. Garibaldi, R.A., et al., *Meatal colonization and catheter-associated bacteriuria.* N Engl J Med, 1980. **303**(6): p. 316-8.

12. Costerton, J.W., G.G. Geesey, and K.J. Cheng, *How bacteria stick.* Sci Am, 1978. **238**(1): p. 86-95.

13. Daifuku, R. and W.E. Stamm, *Bacterial adherence to bladder uroepithelial cells in catheter-associated urinary tract infection.* N Engl J Med, 1986. **314**(19): p. 1208-13.

14. Nickel, J.C., A.G. Gristina, and J.W. Costerton, *Electron microscopic study of an infected Foley catheter.* Can J Surg, 1985. **28**(1): p. 50-1, 54.

15. Costerton, J.W., et al., *Bacterial biofilms in nature and disease.* Annu Rev Microbiol, 1987. **41**: p. 435-64.

16. Donlan, R.M., *Special Issue: Biofilms and Device-Associated Infections.* Emerging Infectious Diseases, 2001. **7**(2): p. 277-281.

17. Stoodley, P., et al., *Orthopaedic biofilm infections.* Curr Orthop Pract, 2011. **22**(6): p. 558-563.

18. Busscher, H.J., et al., *Biofilm formation on dental restorative and implant materials.* J Dent Res, 2010. **89**(7): p. 657-65.

19. Hu, H., et al., *Bacterial Biofilm Infection Detected in Breast Implant-Associated Anaplastic Large-Cell Lymphoma.* Plast Reconstr Surg, 2016. **137**(6): p. 1659-69.

20. Meslemani, D., K. Yaremchuk, and M. Rontal, *Presence of biofilm on adult tracheostomy tubes.* Ear Nose Throat J, 2010. **89**(10): p. 496-504.

21. Food and Drug Administration, U., *Guidance for the Content of Premarket Notifications for Conventional and Antimicrobial Foley Catheters.* 1997.

22. Malpiedi, P.J., *National and State Healthcare-Associated Infection Standardized Infection Ratio Report.* 2011.

23. Peasah, S.K., et al., *Medicare non-payment of hospital-acquired infections: infection rates three years post implementation.* Medicare Medicaid Res Rev, 2013. **3**(3).

24. Saint, S., et al., *A Program to Prevent Catheter-Associated Urinary Tract Infection in Acute Care.* N Engl J Med, 2016. **374**(22): p. 2111-9.

25. Services, U.D.o.H.a.H., *National Action Plan to Prevent Health Care-Associated Infections: Road Map to Elimination, Executive Summary.* 2013: p. 1-19.

26. Hooton, T.M., *Clinical practice. Uncomplicated urinary tract infection.* N Engl J Med, 2012. **366**(11): p. 1028-37.

27. Ingersoll, M.A. and M.L. Albert, *From infection to immunotherapy: host immune responses to bacteria at the bladder mucosa.* Mucosal Immunol, 2013. **6**(6): p. 1041-53.

28. Parsons, C.L., et al., *Bladder surface glycosaminoglycans: an epithelial permeability barrier.* J Urol, 1990. **143**(1): p. 139-42.

29. Carlsson, S., et al., *Effects of pH, nitrite, and ascorbic acid on nonenzymatic nitric oxide generation and bacterial growth in urine.* Nitric Oxide, 2001. **5**(6): p. 580-6.

30. Raffi, H.S., et al., *Tamm-Horsfall protein acts as a general host-defense factor against bacterial cystitis.* Am J Nephrol, 2005. **25**(6): p. 570-8.

31. Hannan, T.J., et al., *Host-pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic Escherichia coli bladder infection.* FEMS Microbiol Rev, 2012. **36**(3): p. 616-48.

32. Arinzon, Z., et al., *Clinical presentation of urinary tract infection (UTI) differs with aging in women.* Arch Gerontol Geriatr, 2012. **55**(1): p. 145-7.

33. de Lastours, V. and B. Foxman, *Urinary tract infection in diabetes: epidemiologic considerations.* Curr Infect Dis Rep, 2014. **16**(1): p. 389.

34. Lichtenberger, P. and T.M. Hooton, *Complicated urinary tract infections.* Curr Infect Dis Rep, 2008. **10**(6): p. 499-504.

35. Levison, M.E. and D. Kaye, *Treatment of complicated urinary tract infections with an emphasis on drug-resistant gram-negative uropathogens.* Curr Infect Dis Rep, 2013. **15**(2): p. 109-15.

36. Chenoweth, C.E. and S. Saint, *Urinary tract infections.* Infect Dis Clin North Am, 2011. **25**(1): p. 103-15.

37. O'May, G.A., et al., *Complicated Urinary Tract Infections due to Catheters.* 2009. **3**: p. 123-165.

38. Haley, R.W., et al., *Nosocomial infections in U.S. hospitals, 1975-1976: estimated frequency by selected characteristics of patients.* Am J Med, 1981. **70**(4): p. 947-59.

39. Maki, D.G. and P.A. Tambyah, *Engineering out the risk for infection with urinary catheters.* Emerg Infect Dis, 2001. **7**(2): p. 342-7.

40. Jacobsen, S.M., et al., *Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis.* Clin Microbiol Rev, 2008. **21**(1): p. 26-59.

41. W., S., *Catheter Associated Urinary Tract Infections: Epidemiology, Pathogenesis, and Prevention.* Americal J Medicine, 1991. **91**(3B): p. 65S-71S.

42. Tambyah, P.A., K.T. Halvorson, and D.G. Maki, *A prospective study of pathogenesis of catheter-associated urinary tract infections.* Mayo Clin Proc, 1999. **74**(2): p. 131-6.

43. Saint, S. and C.E. Chenoweth, *Biofilms and catheter-associated urinary tract infections.* Infect Dis Clin North Am, 2003. **17**(2): p. 411-32.

44. Nickel, J., S. Grant, and J. Costerton, *Catheter-associated bacterium: An experimental study.* Urology, 1985. **26**(4): p. 369-375.

45. Warren, J.W., *Catheter-associated urinary tract infections.* Int J Antimicrob Agents, 2001. **17**(4): p. 299-303.

46. Barford, J.M., et al., *A model of catheter-associated urinary tract infection inittiated by bacterial contamination of the catheter tip.* BJU International, 2008. **102**(1): p. 67-74.

47. Hidron, A.I., et al., *NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007.* Infect Control Hosp Epidemiol, 2008. **29**(11): p. 996-1011.

48. Mulvey, M.A., *Adhesion and entry of uropathogenic Escherichia coli.* Cell Microbiol, 2002. **4**(5): p. 257-71.

49. Struve, C., M. Bojer, and K.A. Krogfelt, *Characterization of Klebsiella pneumoniae type 1 fimbriae by detection of phase variation during colonization and infection and impact on virulence.* Infect Immun, 2008. **76**(9): p. 4055-65.

50. Stahlhut, S.G., et al., *Biofilm formation of Klebsiella pneumoniae on urethral catheters requires either type 1 or type 3 fimbriae.* FEMS Immunol Med Microbiol, 2012. **65**(2): p. 350-9.

51. Fox-Moon, S.M. and M.E. Shirtliff, *Urinary Tract Infections Caused by Proteus mirabilis.* 2015: p. 1389-1400.

52. Flores-Mireles, A.L., et al., *Urinary tract infections: epidemiology, mechanisms of infection and treatment options.* Nat Rev Microbiol, 2015. **13**(5): p. 269-84.

53. Nickel, J.C., et al., *Bacterial biofilms: influence on the pathogenesis, diagnosis and treatment of urinary tract infections.* J Antimicrob Chemother, 1994. **33 Suppl A**: p. 31-41.

54. Ipe, D.S., E. Horton, and G.C. Ulett, *The Basics of Bacteriuria: Strategies of Microbes for Persistence in Urine.* Front Cell Infect Microbiol, 2016. **6**: p. 14.

55. Justice, S.S., et al., *Morphological plasticity as a bacterial survival strategy.* Nat Rev Microbiol, 2008. **6**(2): p. 162-8.

56. Stewart, P.S., *Theoretical aspects of antibiotic diffusion into microbial biofilms.* Antimicrob Agents Chemother, 1996. **40**(11): p. 2517-22.

57. Hoyle, B.D., J. Alcantara, and J.W. Costerton, *Pseudomonas aeruginosa biofilm as a diffusion barrier to piperacillin.* Antimicrob Agents Chemother, 1992. **36**(9): p. 2054-6.

58. Mobley, H.L., M.D. Island, and R.P. Hausinger, *Molecular biology of microbial ureases.* Microbiol Rev, 1995. **59**(3): p. 451-80.

59. Stickler, D., et al., *Proteus mirabilis biofilms and the encrustation of urethral catheters.* Urol Res, 1993. **21**(6): p. 407-11.

60. Lee, C.R., et al., *Strategies to minimize antibiotic resistance.* Int J Environ Res Public Health, 2013. **10**(9): p. 4274-305.

61. Raad, I., *Antibiotics and Prevention of Microbial Colonization of Catheters.* Antimicrob Agents and Chemotherapy, 1995. **39**(11): p. 2397-2400.

62. Fu, W., et al., *Bacteriophage cocktail for the prevention of biofilm formation by Pseudomonas aeruginosa on catheters in an in vitro model system.* Antimicrob Agents Chemother, 2010. **54**(1): p. 397-404.

63. Gloag, E.S., et al., *Micro-Patterned Surfaces That Exploit Stigmergy to Inhibit Biofilm Expansion.* Front Microbiol, 2016. **7**: p. 2157.

64. Gabriel, M.M., et al., *In vitro evaluation of the efficacy of a silver-coated catheter.* Curr Microbiol, 1996. **33**(1): p. 1-5.

65. Bologna, R.A., et al., *Hydrogel/silver ion-coated urinary catheter reduces nosocomial urinary tract infection rates in intensive care unit patients: a multicenter study.* Urology, 1999. **54**(6): p. 982-7.

66. Karchmer, T.B., et al., *A randomized crossover study of silver-coated urinary catheters in hospitalized patients.* Arch Intern Med, 2000. **160**(21): p. 3294-8.

67. Srinivasan, A., et al., *A prospective trial of a novel, silicone-based, silver-coated foley catheter for the prevention of nosocomial urinary tract infections.* Infect Control Hosp Epidemiol, 2006. **27**(1): p. 38-43.

68. Pickard, R., et al., *Antimicrobial catheters for reduction of symptomatic urinary tract infection in adults requiring short-term catheterisation in hospital: a multicentre randomised controlled trial.* The Lancet, 2012. **380**(9857): p. 1927-1935.

69. Parker, A.E. and M.A. Hamilton, *KSA-SM-10- Assessing Resemblance, Repeatability, and Reproducibility for quantitative methods*, C.f.B.E.a.M.S. University, Editor. 2011: Bozeman, MT.

70. Darouiche, R.O., *In VItro Efficacy of Antimicrobial-Coated Bladder Catheters in Inhibiting Bacterial Migration along Catheter Surface.* Concise communications, 1997(176 (October)): p. 1109-1112.

71. T., G., *Evaluation of the Antimicrobial Efficacy of Urinary Catheters Impregnated with Antiseptics in an In Vitro Urinary Tract Model.* Infection Control and Hospital Epidemiology, 2003. **24**(7): p. 506-513.

72. Sherertz, R.J., D.M. Forman, and D.D. Solomon, *Efficacy of dicloxacillin-coated polyurethane catheters in preventing subcutaneous Staphylococcus aureus infection in mice.* Antimicrob Agents Chemother, 1989. **33**(8): p. 1174-8.

73. Darouiche, R.O., et al., *Efficacy of combination of chlorhexidine and protamine sulphate against device-associated pathogens.* J Antimicrob Chemother, 2008. **61**(3): p. 651-7.

74. Gomes, L.C., et al., *Escherichia coli adhesion, biofilm development and antibiotic susceptibility on biomedical materials.* J Biomed Mater Res A, 2015. **103**(4): p. 1414-23.

75. Minuth, J.N., D.M. Musher, and S.B. Thorsteinsson, *Inhibition of the antibacterial activity of gentamicin by urine.* J Infect Dis, 1976. **133**(1): p. 14-21.

76. Dave, R.N., H.M. Joshi, and V.P. Venugopalan, *Novel biocatalytic polymer-based antimicrobial coatings as potential ureteral biomaterial: preparation and in vitro performance evaluation.* Antimicrob Agents Chemother, 2011. **55**(2): p. 845-53.

77. Dalhoff, A., W. Stubbings, and S. Schubert, *Comparative in vitro activities of the novel antibacterial finafloxacin against selected Gram-positive and Gram-negative bacteria tested in Mueller-Hinton broth and synthetic urine.* Antimicrob Agents Chemother, 2011. **55**(4): p. 1814-8.

78. Hachem, R., et al., *Novel antiseptic urinary catheters for prevention of urinary tract infections: correlation of in vivo and in vitro test results.* Antimicrob Agents Chemother, 2009. **53**(12): p. 5145-9.

79. Roe, D., et al., *Antimicrobial surface functionalization of plastic catheters by silver nanoparticles.* J Antimicrob Chemother, 2008. **61**(4): p. 869-76.

80. Nowatzki, P.J., et al., *Salicylic acid-releasing polyurethane acrylate polymers as anti-biofilm urological catheter coatings.* Acta Biomater, 2012. **8**(5): p. 1869-80.

81. Nickel, J.C., J. Downey, and J.W. Costerton, *Movement of pseudomonas aeruginosa along catheter surfaces. A mechanism in pathogenesis of catheter-associated infection.* Urology, 1992. **39**(1): p. 93-8.

82. Dohnt, K., et al., *An in vitro urinary tract catheter system to investigate biofilm development in catheter-associated urinary tract infections.* J Microbiol Methods, 2011. **87**(3): p. 302-8.

83. Stickler, D.J., N.S. Morris, and C. Winters, *Simple physical model to study formation and physiology of biofilms on urethral catheters.* Methods Enzymol, 1999. **310**: p. 494-501.

84. Tenke, P., et al., *Bacterial biofilm formation on urologic devices and heparin coating as preventive strategy.* Int J Antimicrob Agents, 2004. **23 Suppl 1**: p. S67-74.

85. Chua, R.Y.R., et al., *An in vitro urinary catheterization model that approximates clinical conditions for evaluation of innovations to prevent catheter associated urinary tract infections.* J Hosp Infect, 2017.

86. Morris, N.S., D.J. Stickler, and C. Winters, *Which indwelling urethral catheters resist encrustation by Proteus mirabilis biofilms?* Br J Urol, 1997. **80**(1): p. 58-63.

87. Curtin, J.J. and R.M. Donlan, *Using bacteriophages to reduce formation of catheter-associated biofilms by Staphylococcus epidermidis.* Antimicrob Agents Chemother, 2006. **50**(4): p. 1268-75.

88. Lehman, S.M. and R.M. Donlan, *Bacteriophage-mediated control of a two-species biofilm formed by microorganisms causing catheter-associated urinary tract infections in an in vitro urinary catheter model.* Antimicrob Agents Chemother, 2015. **59**(2): p. 1127-37.

89. Roberts, A.E., et al., *The Limitations of In Vitro Experimentation in Understanding Biofilms and Chronic Infection.* J Mol Biol, 2015. **427**(23): p. 3646-61.

90. Purevdorj, B., J.W. Costerton, and P. Stoodley, *Influence of hydrodynamics and cell signaling on the structure and behavior of Pseudomonas aeruginosa biofilms.* Appl Environ Microbiol, 2002. **68**(9): p. 4457-64.

91. Heydorn, A., et al., *Experimental reproducibility in flow-chamber biofilms.* Microbiology, 2000. **146 (Pt 10)**: p. 2409-15.

92. Tolker-Nielsen, T. and C. Sternberg, *Growing and analyzing biofilms in flow chambers.* Curr Protoc Microbiol, 2011. **Chapter 1**: p. Unit 1B 2.

93. Nickel, J.C., et al., *Antibiotic resistance of Pseudomonas aeruginosa colonizing a urinary catheter in vitro.* Eur J Clin Microbiol, 1985. **4**(2): p. 213-8.

94. Maeyama, R., et al., *Novel bactericidal surface: Catechin-loaded surface-erodible polymer prevents biofilm formation.* J Biomed Mater Res A, 2005. **75**(1): p. 146-55.

95. McCoy, W.F., et al., *Observations of fouling biofilm formation.* Can J Microbiol, 1981. **27**(9): p. 910-7.

96. Turnbull, L. and C.B. Whitchurch, *Motility assay: twitching motility.* Methods Mol Biol, 2014. **1149**: p. 73-86.

97. Irwin, N.J., C.P. McCoy, and L. Carson, *Effect of pH on the in vitro susceptibility of planktonic and biofilm-grown Proteus mirabilis to the quinolone antimicrobials.* J Appl Microbiol, 2013. **115**(2): p. 382-9.

98. Ceri, H., et al., *The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms.* J Clin Microbiol, 1999. **37**(6): p. 1771-6.

99. Meije, Y., et al., *Daptomycin is effective as antibiotic-lock therapy in a model of Staphylococcus aureus catheter-related infection.* J Infect, 2014. **68**(6): p. 548-52.

100. Stickler, D. and G. Hughes, *Ability of Proteus mirabilis to swarm over urethral catheters.* Eur J Clin Microbiol Infect Dis, 1999. **18**(3): p. 206-8.

101. Sabbuba, N., *The Migration of Proteus Mirabilis and other urinary tract pathogens over Foley Catheters.* BJU International, 2002. **89**: p. 55-60.

102. Reddy, S.T., et al., *Micropatterned surfaces for reducing the risk of catheter-associated urinary tract infection: an in vitro study on the effect of sharklet micropatterned surfaces to inhibit bacterial colonization and migration of uropathogenic Escherichia coli.* J Endourol, 2011. **25**(9): p. 1547-52.

103. Bouatra, S., et al., *The human urine metabolome.* PLoS One, 2013. **8**(9): p. e73076.

104. Burns, J.R. and B. Finlayson, *A proposal for a standard reference artificial urine in in vitro urolithiasis experiments.* Invest Urol, 1980. **18**(2): p. 167-9.

105. Brooks, T., *A simple Artificial Urine for the Growth of Urinary Pathogens.* Letters in Applied Microbiology, 1997. **24**: p. 203-206.

106. Chutipongtanate, S. and V. Thongboonkerd, *Systematic comparisons of artificial urine formulas for in vitro cellular study.* Anal Biochem, 2010. **402**(1): p. 110-2.

107. Grases, F. and A. Llobera, *Experimental model to study sedimentary kidney stones.* Micron, 1998. **29**(2-3): p. 105-11.

108. Christmas, K.G., et al., *Aggregation and dispersion characteristics of calcium oxalate monohydrate: effect of urinary species.* J Colloid Interface Sci, 2002. **256**(1): p. 168-74.

109. Shrout, J.D., et al., *The impact of quorum sensing and swarming motility on Pseudomonas aeruginosa biofilm formation is nutritionally conditional.* Mol Microbiol, 2006. **62**(5): p. 1264-77.

110. Palmer, K.L., L.M. Aye, and M. Whiteley, *Nutritional cues control Pseudomonas aeruginosa multicellular behavior in cystic fibrosis sputum.* J Bacteriol, 2007. **189**(22): p. 8079-87.

111. Cotter, P.D. and C. Hill, *Surviving the acid test: responses of gram-positive bacteria to low pH.* Microbiol Mol Biol Rev, 2003. **67**(3): p. 429-53, table of contents.

112. Shepard, B.D. and M.S. Gilmore, *Differential expression of virulence-related genes in Enterococcus faecalis in response to biological cues in serum and urine.* Infect Immun, 2002. **70**(8): p. 4344-52.

113. Barr, D.B., et al., *Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements.* Environ Health Perspect, 2005. **113**(2): p. 192-200.

114. Torffvit, O. and C.D. Agardh, *Tubular secretion of Tamm-Horsfall protein is decreased in type 1 (insulin-dependent) diabetic patients with diabetic nephropathy.* Nephron, 1993. **65**(2): p. 227-31.

CHAPTER TWO

STATISTICAL ANALYSIS OF NOVEL *IN VITRO* METHOD TO EVALUATE THE EFFICACY OF SURFACE MODIFIED URINARY CATHETERS

Methods

Description of the Intraluminal Catheter Model (ICM)

The ICM consisted of a 16 French (Fr), 2-way, all-silicone Foley catheter connected by diameter-type tubing to a 5L reservoir of artificial urine media (AUM) on the influent side. The effluent end was connected to a sampling port, which then connected to an effluent waste carboy, as shown in Figure 4. The entire system was contained in an incubator held at 37°C and AUM was continuously delivered to the catheter at a flow rate of 0.75 mL/minute. The AUM used for this model was a modified version of the recipe developed by Brooks and Kevil [1]. The AUM recipe aims to replicate the characteristics of 'normal' human urine by keeping pH, osmolarity and appropriate chemicals within typical physiological ranges. For full chemical composition and preparation instructions of the AUM see appendix A, Standard Operating Procedure for Preparing Artificial Urine Media. Uropathogenic *Escherichia coli* (UPEC) ATCC 53498, originally isolated from a human UTI, was selected for this study. Stocks were maintained at -80°C and propagated on tryptic soy agar (TSA) plates at 37°C.

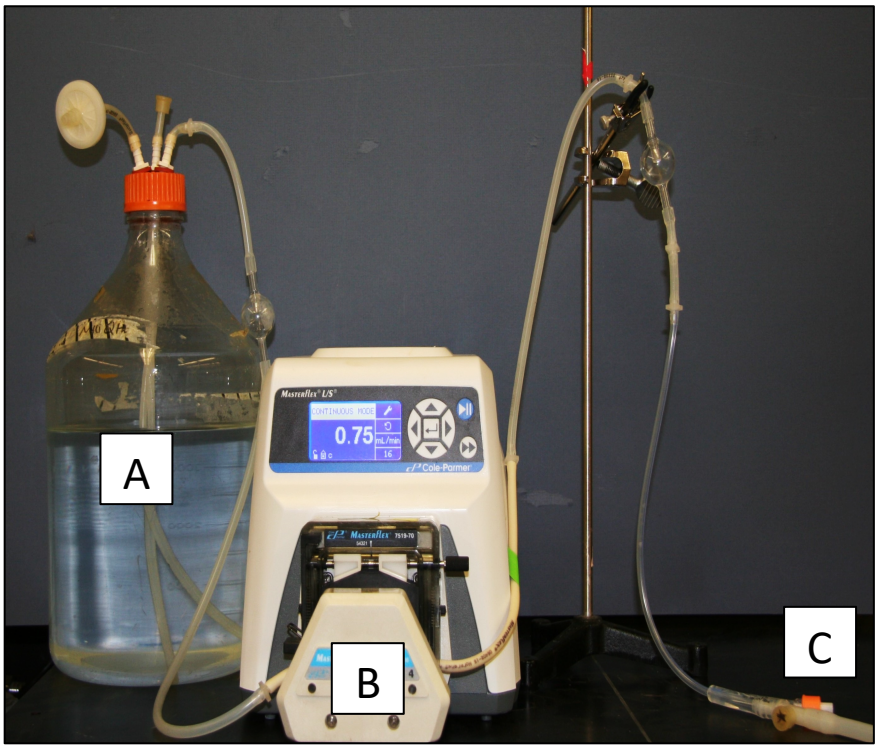

Figure 4. The ICM system, incubated at 37°C. AUM (A) is pumped through tubing at 0.75 mL/min (B) through a 16 Fr, two-way, Foley catheter to sampling port (C) into an effluent waste carboy (not shown here).

Standard Operating Procedure (SOP)

The influent and effluent tubing were assembled and autoclaved. Once the tubing had cooled, the influent tubing was connected to the AUM carboy and fed through the cartridge pump head on the electronic pump. With flame sterilized scissors the tip of the sterile Foley catheter was cut directly below the eyes and attached to the influent tubing. The distal end of the catheter was then connected to the effluent port, which was connected to the waste carboy. The system included up to four catheters in the system at one time. Each additional catheter was attached to the AUM carboy top and fed through their own individual cartridge pump head and attached to separate effluent ports, which then were connected to the effluent waste carboy.

After the system was assembled, AUM was allowed to flow through the sterile catheter at 0.75 mL/min for 2 hours to precoat the catheter surface. The catheter was then inoculated with 2 mL of a 10^3 CFU/mL suspension of UPEC (ATCC 53498). The UPEC suspension was prepared by inoculating 10mL of AUM with 2-3 isolated colonies collected from a bacterial isolation plate. The culture was incubated for 22-24 hours at 37°C. The incubated culture reached a log density of 10^8 CFU/mL, it was serially diluted in AUM to reach a final concertation of 10^3 CFU/mL. Immediately after the addition of UPEC to the catheter, the pump was restarted at 0.75 mL/min. The biofilm was allowed to establish for 96 hours total, the system was sampled at the predetermined time points of 8, 24, 48, 72, and 96 hours.

At each time point a sample of the effluent AUM and a 2-cm segment from the distal end of the catheter were collected. To collect the effluent sample the pump was paused, a sterile syringe was connected to the effluent port, the effluent line was clamped directly below the port and the pump was turned on until a volume of 10 mL was collected. Two effluent samples were collected to ensure uniform flow was achieved, the first sample was disposed of and the second collected and dispensed into 10 mL of buffered dilution water. After effluent collection the pump was paused, and the distal 2-cm segment of the catheter was collected. The catheter segment was rinsed in buffered dilution water and the biofilm was scraped from the surface. In short, to remove the biofilm from the catheter, the segment was cut in half longitudinally was a sterile razor blade. One half was held with sterile forceps, and using a sterile applicator stick, the intraluminal surface was thoroughly scraped 3-4 times. The applicator stick was stirred in

10 mL of buffered dilution water after scraping the surface for approximately 15 seconds to remove attached material. This process was repeated for the second half of the catheter segment. After the biofilm was collected from the surface, both samples, effluent and catheter, were homogenized by a vortex-sonication series. This series consisted of vortexing the samples for 30 seconds followed by 2 minutes of sonication, vortex, sonicate, vortex, to create a uniform cell suspension. The samples were then serially diluted, plated on TSA, incubated for 22-24 hours at 37°C and viable CFUs were enumerated.

The key measurement was the biofilm $\log_{10}$ density (LD). Effluent samples were expressed in CFU/mL and catheter segments in CFU/cm^2. Densities were calculated using the following equations:

$$Effluent\ LD = log_{10}\left(\frac{CFU}{mL}\right) = log_{10}[2 * \left(\frac{10^d CFU}{10\mu L}\right) * \left(\frac{1000\mu l}{1mL}\right)] \quad \text{Eq 1}$$

$$Catheter\ Segment\ LD = log_{10}\left(\frac{CFU}{cm^2}\right) = log_{10}[\left(\frac{10^d CFU}{10\mu L}\right) * (V/SA)] \quad \text{Eq 2}$$

In Equation 2, V is the volume sonicated into (10 mL); and SA is the surface area of one 2-cm long, catheter segment (2.39 cm^2). When no CFUs were observed in all dilutions a ½ CFU was substituted in at the lowest dilution plated, and then scaled up [2]. The 0th dilution was always plated, so after applying the CFU substitution rule for effluent samples,

$$Effluent\ LD = log_{10}\left(2 * \frac{0.5CFU}{0.01mL}\right) = 1.00 \quad \text{Eq 3}$$

was as the substituted value for the effluent LD. For catheter segment samples the lower level of detection was achieved by spread plating the 0th and 1st dilutions, so after applying the CFU substitution rule for catheter samples the LD became:

$$Catheter\ Segment\ LD = log_{10}\left[\left(\frac{0.5CFU}{1\ mL}\right) * \left(\frac{10mL}{2.39cm^2}\right)\right]$$

$$= log_{10}\left[1.05\frac{CFU}{cm^2}\right] = 0.02 \qquad \text{Eq 5}$$

The efficacy of surface modified urinary catheters tested was measured by the log reduction (LR) which is calculated by subtracting the mean LDs for the modified catheter replicates from the mean LDs for control catheter[6]. A high LR indicates the surface modification was effective. LRs were calculated for both effluent and catheter segment samples.

Ruggedness and Repeatability Evaluation Study

A series of experiments were conducted to estimate the repeatability standard deviation, s_r, and the regression coefficients that measure the ruggedness of the ICM SOP. In these experiments, the SOP was not followed exactly, instead small deviations were purposely made to some operational factors. For the ruggedness tests, the settings of five operational factors were altered: inoculum concentration (Inoc), incubator temperature (Temp), pump flow rate (Flow), pH of AUM (pH), and removal technique (Removal). In order to estimate both main effects and two-way interactions among the five factors, experiments were run at low, SOP, and high levels of the factors of interest (Table 6). These low and high levels were chosen in order to span a realistic range of

[6] http://www.biofilm.montana.edu/documents/KSA-SM-07.pdf. Accessed November 26, 2018.

physiological conditions, along with anticipated changes to the protocol when conducted by different technicians and in different labs.

Table 6. The five factors which were analyzed in the ruggedness testing, the short name used in subsequent tables, and the low, medium, and high levels of each factor.

Factor	Short Name	Low	Medium (SOP)	High
Inoculum Concentration	Inoc	10^2 CFU/mL	10^3 CFU/mL	10^4 CFU/mL
Incubator Temperature	Temp	34°C	37°C	40°C
Flow Rate	Flow	0.25mL/min	0.75mL/min	1.25mL/min
pH of AUM	pH	6	6.5	7
Removal Technique	Removal	Sonicate	Scrape + Sonicate	—

Three groups of 16 French (Fr) 2-way, all-silicone Foley catheters were included in the experiments: (i) sterile, uncoated (Bardia Foley Catheters, C.R. Bard Inc., Murray Hill, NJ) (ii) sterile hydrogel-silver coated (Silvertouch Foley Catheters, Medline Industries Inc., Northfield, IL) (iii) sterile silicone catheters that were coated in house with Aledex® solution (Kane Biotech, Winnipeg, Canada).

Experiments at specific combinations of the factors were chosen according to a fractional factorial experimental design and the run order was randomized as outlined in Table 7. Four catheters were run per experiment, one control, one Aledex® coated, and two hydrogel-silver coated catheters. Each pair (i.e. 1A and 1B) were run on the same days in the same lab by one technician in order to minimize day-to-day variability in experimental outcomes, thereby increasing the statistical power to detect the effects of the 5 factors. Data was collected from previous experiments run at standard operating procedure (SOP) values for all of the factors. The results from these experiments provided ‘center points’, allowing for initial estimation of the variability of the control LDs and treated log reductions (LR) when the method was run according to the SOP.

Thus, the ruggedness tests results are based on 19 urinary catheter model runs, all preformed in the same lab by the same technician.

Table 7. Fractional factorial schedule of ruggedness tests. A & B of each run number were separate experiments run side by side.

Run	Inoc(CFU/mL)	Temp(°C)	Flow(mL/min)	pH	Removal
1A	10^4	34	0.25	7	sonicate
1B	10^2	40	1.25	6	scrape
2A	10^2	40	0.25	6	sonicate
2A	10^4	34	1.25	7	scrape
3A	10^2	40	1.25	7	scrape
3B	10^4	34	0.25	6	sonicate
4A	10^2	34	1.25	7	sonicate
4A	10^4	40	0.25	6	scrape
5A	10^2	34	0.25	7	scrape
5B	10^4	40	1.25	6	sonicate
6A	10^2	34	0.25	6	scrape
6B	10^4	40	1.25	7	sonicate
7A	10^2	40	0.25	7	sonicate
8B	10^4	34	1.25	6	scrape
9A	10^2	34	1.25	6	sonicate
9B	10^4	40	0.25	7	scape

Ruggedness was quantified by the coefficients in a linear regression analysis where the response variable was log reduction, for the antimicrobial catheters, and log density, for control catheters. The predictor variables were inoculum concentration, incubator temperature, pump flow rate, pH of AUM, and removal technique. To do the analysis, the five predictor variables were entered as covariates into R Lme4 package.

The ANOVA also provided a variance component analysis to assess the variance between blocks and within blocks. The square root of the sum of the two variances was the repeatability standard deviation, s_r, which was interpreted as the typical difference,

sign neglected, between the log reduction for a single (randomly chosen) block and the mean log reduction across many independent, identical (same operational factor settings) blocks.

Results

Repeatability

For the SOP test experiments, replicate experiments were conducted to allow assessment of the repeatability of the SOP. Table 8 shows the mean log density and standard deviation for control catheters across five SOP experiments. The control catheter segment samples, were estimated to have a resemblance standard deviation of $Cs_{r,catheter} = (0.292+0.56)^{1/2}=0.92$, of which 34% was attributed to within-experiment variation and 66% to between-experiment variation. Control effluent samples had a lower resemblance standard deviation of $Cs_{r,\ effluent} = (0.067+0.046)^{1/2}=0.34$, of which 59% was attributed to within-experiment variation and 41% to between-experiment variation. The repeatability of Kane coated catheters at SOP conditions was determine using LRs from two standard runs. The standard deviation of LR values from Kane catheter segments was calculated to be $s_{r,\ Kane,\ catheter} = (1.74)^{1/2}=1.31$ across all time points. For Kane effluent samples S_r was calculated to be $s_{r,\ Kane,\ effluent}=(1.17)^{1/2}=1.08$ across all time points. For both sample types 100% of the variability was due to between-experiment variation. Silver catheter repeatability was not reported as only one SOP run was completed for the treatment. See Appendix B, Repeatability Analysis, for further detail.

Table 8. Mean log densities and standard deviations (StDev) of SOP controls across five experiments, by sample point. Each experiment had a single replicate at each sample point.

	Catheter Samples		Effluent Samples	
Sample Point(hrs)	Mean $\log_{10}$(CFU/cm^2)	StDev	Mean $\log_{10}$(CFU/mL)	StDev
24	7.07	0.28	7.98	0.36
48	7.42	0.62	8.55	0.24
72	8.46	0.19	8.63	0.29
96	8.69	0.58	8.39	0.36

Ruggedness

Preliminary analysis of the ruggedness data suggested the method was not rugged with respect to the varied operational parameters across time, as shown by Figures 5 and 6. From the ruggedness data a predicted reproducibility standard deviation, s_R, was calculated for each of the three catheter types. The s_R, describes a method's variability when it is run in separate labs. An acceptable level of reproducibility is an s_R of 1.3 or less[7]. To calculate the predicted s_R each run completed for the ruggedness testing, as described in Table 7, was treated as if it was an SOP data set collected from a separate laboratory. The catheter segments were estimated to have s_R's well above the acceptable level of 1.75, 2.47, 2.00 for control, Kane and Silver catheters, respectively, across all time points as reported in Figure 5. The effluent samples showed slightly higher variation with estimated s_R's of 1.49, 2.72, and 2.74 for control, Kane and Silver catheters, respectively, across all time points as reported in Figure 6. To understand which

[7] http://www.biofilm.montana.edu/documents/KSA-SM-03.pdf. Accessed November 6, 2018.

parameters were contributing to high variation of the method further assessment was completed.

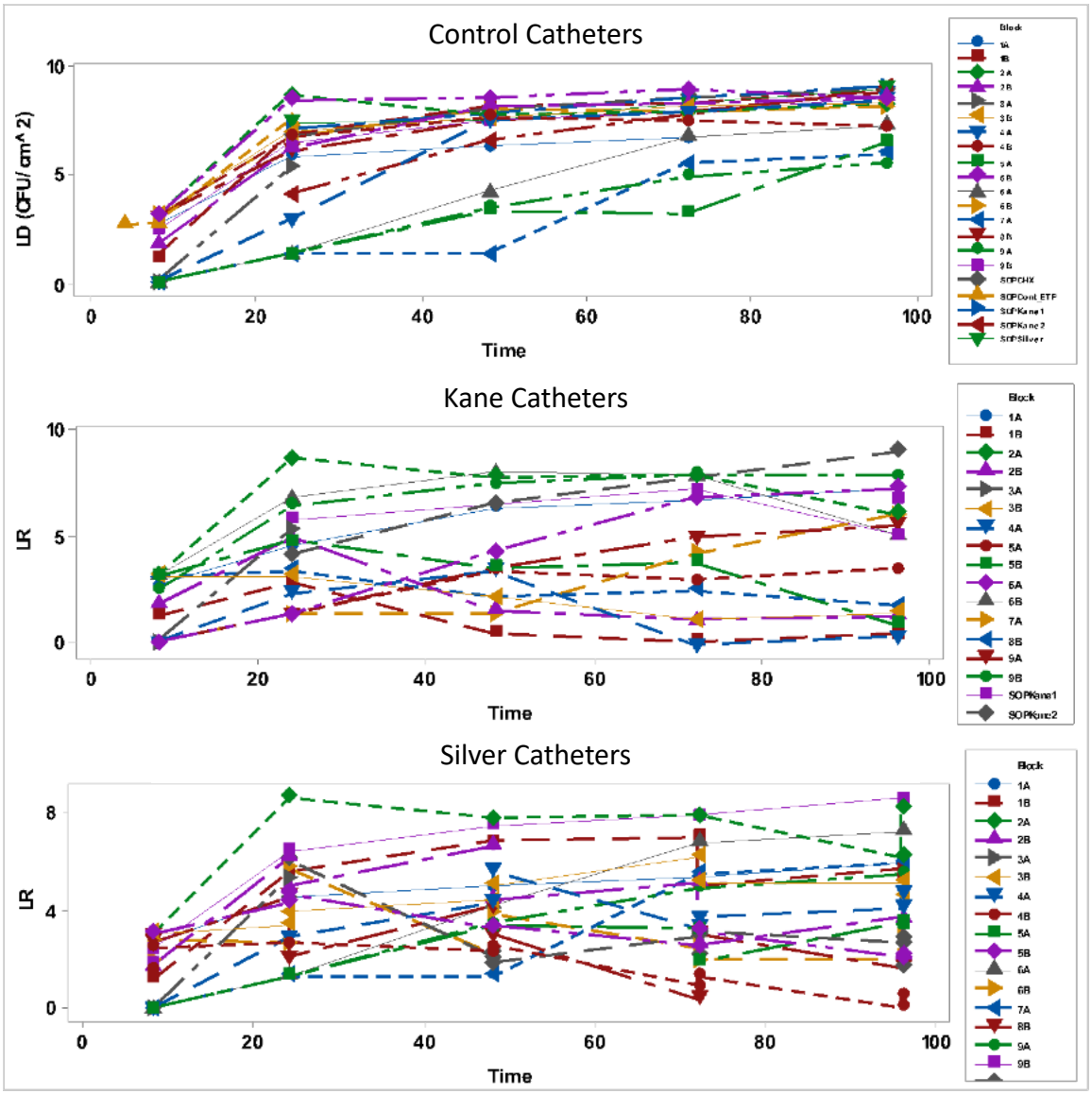

Figure 5. Mean LD's and LR's reported from ruggedness testing for the catheter segment samples of control, Kane, and Silver catheters. A different color and line type is used to visualize each run.

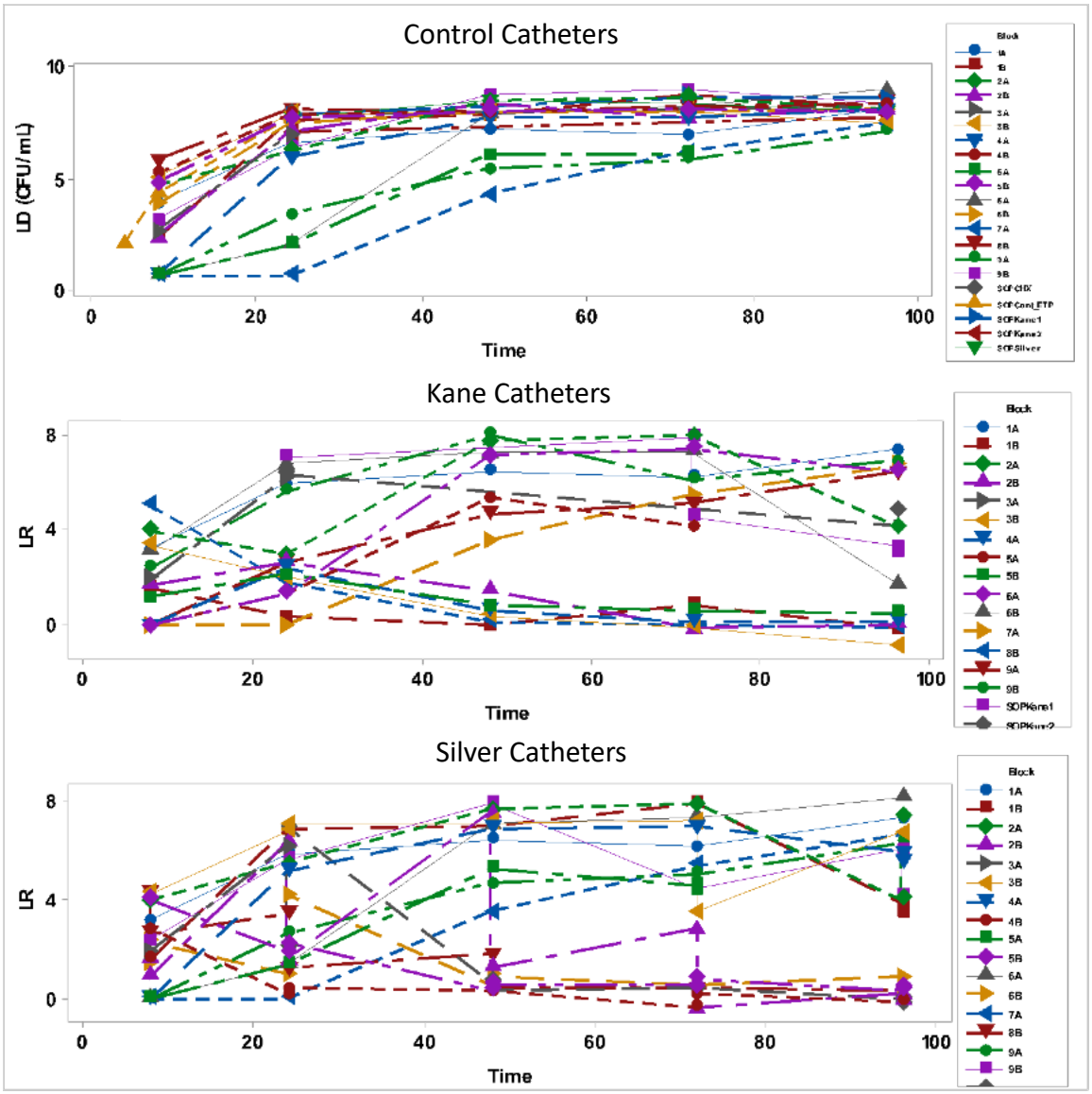

Figure 6. Mean LD's and LR's reported from ruggedness testing for the effluent samples of control, Kane, and Silver catheters. A different color and line type is used to visualize each run.

In order to quantitatively assess the ruggedness of the model a least-squares regression model for the log density for controls and log reduction for the antimicrobial catheters was completed for sample points 8-48 hours. Forward selection was used to determine confounding variables and all models were refined through backwards

selection. The control and silver catheters' regression equations were split by time to decrease model complexity. Full regression equations and partial derivatives are reported in Appendix D, Ruggedness Analysis.

Analysis of the control catheters revealed they were not rugged with respect to any of the varied operational factors. The evaluation showed that for both sample types, effluent and catheter segments, the pH of the AUM had two-way interactions among all the other factors, temperature, flow, removal method, and inoculum concentration.

Analysis of the antimicrobial catheters concluded there were no two-way interactions detected for Kane catheter segment LRs and they were estimated to be rugged with respect to inoculum concentration, temperature, and flow. Catheter samples were not rugged over time which was expected as the system is continuously changing with time, as the biofilm grows, and the coating begins to fail. The effluent samples showed every operational factor had a two-way interaction with time and that flow and temperature each had a two-way interaction with pH. The analysis also detected one three-way interaction among inoculum, pH, and time. Silver catheter LRs for both sample type, effluent and catheter segment, were detected to have two-way interactions between pH of the AUM and all other operational factors.

The ruggedness evaluation highlighted that control and antimicrobial catheters alike were not rugged with respect to biofilm removal technique. Sonication was estimated to always result in at least a log increase in log density or log reduction when compared to scraping the biofilm from the surface. This was true regardless of interactions with other factors.

The pH of the AUM had significant influence on the ruggedness of the control LD and antimicrobial LRs with respect to all other operational factors that had been evaluated in the ruggedness evaluation. Simulations were run to better understand the interactions by varying the ranges of flow rate, inoculum concentration, and temperature of the incubator. The removal method was excluded from the simulation as the regression model showed sonication had a large increase in removal of biofilm with or without the influence of pH. The simulation showed the procedure is rugged when the pH is held at the SOP value of 6.5 and flow rate, inoculum concentration, and temperature are varied within the following ranges: 0.75 mL/min ± 0.14 mL/min, 3 $\log_{10}$(CFU/mL) ± 0.2 $\log_{10}$(CFU/mL), and 36°C ± 1 °C. When the pH is increased to 7 or decreased to 6, this no longer holds true. Therefore, the pH of the AUM must be optimized to increase the ruggedness of the method. Regression coefficients are reported in Tables D-1 through D-3 in Appendix D, Ruggedness Analysis.

Discussion

Statistical Evaluation

A standardized method should be assessed for an acceptable level of the following desirable statistical attributes: reasonableness, relevancy, validity, ruggedness, resemblance, responsiveness, repeatability, and reproducibility[8]. The purpose of the experiments presented here were to evaluate the level of repeatability and ruggedness of the ICM.

[8] http://www.biofilm.montana.edu/documents/KSA-SM-03.pdf. Accessed November 6, 2018.

A quantitative test method is said to be repeatable if it will produce nearly the same LD values in independent tests within the same laboratory. To measure a method's level of repeatability the standard deviation of the LD values is calculated. This is called the repeatability standard deviation and is denoted by s_r. The smaller the s_r the better the repeatability of a method is, a $s_r \leq 1$indicates acceptable repeatability[6].

A method is rugged if the outcome, LR or LD, is insensitive to minor changes of operational factors. No conventional quantitative measure for ruggedness has been set, like s_r but many have been suggested[6]. For the purpose of this ruggedness analysis the method is said to be rugged with respect to a specific operational factor if the effect, calculated from partial derivatives of the regression equation, is less than ±0.30 logs. This effect indicates when a slight change is made to the protocol the new mean LD or LR will be within 0.30 logs of the mean LD or LR expected when the model is run at the SOP. This value was chosen as the threshold for ruggedness as it does not represent a 'meaningful change' [3]. For example, if the LR was originally measured at 0 when the method was run at SOP values and the increased to a LR of 0.3 when the protocol was altered this would not indicate the modified catheter was drastically more effective than the control catheter. However, if the LR increased somewhere closer to a 1, this could indicate the modified catheter is more effective, or suggest a meaningful change. Each threshold for ruggedness is subjective, and one must choose a threshold which accurately reflects their system.

By preforming a ruggedness test, the practitioner can identify which operational parameters must be optimized or that require special attention. A ruggedness test may

also indicate if the method is suitable for a collaborative study (a multi-laboratory study) which assesses a method's reproducibility). An examination of the literature revealed that ruggedness evaluations of the urinary catheter laboratory methods are never performed. However, the experiments presented here demonstrate that quantitative ruggedness analyses of test methods can be very informative and helpful in developing a method that will be predictive of clinical outcomes.

The selection of operational factors to study and the range they were varied was a subjective step in the ruggedness analysis. Experience with the SOP and understanding of the physiological conditions expected during CAUTI were relied on when choosing which factors were the most critical to investigate. The settings for each factor were a combination of those within the range of what might be expected from a clinical standpoint and when competent researchers diligently followed the SOP. Responses were observed at 2 or more levels of each operation factor.

The ICM System

The ruggedness evaluation showed that sonication of catheter segments resulted in the removal of more biofilm from the surface than scraping. For example, if the protocol was followed except that a Kane-coated catheter segment was sonicated, instead of scraped into neutralization broth, as specified by the protocol, the mean log reduction would be estimated to increase by 1.02 log. Therefore, it is recommended that the protocol be modified to include sonication instead of scraping. Following this change, sonication should be compared to other possible methods to further optimize the harvesting of biofilm from the surface of catheters.

The ruggedness analysis also highlighted that the effluent LRs consistently had higher repeatability standard deviations, s_r, than that of the catheter segment LRs. This higher variability may possibly be attributed to biofilm growth in the connector directly proximal to catheter, as shown in Figure 5. This connector has been identified as a constant source of inoculum in the ICM system for both antimicrobial and control catheters. The biofilm density on the interior surface of the connector was found to be 8.64 log (CFU/cm2) and 7.61 log (CFU/cm2) for connectors directly proximal to a control and a silver catheter, respectively, after 72 hours in the system. Future work should be done to understand the influence the constant inoculum has on the performance of surface modified catheters and the overall ruggedness of the model. Further investigation is also required to determine if a constant inoculum source in the model is consistent with challenges the catheter surface will see in the clinical setting.

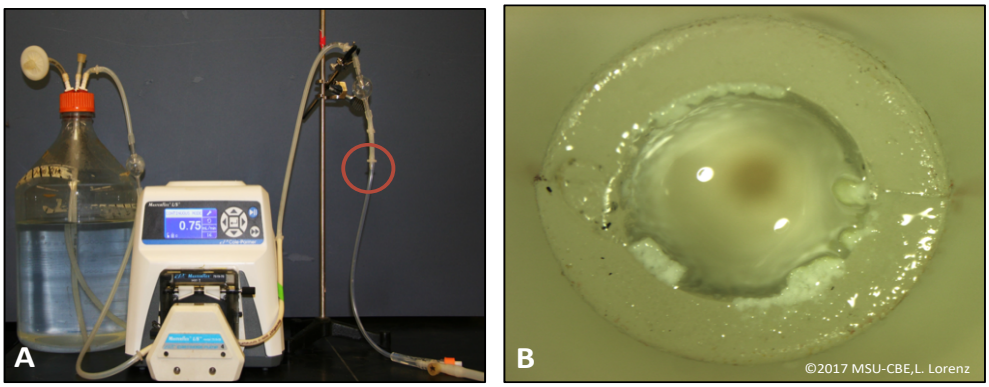

Figure 7. ICM system with connector proximal to the Foley catheter circled in red (A). Epifluorescent image showing UPEC biofilm growth on the inner surface of a connector proximal to an untreated, control Foley catheter after 72 hours in the model (B).

The sensitivity to pH changes as described above indicates that pH is an influential factor on biofilm growth if not carefully controlled. Table 10 shows the

expected changes to LDs of the control catheter segments when pH is varied from high to low and all other operational factors are held at their SOP values. The simulation shows that the difference in pH becomes more pronounced over time. Flow rate at 8 hours, for example, low pH is expected to add 0.125 log to the LD density while a high pH is estimated to add 0.535. This effect is much larger at 48 hours, with a low pH adding 1.095 log to the expected LD and a high pH adding over 3.30 log. This increase in difference with time indicates that pH has an effect as the biofilm is developing.

Table 9. Partial derivatives of control catheter segments with pH varying low to high with all other factors are held at their SOP values. For full table see Table D-4 in Appendix D, Ruggedness Analysis.

		Control Catheter Segments ($log_{10}(CFU/cm^2)$)	
Sample Point (hrs)	Factor	Low pH	High pH
8	inoc	0.69	0.93
	temp	0.077	-0.053
	flow	0.125	0.535
	removal	1.095	1.005
24	inoc	0.63	1.15
	temp	0.41	-0.07
	flow	1.05	2.85
	removal	2.34	0.66
48	inoc	0.455	1.185
	temp	0.225	-0.345
	flow	1.095	3.365
	removal	0.965	0.935

One hypothesis to explain the effect that pH has on the system is that the pH of the growth media may influence gene transcription and pili expression in UPEC. Results from a study by Shwan et. Al. suggest that environmental cues, specifically, osmolarity and pH, regulate the expression of type 1 pili in UPEC [4]. They found that pH alone has

minor effects on the expression of the *fim* promotors, genes which are involved in the production of type 1 pili. A pH of 7 was determined to be the optimal pH for expression of the *fim* promotors, while a shift to acidic pH resulted in an overall decrease in activity. Type 1 pili has been identified as one of UPEC's most critical virulence factors in the establishment of UTI and CAUTI [5]. The pili allow for the bacteria to strongly adhere to uroepithelial cells or catheter surfaces and to withstand the flow of urine. Without pili ability to adhere to a surface and ultimately form biofilms is significantly decreased. It is hypothesized that the variation the pH of the AUM has caused the amount of pili expressed on the surface of the UPEC to vary ultimately causing the variation in biofilm LD seen in the system. Further ruggedness analysis should be completed to determine if other uropathogens would be as sensitive to pH change in the AUM as UPEC. Future studies may reveal that an optimal pH will need to be determined for individual uropathogens in order to create a rugged model.

The ICM was developed to simulate the real-world scenario in order to produce clinically predictive results. With further optimization and modification to the SOP, it may be a useful tool when developing and regulating novel surface modified urinary catheters.

Literature Cited

1. Brooks, T., *A simple Artificial Urine for the Growth of Urinary Pathogens.* Letters in Applied Microbiology, 1997. **24**: p. 203-206.

2. Hamilton, M.A., et al., *Guidelines for the Statistical Analysis of a Collaborative Study of a Laboratory Method for Testing Disinfectant Product Performance.* Journal of Aoac International, 2013. **96**(5): p. 1138-1151.

3. Thompson, M., S.L.R. Ellison, and R. Wood, *Harmonized guidelines for single-laboratory validation of methods of analysis - (IUPAC technical report).* Pure and Applied Chemistry, 2002. **74**(5): p. 835-855.

4. Schwan, W.R., et al., *Osmolarity and pH growth conditions regulate fim gene transcription and type 1 pilus expression in uropathogenic Escherichia coli.* Infection and Immunity, 2002. **70**(3): p. 1391-1402.

5. Wiles, T.J., R.R. Kulesus, and M.A. Mulvey, *Origins and virulence mechanisms of uropathogenic Escherichia coli.* Exp Mol Pathol, 2008. **85**(1): p. 11-9.

CHAPTER THREE

PROSPECTIVE STUDIES AND CONCLUSIONS

Validation of Artificial Urine Media (AUM)

An extensive literature review, summarized in Chapter 1, revealed for an *in vitro* biofilm model to produce clinically relevant data it must incorporate nutrients reflective of the environment of interest [1-3]. Therefore, urine must be used as the growth media when modelling biofilm growth in urinary catheters. The artificial urine media (AUM) recipe proposed to be included in the standard ICM test method is a modified version of the recipe originally proposed by Brooks et. al. [4]. This recipe was chosen due to its resemblance to 'normal' human urine [5]. The SOP for the preparation of the AUM is described extensively in Appendix A.

Before the method is submitted for standardization the AUM recipe must be validated. In order to validate the media, the following studies must be done at a minimum. First, it must be shown to support the growth of broad spectrum of uropathogens. The FDA requires the following microorganisms be included in the *in vitro* testing of any antimicrobial Foley catheter: *Candida sp., Citrobacter diversus, Enterobacter cloacae, Enterococcus, Escherichia coli, Klebsiellae pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus saprophyticus*, and *Streptococcus fecalis*[9]. Second, the growth of the specified uropathogens in AUM must be validated against their growth in standardized human urine to insure similar log densities are

[9] https://www.fda.gov/MedicalDevices/ucm080884.htm. Accessed October 30, 2018.

achievable. Third, the pH and osmolarity of the AUM must be optimized. The SOP currently specifies for a pH of 6.5 and an osmolarity of 0.34 mol/kg [4]. The pH of human urine may range from 4.5-8, with 'normal' urine typically measuring at a pH of approximately 6 and osmolarity may range from 0.038- 1.4mol/kg [10] [11]. The SOP is accurate with respect to expected physiological conditions, but it must optimized for each uropathogen grown in the ICM.

Having a validated and standardized artificial urine recipe would be of significant benefit to FDA reviewers as well as researchers as it would help to decrease variation across studies allowing efficacies to be compared more accurately.

Development of an Extraluminal Test Model

As discussed previously, CAUTIs may occur one of two ways, either by contamination of the intraluminal surface or by migration along the external surface. Studies have suggested if strict sterility of the collection system is kept the extraluminal route becomes the major pathway for bacteria to gain access to the bladder [6, 7]. Therefore, to fully assess how a surface modified urinary catheter will perform clinically its efficacy must be evaluated under both extraluminal and intraluminal conditions. Chapter 2 establishes and validates a standardized test method to evaluate the efficacy of intraluminal surface modifications under relevant environmental conditions. Several extraluminal models proposed in the literature were reviewed and are summarized in Chapter One. Of those, three, which appeared to be the most reasonable and relevant

[10] https://www.rnceus.com/ua/uaph.html. Accessed October 30, 2018.
[11] https://iai.asm.org/content/70/3/1391. Accessed November 8,2018.

models, were tested in the lab in an effort to develop a complimentary method to the intraluminal model. All of the selected models were designed to evaluate the ability of surface modifications to inhibit the migration of bacteria along the external surface of the catheter.

The first model attempted was an *in vitro* model designed by Darouiche et. al.[8]. The model attempts to duplicate the catheterized urinary system *in vitro* by simulating the flow of urine from the kidney into the catheterized bladder, a small hole in the bladder allows a film of urine to develop along the external surface of the catheter, shown in Figure 6. The catheter's tip is sealed so only extraluminal migration may occur. The distal end of the catheter rests in a funnel, which represents the meatus, and a constant bacterial inoculum is pumped onto the end catheter, simulating meatal colonization. Small samples of urine are obtained from the bladder daily to determine the ability of a surface modified catheter to inhibit the migration of bacteria along the extraluminal surface to the bladder.

When this model was repeated in the lab, it was determined that the initial set up was too complex for the method to be standardized. When developing a standard method, researchers must consider the reproducibility of the method. It was reasonable to assume for this method that the complexity of the set up would be a large source of variability between technician and laboratory. The researcher must also consider the relevance of a model, and upon further investigation it was determined that this model did not accurately represent the clinical environment. If urine is bypassing the catheter, leaking between the catheter shaft and the urethra, as simulated by this model, it is often an

indication that the device if not functioning properly. Catheter bypassing is most commonly associated with occlusion of the eyes of the catheter due to either a kink in the tip or encrustation of the catheter eyes [9]. Therefore, this model was deemed to not be reasonable or reflective of the physiological environment seen by the extraluminal surface of the catheter *in vivo*.

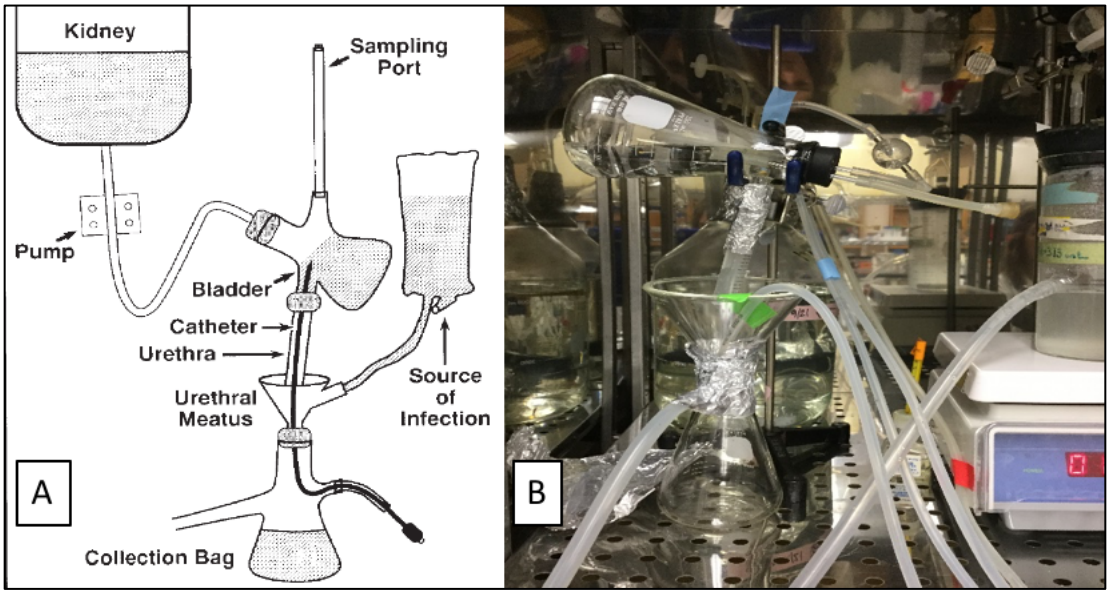

Figure 8. Extraluminal model developed by Dariouche et al. (A) Schematic depiction of experimental set up, originally published in *[8]*. (B) Photo of experimental set up in the SBM laboratory.

The second model attempted was originally described by Gaonkar et al., and was designed to also model the catheterized urinary system [10]. The Gaonkar model uses two conical tubes to simulate the bladder, catheterized urethra and collection bag, shown in Figure 7. The smaller tube contains a sealed, sterile segment of the catheter encased by an agar track. At the top of the small tube there is a small space left which is filled with sterile artificial urine, representing the bladder. At the bottom of the agar track a portion of the catheter protrudes from the agar tract and remains exposed in the larger tube,

simulating the catheterized meatus. The model is inoculated by swabbing the exposed end of the catheter and bottom of the agar track with a cotton swab which has been dipped in a bacterial culture. The bladder is sampled daily until bacteria are detected, indicating the failure of the surface modification. This model showed significant promise to be developed into a standard test method, as the experimental set-up was simple, and it attempted to simulate the *in vivo* environment unlike many others in the literature had. The Gaonkar model uses agar to represent the urethra instead of a glass tube as seen in the previous model. By surrounding the catheter with agar, the researchers simulate the periurethral space between the catheter and agar that is seen between the shaft of the catheter and urethra *in vivo*. However, it was found that there were several structural issues with the agar tract that decreased the model's repeatability. For example, the agar tract frequently slipped out of the smaller tube into the collection tube and the probability depended on the location of the laboratory. When the tract would remain in place the bacteria did not necessarily only migrate along the external surface of the catheter to the bladder but also along the outside of agar tract and the tube wall resulting in false positive urine cultures. After a year of attempting to solve these design issues the model was abandoned.

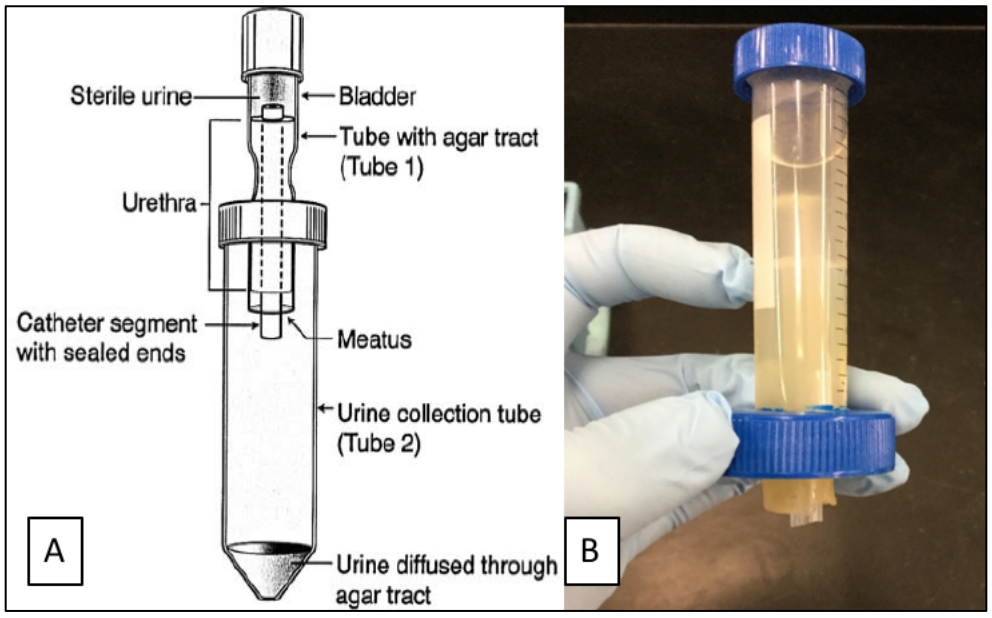

Figure 9. Extraluminal model developed by Gaonkar et al. (A) Schematic depiction of experimental set up, originally published in [10]. (B) Photo of reactor prototype developed in the SBM laboratory.

The third model tested was the plate migration assay which was originally proposed by Stickler et. al. in 1999 to study the ability of *P. mirabilis* to swarm over catheter surfaces [11]. Since then the assay has been utilized to evaluate the efficacy of novel surface modifications to prevent bacterial migration along the catheter surface [12, 13]. The assay, shown in Figure 8, consists of a sealed catheter segment placed between two halves of agar in a petri dish. The agar is inoculated with a small volume of bacterial culture on one side of the catheter segment and the plate is incubated. The catheter has failed when bacteria appear on the other side of the segment. This assay is simple, repeatable, reproducible and studies suggest it may be predictive of surface modifications' clinical performances. A study performed by Sabbuda et. al. used the plate migration assay to examine the effectiveness of hydrogel-coated and hydrogel-silver coated catheters to prevent the migration of uropathogens over their surface [12]. The

results suggested that hydrogel coatings, with or without silver incorporated, enhanced an organism's ability to migrate across the catheter surface. The findings of this study were supported by two clinical trials which examined the efficacy of hydrogel-silver coated urinary catheters [14, 15]. Both trials concluded there was not enough evidence to suggest these catheters gave greater protection than control catheters. However, other trails are in opposition with these, reporting positive trends towards decreasing CAUTI [16]. Therefore, it cannot be stated that this assay is predictive of clinical outcomes. It may be stated, however, that the assay is a helpful tool in the beginning stages of a surface modifications development, similar to the function of the ZOI, allowing for the researcher to understand how for the modification may interact with relevant uropathogens. This assay cannot serve as a prerequisite to clinical trials as none of relevant environmental conditions the catheter would be exposed to are represented.

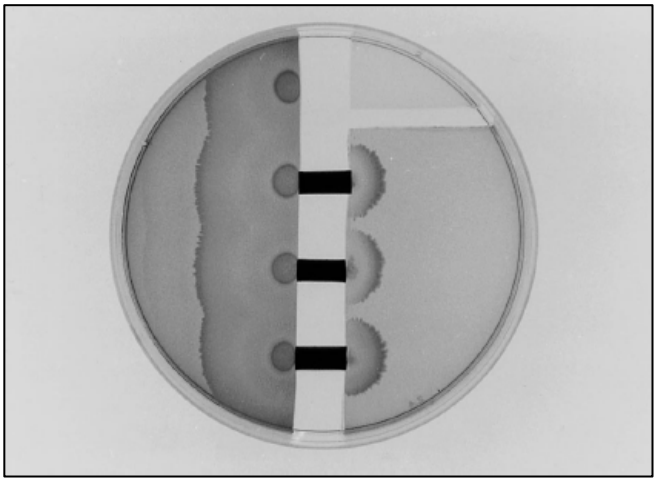

Figure 10. Photo of the plate migration assay developed by Stickler et. al. The photo shows the swarming of *Proteus mirabilis* NSM42 over hydrogel-coated latex catheter segments. Image originally published in [11].

As development of an extraluminal model moves forward, the researchers must keep some critical parameters in mind to insure the method will yield relevant results for translation into patients. First, it is not accurate to use AUM as the growth media, as discussed above, because the extraluminal surface should not be exposed to urine if the device is functioning properly. The host-device interaction must be investigated and determined how it will influence the properties of the growth media. For example, it has been shown that when a catheter is placed in a patient there can be significant damage to the urethral tissue causing bleeding, the presence of blood allows bacteria to use iron scavenging genes as a way to obtain nutrients [17]. Upon insertion it is also assumed the catheter is coated with fibrinogen, will this be included [18]? To attempt to fully model the host immune response the method will not be reasonable, reproducible or feasible at this point. Second, the method cannot be biased towards a specific type of surface modification. Modifications have included contact killing surfaces, antimicrobial-releasing coatings, Nano printed surfaces, etc. and new technologies are being invented all the time. The method must be designed to not be biased against any surface. Third, the FDA requires a full segment of the final device to be used in the final testing[12]. Although, the development of an extraluminal model is crucial in the effort to bring effective and innovation solutions to patients.

[12] https://www.fda.gov/MedicalDevices/ucm080884.htm. Accessed October 30, 2018.

Conclusion

Catheter associated infections continue to one of the most common healthcare associated infections, with CAUTIs accounting for over a third of all HAIs. As novel surface modified urinary catheters continue to be developed the FDA needs standard testing methods which will accurately predict the clinical performance of products and allow for the comparison of efficacies across products. This research proposes a standard test method which aims to replicate the *in vivo* infection with two quantitative, *in vitro* methods. The ICM shows great promise for becoming a useful tool in evaluating the ability of intraluminal modifications to delay the development of biofilm on the catheter surface. Future research should focus on optimizing the biofilm harvesting technique used and the artificial urine media recipe. Development of an extraluminal model is yet to be completed but should be an important goal for the grant moving forward. Extraluminal infections account for 66% of all CAUTIs, therefore the evaluation of a surface modification's ability to inhibit migration of bacteria along the extraluminal surface of a catheter should be considered a critical portion of *in vitro* testing. The development of a standard *in vitro* method which simulates the physiological conditions of both routes of infection will be a significant advancement and will help to bring innovative and effective solutions to patients.

Literature Cited

1. Roberts, A.E., et al., *The Limitations of In Vitro Experimentation in Understanding Biofilms and Chronic Infection.* J Mol Biol, 2015. **427**(23): p. 3646-61.

2. Shrout, J.D., et al., *The impact of quorum sensing and swarming motility on Pseudomonas aeruginosa biofilm formation is nutritionally conditional.* Mol Microbiol, 2006. **62**(5): p. 1264-77.

3. Palmer, K.L., L.M. Aye, and M. Whiteley, *Nutritional cues control Pseudomonas aeruginosa multicellular behavior in cystic fibrosis sputum.* J Bacteriol, 2007. **189**(22): p. 8079-87.

4. Brooks, T., *A simple Artificial Urine for the Growth of Urinary Pathogens.* Letters in Applied Microbiology, 1997. **24**: p. 203-206.

5. Ipe, D.S., E. Horton, and G.C. Ulett, *The Basics of Bacteriuria: Strategies of Microbes for Persistence in Urine.* Front Cell Infect Microbiol, 2016. **6**: p. 14.

6. Garibaldi, R.A., et al., *Meatal colonization and catheter-associated bacteriuria.* N Engl J Med, 1980. **303**(6): p. 316-8.

7. Tambyah, P.A., K.T. Halvorson, and D.G. Maki, *A prospective study of pathogenesis of catheter-associated urinary tract infections.* Mayo Clin Proc, 1999. **74**(2): p. 131-6.

8. Darouiche, R.O., *In VItro Efficacy of Antimicrobial-Coated Bladder Catheters in Inhibiting Bacterial Migration along Catheter Surface.* Concise communications, 1997(176 (October)): p. 1109-1112.

9. Theriault, R., P. Ward-Smith, and C. Soper, *Leakage associated with urinary catheter usage: a design challenge.* Urol Nurs, 2012. **32**(6): p. 307-12.

10. Gaonkar, T., *Evaluation of the Antimicrobial Efficacy of Urinary Catheters Impregnated with Antiseptics in an In Vitro Urinary Tract Model.* Infection Control and Hospital Epidemiology, 2003. **24**(7): p. 506-513.

11. Stickler, D. and G. Hughes, *Ability of Proteus mirabilis to swarm over urethral catheters.* Eur J Clin Microbiol Infect Dis, 1999. **18**(3): p. 206-8.

12. Sabbuba, N., *The Migration of Proteus Mirabilis and other urinary tract pathogens over Foley Catheters.* BJU International, 2002. **89**: p. 55-60.

13. Reddy, S.T., et al., *Micropatterned surfaces for reducing the risk of catheter-associated urinary tract infection: an in vitro study on the effect of sharklet micropatterned surfaces to inhibit bacterial colonization and migration of uropathogenic Escherichia coli.* J Endourol, 2011. **25**(9): p. 1547-52.

14. Srinivasan, A., et al., *A prospective trial of a novel, silicone-based, silver-coated foley catheter for the prevention of nosocomial urinary tract infections.* Infect Control Hosp Epidemiol, 2006. **27**(1): p. 38-43.

15. Thibon, P., et al., *Randomized multi-centre trial of the effects of a catheter coated with hydrogel and silver salts on the incidence of hospital-acquired urinary tract infections.* J Hosp Infect, 2000. **45**(2): p. 117-24.

16. Bologna, R.A., et al., *Hydrogel/silver ion-coated urinary catheter reduces nosocomial urinary tract infection rates in intensive care unit patients: a multicenter study.* Urology, 1999. **54**(6): p. 982-7.

17. Jacobsen, S.M., et al., *Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis.* Clin Microbiol Rev, 2008. **21**(1): p. 26-59.

18. Flores-Mireles, A.L., et al., *Urinary tract infections: epidemiology, mechanisms of infection and treatment options.* Nat Rev Microbiol, 2015. **13**(5): p. 269-84.

REFERENCES CITED

Arinzon, Z., Shabat, S., Peisakh, A., & Berner, Y. (2012). Clinical presentation of urinary tract infection (UTI) differs with aging in women. *Arch Gerontol Geriatr, 55*(1), 145-147. doi:10.1016/j.archger.2011.07.012

Barford, J. M., Anson, K., Hu, Y., & Coates, A. R. (2008). A model of catheter-associated urinary tract infection inittiated by bacterial contamination of the catheter tip. *BJU International, 102*(1), 67-74.

Barr, D. B., Wilder, L. C., Caudill, S. P., Gonzalez, A. J., Needham, L. L., & Pirkle, J. L. (2005). Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environ Health Perspect, 113*(2), 192-200.

Bologna, R. A., Tu, L. M., Polansky, M., Fraimow, H. D., Gordon, D. A., & Whitmore, K. E. (1999). Hydrogel/silver ion-coated urinary catheter reduces nosocomial urinary tract infection rates in intensive care unit patients: a multicenter study. *Urology, 54*(6), 982-987.

Bouatra, S., Aziat, F., Mandal, R., Guo, A. C., Wilson, M. R., Knox, C., . . . Wishart, D. S. (2013). The human urine metabolome. *PLoS One, 8*(9), e73076. doi:10.1371/journal.pone.0073076

Brooks, T. (1997). A simple Artificial Urine for the Growth of Urinary Pathogens. *Letters in Applied Microbiology, 24*, 203-206.

Burns, J. R., & Finlayson, B. (1980). A proposal for a standard reference artificial urine in in vitro urolithiasis experiments. *Invest Urol, 18*(2), 167-169.

Busscher, H. J., Rinastiti, M., Siswomihardjo, W., & van der Mei, H. C. (2010). Biofilm formation on dental restorative and implant materials. *J Dent Res, 89*(7), 657-665. doi:10.1177/0022034510368644

Carlsson, S., Wiklund, N. P., Engstrand, L., Weitzberg, E., & Lundberg, J. O. (2001). Effects of pH, nitrite, and ascorbic acid on nonenzymatic nitric oxide generation and bacterial growth in urine. *Nitric Oxide, 5*(6), 580-586. doi:10.1006/niox.2001.0371

Ceri, H., Olson, M. E., Stremick, C., Read, R. R., Morck, D., & Buret, A. (1999). The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J Clin Microbiol, 37*(6), 1771-1776.

Chenoweth, C. E., & Saint, S. (2011). Urinary tract infections. *Infect Dis Clin North Am, 25*(1), 103-115. doi:10.1016/j.idc.2010.11.005

Christmas, K. G., Gower, L. B., Khan, S. R., & El-Shall, H. (2002). Aggregation and dispersion characteristics of calcium oxalate monohydrate: effect of urinary species. *J Colloid Interface Sci, 256*(1), 168-174.

Chua, R. Y. R., Lim, K., Leong, S. S. J., Tambyah, P. A., & Ho, B. (2017). An in vitro urinary catheterization model that approximates clinical conditions for evaluation of innovations to prevent catheter associated urinary tract infections. *J Hosp Infect*. doi:10.1016/j.jhin.2017.05.006

Chutipongtanate, S., & Thongboonkerd, V. (2010). Systematic comparisons of artificial urine formulas for in vitro cellular study. *Anal Biochem, 402*(1), 110-112. doi:10.1016/j.ab.2010.03.031

Control, C. f. D. (2018). Urinary Tract Infection (Catheter-Associated Urinary Tract Infection [CAUTI] and Non-Catheter-Associated Urinary Tract Infection [UTI] and Other Urinary System Infection [USI] Events. (Device-associated Module-UTI).

Costerton, J. W., Cheng, K. J., Geesey, G. G., Ladd, T. I., Nickel, J. C., Dasgupta, M., & Marrie, T. J. (1987). Bacterial biofilms in nature and disease. *Annu Rev Microbiol, 41*, 435-464. doi:10.1146/annurev.mi.41.100187.002251

Costerton, J. W., Geesey, G. G., & Cheng, K. J. (1978). How bacteria stick. *Sci Am, 238*(1), 86-95.

Cotter, P. D., & Hill, C. (2003). Surviving the acid test: responses of gram-positive bacteria to low pH. *Microbiol Mol Biol Rev, 67*(3), 429-453, table of contents.

Curtin, J. J., & Donlan, R. M. (2006). Using bacteriophages to reduce formation of catheter-associated biofilms by Staphylococcus epidermidis. *Antimicrob Agents Chemother, 50*(4), 1268-1275. doi:10.1128/AAC.50.4.1268-1275.2006

Daifuku, R., & Stamm, W. E. (1986). Bacterial adherence to bladder uroepithelial cells in catheter-associated urinary tract infection. *N Engl J Med, 314*(19), 1208-1213. doi:10.1056/NEJM198605083141902

Dalhoff, A., Stubbings, W., & Schubert, S. (2011). Comparative in vitro activities of the novel antibacterial finafloxacin against selected Gram-positive and Gram-negative bacteria tested in Mueller-Hinton broth and synthetic urine. *Antimicrob Agents Chemother, 55*(4), 1814-1818. doi:10.1128/AAC.00886-10

Darouiche, R. O. (1997). <US5624704.pdf>.

Darouiche, R. O., Mansouri, M. D., Gawande, P. V., & Madhyastha, S. (2008). Efficacy of combination of chlorhexidine and protamine sulphate against device-associated pathogens. *J Antimicrob Chemother, 61*(3), 651-657. doi:10.1093/jac/dkn006

Dave, R. N., Joshi, H. M., & Venugopalan, V. P. (2011). Novel biocatalytic polymer-based antimicrobial coatings as potential ureteral biomaterial: preparation and in vitro performance evaluation. *Antimicrob Agents Chemother, 55*(2), 845-853. doi:10.1128/AAC.00477-10

de Lastours, V., & Foxman, B. (2014). Urinary tract infection in diabetes: epidemiologic considerations. *Curr Infect Dis Rep, 16*(1), 389. doi:10.1007/s11908-013-0389-2

Dohnt, K., Sauer, M., Muller, M., Atallah, K., Weidemann, M., Gronemeyer, P., . . . Krull, R. (2011). An in vitro urinary tract catheter system to investigate biofilm development in catheter-associated urinary tract infections. *J Microbiol Methods, 87*(3), 302-308. doi:10.1016/j.mimet.2011.09.002

Donlan, R. M. (2001). Special Issue: Biofilms and Device-Associated Infections. *Emerging Infectious Diseases, 7*(2), 277-281.

Feneley, R. C., Hopley, I. B., & Wells, P. N. (2015). Urinary catheters: history, current status, adverse events and research agenda. *J Med Eng Technol, 39*(8), 459-470. doi:10.3109/03091902.2015.1085600

Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol, 13*(5), 269-284. doi:10.1038/nrmicro3432

Foley, F. E. B. (1937). A Hemostatic Bag Catheter. *The Journal of Urology, 38*(1), 134-139.

Food and Drug Administration, U. (1997). Guidance for the Content of Premarket Notifications for Conventional and Antimicrobial Foley Catheters.

Fox-Moon, S. M., & Shirtliff, M. E. (2015). Urinary Tract Infections Caused by Proteus mirabilis. 1389-1400. doi:10.1016/b978-0-12-397169-2.00077-9

Fu, W., Forster, T., Mayer, O., Curtin, J. J., Lehman, S. M., & Donlan, R. M. (2010). Bacteriophage cocktail for the prevention of biofilm formation by Pseudomonas aeruginosa on catheters in an in vitro model system. *Antimicrob Agents Chemother, 54*(1), 397-404. doi:10.1128/AAC.00669-09

Gabriel, M. M., Mayo, M. S., May, L. L., Simmons, R. B., & Ahearn, D. G. (1996). In vitro evaluation of the efficacy of a silver-coated catheter. *Curr Microbiol, 33*(1), 1-5.

Gaonkar, T. (2003). Evaluation of the Antimicrobial Efficacy of Urinary Catheters Impregnated with Antiseptics in an In Vitro Urinary Tract Model. *Infection Control and Hospital Epidemiology, 24*(7), 506-513.

Garibaldi, R. A., Burke, J. P., Britt, M. R., Miller, M. A., & Smith, C. B. (1980). Meatal colonization and catheter-associated bacteriuria. *N Engl J Med, 303*(6), 316-318. doi:10.1056/NEJM198008073030605

Garibaldi, R. A., Burke, J. P., Dickman, M. L., & Smith, C. B. (1974). Factors predisposing to bacteriuria during indwelling urethral catheterization. *N Engl J Med, 291*(5), 215-219. doi:10.1056/NEJM197408012910501

Gillespie, W. A., Linton, K. B., Miller, A., & Slade, N. (1960). The diagnosis, epidemiology and control of urinary infection in urology and gynaecology. *J Clin Pathol, 13*, 187-194.

Gloag, E. S., Elbadawi, C., Zachreson, C. J., Aharonovich, I., Toth, M., Charles, I. G., . . . Whitchurch, C. B. (2016). Micro-Patterned Surfaces That Exploit Stigmergy to Inhibit Biofilm Expansion. *Front Microbiol, 7*, 2157. doi:10.3389/fmicb.2016.02157

Gomes, L. C., Silva, L. N., Simoes, M., Melo, L. F., & Mergulhao, F. J. (2015). Escherichia coli adhesion, biofilm development and antibiotic susceptibility on biomedical materials. *J Biomed Mater Res A, 103*(4), 1414-1423. doi:10.1002/jbm.a.35277

Grases, F., & Llobera, A. (1998). Experimental model to study sedimentary kidney stones. *Micron, 29*(2-3), 105-111.

Hachem, R., Reitzel, R., Borne, A., Jiang, Y., Tinkey, P., Uthamanthil, R., . . . Raad, I. (2009). Novel antiseptic urinary catheters for prevention of urinary tract infections: correlation of in vivo and in vitro test results. *Antimicrob Agents Chemother, 53*(12), 5145-5149. doi:10.1128/AAC.00718-09

Haley, R. W., Hooton, T. M., Culver, D. H., Stanley, R. C., Emori, T. G., Hardison, C. D., . . . Schatz, G. D. (1981). Nosocomial infections in U.S. hospitals, 1975-1976: estimated frequency by selected characteristics of patients. *Am J Med, 70*(4), 947-959.

Hanafy, H. M., Saad, S. M., & Al-Ghorab, M. M. (1974). Ancient Egyptian medicine: contribution to urology. *Urology, 4*(1), 114-120.

Hannan, T. J., Totsika, M., Mansfield, K. J., Moore, K. H., Schembri, M. A., & Hultgren, S. J. (2012). Host-pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic Escherichia coli bladder infection. *FEMS Microbiol Rev, 36*(3), 616-648. doi:10.1111/j.1574-6976.2012.00339.x

Heydorn, A., Ersboll, B. K., Hentzer, M., Parsek, M. R., Givskov, M., & Molin, S. (2000). Experimental reproducibility in flow-chamber biofilms. *Microbiology, 146 (Pt 10)*, 2409-2415. doi:10.1099/00221287-146-10-2409

Hidron, A. I., Edwards, J. R., Patel, J., Horan, T. C., Sievert, D. M., Pollock, D. A., . . . Participating National Healthcare Safety Network, F. (2008). NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. *Infect Control Hosp Epidemiol, 29*(11), 996-1011. doi:10.1086/591861

Hooton, T. M. (2012). Clinical practice. Uncomplicated urinary tract infection. *N Engl J Med, 366*(11), 1028-1037. doi:10.1056/NEJMcp1104429

Hoyle, B. D., Alcantara, J., & Costerton, J. W. (1992). Pseudomonas aeruginosa biofilm as a diffusion barrier to piperacillin. *Antimicrob Agents Chemother, 36*(9), 2054-2056.

Hu, H., Johani, K., Almatroudi, A., Vickery, K., Van Natta, B., Kadin, M. E., . . . Deva, A. K. (2016). Bacterial Biofilm Infection Detected in Breast Implant-Associated Anaplastic Large-Cell Lymphoma. *Plast Reconstr Surg, 137*(6), 1659-1669. doi:10.1097/PRS.0000000000002010

Ingersoll, M. A., & Albert, M. L. (2013). From infection to immunotherapy: host immune responses to bacteria at the bladder mucosa. *Mucosal Immunol, 6*(6), 1041-1053. doi:10.1038/mi.2013.72

Ipe, D. S., Horton, E., & Ulett, G. C. (2016). The Basics of Bacteriuria: Strategies of Microbes for Persistence in Urine. *Front Cell Infect Microbiol, 6*, 14. doi:10.3389/fcimb.2016.00014

Irwin, N. J., McCoy, C. P., & Carson, L. (2013). Effect of pH on the in vitro susceptibility of planktonic and biofilm-grown Proteus mirabilis to the quinolone antimicrobials. *J Appl Microbiol, 115*(2), 382-389. doi:10.1111/jam.12241

Jacobsen, S. M., Stickler, D. J., Mobley, H. L., & Shirtliff, M. E. (2008). Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. *Clin Microbiol Rev, 21*(1), 26-59. doi:10.1128/CMR.00019-07

Justice, S. S., Hunstad, D. A., Cegelski, L., & Hultgren, S. J. (2008). Morphological plasticity as a bacterial survival strategy. *Nat Rev Microbiol, 6*(2), 162-168. doi:10.1038/nrmicro1820

Karchmer, T. B., Giannetta, E. T., Muto, C. A., Strain, B. A., & Farr, B. M. (2000). A randomized crossover study of silver-coated urinary catheters in hospitalized patients. *Arch Intern Med, 160*(21), 3294-3298.

Lee, C. R., Cho, I. H., Jeong, B. C., & Lee, S. H. (2013). Strategies to minimize antibiotic resistance. *Int J Environ Res Public Health, 10*(9), 4274-4305. doi:10.3390/ijerph10094274

Lehman, S. M., & Donlan, R. M. (2015). Bacteriophage-mediated control of a two-species biofilm formed by microorganisms causing catheter-associated urinary tract infections in an in vitro urinary catheter model. *Antimicrob Agents Chemother, 59*(2), 1127-1137. doi:10.1128/AAC.03786-14

Levison, M. E., & Kaye, D. (2013). Treatment of complicated urinary tract infections with an emphasis on drug-resistant gram-negative uropathogens. *Curr Infect Dis Rep, 15*(2), 109-115. doi:10.1007/s11908-013-0315-7

Lichtenberger, P., & Hooton, T. M. (2008). Complicated urinary tract infections. *Curr Infect Dis Rep, 10*(6), 499-504.

Lo, E., Nicolle, L. E., Coffin, S. E., Gould, C., Maragakis, L. L., Meddings, J., . . . Yokoe, D. S. (2014). Strategies to prevent catheter-associated urinary tract infections in acute care hospitals: 2014 update. *Infect Control Hosp Epidemiol, 35 Suppl 2*, S32-47.

Maeyama, R., Kwon, I. K., Mizunoe, Y., Anderson, J. M., Tanaka, M., & Matsuda, T. (2005). Novel bactericidal surface: Catechin-loaded surface-erodible polymer prevents biofilm formation. *J Biomed Mater Res A, 75*(1), 146-155. doi:10.1002/jbm.a.30346

Maki, D. G., & Tambyah, P. A. (2001). Engineering out the risk for infection with urinary catheters. *Emerg Infect Dis, 7*(2), 342-347. doi:10.3201/eid0702.700342

Malpiedi, P. J. (2011). National and State Healthcare-Associated Infection Standardized Infection Ratio Report.

Marzo, A., Melis, A., Unger, J., Sablotni, R., Pistis, M., & McCarthy, A. D. (2018). An engineering approach towards a more discrete and efficient urinary drainage system. *Proc Inst Mech Eng H*, 954411918790286. doi:10.1177/0954411918790286

McCoy, W. F., Bryers, J. D., Robbins, J., & Costerton, J. W. (1981). Observations of fouling biofilm formation. *Can J Microbiol, 27*(9), 910-917.

Meije, Y., Almirante, B., Del Pozo, J. L., Martin, M. T., Fernandez-Hidalgo, N., Shan, A., . . . Gavalda, J. (2014). Daptomycin is effective as antibiotic-lock therapy in a model of Staphylococcus aureus catheter-related infection. *J Infect, 68*(6), 548-552. doi:10.1016/j.jinf.2014.01.001

Meslemani, D., Yaremchuk, K., & Rontal, M. (2010). Presence of biofilm on adult tracheostomy tubes. *Ear Nose Throat J, 89*(10), 496-504.

Miller, A., Gillespie, W. A., Linton, K. B., Slade, N., & Mitchell, J. P. (1958). Postoperative infection in urology. *Lancet, 2*(7047), 608-612.

Minuth, J. N., Musher, D. M., & Thorsteinsson, S. B. (1976). Inhibition of the antibacterial activity of gentamicin by urine. *J Infect Dis, 133*(1), 14-21.

Mobley, H. L., Island, M. D., & Hausinger, R. P. (1995). Molecular biology of microbial ureases. *Microbiol Rev, 59*(3), 451-480.

Morris, N. S., Stickler, D. J., & Winters, C. (1997). Which indwelling urethral catheters resist encrustation by Proteus mirabilis biofilms? *Br J Urol, 80*(1), 58-63.

Mulvey, M. A. (2002). Adhesion and entry of uropathogenic Escherichia coli. *Cell Microbiol, 4*(5), 257-271.

Nickel, J., Grant, S., & Costerton, J. (1985). Catheter-associated bacterium: An experimental study. *Urology, 26*(4), 369-375.

Nickel, J. C., Costerton, J. W., McLean, R. J., & Olson, M. (1994). Bacterial biofilms: influence on the pathogenesis, diagnosis and treatment of urinary tract infections. *J Antimicrob Chemother, 33 Suppl A*, 31-41.

Nickel, J. C., Downey, J., & Costerton, J. W. (1992). Movement of pseudomonas aeruginosa along catheter surfaces. A mechanism in pathogenesis of catheter-associated infection. *Urology, 39*(1), 93-98.

Nickel, J. C., Gristina, A. G., & Costerton, J. W. (1985). Electron microscopic study of an infected Foley catheter. *Can J Surg, 28*(1), 50-51, 54.

Nickel, J. C., Wright, J. B., Ruseska, I., Marrie, T. J., Whitfield, C., & Costerton, J. W. (1985). Antibiotic resistance of Pseudomonas aeruginosa colonizing a urinary catheter in vitro. *Eur J Clin Microbiol, 4*(2), 213-218.

Nowatzki, P. J., Koepsel, R. R., Stoodley, P., Min, K., Harper, A., Murata, H., . . . Russell, A. J. (2012). Salicylic acid-releasing polyurethane acrylate polymers as anti-biofilm urological catheter coatings. *Acta Biomater, 8*(5), 1869-1880. doi:10.1016/j.actbio.2012.01.032

O'May, G. A., Jacobsen, S. M., Stickler, D. J., Mobley, H. L. T., & Shirtliff, M. E. (2009). Complicated Urinary Tract Infections due to Catheters. *3*, 123-165. doi:10.1007/978-3-540-68119-9_6

Palmer, K. L., Aye, L. M., & Whiteley, M. (2007). Nutritional cues control Pseudomonas aeruginosa multicellular behavior in cystic fibrosis sputum. *J Bacteriol, 189*(22), 8079-8087. doi:10.1128/JB.01138-07

Parker, A. E., & Hamilton, M. A. (2011, June 23,2011). *KSA-SM-10- Assessing Resemblance, Repeatability, and Reproducibility for quantitative methods*. Bozeman, MT.

Parsons, C. L., Boychuk, D., Jones, S., Hurst, R., & Callahan, H. (1990). Bladder surface glycosaminoglycans: an epithelial permeability barrier. *J Urol, 143*(1), 139-142.

Peasah, S. K., McKay, N. L., Harman, J. S., Al-Amin, M., & Cook, R. L. (2013). Medicare non-payment of hospital-acquired infections: infection rates three years post implementation. *Medicare Medicaid Res Rev, 3*(3). doi:10.5600/mmrr.003.03.a08

Pickard, R., Lam, T., MacLennan, G., Starr, K., Kilonzo, M., McPherson, G., . . . N'Dow, J. (2012). Antimicrobial catheters for reduction of symptomatic urinary tract infection in adults requiring short-term catheterisation in hospital: a multicentre randomised controlled trial. *The Lancet, 380*(9857), 1927-1935. doi:10.1016/s0140-6736(12)61380-4

Purevdorj, B., Costerton, J. W., & Stoodley, P. (2002). Influence of hydrodynamics and cell signaling on the structure and behavior of Pseudomonas aeruginosa biofilms. *Appl Environ Microbiol, 68*(9), 4457-4464.

Raffi, H. S., Bates, J. M., Jr., Laszik, Z., & Kumar, S. (2005). Tamm-Horsfall protein acts as a general host-defense factor against bacterial cystitis. *Am J Nephrol, 25*(6), 570-578. doi:10.1159/000088990

Reddy, S. T., Chung, K. K., McDaniel, C. J., Darouiche, R. O., Landman, J., & Brennan, A. B. (2011). Micropatterned surfaces for reducing the risk of catheter-associated urinary tract infection: an in vitro study on the effect of sharklet micropatterned surfaces to inhibit bacterial colonization and migration of uropathogenic Escherichia coli. *J Endourol, 25*(9), 1547-1552. doi:10.1089/end.2010.0611

Roberts, A. E., Kragh, K. N., Bjarnsholt, T., & Diggle, S. P. (2015). The Limitations of In Vitro Experimentation in Understanding Biofilms and Chronic Infection. *J Mol Biol, 427*(23), 3646-3661. doi:10.1016/j.jmb.2015.09.002

Roe, D., Karandikar, B., Bonn-Savage, N., Gibbins, B., & Roullet, J. B. (2008). Antimicrobial surface functionalization of plastic catheters by silver nanoparticles. *J Antimicrob Chemother, 61*(4), 869-876. doi:10.1093/jac/dkn034

Sabbuba, N. (2002). The Migration of Proteus Mirabilis and other urinary tract pathogens over Foley Catheters. *BJU International, 89*, 55-60.

Saint, S., & Chenoweth, C. E. (2003). Biofilms and catheter-associated urinary tract infections. *Infect Dis Clin North Am, 17*(2), 411-432.

Saint, S., Greene, M. T., Krein, S. L., Rogers, M. A., Ratz, D., Fowler, K. E., . . . Fakih, M. G. (2016). A Program to Prevent Catheter-Associated Urinary Tract Infection in Acute Care. *N Engl J Med, 374*(22), 2111-2119. doi:10.1056/NEJMoa1504906

Services, U. D. o. H. a. H. (2013). National Action Plan to Prevent Health Care-Associated Infections: Road Map to Elimination, Executive Summary. 1-19.

Shepard, B. D., & Gilmore, M. S. (2002). Differential expression of virulence-related genes in Enterococcus faecalis in response to biological cues in serum and urine. *Infect Immun, 70*(8), 4344-4352.

Sherertz, R. J., Forman, D. M., & Solomon, D. D. (1989). Efficacy of dicloxacillin-coated polyurethane catheters in preventing subcutaneous Staphylococcus aureus infection in mice. *Antimicrob Agents Chemother, 33*(8), 1174-1178.

Shrout, J. D., Chopp, D. L., Just, C. L., Hentzer, M., Givskov, M., & Parsek, M. R. (2006). The impact of quorum sensing and swarming motility on Pseudomonas aeruginosa biofilm formation is nutritionally conditional. *Mol Microbiol, 62*(5), 1264-1277. doi:10.1111/j.1365-2958.2006.05421.x

Srinivasan, A., Karchmer, T., Richards, A., Song, X., & Perl, T. M. (2006). A prospective trial of a novel, silicone-based, silver-coated foley catheter for the prevention of nosocomial urinary tract infections. *Infect Control Hosp Epidemiol, 27*(1), 38-43. doi:10.1086/499998

Stahlhut, S. G., Struve, C., Krogfelt, K. A., & Reisner, A. (2012). Biofilm formation of Klebsiella pneumoniae on urethral catheters requires either type 1 or type 3 fimbriae. *FEMS Immunol Med Microbiol, 65*(2), 350-359. doi:10.1111/j.1574-695X.2012.00965.x

Stamm, W. (1991). Catheter Associated Urinary Tract Infections: Epidemiology, Pathogenesis, and Prevention. *Americal J Medicine, 91*(3B), 65S-71S.

Stewart, P. S. (1996). Theoretical aspects of antibiotic diffusion into microbial biofilms. *Antimicrob Agents Chemother, 40*(11), 2517-2522.

Stickler, D., Ganderton, L., King, J., Nettleton, J., & Winters, C. (1993). Proteus mirabilis biofilms and the encrustation of urethral catheters. *Urol Res, 21*(6), 407-411.

Stickler, D., & Hughes, G. (1999). Ability of Proteus mirabilis to swarm over urethral catheters. *Eur J Clin Microbiol Infect Dis, 18*(3), 206-208.

Stickler, D. J., Morris, N. S., & Winters, C. (1999). Simple physical model to study formation and physiology of biofilms on urethral catheters. *Methods Enzymol, 310*, 494-501.

Struve, C., Bojer, M., & Krogfelt, K. A. (2008). Characterization of Klebsiella pneumoniae type 1 fimbriae by detection of phase variation during colonization and infection and impact on virulence. *Infect Immun, 76*(9), 4055-4065. doi:10.1128/IAI.00494-08

Tambyah, P. A., Halvorson, K. T., & Maki, D. G. (1999). A prospective study of pathogenesis of catheter-associated urinary tract infections. *Mayo Clin Proc, 74*(2), 131-136. doi:10.4065/74.2.131

Tenke, P., Riedl, C. R., Jones, G. L., Williams, G. J., Stickler, D., & Nagy, E. (2004). Bacterial biofilm formation on urologic devices and heparin coating as preventive strategy. *Int J Antimicrob Agents, 23 Suppl 1*, S67-74. doi:10.1016/j.ijantimicag.2003.12.007

Theriault, R., Ward-Smith, P., & Soper, C. (2012). Leakage associated with urinary catheter usage: a design challenge. *Urol Nurs, 32*(6), 307-312.

Thibon, P., Le Coutour, X., Leroyer, R., & Fabry, J. (2000). Randomized multi-centre trial of the effects of a catheter coated with hydrogel and silver salts on the incidence of hospital-acquired urinary tract infections. *J Hosp Infect, 45*(2), 117-124. doi:10.1053/jhin.1999.0715

Thompson, M., S.L.R. Ellison, and R. Wood, *Harmonized guidelines for single-laboratory validation of methods of analysis - (IUPAC technical report)*. Pure and Applied Chemistry, 2002. **74**(5): p. 835-855

Tolker-Nielsen, T., & Sternberg, C. (2011). Growing and analyzing biofilms in flow chambers. *Curr Protoc Microbiol, Chapter 1*, Unit 1B 2. doi:10.1002/9780471729259.mc01b02s21

Torffvit, O., & Agardh, C. D. (1993). Tubular secretion of Tamm-Horsfall protein is decreased in type 1 (insulin-dependent) diabetic patients with diabetic nephropathy. *Nephron, 65*(2), 227-231. doi:10.1159/000187479

Turnbull, L., & Whitchurch, C. B. (2014). Motility assay: twitching motility. *Methods Mol Biol, 1149*, 73-86. doi:10.1007/978-1-4939-0473-0_9

Warren, J. W. (2001). Catheter-associated urinary tract infections. *Int J Antimicrob Agents, 17*(4), 299-303.

Weiner, L. M., Webb, A. K., Limbago, B., Dudeck, M. A., Patel, J., Kallen, A. J., . . . Sievert, D. M. (2016). Antimicrobial-Resistant Pathogens Associated With Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011-2014. *Infect Control Hosp Epidemiol, 37*(11), 1288-1301. doi:10.1017/ice.2016.174

APPENDICES

APPENDIX A

STANDARD OPERATING PROCEDURE FOR PREPARING ARTIFICAL URINE MEDIA

1.0 PURPOSE AND APPLICABILITY

1.1 The purpose of this method is to prepare artificial urine media for the use in the Intraluminal Catheter Model (ICM).

2.0 METHOD SUMMARY

2.1 This method describes the preparation of sterile chloride-sulfate solution, six stock solutions, and a 1M HCl solution that are mixed together to make sterile artificial urine media.

2.2 This procedure is based upon Brooks, T. (1997). "A simple Artificial Urine for the Growth of Urinary Pathogens." Letters in Applied Microbiology **24**: 203-206.

3.0 DEFINITIONS

3.1 Preparation No.= number assigned to artificial urine media stock solutions.

3.2. MQ water = MilliQ water

3.3 RT = room temperature (20 ± 5°C)

3.4 AUM= Artificial Urine Media

3.5 BSC= Biosafety Cabinet

3.6 HCl= Hydrochloric acid

4.0 HEALTH AND SAFETY WARNINGS

4.1 It is required that personnel wear lab coat, gloves, and safety glasses when working with hazardous chemicals.

5.0 CAUTIONS

5.1 Check the all stock solutions for precipitation, contamination or other characteristics that could indicate chemical degradation or compromise reagent quality.

6.0 PERSONNEL QUALIFICATIONS

6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

6.2 Personnel are required to be knowledgeable of the procedure in SOP QC 25 Performance Assessment and Sterility Verification of Prepared Media and Reagents, SOP QC 18 Performance Verification of Autoclaves and SOP QC 17 Maintenance and Use of Biological Safety Cabinets.

7.0 EQUIPMENT AND SUPPLIES

7.1 1 L glass bottles (6)

7.2 5 L glass bottles

7.3 Autoclave

7.4 Filtration units (6)

7.5 1 L flasks with vacuum port (6)

7.6 Non-sterile 0.22um nylon filters

7.7 MQ water

7.8 Flasks with a capacity of 1 L flask (volumetric flask or Erlenmeyer flask pre-measured and marked at 1 L mark)

7.9 Autoclave pouches

7.10 Stir bars (7)

7.11 Norprene tubing, size 15

7.12 Vacuum line/unit

7.13 1 L graduated cylinders

7.14 Stir + Hot plate

7.15 50mL serological pipettes

7.16 Aluminum foil

7.17 Serological pipette

7.18 Incubator set at 36 ± 2°C

7.19 10mL sterile syringe

7.20 Carboy top for 5 L glass bottle with silicone tubing and female leur lock syringe port

7.21 Small autoclave tray

7.22 1M HCl

7.23 Filtration Unit Clamps (7)

7.24 BSC

7.25 Chemical Components:

Table A-1. Chemical components of AUM

Component	Concentration(g/L)
Calcium Chloride ($CaCl_2x2H_2O$)	0.37
Sodium Chloride (NaCl)	5.2
Sodium Sulfate ($Na_2SO_4x7H_2O$)	3.2
Potassium Dihydrogen Phosphate (KH_2PO_4)	0.95
Ammonium Chloride (NH_4Cl)	1.3
Urea (CH_4N_4O)	8
Creatinine ($C_4H_7N_3O$)	0.25
Peptone I.37	1
Yeast	0.005
Lactic Acid ($C_3H_6O_3$)	0.05
Citric Acid ($C_6H_8O_7$)	0.4
Sodium Bicarbonate ($NaHCO_3$)	2.1
Iron II Sulfate ($FeSO_4$ *$7H_2O$)	0.0006
Magnesium sulfate ($MgSO_4$*$7H_2O$)	0.49
Dipotassium hydrogen phosphate (K_2HPO_4)	1.2

8.0 PROCEDURE

8.1 Prepare Chloride-Sulfate Solution

8.1.1 Fill a non-sterile 5 L glass bottle with 4580mL of non-sterile MQ water.

8.1.2 Place the filled bottle on stir plate, add a stir bar to the bottle and set the plate to approximately 300rpm.

8.1.3 Combine the following in the bottle:

a. Calcium Chloride ($CaCl_2$ *2 H_2O) ……1.85g

b. Sodium Chloride (NaCl)……..26g

c. Magnesium Sulfate ($MgSO_4$ * $7H_2O$) ……...…2.45g

d. Sodium Sulfate (Na_2SO_4 * 7 H_2O) ……17g

e. Ammonium Chloride (NH_4Cl) ……..…….6.5g

f. Peptone……...5g

8.1.4 Once dissolved, cap the bottle loosely. Place a piece of autoclave tape marked with the contents and date of preparation.

8.1.5 Autoclave bottle for a minimum of 1 hour according to SOP QC 18 Performance Verification of Autoclaves.

8.1.6 Allow chloride-sulfate solution to cool to room temperature (20 ± 5°C) prior to adding stock solutions.

8.2 Autoclave Glassware, Carboy Top, and Filters

8.2.1 Gather clean filtration units, 1 L flasks with vacuum port, and 1 L glass bottles.

8.2.2 Individually wrap each filtration unit with aluminum foil. Place a piece of autoclave tape on each, labeled ‘clean’. Place in autoclave tray. Cover the mouth and vacuum port of 1 L flasks with aluminum foil. Place a piece of autoclave tape on each, labeled ‘clean’. Place in autoclave tray with filtration units.

8.2.3 Attach silicone tubing to carboy top, such that the tubing will reach the bottom of the 5 L glass bottles. On the top of the carboy top attach a female leur lock port and cover with a rubber cap. Cover all other openings with aluminum foil. Wrap the entire carboy top with aluminum foil and place a piece of autoclave tape on it,

labeled ‘clean’. Place in autoclave tray with filtration units and 1 L flasks.

8.2.4 Cap the 1 L bottles loosely and place a piece of autoclave tape on each, labeled ‘clean’. Place in autoclave tray with filtration units, 1 L flasks, and carboy top.

8.2.5 With a gloved hand, place 10 non-sterile nylon filters in an autoclave pouch and seal. Place in autoclave tray with filtration units, 1 L flasks, 1 L glass bottle, and carboy top.

8.2.6 Autoclave for a minimum of 20 minutes according to SOP QC 18 Performance Verification of Autoclaves.

8.2.7 Allow glassware to cool to room temperature (20 ± 5°C) prior to using.

8.3 Prepare Yeast- Lactic-Citric Acid Stock Solution (Preparation no. 2)

8.3.1 Add 0.5 g yeast extract, 5.0 g lactic acid ($C_3H_6O_3$), 40 g citric acid ($C_6H_8O_7$) to 500 mL fresh, non-sterile MQ water.

8.3.2 Add a stir bar and place on magnetic stir plate until fully dissolved.

8.3.3 Remove the stir bar and add MQ water for a final volume of 1000 mL. Add the stir bar and mix well.

8.3.5 Vacuum filter into a sterile 1 L flask with vacuum port. Remove flask from vacuum source and move into BSC.

8.3.6 Aseptically remove filtration unit, place to the side, and pour filter sterilized solution into sterile 1 L glass bottle and tighten cap.

8.3.7 Label the bottle with contents and preparation number. Store at RT. Record appropriate information on a new Media/Reagent Preparation Sheet.

8.4 Prepare Creatinine Stock Solution and Iron (II) Sulfate Stock Solution (Preparation no. 3)

8.4.1 Add 25 g creatinine ($C_4H_7N_3O$) and 0.06 g iron (II) sulfate ($FeSO_4$ *7 H_2O) to 500 mL fresh, non-sterile MQ water.

8.4.2 Add a stir bar and place on magnetic stir plate until fully dissolved.

8.4.3 Remove the stir bar and add MQ water for a final volume of 1000 mL. Add the stir bar and mix well.

8.4.5 Vacuum filter into a sterile 1 L flask with vacuum port. Remove flask from vacuum source and move into BSC.

8.4.6 Aseptically remove filtration unit, place to the side, and pour filter sterilized solution into sterile 1 L glass bottle and tighten cap.

8.4.7 Label the bottle with contents and preparation number. Store at RT. Record appropriate information on a new Media/Reagent Preparation Sheet.

8.5 Prepare Potassium Dihydrogen Phosphate Stock Solution(Preparation no4)

8.5.1 Add 95 g of potassium dihydrogen phosphate (KH_2PO_4) to 500 mL fresh, non-sterile MQ water.

8.5.2 Add a stir bar and place on magnetic stir plate until fully dissolved.

8.5.3 Remove the stir bar and add MQ water for a final volume of 1000 mL. Add the stir bar and mix well.

8.5.5 Vacuum filter into a sterile 1 L flask with vacuum port. Remove flask from vacuum source and move into BSC.

8.5.6 Aseptically remove filtration unit, place to the side, and pour filter sterilized solution into sterile 1 L glass bottle and tighten cap.

8.5.7 Label the bottle with contents and preparation number. Store at RT. Record appropriate information on a new Media/Reagent Preparation Sheet.

8.6 Prepare Dipotassium hydrogen phosphate Stock Solution(Preparation no5)

8.6.1 Add 120 g of dipotassium hydrogen phosphate (K_2HPO_4) to 500 mL fresh, non-sterile MQ water.

8.6.2 Add a stir bar and place on magnetic stir plate until fully dissolved.

8.6.3 Remove the stir bar and add MQ water for a final volume of 1000 mL. Add the stir bar and mix well.

8.6.5 Vacuum filter into a sterile 1 L flask with vacuum port. Remove flask from vacuum source and move into BSC.

8.6.6 Aseptically remove filtration unit, place to the side, and pour filter sterilized solution into sterile 1 L glass bottle and tighten cap.

8.6.7 Label the bottle with contents and preparation number. Store at RT. Record appropriate information on a new Media/Reagent Preparation Sheet.

8.7 Prepare Urea Stock Solution (Preparation no. 6)

8.7.1 Add 400 g of urea (CH_4N_4O) to 500 mL fresh, non-sterile MQ water.

8.7.2 Add a stir bar and place on magnetic stir plate until fully dissolved.

8.7.3 Remove the stir bar and add MQ water for a final volume of 1000 mL. Add the stir bar and mix well.

8.7.5 Vacuum filter into a sterile 1 L flask with vacuum port. Remove flask from vacuum source and move into BSC.

8.7.6 Aseptically remove filtration unit, place to the side, and pour filter sterilized solution into sterile 1 L glass bottle and tighten cap.

8.7.7 Label the bottle with contents and preparation number. Store at RT. Record appropriate information on a new Media/Reagent Preparation Sheet.

8.8 Prepare Sodium Bicarbonate Stock Solution (Preparation no. 7)

8.8.1 Add 105 g of sodium bicarbonate ($NaHCO_3$) to 500 mL fresh, non-sterile MQ water.

8.8.2 Add a stir bar and place on magnetic stir + hot plate until fully dissolved. Set heat to approximately 70°C.

8.8.3 Remove the stir bar and add MQ water for a final volume of 1000 mL. Add the stir bar and mix well.

8.8.5 Vacuum filter into a sterile 1 L flask with vacuum port. Remove flask from vacuum source and move into BSC.

8.8.6 Aseptically remove filtration unit, place to the side, and pour filter sterilized solution into sterile 1 L glass bottle and tighten cap.

8.8.7 Label the bottle with contents and preparation number. Store at RT. Record appropriate information on a new Media/Reagent Preparation Sheet.

8.9 Prepare AUM and Attach Carboy Top

8.9.1 Turn on BSC and allow it to run for at least 3 minutes. Wipe the BSC work surface with a paper towel moistened with 70% ethanol. Record appropriate information on the BSC Monitoring Record Form (SOP QC 17 Maintenance and Use of Biological Safety Cabinets).

8.9.2 Wipe down the outside of 5 L sterile MQ water bottle with a paper towel moistened with 70% ethanol and place in BSC.

8.9.3 Aseptically add 50 mL of stock solutions preparation no. 2,3,4 and 5 per 5 L sterile Chloride-Sulfate base solution using a new sterile 50 mL serological pipette per stock solution. Aseptically add 100 mL of stock solutions preparation no. 6 and 7 per 5 L sterile Chloride-Sulfate base solution using a new sterile 50 mL serological pipette per stock solution. Cap the bottle and place on stir plate to mix.

8.9.4 Adjust pH to 6.5 using 1M HCl. Record the volume added on the Media/Reagent Preparation Sheet.

8.9.5 Place label on 5 L glass bottle marked with final composition (a.k.a AUM) and date of preparation.

8.9.6 Place the sterile carboy top in the BSC and aseptically remove the foil.

8.9.7 Remove the foil and place the top in the dilution water bottle taking care not to touch the filling tube to any surfaces. Screw the top onto the bottle.

8.10 Fill the Inoculum Tubes

8.10.1 Wipe the work surface with paper towels wetted with 70% ethanol.

8.10.2 Wipe the bottom of the rack(s) of sterile 15mL falcon tubes with paper towels wetted with 70% ethanol and place them in the BSC.

8.10.3 With gloved hands, remove rubber cap from leur lock port, place to the side, attach sterile 10mL leur lock syringe. Slowly collect 10mL of AUM into syringe, once collected remove syringe and dispense into falcon tube.

8.10.4 Carefully replace the rubber cap.

8.10.5 Remove the rack(s) from the BSC, wipe the work surface with ethanol wetted paper towels and turn the BSC off.

9.0 REFERENCES

9.1 Brooks, T. (1997). "A simple Artificial Urine for the Growth of Urinary Pathogens." Letters in Applied Microbiology **24**: 203-206.

APPENDIX B

STANDARD OPERATING PROCEDURE FOR INTRALUMINAL CATHETER MODEL FOR GROWING AN *ESCHERICHIA COLI* BIOFILM

1.0 PURPOSE AND APPLICABILITY

1.1 The purpose of this method is to guide a researcher in growing a standard *Escherichia coli* biofilm in a catheter under flow.

1.2 This test method is used for growing a repeatabile *Escherichia coli* ATCC 53498 biofilm in a Foley catheter. Prior to inoculation with an overnight culture sterile nutrient media are flowed through the catheter for 2 hours. During the entire test, the biofilm is exposed to continuous low shear flow of nutrients through the Foley catheter. At the end of each 24-hour increment the biofilm accumulation is quantified by collecting 10 mL of effluent flow and collecting a 2 cm segment from the distal end of the catheter. The biofilm is harvested by rinsing the catheter segment in buffered dilution water then placing segment into 10mL of neutralization broth to be processed. Both samples are vortexed and sonicated to disaggregate the clumps and diluted and plated for viable cell enumeration.

2.0 METHOD SUMMARY

2.1 The method explains how the model, nutrient media and sampling materials are prepared.

2.2 The method is broken down into separate tasks including streaking a plate for isolated colonies, growing an inoculum, inoculating the model for continuous flow conditions, sampling and cell enumeration. The method

also includes calculations for determining inoculum broth and biofilm densities.

3.0 DEFINITIONS

3.1 ICM = Intraluminal Catheter Model

3.2 TSA = Tryptic soy agar

3.3 DI water = deionized water

3.4 MQ water = MilliQ water

3.5 BSC = Biosafety Cabinet

3.6 AUM = Artificial Urine Media

3.7 Continuous flow (CF) phase = media flows in and spent media and planktonic cells flow out

3.8 Residence time = the amount of time that substrate (media) is present in the catheter before it is washed out of the reactor. Residence time is calculated by dividing the reactor volume (mL) by the flow rate (mL/min). For example, *Escherichia coli* ATCC 53498 has a doubling time of 2.62 hours at room temperature. Planktonic cell washout conditions will ensure that remaining bacteria in the model are those adhered to catheter surface.

4.0 HEALTH AND SAFETY WARNINGS

4.1 It is required that personnel wear lab coat, gloves, and safety glasses when working with Biosafety Level 2 bacteria.

4.2 Proper sterilization and disposal of any solution containing bacteria is mandatory.

5.0 CAUTIONS

5.1 When collecting effluent samples, the technician must ensure the pump is off and effluent line is unclamped before removing collection syringe. If this is not done the pressure will cause the effluent flow to spray out of the collection port.

6.0 INTERFERENCES

6.1 Any changes to this protocol such as media pH (for inoculum or continuous flow phase), flow rate, inoculum serotype, incubation temperature, etc. will result in a different biofilm with respect to phenotype and possibly genotype.

6.2 When biofilm is removed from the catheter surface, it is in the form of clumps. It is important that biofilm samples that have been disaggregated into a uniform suspension to avoid artificially low viable cell counts.

6.3 This SOP reflects changes to the harvesting method as determined necessary by ruggedness analysis.

6.4 Replace bacterial air vents when they are soiled or as necessary.

6.5 Pump flow rates must be set as accurately as possible. Too much deviation (greater than 0.14 mL/min) from the target flow rate could result in change of the biofilm growth conditions.

6.6 A pump flow rate is dependent upon the pump setting and the inner diameter of the tubing used to calibrate the pump. It is important to use the

same size tubing to calibrate the pump that will be used during an experiment.

6.7 Use tubing designed for use with pump heads.

6.8 Calibrate the pump with the same number of pump heads that will be used during the experiment.

6.9 Check the chemical compatibility of pump grade tubing to ensure it is resistant to the liquids being pumped.

7.0 PERSONNEL QUALIFICATIONS

7.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.2 Personnel are required to be knowledgeable of SOP #2 “Preparing Artificial Urine Media”.

7.3 Personnel are required to be knowledgeable of dilution and plating SOPs.

7.4 Personnel are required to be knowledgeable of Biosafety and spill SOPs.

8.0 EQUIPMENT AND SUPPLIES

8.1 TSA plates

8.2 Inoculating loop

8.3 *Escherichia coli* ATCC 53498 frozen stock culture

8.4 Incubator set at 37 ± 2°C

8.5 Artificial Urine Media (AUM)

8.6 15mL and 50mL falcon tube

8.7 Female luer lock connector (sized to match reactor tubing)

8.8 10mL luer lock syringes

8.9 Sterile dilution tubes

8.10 MasterFlex peristaltic pump with 1 Easy Load pump head and 1 pump controller for continuous flow phase

8.11 Bacterial air vents, 0.22 μm pore size, < 25 mm diameter

8.12 5L carboy of sterile AUM, lid plumbed with at least 2 fittings (1 for bacterial air vent and 1 for continuous flow media)

8.13 20 L carboy for effluent (waste), lid plumbed with at least 2 fittings (1 for bacterial air vent and 1 for effluent waste)

8.14 C-clamp

8.15 100 mL graduated cylinder

8.16 NIST-certified timer

8.17 500 mL graduated cylinder

8.18 Sterile 1 mL pipette tips

8.19 Sterile 200 μL pipette tips

8.20 1 mL pipette

8.21 200 μL electronic pipette

8.22 Sterile dilution tubes containing 9 mL sterile dilution water

8.23 Vortexer

8.24 Sterile Petri dishes, 100 x 15 mm

8.25 Sonicating water bath with 50 – 60 Hz power setting

8.26 Ruler

8.27 Silicone tubing for reactor and continuous flow phase (size 15, 16, 25)

8.28 Norprene tubing for peristaltic pump head (size 16)

8.29 Glass flow break

8.30 Autoclavable straight and reducing tubing connectors (sized to match reactor tubing)

8.31 Clamp and clamp stand to hold glass flow break

8.32 Large waste tray for 20L effluent (waste) carboy

8.33 Aluminum foil

8.34 Autoclave tape

8.35 ~100 mL 95% ethanol in 250 mL glass beaker to flame-sterilize hemostat

8.36 Bunsen or alcohol burner

8.37 Aluminum foil wrapped test tube rack or block to support tools being flame-sterilized

8.38 Kimwipes

8.39 70% ethanol in squeeze or spray bottle

8.40 Sterile 50 mL centrifuge tubes containing 10 mL sterile dilution water

8.41 200 mL beaker

8.42 Luer lock collection port

9.0 PROCEDURE

9.1 Assemble Model

9.1.1 Preparation of Influent Line

9.1.1.1 *From media side to catheter*- See Fig. 1. Connect size 16 silicone tubing with flow break spliced in it, to segment of neoprene tubing. On the opposite side of the neoprene tubing connect another segment of silicone tubing with flow break spliced in it and a free connector at the end.

9.1.2 Preparation of Effluent Line

9.1.2.1 Connect luer lock port to size 15 silicone tubing- See Fig. 1. Ensure tubing is long enough to reach waste carboy. Place an extra connector in beaker and cover the top of beaker with aluminum foil.

9.1.3 Sterilization of model:

9.1.3.1 Cover the ends of influent tubing with aluminum foil.

9.1.3.2 Place rubber cap on exposed luer lock port, see Fig. 1, and aluminum foil on the exposed ends of the effluent tubing.

9.1.3.3 Sterilize the model tubing and extra connector for 20 min on dry cycle of a steam sterilizer.

9.1.4 Model Setup:

9.1.4.1 Connect influent tubing to nutrient carboy so flow breaks are oriented correctly with the direction of flow, see Fig. B-1.

9.1.4.2 Feed neoprene tubing through pump and close in place.

9.1.4.3 Clamp second flow break in upright position, so free connector is pointed downward.

9.1.4.4 Open sterile catheter, with flame sterilized scissors cut the proximal tip off the catheter, directly below the drainage hole.

9.1.4.5 Connect the proximal end of the catheter to the free connector on the end of the influent tubing.

9.1.4.6 Connect the distal end of the catheter (drainage port) to the luer lock port.

9.1.4.7 Connect the exposed end of the effluent tubing to the waste carboy.

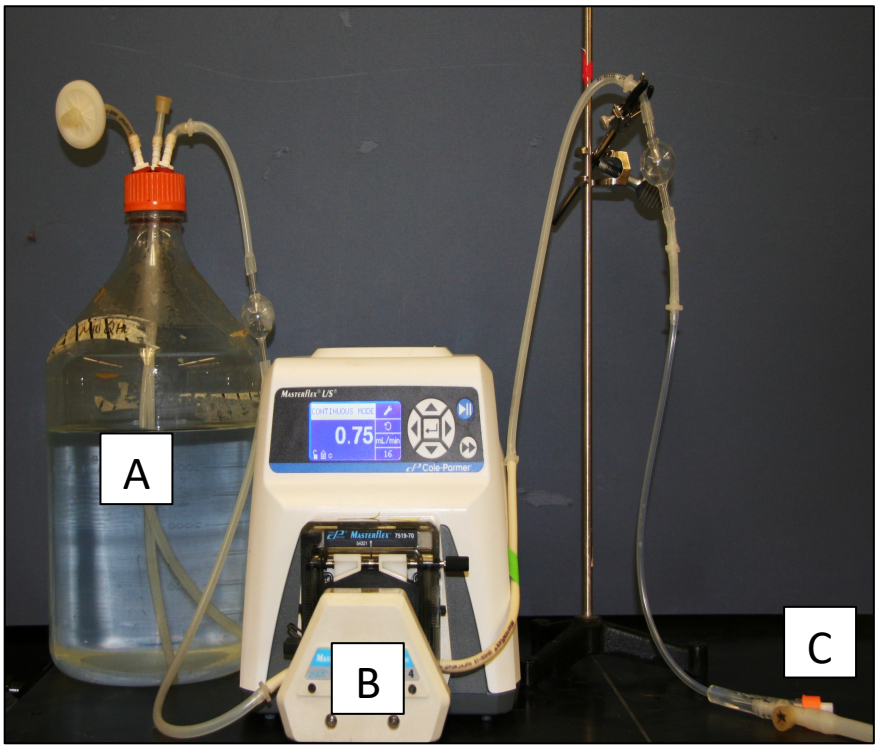

Figure B-1. Experimental set-up used to monitor biofilm formation on the intraluminal surface of urinary catheter. AUM (A) is pumped through tubing at 0.75mL/min (B) through a 16 French (Fr) urinary catheter to sampling port (C).

9.2 Calibrate pump for continuous flow

9.2.1 Attach one pump head to pump with appropriate hardware.

9.2.2 Connect the pump controller to the pump and plug into an electrical outlet.

9.2.3 Close the pump head to cover the moving rollers, turn the pump on and let warm up for 1 – 2 hours.

9.2.4 Turn pump off and open pump head.

9.2.5 Position the tubing through the pump head so that the tubing is in the center of the upside-down V on each side of the pump head.

9.2.6 Close the pump head and seat firmly to prevent creeping by adjusting the black adjustment buttons on each side of the pump head.

9.2.7 Place one end of the tubing into beaker of water and the other end into the graduated cylinder.

9.2.8 Turn the pump on to the approximate flow setting desired (or start with 1 on the dial).

9.2.9 Let the water flow through the tubing. Start the timer when drops begin to fall into the graduated cylinder.

9.2.10 Time for 2 - 3 minutes. Divide the volume in the cylinder by the number of minutes to determine the flow rate in mL/min. Continue to fine tune by slightly adjusting the tightness of the pump head until the flow rate is within the flow rate range. (The flow rate should be within +/- 0.2 mL/min.)

9.2.11 Confirm that flow rate is accurate by emptying the cylinder and timing for 10 minutes. Continue to adjust the flow rate, if necessary until it is within the appropriate range.

9.2.12 Calibrate the pump prior to each experiment to confirm that the desired flow rate is achieved. If appropriate, confirm the flow rate after completion of the experiment by repeating steps 9.2.7 through 9.2.12.

9.3 Prepare media and reagents

9.3.1 Prepare TSA plates.

9.3.2 Prepare sterile dilution tubes.

9.3.5 Prepare AUM.

9.4 Running an ICM Experiment

9.4.1 Culture Preparation

9.4.1.1 Inoculate 10mL of AUM in 15mL falcon tube with 2-3 isolated colonies from streak plate that was incubated at 36± 2°C for 22 ± 2 hours.

9.4.1.2 Incubate the falcon tube for 22 ± 2 hours in an incubator set at 36 ± 2°C.

9.4.1.3 Reactor will be inoculated with 10^3 CFU/mL, this concentration can be achieved through serial dilution of the overnight culture to the 5th dilution. This serial dilution should be done in AUM. The 5th dilution will become the inoculum for the model and its density should be checked through serial dilution and plating.

9.4.2 Sterile Flow Phase

9.4.2.1 Prime the tubing with AUM by pressing the fast forward (>>) button on the pump until AUM has reached the proximal end of the catheter.

9.4.2.2 Pump a continuous flow of nutrient into the catheter at a flow rate equal to 0.75ml/min.

9.4.2.3 Run sterile flow for 2 hours.

9.4.3 Inoculation of ICM

9.4.3.1 Pause the pump.

9.4.3.2 Aseptically remove the proximal end of catheter from connector and pipette 2mL of bacteria from overnight culture, prepared previously (see Section 9.4.1), through the catheter.

9.4.3.3 Aseptically reconnect the catheter to the connector and restart the pump. Operate the model in Continuous Flow Mode until desired sample time points have been completed.

9.4.4 Inoculum Log Density Calculation

9.4.4.1 Count the inoculum plates from step 9.4.1.3 after they have been incubated at 36± 2°C for 22 ± 2 hours on the dilution with 3 – 30 CFU/drop and record the counts in data spreadsheet.

9.4.4.2 Calculate LOG_{10} CFU/mL using the following equation:

$LOG_{10}(CFU/mL) = LOG_{10}[(\text{average CFU per drop/drop volume}) / (\text{Dilution})]$

Example: average CFU per drop = 3.6

Volume per drop: 0.01 mL

Dilution = 10^{-0}

LOG_{10}(CFU/mL) =

$LOG_{10}[(6.6/0.01) / (10^{-0})] = 3.16$

9.4.4.3 If the inoculum density is not approximately LOG_{10} 3.0 CFU/mL, terminate the experiment.

9.4.5 Collection of Samples at 24, 48, 72, and 96 hours

9.4.5.1 Prepare sampling materials: vortex, sonicator, culture tubes, rinse tubes, collection tubes, pipettes, empty sterile petri dish, flame sterilized forceps and scissors, 10mL luer lock syringes, screw clamp, waste media jar, timer, ruler.

9.4.5.2 Pause the pump, remove the rubber cap from luer lock port and connect the 10mL syringe to the port. With screw clamp, clamp effluent tubing directly below end of port.

9.4.5.3 Restart the pump and set timer for 15 mins.

9.4.5.4 After 15 minutes, pause pump, unscrew clamp (AWAYS DO BEFORE REMOVING SYRINGE). Remove syringe and dispense in waste media jar.

9.4.5.5 Repeat steps 9.4.5.2-4. After 15 mins, pause pump, unscrew clamp, remove syringe and dispense effluent sample into 10 mL of buffered dilution water giving a final volume of 20mL.

9.4.5.5.1 If working with a treated catheter, reference ASTM E-1054 to determine appropriate neutralization broth.

9.4.5.6 With pump still paused, use 70% ethanol to clean the outside of the catheter. Then measure 2cm from distal end and mark catheter - See Fig. B-2.

9.4.5.7 With flame sterilized scissors cut the catheter at distal end and on 2 cm mark, place segment in empty sterile petri dish. Connect catheter to distal end with extra connector that was sterilized with tubing. Restart the pump.

9.4.5.7.1 On all times past the first sample point, the 2 cm segment will be measured from the end of the connector.

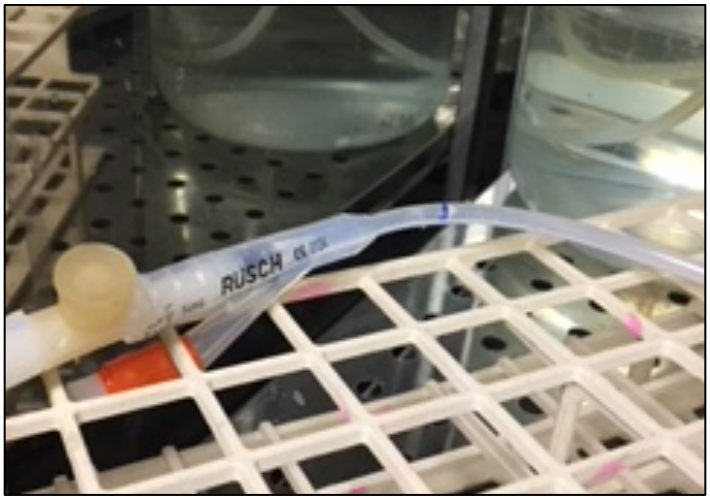

Figure B-2. Collection of catheter segment. Blue line indicates the 2 cm mark from the distal end of the catheter.

9.4.6 Collect Biofilm from catheter segment:

9.4.6.1 Rinse the catheter segment to remove planktonic cells: With flame sterilized forceps, gently immerse catheter segment into 20mL sterile dilution water then immediately remove.

9.4.6.2 Place segment into 10mL of buffered dilution water or neutralization broth if testing treated catheters.

9.4.7 Disaggregate and Plate Samples:

9.4.7.1 Vortex each tube on the highest setting, ensuring a complete vortex for 30 seconds.

9.4.7.2 Place both tubes into a wire or plastic conical tube rack and suspend the rack in the ultrasonic water bath so that the liquid level in the tubes is even with the water level in the tank of the bath. Do not allow the tubes of the rack to touch

the bottom of the sides of the ultrasonic water bath. Sonicate the tubes at 45kHz for 2 minutes on the normal function.

9.4.7.3 Vortex the tubes as described in 9.4.7.1.

9.4.7.4 Sonicate the tubes as described in 9.4.7.2.

9.4.7.5 Vortex the tubes as described in 9.4.7.1.

9.4.7.6 Dilute and Recover Disaggregated Samples:

9.4.7.6.1 Serially dilute the samples, vortexing between each dilution. Continue the dilution series out to 10^{-7}.

9.4.7.6.2 Drop plate the dilution series according to SOP SBM 5 Drop Plate Method. Allow the plates to dry, invert them and incubate for 17-20 hr at 36 ± 2°C.

9.4.8 Cell Enumeration

9.4.8.1 Remove plates from incubator (see step 9.4.7.6.2.). Count dilution containing between 3 and 30 CFU per drop.

9.7.5.2 Record the counts and the corresponding dilutions on Viable Plate Count Sheet and in data spreadsheet.

9.7.5.3 Calculate the log density according to the following equation:

9.7.5.3.1 Catheter Segment LD:

$LOG_{10}(CFU/cm^2) = LOG_{10}[((average\ CFU/drop\ volume)\ /(Dilution))\ x\ (Volume\ scraped\ into/Surface\ area)]$

or

$LOG_{10}\ (CFU/cm^2) = LOG_{10}\ [((X/B)/(D))\ (V/A)]$

X = mean CFU

B = volume plated

V = volume scraped into

A = surface area scraped

D = dilution

Example: X (mean CFU) = 6.5

B (Volume plated) = 0.01 mL

V (Volume scraped into) = 10 mL

A (catheter segment surface area)* = 2.38 cm^2

D (dilution) = 10^{-5}

$LOG_{10}(CFU/cm^2)$ =

$=LOG_{10}[((6.5/0.01)/(10^{-5}))\ (10/2.38)] = 7.83$

*Surface Area specific for 2 cm segment of a 16 French Foley catheter.

9.7.5.3.2 Effluent Sample LD:

$LOG_{10}(CFU/mL) = LOG_{10}(2*(\text{average CFU/drop volume}) /(\text{Dilution}))$

or

$LOG_{10}(CFU/mL) = LOG_{10}(2*(X/B)/(D))$

X = mean CFU

B = volume plated

D = dilution

Example: X (mean CFU) = 5.5

B (Volume plated) = 0.01 mL

D (dilution) = 10^{-5}

$LOG_{10}(CFU/mL) = LOG_{10}(2*(5.5/0.01)/(10^{-5}))$

$= 7.91$

*Note: the factor of 2 accounts for the 1:2 dilution of the original sample.

9.4.9 Repeat Sections 9.4.5-8 for every sample time point. Typically sample the model at 24, 48, 72, and 96 hours.

9.5 Experiment clean up

9.5.1 Autoclave the tubing, dilution tubes, collected samples and any other materials contaminated with bacteria for at least 20 min on liquid exhaust cycle.

9.5.2 Clean the dilution tubes according to SOP QC 1 Cleaning Dilution Tubes and Glassware.

9.5.3 Remove catheter from tubing and dispose of into biohazard waste bin. Rinse the influent and effluent tubing with DI water, drain and allow to dry.

9.5.4 Remove flow breaks from tubing and rinse with DI water until AUM residue is removed. If necessary, place flow breaks in beaker with DI and place in jewelry sonicator for approximately 2 minutes. Rinse again and allow to dry.

9.5.5 Rinse the continuous flow media carboy, carboy top and tubing with DI water (3x), drain and allow to dry. Insure that the bacterial air vent on the carboy top doesn't get wet.

9.5.6 Add bleach to the 20L waste carboy to achieve a final concentration of 10% v/v (~1/2 gallon of bleach per 20L waste).

9.5.7 Label the carboy with label tape stating that the carboy was bleached, the date and your initials. Allow the carboy to sit for 24 hours. To confirm that the bacteria in the waste carboy are not viable, plate 1 mL on TSA agar to confirm that no growth is present prior to dumping the carboy contents. If no growth is present on the plate, dispose of the contents down the drain.

9.5.7.1 If growth is present on the plate repeat step 9.5.7 until no growth is detected.

10.0 DATA AND RECORDS MANAGEMENT

10.1 Data will be recorded promptly, legibly and in indelible ink on the appropriate forms. Completed forms are archived in secured file cabinets in EPS 305. Only authorized personnel have access to the secured files. Archived data is subject to the retention schedule contained in SOP ADM 2 Record and Archives.

10.2 Any additional information such as observations, notes or other items not included on the data forms will be recorded in the project laboratory notebook. Laboratory notebooks are archived in secured file cabinets in EPS 305. Only authorized personnel have access to the secured files. Archived data is subject to the retention schedule contained in SOP ADM 2 Record and Archives.

10.3 Completed data forms are archived in secured file cabinets. Only authorized personnel have access to the secured files. Archived data is subject to the retention schedule contained in SOP ADM 2 Record and Archives.

11.0 QUALITY ASSURANCE AND QUALITY CONTROL

11.1 The SBML is consistent with 40 CFR Part 160 Good Laboratory Practice Standards. Appropriate quality control measures are integrated into each SOP.

11.2 For quality control purposes, the required information is documented in the laboratory notebook or on the appropriate record form(s).

11.3 Calibration data will be recorded promptly, legibly and in indelible ink on the appropriate biofilm reactor data sheet, if applicable. Completed data forms are archived in secured file cabinets. Only authorized personnel have access to the secured files. Archived data is subject to the retention schedule contained in SOP ADM 2 Record and Archives.

APPENDIX C

REPEATABILITY ANALYSIS

Mean Log Densities Over Time

Table C-1. Mean log densities and standard deviations (StDev) of SOP controls over 5 experiments, by sample point. Each experiment had a single replicate at each sample point.

	Catheter Samples		Effluent Samples	
Sample Point(hrs)	Mean $\log_{10}(CFU/cm^2)$	StDev	Mean $\log_{10}(CFU/mL)$	StDev
24	7.07	0.28	7.98	0.36
48	7.42	0.62	8.59	0.24
72	8.46	0.19	8.63	0.29
96	8.69	0.58	8.39	0.36
120	8.63	1.33	8.35	0.02
144	8.93	0.75	8.12	0.29
168	9.23	0.05	8.17	0.41

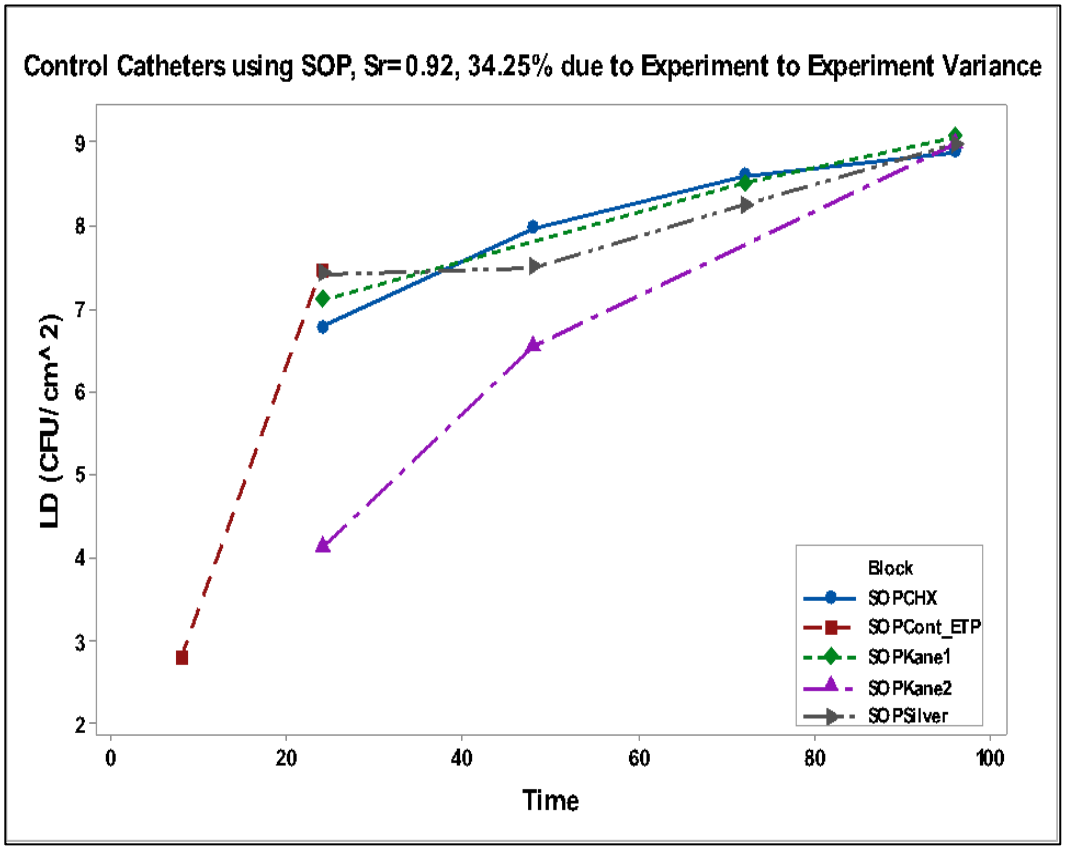

Figure C-1. Log densities of SOP control catheter segments over 5 experiments by sample point. Each experiment had a single replicate at each sample point.

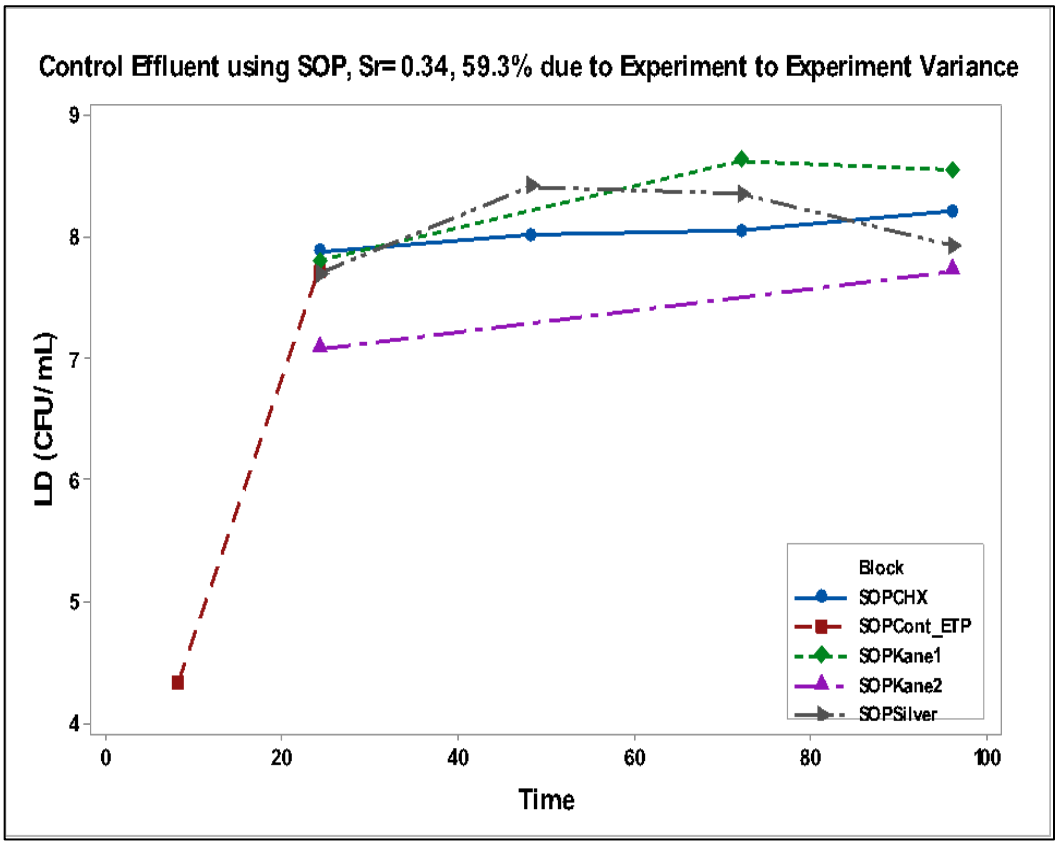

Figure C-2. Log densities of SOP control effluent samples over five experiments by sample point. Each experiment had a single replicate at each sample point.

Resemblance Standard Deviation for SOP Controls

Table C-2. Variance components of control catheter segments.

Source	Variance	% of Total
Block	0.292	34.25%
Error	0.561	65.75%
Total	0.854	

$Cs_{r,\ catheter} = (0.292+0.56)^{1/2} = 0.92$

Table C-3. Variance components of control effluent samples.

Source	Variance	% of Total
Block	0.067	59.30%
Error	0.046	40.70%
Total	0.113	

$Cs_{r,\ effluent} = (0.067+0.046)^{1/2}=0.34$

Repeatability Standard Deviations for Kane Coated Catheters

Table C-4. Variance components of Kane catheter segment samples.

Source	Variance	% of Total
Block	0.000	0%
Error	1.75	100%
Total	1.75	

$s_{r,\ Kane,\ catheter} = (1.74)^{1/2}=1.31$

Table C-5. Variance components of Kane effluent samples.

Source	Variance	% of Total
Block	0.000	0%
Error	1.17	100%
Total	1.17	

$s_{r,\ Kane,\ effluent} = (1.17)^{1/2}=1.08$

APPENDIX D

RUGGEDNESS ANALYSIS

Control Catheters

Control models were fit with a quadratic fixed-effect linear regression model. With a quadradic term for temperature and all split by time.

Catheter Samples

8-hour regression equation

$$\begin{aligned}LD= {} & 0.81(\text{Inoc-3}) -1.4(\text{pH-6.5}) + 0.012(\text{Temp-37}) -0.18*\text{I(Tempc\^{}2)} \\ & +0.33(\text{Flow-0.75}) + 1.05*\text{RemovalMethod} -0.13[(\text{Temp-37}) (\text{pH-6.5})] \\ & +0.41[(\text{Flow-0.75})(\text{pH-6.5})] - 0.09[\text{RemovalMethod} *(\text{pH-6.5})] \\ & +0.24[(\text{Inoc-3})(\text{pH-6.5})]+2.77\end{aligned}$$

8-hour partial derivatives

$$\frac{\mathrm{df}}{\mathrm{dInoc}} = 0.89 + 0.24(\mathrm{pH} - 6.5)$$

$$\begin{aligned}\frac{\mathrm{df}}{\mathrm{dpH}} = {} & -1.39 - 0.13(\mathrm{Temp} - 37) + 0.41(\mathrm{Flow} - 0.75) \\ & - 0.09(\text{Removal Method} = \text{sonicate}) + 0.24(\mathrm{Inoc} - 3)\end{aligned}$$

$$\frac{\mathrm{df}}{\mathrm{dTemp}} = 0.012 - 0.36(\mathrm{Temp} - 37) - 0.13(\mathrm{pH} - 6.5)$$

$$\frac{\mathrm{df}}{\mathrm{dFlow}} = 0.33 + 0.41(\mathrm{pH} - 6.5)$$

$$\frac{\mathrm{df}}{\mathrm{d(sonicate - scrape)}} = 1.05 - 0.09(\mathrm{pH} - 6.5)$$

24-hour regression equation

LD= 0.89(Inoc-3) -1.73(pH-6.5) + 0.175(Temp-37) -0.23*I(Tempc^2)

+1.95(Flow-0.75) +1.5*RemovalMethod -0.48[(Temp-37) (pH-6.5)]

+1.8[(Flow-0.75)(pH-6.5)]– 1.68[RemovalMethod *(pH-6.5)]

+ 0.53[(Inoc-3)(pH-6.5)]+6.52

24-hour partial derivatives

$$\frac{\mathrm{df}}{\mathrm{dInoc}} = 0.89 + 0.52(\mathrm{pH} - 6.5)$$

$$\frac{\mathrm{df}}{\mathrm{dpH}} = -1.73 - 0.48(\mathrm{Temp} - 37) + 1.80(\mathrm{Flow} - 0.75) - 1.68(\mathrm{Removal\ Method} = \mathrm{sonicate}) + 0.52(\mathrm{Inoc} - 3)$$

$$\frac{\mathrm{df}}{\mathrm{dTemp}} = 0.17 - 0.47(\mathrm{Temp} - 37) - 0.48(\mathrm{pH} - 6.5)$$

$$\frac{\mathrm{df}}{\mathrm{dFlow}} = 1.95 + 1.80(\mathrm{pH} - 6.5)$$

$$\frac{\mathrm{df}}{\mathrm{d(sonicate - scrape)}} = 1.50 - 1.68(\mathrm{pH} - 6.5)$$

48-hour regression equation

LD= 0.82(Inoc-3) -2.04(pH-6.5) -0.06(Temp-37) -0.13*I(Tempc^2)

+ 2.23(Flow-0.75) + 0.95*RemovalMethod -0.57[(Temp-37) (pH-6.5)]

+ 2.27[(Flow-0.75)(pH-6.5)]– 0.027[RemovalMethod *(pH-6.5)]

+ 0.73[(Inoc-3)(pH-6.5)]+7.015

48-hour partial derivatives

$$\frac{\mathrm{df}}{\mathrm{dInoc}} = 0.82 + 0.73(\mathrm{pH} - 6.5)$$

$$\frac{\mathrm{df}}{\mathrm{dpH}} = -2.04 - 0.57(\mathrm{Temp} - 37) + 2.27(\mathrm{Flow} - 0.75) - 0.03(\mathrm{Removal\ Method} = \mathrm{sonicate}) + 0.73(\mathrm{Inoc} - 3)$$

$$\frac{\mathrm{df}}{\mathrm{dTemp}} = -0.06 - 0.26(\mathrm{Temp} - 37) - 0.57(\mathrm{pH} - 6.5)$$

$$\frac{df}{dFlow} = 2.23 + 2.27(pH - 6.5)$$
$$\frac{df}{d(sonicate - scrape)} = 0.95 - 0.03(pH - 6.5)$$

Effluent Samples

8-hour regression equation

$$LD = (Inoc-3) -2.01(pH-6.5) + 0.05(Temp-37) -0.15331*I(Tempc^2) + 0.5(Flow-0.75) -0.48[(Inoc-3)(pH-6.5)]-0.09[(Temp-37) (pH-6.5)] + 0.81[(Flow-0.75)(pH-6.5)] +4.62$$

8-hour partial derivatives

$$\frac{df}{dInoc} = 1.00 - 0.48(pH - 6.5)$$
$$\frac{df}{dpH} = -2.01 - 0.09(Temp - 37) + 0.81(Flow - 0.75) - 0.48(Inoc - 3)$$
$$\frac{df}{dTemp} = 0.05 - 0.31(Temp - 37) - 0.09(pH - 6.5)$$
$$\frac{df}{dFlow} = 0.50 + 0.81(pH - 6.5)$$

24-hour regression equation

$$LD = 1.14(Inoc-3) -1.7(pH-6.5) -0.04(Temp-37) -0.19*I(Tempc^2) + 2.54(Flow-0.75) -0.02[(Inoc-3)(pH-6.5)]-0.35[(Temp-37) (pH-6.5)] + 2.21[(Flow-0.75)(pH-6.5)] +7.86$$

24-hour Partial Derivatives

$$\frac{df}{dInoc} = 1.14 - 0.02(pH - 6.5)$$
$$\frac{df}{dpH} = -1.70 - 0.35(Temp - 37) + 2.21(Flow - 0.75) - 0.02(Inoc - 3)$$

$$\frac{df}{dTemp} = 0.04 - 0.38(Temp - 37) - 0.35(pH - 6.5)$$
$$\frac{df}{dFlow} = 2.54 + 2.21(pH - 6.5)$$

48-hour regression equation

LD= 0.23(Inoc-3) -1.44(pH-6.5) -0.12(Temp-37) -0.12*I(Tempc^2)

+ 0.97(Flow-0.75)+0.69[(Inoc-3)(pH-6.5)]-0.21[(Temp-37) (pH-6.5)]

+ 1.74[(Flow-0.75)(pH-6.5)] +8.71

48-hour partial derivatives

$$\frac{df}{dInoc} = 0.23 - 0.69(pH - 6.5)$$
$$\frac{df}{dpH} = -1.44 - 0.21(Temp - 37) + 1.74(Flow - 0.75) - 0.69(Inoc - 3)$$
$$\frac{df}{dTemp} = -0.12 - 0.23(Temp - 37) - 0.21(pH - 6.5)$$
$$\frac{df}{dFlow} = 0.97 + 1.74(pH - 6.5)$$

Kane Biotech Catheters

Kane Biotech catheters were fit with a mixed-effect linear regression model, with a random effect for block using all data through 48 hours.

Catheter Samples

Regression equation

LR= 0.52(Flow-0.75) + 0.06(Time-8)+0.07(Temp-37) +0.3(Inoc-3)

-0.74(pH-6.5) +1.02*RemovalMethod +(1 Block) +1.85

Partial derivatives

$$\frac{df}{dInoc} = 0.30$$

$$\frac{df}{dpH} = -0.74$$

$$\frac{df}{dTemp} = 0.07$$

$$\frac{df}{dFlow} = 0.52$$

$$(sonicate - scrape) = 1.02$$

$$\frac{df}{dTime} = 0.06$$

Effluent Samples

Regression equation

LR= 1.08(Flow-0.75)-0.02(Temp-37)+0.79(Inoc-3)

-0.72(pH-6.5)+0.05(Time-8) + 0.18[(Inoc-3)(ph-6.5)]

-0.06[(Flow- 0.75)(Time-8)] +0.001[(Temp-37)(Time-8)]

-0.04[(Inoc-3)(Time-8)] +0.06[(pH-6.5)(Time-8)]

+1.1[(Flow-0.75)(pH-6.5)]+0.28[(Temp-37)(pH-6.5)]

+0.07[(Inoc-3)(pH-6.5)(Time-8)] +(1 Block) +2.43

Partial Derivatives

$$\frac{df}{dInoc} = 0.79 + 0.18(pH - 6.5) - 0.04(Time - 8) + 0.07(pH - 6.5)(Time - 12)$$

$$\frac{df}{dpH} = -0.72 + 0.28(Temp - 37) + 1.10(Flow - 0.75) + 0.18(Inoc - 3) + 0.06(Time - 8) + 0.07(Time - 12)(Inoc - 3)$$

$$\frac{df}{dTemp} = -0.02 + 0.002(Time - 8) + 0.28(pH - 6.5)$$

$$\frac{df}{dFlow} = 1.08 + 1.10(pH - 6.5) - 0.06(Time - 8)$$

$$\frac{\mathrm{df}}{\mathrm{dTime}} = 0.05 + 0.002(\mathrm{Temp} - 37) - 0.06(\mathrm{Flow} - 0.75) - 0.04(\mathrm{Inoc} - 3) + 0.06(\mathrm{pH} - 6.5) + 0.07(\mathrm{pH} - 6.5)(\mathrm{Inoc} - 3)$$

Silver Catheters

Silver catheters were fit with a weighted mixed effect linear regression model, with a random effect for block and split by time.

Catheter Segments

8-hour regression equation

$$\mathrm{LR} = 0.5(\mathrm{Flow}\text{-}0.75) + .004(\mathrm{Temp}\text{-}37) + 0.83(\mathrm{Inoc}\text{-}3) - 1.1(\mathrm{pH}\text{-}6.5) + 1.26*\mathrm{RemovalMethod} + 0.07[(\mathrm{Inoc}\text{-}3)(\mathrm{pH}\text{-}6.5)] - 0.18[(\mathrm{Temp}\text{-}37)(\mathrm{pH}\text{-}6.5)] - 0.29[(\mathrm{Flow}\text{-}0.75)(\mathrm{pH}\text{-}6.5)] - 0.79[(\mathrm{pH}\text{-}6.5)*\mathrm{RemovalMethod})] + (1\mathrm{Block}) + 0.97$$

8-hour partial derivatives

$$\frac{\mathrm{df}}{\mathrm{dInoc}} = 0.83 + 0.07(\mathrm{pH} - 6.5)$$

$$\frac{\mathrm{df}}{\mathrm{dpH}} = -1.09 - 0.18(\mathrm{Temp} - 37) - 0.29(\mathrm{Flow} - 0.75) - 0.79(\mathrm{Removal\ Method} = \mathrm{sonicate}) + 0.07(\mathrm{Inoc} - 3)$$

$$\frac{\mathrm{df}}{\mathrm{dTemp}} = 0.004 - 0.18(\mathrm{pH} - 6.5)$$

$$\frac{\mathrm{df}}{\mathrm{dFlow}} = 0.50 - 0.29(\mathrm{pH} - 6.5)$$

$$\frac{\mathrm{df}}{\mathrm{d(sonicate - scrape)}} = 1.26 - 0.79(\mathrm{pH} - 6.5)$$

24-hour regression equation

LR= 2.06(Flow-0.75) +.01(Temp-37)+0.05(Inoc-3)-0.19(pH-6.5)

+0.45*RemovalMethod+1.12[(Inoc-3)(pH-6.5)]

-0.21[(Temp-37)(pH-6.5)] +0.54[(Flow-0.75)(pH-6.5)]

-1.83[(pH-6.5)*RemovalMethod)] +(1 Block)+3.60

24-hour partial derivatives

$$\frac{\mathrm{df}}{\mathrm{dInoc}} = 0.05 + 1.12(\mathrm{pH} - 6.5)$$

$$\frac{\mathrm{df}}{\mathrm{dpH}} = 0.19 - 0.21(\mathrm{Temp} - 37) + 0.54(\mathrm{Flow} - 0.75) - 1.83(\mathrm{Removal\ Method} = \mathrm{sonicate}) + 1.12(\mathrm{Inoc} - 3)$$

$$\frac{\mathrm{df}}{\mathrm{dTemp}} = 0.01 - 0.21(\mathrm{pH} - 6.5)$$

$$\frac{\mathrm{df}}{\mathrm{dFlow}} = 2.06 - 0.54(\mathrm{pH} - 6.5)$$

$$\frac{\mathrm{df}}{\mathrm{d(sonicate - scrape)}} = 0.45 - 1.83(\mathrm{pH} - 6.5)$$

48-hour regression equation

LR=2.06(Flow-0.75) +.01(Temp-37)+0.05(Inoc-3)-0.19(pH-6.5)

+0.45*RemovalMethod+1.12[(Inoc-3)(pH-6.5)]

-0.21[(Temp-37)(pH-6.5)] +0.54[(Flow-0.75)(pH-6.5)]

-1.83[(pH-6.5)*RemovalMethod)] +(1 Block)+3.60

48-hour Partial Derivatives

$$\frac{\mathrm{df}}{\mathrm{dInoc}} = -0.44 + 2.17(\mathrm{pH} - 6.5)$$

$$\frac{\mathrm{df}}{\mathrm{dpH}} = -0.72 - 0.39(\mathrm{Temp} - 37) - 0.04(\mathrm{Flow} - 0.75) - 1.42(\mathrm{Removal\ Method} = \mathrm{sonicate}) + 2.17(\mathrm{Inoc} - 3)$$

$$\frac{df}{dTemp} = -0.18 - 0.39(pH - 6.5)$$
$$\frac{df}{dFlow} = 0.51 - 0.04(pH - 6.5)$$
$$\frac{df}{d(sonicate - scrape)} = 1.05 - 1.42(pH - 6.5)$$

Effluent Samples

8-hour regression equation

LR=0.57(Flow-0.75)+0.05(Temp-37)+0.54(Inoc-3)-1.46(pH-6.5)

+0.01[(Inoc-3)(pH-6.5)] -0.23[(Flow-0.75)(pH-6.5)

-0.16[(Temp-37)(pH-6.5)]+(1 Block)+1.82

8-hour partial derivatives

$$\frac{df}{dInoc} = 0.54 + 0.01(pH - 6.5)$$
$$\frac{df}{dpH} = -1.46 - 0.16(Temp - 37) - 0.23(Flow - 0.75) + 0.01(Inoc - 3)$$
$$\frac{df}{dTemp} = 0.05 - 0.16(pH - 6.5)$$
$$\frac{df}{dFlow} = 0.57 - 0.23(pH - 6.5)$$

24-hour regression equation

LR=0.75(Flow-0.75)-0.08(Temp-37)-0.43(Inoc-3)-0.44(pH-6.5)

+0.68[(Inoc-3)(pH-6.5)]+0.89[(Flow-0.75)(pH-6.5)

-0.008[(Temp-37)(pH-6.5)] +(1 Block)+3.73

24-hour partial derivatives

$$\frac{df}{dInoc} = -0.43 + 0.68(pH - 6.5)$$
$$\frac{df}{dpH} = 0.44 - 0.008(Temp - 37) - 0.89(Flow - 0.75) + 0.68(Inoc - 3)$$

$$\frac{df}{dTemp} = -0.08 - 0.008(pH - 6.5)$$
$$\frac{df}{dFlow} = 0.75 - 0.89(pH - 6.5)$$

48-hour regression equation

LR= -1.47(Flow-0.75)-0.42(Temp-37)-1.39(Inoc-3)-0.63(pH-6.5)

+2.43[(Inoc-3)(pH-6.5)]-0.1[(Flow-0.75)(pH-6.5)

-0.19[(Temp-37)(pH-6.5)]+(1 Block)+ 4.33

48-hour partial derivatives

$$\frac{df}{dInoc} = -1.39 + 2.43(pH - 6.5)$$
$$\frac{df}{dpH} = -0.63 - 0.19(Temp - 37) - 0.10(Flow - 0.75) + 2.43(Inoc - 3)$$
$$\frac{df}{dTemp} = -0.42 - 0.19(pH - 6.5)$$
$$\frac{df}{dFlow} = -1.47 - 0.10(pH - 6.5)$$

Simulations with Partial Derivatives

Table D-1. Flow simulations completed with partial derivatives of regression equations. Simulations show that if pH is held at SOP value of 6.5, the method is rugged when flow varies from 0.61mL/min to 0.89mL/min. The method is said to be rugged with respect to flow if the effect is calculated to be > ±0.3.

		Control						**Silver**						**Kane**	
		Biofilm Sample			Effluent			Biofilm Sample			Effluent			Biofilm Sample	Effluent
		8	24	48	8	24	48	8	24	48	8	24	48		
pH 7	0.89ml/min	0.25	1.17	1.45	0.48	1.46	1.01	-0.08	0.02	0.05	-0.04	-0.34	-0.26	0.07	0.15
	0.61ml/min	0.16	0.63	0.82	0.34	0.75	0.73	-0.22	-0.56	-0.09	-0.19	-0.55	0.16	-0.07	-0.15
SOP	0.89ml/min	0.05	0.27	0.31	0.07	0.36	0.14	0.07	0.29	0.07	0.08	0.11	-0.21	0.07	0.15
	0.61ml/min	-0.05	-0.27	-0.31	-0.07	-0.36	-0.14	-0.07	-0.29	-0.07	-0.08	-0.11	0.21	-0.07	-0.15
pH 6	0.89ml/min	-0.16	-0.63	-0.82	-0.34	-0.75	-0.73	0.22	0.56	0.09	0.19	0.55	-0.16	0.07	0.15
	0.61ml/min	-0.25	-1.17	-1.45	-0.48	-1.46	-1.01	0.08	-0.02	-0.05	0.04	0.34	0.26	-0.07	-0.15

Table D-2. Inoculum simulations completed with partial derivatives of regression equations. Simulations show that if pH is held at SOP value of 6.5, the method is rugged when inoculum concentration varies from 2.8 log(CFU/mL) to 3.2 log(CFU/mL). The method is said to be rugged with respect to inoculum concertation if the effect is calculated to be > ±0.3.

		Control						**Silver**						**Kane**	
		Biofilm Sample			Effluent			Biofilm Sample			Effluent			Biofilm Sample	Effluent
		8	24	48	8	24	48	8	24	48	8	24	48		
pH 7	3.2 log CFU/mL	0.28	0.44	0.53	-0.04	0.22	-0.30	0.20	0.57	1.00	0.11	0.25	0.94	0.06	0.16
	2.8 log CFU/mL	-0.04	0.08	0.20	-0.44	-0.24	-0.39	-0.13	0.55	1.17	-0.10	0.43	1.49	-0.06	-0.16
SOP	3.2 log CFU/mL	0.16	0.18	0.16	0.20	0.23	0.05	0.17	0.01	-0.09	0.11	-0.09	-0.28	0.06	0.16
	2.8 log CFU/mL	-0.16	-0.18	-0.16	-0.20	-0.23	-0.05	-0.17	-0.01	0.09	-0.11	0.09	0.28	-0.06	-0.16
pH 6	3.2 log CFU/mL	0.04	-0.08	-0.20	0.44	0.24	0.39	0.13	-0.55	-1.17	0.10	-0.43	-1.49	0.06	0.16
	2.8 log CFU/mL	-0.28	-0.44	-0.53	0.04	-0.22	0.30	-0.20	-0.57	-1.00	-0.11	-0.25	-0.94	-0.06	-0.16

Table D-3. Temperature simulations completed with partial derivatives of regression equations. Simulations show that if pH is held at SOP value of 6.5, the method is rugged when inoculum concentration varies from 36°C to 38°C. The method is said to be rugged with respect to temperature if the effect is calculated to be > ±0.3.

		Control						**Silver**						**Kane**	
		Biofilm Sample			Effluent			Biofilm Sample			Effluent			Biofilm Sample	Effluent
		8	24	48	8	24	48	8	24	48	8	24	48		
pH 7	38°C	-0.41	-0.54	-0.61	-0.31	-0.52	-0.46	-0.09	-0.10	-0.38	-0.03	-0.08	-0.52	-0.07	-0.02
	36°C	0.28	0.06	0.04	0.22	0.17	0.25	-0.09	-0.12	-0.02	-0.13	0.08	0.33	0.07	0.02
SOP	38°C	-0.35	-0.30	-0.32	-0.26	-0.34	-0.35	0.00	0.01	-0.18	0.05	-0.08	-0.42	-0.07	-0.02
	36C	0.35	0.30	0.32	0.26	0.34	0.35	0.00	-0.01	0.18	-0.05	0.08	0.42	0.07	0.02
pH 6	38°C	-0.28	-0.06	-0.04	-0.22	-0.17	-0.25	0.09	0.12	0.02	0.13	-0.08	-0.33	-0.07	-0.02
	36°C	0.41	0.54	0.61	0.31	0.52	0.46	0.09	0.10	0.38	0.03	0.08	0.52	0.07	0.02

Table D-4. The calculated effect from the partial derivatives of control catheters when pH varies, and all other operational factors are held at their SOP values.

		Low pH		SOP		High pH	
Time	Factor	CS	E	CS	E	CS	E
8	inoc	0.69	0.24	0.81	1	0.93	-0.24
	temp	0.077	0.095	0.012	0.05	-0.053	0.005
	flow	0.125	-0.405	0.33	0.5	0.535	0.405
	removal	1.095		1.05		1.005	
24	inoc	0.63	0.01	0.89	1.14	1.15	-0.01
	temp	0.41	0.215	0.17	0.04	-0.07	-0.135
	flow	1.05	-1.105	1.95	2.54	2.85	1.105
	removal	2.34		1.5		0.66	
48	inoc	0.455	0.345	0.82	0.23	1.185	-0.345
	temp	0.225	-0.015	-0.06	-0.012	-0.345	-0.225
	flow	1.095	-0.87	2.23	0.97	3.365	0.87
	removal	0.965		0.95		0.935	