Analysis of Sterile Practice in the Department of Cardiothoracic Surgery at Aurora St. Luke's Medical Center, Milwaukee, Wisconsin

by

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Abstract

The process of cardiopulmonary bypass (CPB) is essential for many cardiac procedures. A major risk of cardiac surgery is the risk of postoperative infection, specifically surgical site infections (SSI), which lead to increased patient morbidity and mortality, increased length of hospital stay, and increased cost of surgical procedures. In order to prevent SSI and hospital-acquired infection (HAI), sterile technique is used throughout the processes of assembling, priming, and storing circuits used for CPB. Safe CPB circuit storage is a multi-faceted process, and it is the responsibility of the perfusionist to ensure that these processes have been carried out in sterile fashion.

The purpose of this study had three aims, and took place at Aurora St. Luke's Medical Center in Milwaukee, Wisconsin. The first aim was to analyze potential contaminants in a primed circuit from the initial time of priming until seven days of standby. The second aim was to analyze potential contaminants on a sterile gown, which was used and re-used to cover stored CPB circuits for seven days. Additionally, potential contaminants were enumerated from an uncovered CPB circuit at the time of assembly and after 24 hours. The final aim was to assess circuit vulnerability to contaminants by determining the air quality in both the perfusion room and operating room (OR). All contaminants were measured by counting colony forming units (CFU) on tryptic soy agar (TSA), and appropriate statistical analysis was performed on the data.

Results illustrated that the CPB circuit on standby for seven days was free of microbiological contaminants. All sampling points resulted in 0 CFU, indicating that the circuit was safe for use in terms of sterility. The sterile gown was free of contaminants at the first sampling point, but after 24 hours tested for significant levels of contamination (20.4 ± 2.97 CFU/100 μL, $p<0.001$). However, data from an uncovered CPB circuit were not significant after 24 hours. Finally, environmental sampling indicates that time was a significant factor in the number of CFU in the perfusion room ($p=0.006$), but not in the operating room ($p=0.44$). Additionally, in the perfusion room, CFU increased from 1.3 ± 0.52 to 4.2 ± 4.2 at the 15-minute and 30-minute sampling points.

This study not only demonstrates that an open CPB circuit is safe for use after seven days on standby, but the study also addresses concerns pertaining to CPB circuit storage. For storage of CPB circuits, a new, sterile cover should be used immediately after assembly. Furthermore, any open medical device should not be used after 15 minutes of exposure to the perfusion room environment.

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1. Introduction

A major risk to cardiac surgery patients is postoperative infection, which directly correlates with patient morbidity and mortality [1]. Although infection rates likely vary by surgical center, one European report found that 3.5% of coronary artery bypass graft patients developed a surgical site infection (SSI), making it the second most common type of postoperative infection among all surgeries [2]. In fact, healthcare-associated infections are one of the top 10 leading causes of deaths in the United States [3]. In 2002, the Centers for Disease Control (CDC) estimated that hospital-acquired infections totaled nearly 1.7 million infections, with almost 99,000 associated deaths in the United States [4]. Of these 1.7 million infections, roughly 20% were SSI's [4]. Many factors have been implicated in the risk of SSI, and prevention of these risks is a complex issue that requires preoperative, intraoperative, and postoperative considerations. Until recently, there have not been global guidelines for safe surgical practices specifically targeted to reduce the risk of SSI and decrease patient morbidity and mortality. In 2016, the World Health Organization filled this void in global medicine, and published international guidelines to be implemented in the operating rooms of all nations, regardless of economic conditions. It is the aim of these guidelines to provide recommendations that will result in the reduction of patient morbidity, mortality, while increasing hospital savings resulting from infection prevention [5].

Sterile technique is a fundamental concept in safe surgical practice and the reduction of SSI. The use of sterile technique ensures that the surgical field does not become contaminated with microbes that might cause SSI [5]. The best practices for setting up, maintaining, and monitoring the sterile field within the operating room are

well documented, with strict protocols in place to prevent gross contamination. However, the cardiopulmonary bypass (CPB) circuit, utilized in many heart surgeries, provides unique challenges to ensuring circuit sterility. With the bypass circuit, there is risk of contamination in the assembly, storage, and priming periods. During cardiac surgery, the patient is exposed to the circuit, which could result in infection if the circuit is not properly sterilized.

The purpose of this study was to investigate the cardiopulmonary bypass circuit for risk of infectious contamination. This investigation included three aims. The first was to analyze potential contaminants in a primed circuit from the first day of priming with crystalloid solution until the seventh day of circuit standby. The second aim was to analyze a dry, sterile-assembled circuit for proper storage conditions. The final aim was to assess circuit vulnerability to contamination during the period of drug administration in the perfusion room. By analyzing these three periods of circuit vulnerability, possible contamination risks, if any, can be identified. If contamination is found, systems could then be developed to lessen these risks.

2. Background

2.1 Sterile Technique

2.1.2 Introduction to Sterile Technique

The goal of sterile technique is to ensure that the operating environment contains as few microbes as possible and to reduce the risk of surgical site infections [5]. Surgical site infections, or SSI, can increase the morbidity and mortality risks associated with surgery, and increase the incidence of postoperative complications and length of hospital stay [5]. Therefore, significant effort is put into optimizing the surgical patient and the operating room to reduce these risks. While some techniques may vary from institution to institution, many common practices include environmental cleaning, hand hygiene, patient preparation with aseptic solution, and the use of sterile surgical attire [5]. The National Guideline Clearinghouse (NGC) has laid out recommendations and best practices for maintenance of a sterile field during surgery. These practices include the use of sterile surgical gowns and sterile gloves, as well as guidance on the way surgical instruments should be handled in order to maintain sterility at the operating field [1].

Until recently, recommendations for best practices to prevent SSI have largely been based on local, state, or national guidelines. In 2016, however, as previously indicated, the World Health Organization (WHO) established international guidelines for sterile technique and the prevention of SSI, providing preoperative, intraoperative, and postoperative suggestions. Many of these recommendations focus on the importance of maintaining a sterile environment and the decontamination of surgical equipment and devices [5]. Additionally, the WHO guidelines outline the proper storage of sterile equipment. The WHO recommends that sterile packs be stored in a clean, dry

environment, with minimal moisture sources [5]. The storage area should be free of temperature fluctuations and it should additionally provide adequate air circulation. The storage area should also have limited access to it. Storage of the sterile equipment should be on racks at least 10 cm off the floor, and 10 cm from the ceiling, with open single-layer racks in favor of closed shelving. The packs should all be labeled visibly and clearly. Finally, all equipment should be inspected to ensure that it meets the requirements of a sterile product before its use, and any equipment that is beyond the best-used-by-date must be discarded. From safe storage, it is then the responsibility of the operating room personnel to ensure that devices are visibly clean and opened in a sterile fashion; no unnecessary delay of surgery should take place because of a lack of sterile instruments [5].

During the preoperative period, there are also strict guidelines that must be maintained. Many times, these guidelines are altered because of the type of operation being performed. In general, these preoperative measures consist of preoperative bathing of the patient, the use of mupirocin ointment (possibly with chlorhexidine gluconate body wash), optimal time administration of a prophylactic antibiotic, hair removal with a clipper, and surgical site preparation with an alcohol-based antiseptic solution [5]. Additionally, skin adhesives have become commonplace to prevent the spread of skin microbiota into the site of incision, although there is limited evidence to show their effectiveness [1].

Intraoperatively, there are several considerations that are directly pertinent to cardiac surgery and the cardiopulmonary bypass circuit. According to the WHO recommendations, the patient should receive an 80% fraction of inspired oxygen (FiO2)

during the operative period, and for 2 to 6 hours postoperatively, to reduce the risk of SSI [5]. The WHO also recommends maintaining normothermia, maintenance of blood glucose at levels of <150mg/dL, and maintenance of adequate circulating volume [5]. The guidelines for maintaining a sterile field suggest that doors be kept closed, and only opened for essential personnel to enter or exit [5]. The operating room should also have high efficiency air filters, positive air pressure, and directional air flow [6]. Additionally, the prophylactic antibiotic therapy should be continued into the postoperative period and appropriate dressings must be used to close the surgical site [5].

2.1.2 The Cycle of Decontamination and Classification of Medical Devices

The cycle of decontamination for reusable surgical instruments is depicted in Figure 1. In Figure 1, the features of decontamination are outlined, with each step as important as the next. Each step must be handled with care to protect the medical device and to prevent the risk of exposure to microorganisms. In the case of cardiopulmonary bypass (CPB) equipment, sterile packs are disposed of between each use, and are received from the manufacturer after the “Sterilization” step [7].

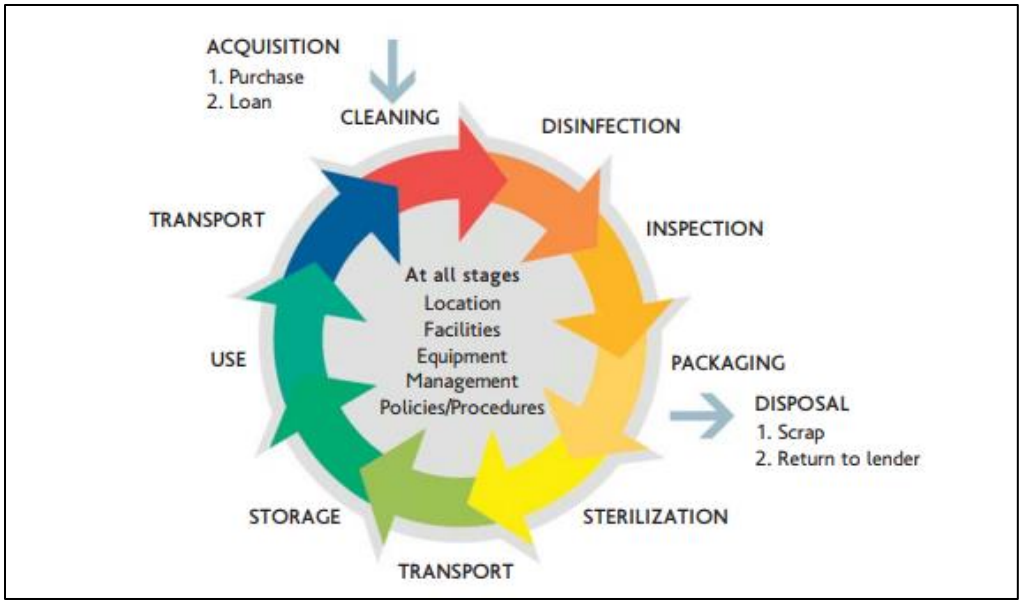

Figure 1: Depiction of the Life Cycle of Decontamination [7].

Additionally, the Spaulding classification of medical devices designates medical instruments as critical, semi-critical, or non-critical according to their degree of risk of infection [8]. Critical medical instruments are those that come in contact with the mucous membranes, or that enter a sterile body cavity. Under the Spaulding classification, the CPB tubing circuit is a critical medical instrument that requires all microorganisms be killed prior to its use [8]. The mechanism of sterilization of the CPB circuit is outlined in detail in Section 2.3.1.

2.1.3 Time-Dependent Contamination of the Sterile Field

Despite all efforts to maintain a sterile environment, there is an inherent time-dependent contamination that must also be considered. Studies have demonstrated that even in operating rooms that use laminar flow and positive pressure, there is still contamination that occurs with operating room traffic and other uncontrollable factors

[9]. Andersson *et al.* had found that in procedures lasting at least one hour, 62% of irrigation solutions become contaminated [9]. Similarly, Baird *et al.* found that in orthopedic surgery, 74% of surgical splash bin samples tested positive for bacterial culture [10]. Regarding the operating room itself as a source of contamination, Ritter *et al.* found that bacterial colony count increases significantly with doors left open, and when five or more people are added into the operating room [11]. Meanwhile, some equipment, such as surgical face shields, do not have a direct effect on bacterial colony count [11]. In many respects, the OR staff themselves remain a large source of bacteria in the operative setting, with skin flora appearing to be a prominent source of contamination [11]. Thus, in situations that cause a delay before the beginning of an operation, there are not guidelines for how long a room can remain free of contamination risk. However, it is recognized that sterilization of equipment does not produce absolute sterility throughout the entire duration of the operation [11]. Through some simple, practical actions, such as covering sterile equipment with a sterile towel, OR staff can reduce the risk of time-dependent contamination [12].

2.2 The Implications of Breaking Sterile Technique

While the concept of sterile technique is well documented and dates back to the 19th century [13], cardiac surgery has become increasingly complex, with new techniques and equipment being constantly introduced. These progressively complex surgeries can require new technologies such as robotics, extracorporeal membrane oxygenation (ECMO), and CPB. While these new technologies aid in the progression of healthcare outcomes, they may also introduce unique threats to the sterile field. When working with

such equipment, it is imperative that the OR personnel understand the implications of breaking sterile technique. The actions taken immediately after sterile technique is broken are critical for good patient outcomes, and methods should be implemented to prevent future breaks in sterile technique. If these procedures are not properly implemented, it could result in higher costs to the patient, and the patient may have significant risk of morbidity and mortality.

2.2.1 Containing the Threat of a Broken Sterile Field

The first step in preventing a desterilized field is containing the risk that threatens the sterile field. In the event that sterile technique is broken, precautions need to be taken to ensure that the patient is not exposed to contaminated instruments or surfaces. As mentioned previously, there are countless sources of microbial contamination in the OR. These sources can vary from endogenous bacteria that can be removed by simple handwashing, to exogenous microorganisms that are of an unknown source that become prevalent in a time-dependent manner [1, 9].

Breaks in sterile technique are classified into four categories. The most obvious break in technique is classified as Category One. A Category One technique is caught immediately, and does not pose an inherent threat to the patient. Category Two is a threat identified shortly after it occurs, and Category Three is a threat that is recognized later in the surgery. Finally, a Category Four is a break in technique that is not recognized at all, and can pose a great threat to patient well-being [14]. By minimizing the duration of the break in sterile technique, the break is less likely to transmit a pathogenic microorganism to the patient. Aside from the duration of the break in sterile technique, the type of

contaminant could also lead to increased complications in the postoperative period. More specifically, the risk of antimicrobial resistant pathogens poses the largest threat of bacterial infections [15]. According the National Healthcare Safety Network, over 16% of hospital-acquired infections (HAI's) were associated with multidrug-resistant pathogens, as outlined in Table 1 [15]. A list of the most common pathogens in HAI's is found in Table 2. When a break in sterile technique occurs and the patient's safety is at risk, the break in technique should be corrected as soon as it is deemed safe by the operating room staff. In these instances, it is up to the healthcare facility to have policies and procedures in place for reporting breaks in sterile technique [16]. Additionally, the appropriate risk management personnel should be notified [16].

Table 1: Most Common Multidrug Resistant Pathogens and Percent of Resulting HAI's [15].

Most Common Multidrug Resistant Pathogens	Percent of Hospital-Acquired Infections
Methicillin-resistant *S aureus*	8%
Vancomycin-resistant *Enterococcus faecium*	4%
Carbapenem-resistant *P aerugenosa*	2%
Extended-spectrum cephalosporin-resistant *K pneumoniae*	1%
Extended-spectrum cephalosporin-resistant *E Coli*	0.5%
Carbapenem-resistant *A Baumannii, K pneuumoniae, K oxytoca,* and *E coli*	0.5%

Table 2: Most Common Pathogens and Percent of Resulting HAI's [15].

10 Most Common pathogens in Hospital-Acquired Infections	Percent of Hospital-Acquired Infections
Coagulase-negative staphylococci	15%
Staphylococcus aureus	15%
Enterococcus species	12%
Candida species	11%
Escherichia coli	10%
Pseudomonas aeruginosa	8%
Klebsiella pneumoniae	6%
Enterobacter species	5%
Acinetobacter baumannii	3%
Klebsiella oxytoca	2%

2.2.2 Financial Implications of Infection

If the risk of infection is not contained and an HAI occurs, a major implication that the patient and hospital would have to endure is the financial impact. HAI's are a significant source of economic burden, largely due to prolonged hospitalization and readmission costs. In the United States, additional hospital expenditures due to HAI cost $6.5 billion annually [17]. However, the risk of HAI's and SSI's has decreased in recent years. In the United States, the rate of SSI after cardiac procedures decreased by 30% over the course of 2008 to 2012 [18]. This drop in rate of SSI is largely attributed to healthcare policymakers developing programs to target the prevention of hospital-acquired infections, including denial of reimbursement of extra costs associated with preventable infection, mandatory public reporting of hospital HAI rates, and institution adherence to national quality measures [19]. Typically, the studies that address the economic impact of HAI's strictly rely on billing datasets [20]. For example, a coronary artery bypass graft (CABG) performed on Medicare beneficiaries cost $32,201 ± $23,059, with a length of stay of 9.9 days ± 7.8 days [20]. However, patients that experienced postoperative complications and infection cost an average additional $15,468, and had an increased length of stay of 5.3 days [20]. The disadvantage of the studies that use strict billing data is that they do not account for the patient's baseline clinical status, which results in large variation in their clinical outcomes.

In comparison, studies have been done that compare demographics, baseline laboratory results, and comorbidities in the patients. By including these other factors, it can be predicted that patients with congestive heart failure, hypertension, and history of stroke have a higher incidence of HAI, and therefore a higher cost of care [21]. In a study

by Greco *et al*., the average length of stay for patients with HAI increased to 33 days, versus 9 days for patients without an HAI [21]. The full analysis of the cost of care for patients with and without major infection is found in Figure 2. In Figure 2, patients without infection peak at a cost of roughly $5,000 per day, which quickly drops to about $2,000 per day. In contrast, patients with infection sustain a higher daily cost of care for a longer length of stay, with a final cost totaling $38,000 higher than those without infection [21]. The bulk of this increased hospital cost results from ICU costs (47%), medications (12%), hospital supplies (12%), and lab costs (10%) [21]. These additional costs are outlined in Figure 3.

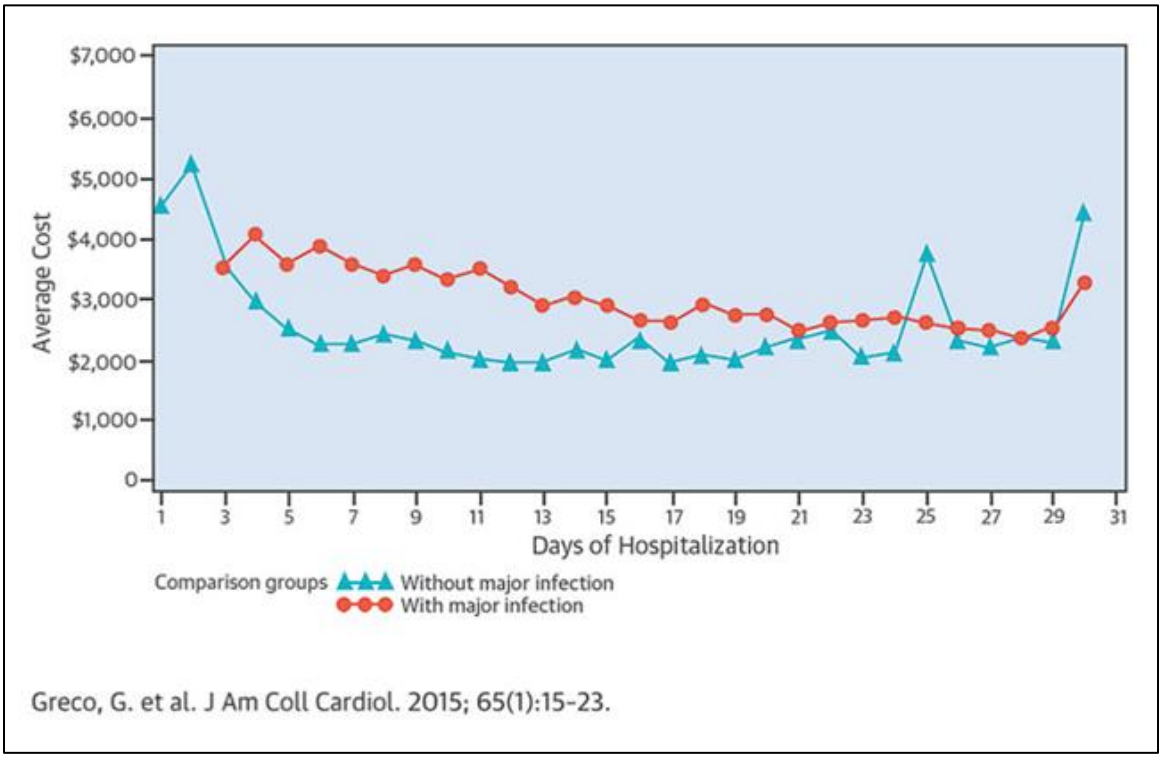

Figure 2: The Increase in Hospital Cost and Increased Length of Hospitalization for Patients With and Without an HAI [21].

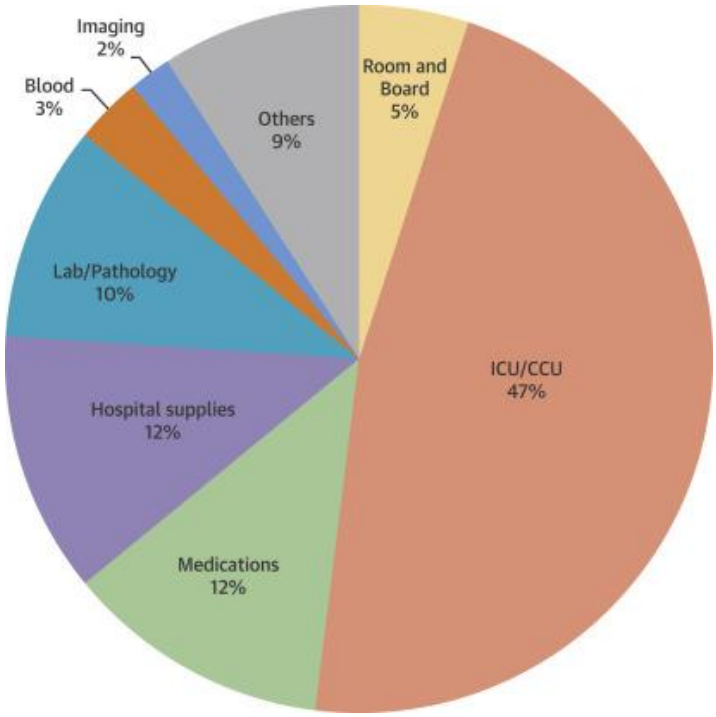

Figure 3: The Distribution of Costs Associated With HAI Patients [21].

2.2.3 Morbidity and Mortality Implications of Infection

The most detrimental risk associated with a break in sterile practice is the increased risk of patient morbidity and mortality. Hospital-acquired infections have been implicated in the deaths of over 99,000 individuals annually in the United States [17]. In a study by Gelijns *et al*., it was found that most common infection sites that increase patient morbidity are incisional SSI, secondary incision sites (such as for vein harvesting in CABG patients), mediastinitis, infectious myocarditis, endocarditis, cardiac device infection, pneumonia, *Clostridium difficile* colitis, and septic blood infection [17]. These common infection types are outlined in Table 3.

Table 3: Most Common Infection Types Among Patients with HAI's [17].

Type of Infection	# of Events	# of Patients	% of Patients (N=5158)	Days From Surgery to First Infection		
				Median	Min	Max
Pneumonia	125	123	2.38	8	1	62
Bloodstream Infection	59	56	1.09	15	0	65
C. Difficile Colitis	52	50	0.97	17	3	63
Deep Incision Surg site infection (chest)	26	26	0.56	20.5	5	54
Mediastinitis	12	12	0.23	24.5	6	60
Deep Incision Surg site infection (groin)	10	10	0.21	26	6	49
Myocarditis or Pericarditis	5	4	0.08	16	14	27
Empyema	4	3	0.06	56	13	63
Endocarditis	3	3	0.06	25	25	51
Device-related precut site infection	3	3	0.06	54	9	62
Pocket Infection	2	2	2.33	38.5	15	62

Gelijns *et al*. also suggest that left ventricular assist device (LVAD) and transplant patients have higher risk of infection than other cardiac patients [17]. Patients with chronic lung disease, heart failure, and elevated creatinine also have a higher risk of postoperative infection [17]. In the majority of cases of infection in the investigation by Gelijns *et al.*, the onset of symptoms occurred most frequently several weeks to one month after surgery [17]. Despite the administration of prophylactic cephalosporin, patients were still infected with Gram-positive and Gram-negative microbial isolates [17]. For mortality, the 65-day mortality rate for infected patients was 5%, while for non-infected patients, the mortality rate was 0.7%. Additionally, the mortality rate for men was half of the mortality rate for women [17]. A Mantel-Byar test was performed and a curve was constructed to illustrate the increased risk of mortality associated with infection during the postoperative period, which is depicted in Figure 4.

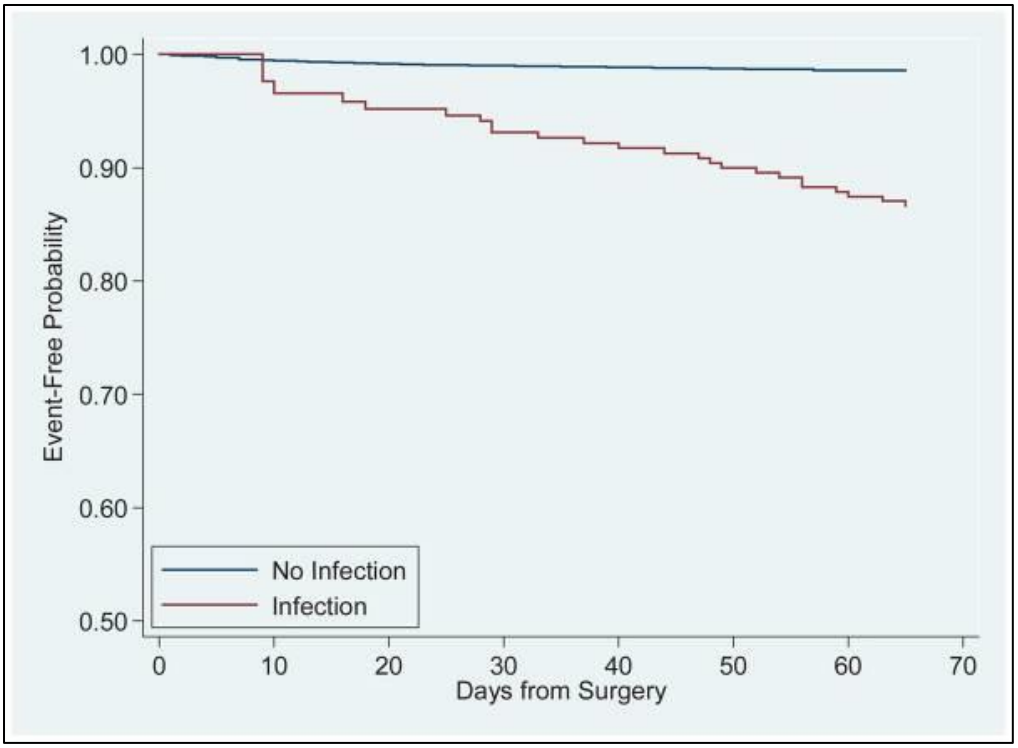

Figure 4: Mantel Byar Survival Curve for Patients With and Without an HAI [17]. Over the 65-day post-surgical follow-up period, 97 deaths occurred.

2.3 Sterility and the Extracorporeal Circuit

Because of the detrimental effects that infection has, it is of utmost concern that the risk of infection is controlled and minimized. However, the dynamic nature of the operating room may pose threats to patient and field sterility. With many people moving throughout the operating room, machines being moved towards and away from the operating table, there are seemingly an infinite number of ways that the sterile field may become compromised. With respect to the cardiopulmonary bypass circuit, another set of unique challenges to sterility are presented. While the general principles of aseptic technique are established and followed, there is a high level of hospital-to-hospital variation in regards to circuit setup, priming, and storage. In many instances, the protocols that ensure CPB circuit sterility are set up and maintained by the perfusion staff

themselves. The CPB equipment, including cardioplegia delivery system and heater-cooler device, all require special attention to ensure their sterility.

2.3.1 Sterilization of the CPB Circuit

According to the life cycle of decontamination in Figure 1, the CPB circuit is received after the “Transport” stage, and terminates after the “Use” stage. As stated by WHO standards, it is the duty of personnel receiving and using the sterile equipment to report if there is any indication that the equipment received may not be sterile [5]. The primary technique used to sterilize disposable equipment used in CPB circuits is gas treatment by ethylene oxide, which has bactericidal, sporicidal, and virucidal activity [22].

For many pieces of sensitive medical equipment, ethylene oxide (EO) treatment is the only acceptable sterilization method [23]. The mechanism behind EO treatment is simple and efficient. EO is a direct alkylating agent that adds alkyl groups to DNA, proteins, and RNA in microorganisms [23]. By binding to the hydroxyl, amino, and carboxyl groups in microorganisms, it prevents normal cellular metabolism and renders bacteria, viruses, and spores nonviable [22]. On the other hand, disposable medical devices, such as polyvinyl chloride (PVC) tubing used in the CPB circuit, do not have a structural change in reaction to EO [22]. This makes EO treatment a desirable process to sterilize the disposable CPB circuit.

There are many advantages of EO sterilization over other methods, such as steam or dry heat. First, the use of EO is widely advantageous in the sterilization of medical devices because many materials are sensitive to high temperature and moisture [23].

Steam and gamma irritation may cause degradation of PVC polymers, which can lead to diminished physical properties that would be detrimental to the performance of the material [24]. The cost of EO sterilization is also an advantage, leading to minimal increased cost of the manufacturing costs of medical equipment [23]. EO sterilization has rapid activity, strong penetrability, and material compatibility that make it an effective and cost-effective way to sterilize sensitive medical equipment.

2.3.2 Pump Assembly, Priming, and Storage

When a sterile pack is received and stored for future use, it is typically the task of the perfusionist or perfusion assistant to assemble the circuit in a sterile manner. In addition, it is imperative that those responsible ensure that any equipment is not stored past the date of expiration and does not feature a defective or abnormal appearance [5]. Any product that is found to have questionable sterility should be returned to the vendor or manufacturer [5]. If all equipment is deemed sterile, the perfusionist will undergo a thorough handwashing before opening the extracorporeal circuit components. The trained perfusionist or perfusion assistant will then use an aseptic technique throughout the assembly of the CPB circuit.

If the circuit is left assembled for future priming, it is the task of the perfusion team to maintain the sterility of the circuit before its priming and eventual use. Ultimately, it is the duty of the healthcare provider to provide protocols for the hospital's standards related to storage of dry circuits. In a study to determine exactly how long a circuit with a vented reservoir can remain sterile before priming, Searles *et al*. determined that the level of contamination within 72 hours of dry assembly is insignificant [25].

Furthermore, in an investigation of 100 circuits, Lonský *et al.* determined that a dry circuit can remain sterile for up to 90 hours before use [26]. In literature regarding closed systems, Tan *et al.* established that Extracorporeal Membrane Oxygenation (ECMO) circuits can maintain dry sterility for up to 65 days [27]. Thus, data suggest that extracorporeal circuits may remain unused for a period of time, given that they are assembled in a sterile fashion.

Once the circuit has been assembled in a sterile manner, the perfusionist then proceeds to prime the circuit. The extracorporeal circuit manufacturer provides guidelines on the proper addition of fluid to the circuit. Typically, the circuit is primed with an appropriate crystalloid solution. The fluid is then circulated through an inline pre-bypass filter for a minimum of five minutes (local protocol). The circuit is then meticulously inspected for air and gaseous microemboli (local protocol). After priming with fluid, the circuit can either be used in cardiac surgery, or stored for future use.

If the circuit is primed and stored for future use, the healthcare provider should maintain protocols that outline the storage duration and conditions that maintain the sterility of the CPB circuit. Similar to studies on the safe storage duration of dry circuits, the safe storage duration of primed circuits has also been investigated. In a study using a hard-shell open-reservoir, Tagaya *et al.* established that the primed CPB circuit can maintain sterility for up to 144 hours after initial priming [28]. Further, the study also determined that there were no bacterial endotoxins present in the circuit samples [28]. Apart from the risk of microbial contamination, this study also investigated the possibility of release of chemical substances from prolonged standby. By using the molecular weight spreads of their samples, Tagaya *et al.* determined that there was no

chemical release from the circuit when placed on standby for 144 hours [28]. Finally, Young *et al.* determined that open-reservoir CPB circuits set up and stored in an open operating room can maintain sterility for up to 168 hours, or 7 days [29].

2.3.3 Heater-Cooler Contamination, Cardioplegia Contamination

Despite the efforts that are put forward in maintaining circuit sterility during the immediate preoperative period, it is possible that the advent of new technologies and devices and ancillary devices may come with a threat to patient safety. *Nontuberculous mycobacteria* is an opportunistic pathogen that causes infection in immunocompromised patients, and has historically been linked to hospital-acquired infections [30]. Outbreaks of nontuberculous mycobacteria (NTM) have been reported as recently as 2015, causing infection in patients who recently underwent heart surgery [30]. In spring 2015, researchers in Switzerland reported an outbreak of the infection with a distinct species, *Mycobacterium avium,* and were able to isolate the bacteria from heater-cooler devices (HCD) used by the perfusionist in cardiac surgery [31]. In July 2015, another outbreak of infections arose, this time in the United States. The Wellspan York Hospital in York, Pennsylvania performed a case-control study to identify sources of the infection [32]. In a study of 11 infected patients, eight of the patients had *Mycobacterium avium* isolated from their sites of infection [32]. In an infection control and environmental assessment, it was determined that the Stockert Heater-Cooler 3T system was the source of contamination [32]. The low infection rate, affecting 0.4% of patients undergoing heart surgery at the Wellpsan York Hopsital, made identification of the contamination source difficult [32]. However, laboratory testing and manufacturer literature confirmed that *M.*

avium is subject to aerolization with the Stockert 3T HCD venting system [32]. The same equipment was implicated in the infections found in European studies [31]. Based on findings from the global crisis that ensued, the CDC made several recommendations to hospitals using the Stockert 3T HCD to enhance detection and surveillance of HCD-related infections [32].

Another source of perfusion-related contamination that has been reported is the cardioplegia delivery system. Reports of contaminated cardioplegia solution date back to 1986, with five patients dying because of solution contaminated with *Enterobacter cloacae* [33]. Other patients, while not dying from infection, incurred early bleeding, needing reoperation, and additionally experienced mycotic aneurysms during the postoperative period [33]. Other complications included sternal infections in six patients, adult respiratory distress syndrome (ARDS) in three patients, renal failure in four patients, and organic brain syndrome in five patients [33]. More recently, a 2005 outbreak due to contaminated cardioplegia in Virginia resulted in the deaths of three patients over the course of eight days because of multiple Gram-negative Bacilli contamination [34]. An additional eight patients suffered from systemic inflammatory response syndrome (SIRS), but made full recoveries [34].

Thus, contamination of the cardiopulmonary bypass circuit is not only limited to the circuit itself. Ancillary devices such as the heater-cooler or cardioplegia delivery system are also potential sources of contamination, and have been implicated as sources of bacterial contamination in the past [30, 33]. By analyzing all aspects of the CPB circuit, the perfusionist can maintain sterility of all equipment and reduce the risk of infection to the patient.

3. Project Statement

With the news of contaminated heater-cooler devices implicated in numerous deaths in the United States and Europe, there is renewed interest on the sterility of CPB circuits and associated devices. The goal of this study had three aims, which seek to investigate the CPB circuit for potential infectious contaminants. These potential contaminants were enumerated by counting colony forming units, or CFU.

The first aim was to analyze potential contaminants in a primed circuit from the first day of priming with crystalloid solution until the seventh day of circuit standby. Based on previous studies, the following hypothesis was tested:

1. After seven days, the crystalloid fluid from a primed circuit will not contain any detectable CFU.

The second aim was to analyze a dry, sterile-assembled circuit for proper storage conditions. At Aurora St. Luke's Medical Center (ASLMC) in Milwaukee, Wisconsin, CPB circuits are assembled in a sterile fashion and stored for future use in a perfusion room for up to 30 days (local protocol). During the storage period, a semi-permeable sterile gown is placed on the circuit, protecting the circuit from environmental contamination. The goal of the second experimental setup was to establish how long a sterile gown can be used to protect the CPB circuit from the environment, and subsequently to determine if the sterile gown becomes more contaminated than an uncovered CPB circuit. Thus, the following hypotheses were tested:

2. The amount of time after opening a sterile gown will directly correlate with the amount of CFU detected on the gown.

3. If a circuit is left uncovered and open to the perfusion room environment for 24 hours, it will contain fewer CFU than a re-used sterile gown after 24 hours.

The final aim was to assess circuit vulnerability to contamination during the period of drug administration. At ASLMC, pharmacological agents are commonly added to the CPB circuit through an open 60 cc syringe placed at the top of the cardiotomy. By testing the environmental air for contaminants by using “settle plates”, the environment that the 60 cc syringe is exposed to is also tested. Thus, the final hypothesis was:

4. Settle plates in the perfusion room will increase in CFU in a time-dependent manner.

4. Materials and Methods

4.1 Sterility after 168 Hours of Standby

Previous studies have shown that an open system can maintain sterility for up to 168 hours [29]. This study sought to confirm these results using an open system at ASLMC. In this experiment, a Terumo System 1 pump was assembled using sterile technique. The pump consisted of a roller pumphead, Terumo Capiox FX25 oxygenator with integrated arterial filter, Terumo Capiox 4,000 mL hard-shell reservoir, and 3/8 inch arterial line and ½ inch venous line polyvinyl chloride tubing with X-coating. The cardiopulmonary bypass circuit was then primed with 1500 mL of Plasmalyte, which was recirculated through a 0.2 micron pre-bypass filter to remove manufacturing debris and potential contaminants. After sterile removal of the pre-bypass filter, the pump was shut down by placing clamps on the superior and inferior venous lines and closing all purges. A system pressure of 100 mmHg was maintained during shutdown periods, and the pump was stored with a sterile cover.

Prior to sampling periods, the pump was run at 5 L/minute at a system pressure of 100 mmHg for 5 minutes. Thirty mL of fluid was then sampled from a sampling manifold at 0, 24, 48, 72, 96, 120, 144, and 168 hours. The same sampling manifold was used for all successive sampling, swabbing with alcohol before and after sampling. A new sterile syringe was subsequently placed on the sampling manifold to maintain sterility during storage periods. Within 4 hours of attaining samples, they were plated on Tryptic Soy Agar (TSA) (Becton Dickinson, Cat. No. 257106). The samples were plated in duplicate in 0.1 mL and 1 mL aliquots, for a total of 32 plates. If samples were unable to be plated within 4 hours, they were placed in a 4° C cooler until plating. Inoculated plates and one

control plate were then inverted and incubated for 48 hours at 37° C. After incubation, plates were enumerated by counting CFU.

TSA was used because it is a general purpose growth medium that supports the growth of non-fastidious microorganisms, such as *Enterobacteriaceae*, *Pseudomonas*, enterococci, staphylococci, and *Bacilli* [35]. Many of these bacteria are found in Table 1 and Table 2, as they are commonly implicated in HAI [15]. Since TSA is able to support a wide range of bacteria, it is commonly used in the methods for the examination of water, waste-water, and foods [35].

4.2 Sterile Gown and Reservoir Sampling

In many perfusion practices, CPB circuits are assembled and stored for future use. Depending on the practice, the circuit may be stored in an operating room or in a separate perfusion room for several days. At ASLMC, the perfusionist assembles the circuit in a perfusion room, and places a sterile gown on the pump as a barrier between the circuit and the environment. This sterile gown acts as a last line of defense if the pump becomes erroneously disassembled, which could result in contamination of the circuit. However, it is commonplace that sterile gowns are re-used from circuit to circuit, and are therefore no longer sterile after the first use. Additionally, these gowns are semi-permeable to the environment. Thus, it was the goal of this testing to determine if a re-used sterile gown contains more contaminants than an uncovered CPB venous reservoir.

In this experiment, microbiological swabs (3M, Cat. No. RS96010BPW) containing 10 mL buffered peptone water broth were used to determine the microbiological makeup of a sterile gown after 0, 24, 48, 72, 96, 120, 144, and 168 hours.

The gown was used and re-used as a pump cover for the duration of the testing period, without additional sterilization between sampling periods. Sampling of the sterile gown consisted of removing the swab from the sampling tube, and placing the tip of the swab on a 10 x 10 cm area of the gown that comes into direct contact with the circuit. The swab was streaked in a horizontal fashion, ensuring that the entire swab tip comes into contact with the gown. The swab was placed back in the tube, and then re-sampled the area in a vertical fashion. Finally, the swab was once again placed in the tube and sampled the gown in a horizontal fashion again. The sampling procedure is shown in Figure 5. For the period of 7 days, five plates were inoculated on TSA with 0.1 mL of aliquots, for a total of 40 plates.

Figure 5: Swabbing Technique Used for Sterile Gown and Reservoir Sampling.

The same swabbing procedure was then applied to a venous reservoir in the perfusion room. The reservoir was swabbed immediately after assembly, and then stored without a sterile gown and left open to the environment. After 24 hours of storage, the reservoir was swabbed again. This methodology was used to ascertain if a pump without a re-used gown would contain the same amount of CFU as the gown itself. Aseptic

technique was used when swabbing, and seven plates were inoculated with 0.1 mL aliquots from each swabbing period, for a total of 14 plates.

All samples were plated within 4 hours of sampling. If samples were unable to be plated within 4 hours, they were placed in a 4° C cooler until plating. Inoculated plates and a control plate were then inverted and incubated for 48 hours at 37° C. After incubation, plates were enumerated by counting CFU.

4.3 Environmental Sampling

Previous studies have demonstrated that time-dependent contamination occurs in operating rooms, even when laminar flow and positive pressure are employed [9]. Additionally, in the perfusion room at ASLMC, an open 60 cc syringe is commonly used for the addition of pharmacological agents into the CPB circuit. This syringe typically remains open to the perfusion room environment, and is used throughout the duration of the surgery.

In this experiment, “settle plates” were used to determine how many CFU result from the settling of airborne contamination in an environment. Settle plates are used in medical and pharmaceutical industries to monitor air quality by detecting bacteria and fungi that descend onto the plate [36]. The settle plate is then incubated, and the number of CFU is determined. Here, TSA settle plates were used to determine the amount of time-dependent contamination that occurs in both the operating room and perfusion room. In the perfusion room, settle plates were left open on the East and West sides for 5, 15, 30, 60, and 120 minutes. In the operating rooms, settle plates were left in OR three, seven, and four for 5, 15, 30, 60, and 120 minutes. Additionally, the surgeon, procedure,

and date of sampling were noted. A total of 20 samples were taken from the OR, and 30 samples were taken from the perfusion room. After the exposure periods, the plates were closed, inverted, and incubated with a control plate for 48 hours at 37° C. After incubation, plates were enumerated by counting CFU.

4.4. Statistical Analysis

Statistical analysis was performed with Minitab 17 software. All values are expressed as mean CFU/100 μL ± standard deviation. A *p* value less than 0.05 was considered significant for all statistical tests. The R^2 value was used to determine the degree in variability that could be explained by the predictor variables.

To compare CFU on a used sterile gown over the course of seven days, a one-way ANOVA was used with a Tukey Pairwise comparison. Additionally, a one-way ANOVA was used to compare CFU on an uncovered venous reservoir from the time of assembly until 24 hours. For the air quality samples, a regression was performed to analyze data found between the operating room settle plates and perfusion room settle plates. To compare within-group samples from the perfusion room and operating room, one-way ANOVA was used. For operating room settle plates, a multiple regression was used to compare CFU to varying operating conditions. For perfusion room settle plates, a Tukey Pairwise comparison was used to compare the mean CFU from each time period. Assumptions of the data for statistical testing include normality, equal variance, independence, and multicollinearity.

5. Results

5.1 Sterility after 168 Hours of Standby

The CPB circuit was maintained within a controlled environment for 168 hours, or 7 days. There was no microbiological growth detected in any samples or controls, as shown in Table 4.

Table 4: CFU Counts from CPB Circuit on Standby 168 Hours after Priming.

Sampling Date	Hours Since Priming	100 μL CFU	1 mL CFU	Control
1/15/2018	0	0	0	0
1/16/2018	24	0	0	0
1/17/2018	48	0	0	0
1/18/2018	72	0	0	0
1/19/2018	96	0	0	0
1/20/2018	120	0	0	0
1/21/2018	144	0	0	0
1/22/2018	168	0	0	0

5.2 Sterile Gown and Reservoir Sampling

The sterile gown was maintained in rotation for 168 hours, or 7 days. Inoculated and control TSA plates were evaluated for microbial growth after the 48-hour incubation period. As time since the opening of the gown increases, the number of CFU/100 µL increases. This is illustrated in Figure 6. One-way ANOVA analysis shows that time is a predictor variable for increase in CFU in the samples (R^2 of 83%). A Tukey Pairwise comparison indicates that time significantly affects CFU/100 µL between time 0 and all other tested time points (p<0.001).

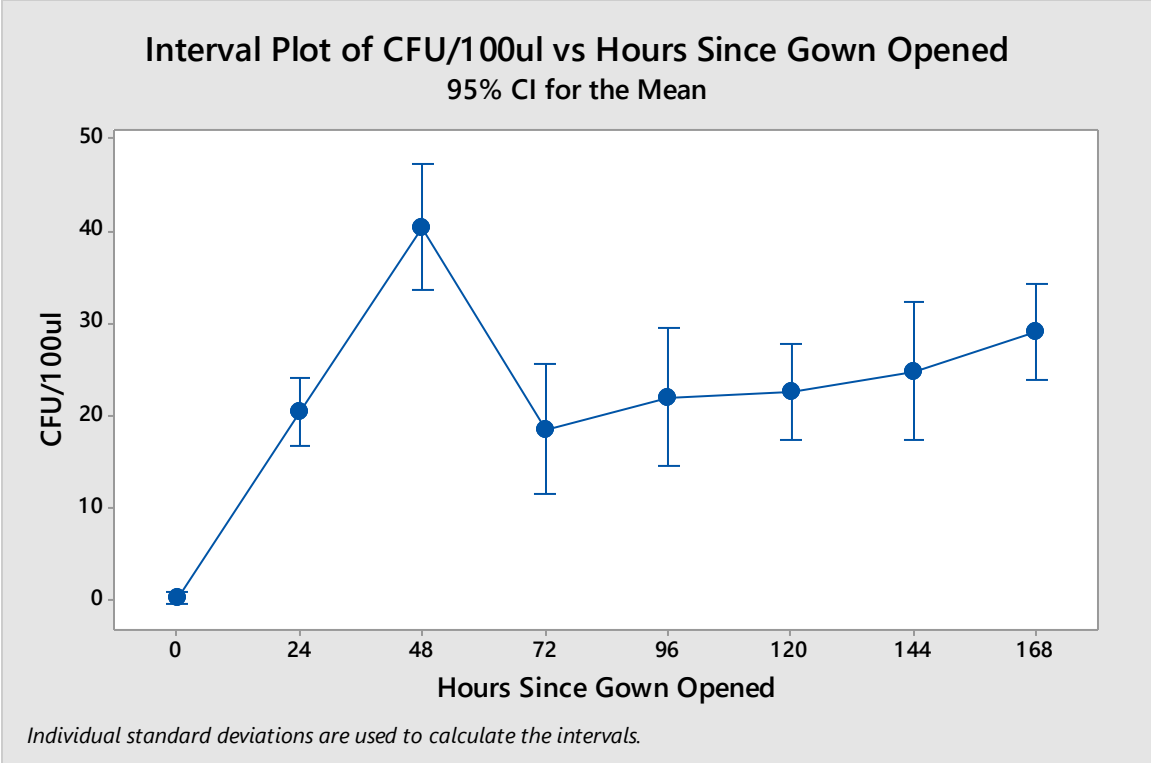

Figure 6: Interval Plot of CFU/100 µL versus Time in Hours since Opening the Sterile Gown (p<0.001).

As a follow-up to the sterile gown testing, the same sampling procedure was applied to a venous reservoir in the perfusion room, which was stored without a sterile gown and open to the perfusion room environment. One-way ANOVA analysis shows that time is not a predictor variable for increase in CFU in these samples (R^2 = 3%). These data are illustrated in Figure 7.

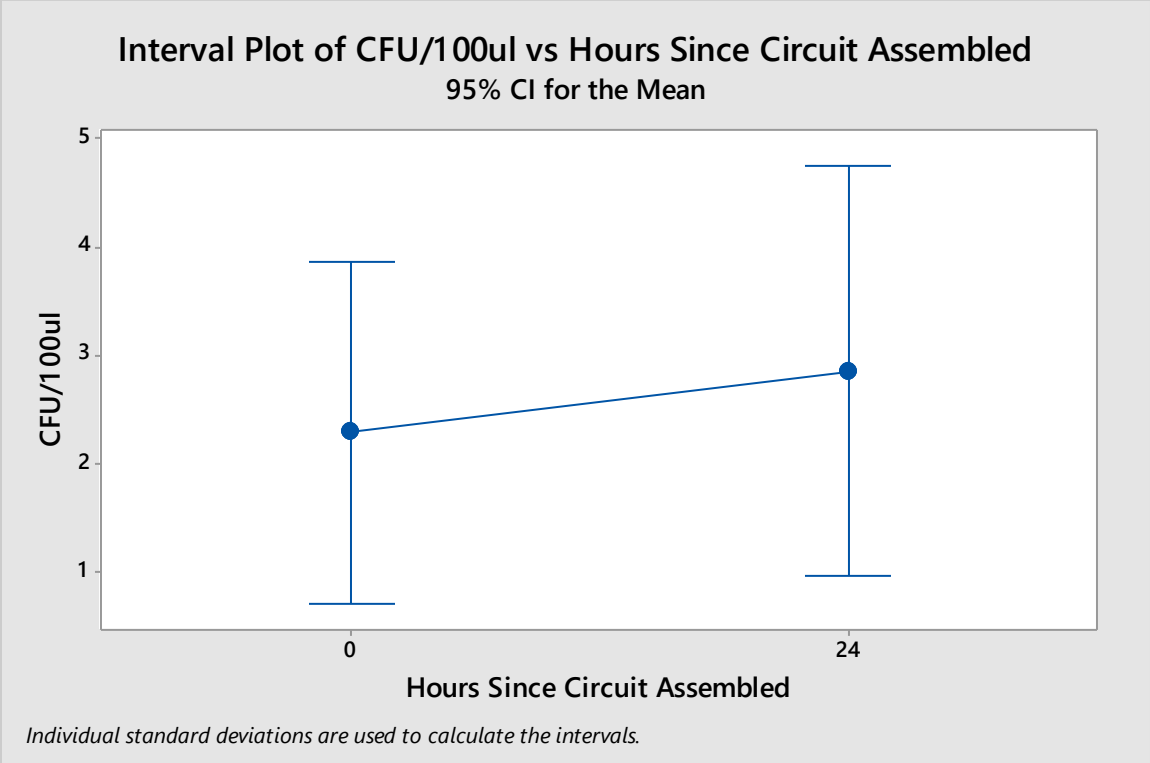

Figure 7: Interval Plot of CFU/100 μL versus Time in Hours since Circuit Assembly (*p*=0.58).

5.3 Environmental Sampling

Settle plates from the OR and perfusion room were evaluated for microbial growth after the 48-hour incubation period. The interaction plot for CFU and sampling location indicates that the perfusion room samples have a larger mean CFU count than corresponding OR samples for all time points, as illustrated in Figure 8. Additionally, regression analysis indicates that the interaction between location and time of sampling is statistically significant (p=0.015).

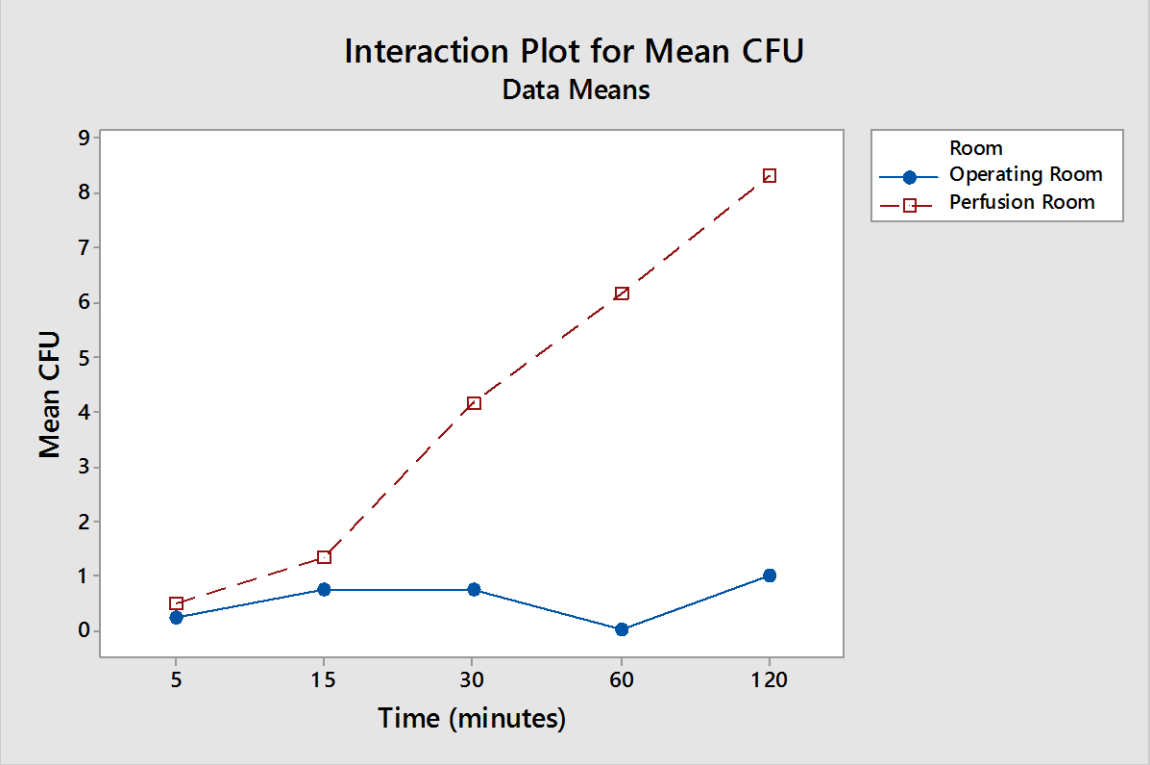

Figure 8: Interaction Plot for Mean CFU and Time in the OR and Perfusion Room.

A one-way ANOVA indicates that time is a significant factor on the number of CFU in the perfusion room (p=0.006). However, time is not a significant factor on the number of CFU in the operating room (p=0.44). Additionally, a multiple regression reveals that the surgeon (p=1.0) and day of sampling (p=0.162) were also insignificant factors on the number of CFU in the operating room. A Tukey Pairwise Comparison indicated that time does not significantly affect CFU for any time points in the OR. Subsequently, a Tukey Pairwise Comparison was used to compare the mean CFU for each time period in the perfusion room. The analysis indicates that time affected CFU significantly between the 120-minute and 5-minute periods, as well as the 120-minute and 15-minute periods.

Finally, the scatterplot with regression line of CFU in the operating room versus CFU in the perfusion room indicates that there is a high level of variability in the perfusion room samples for the 30-, 60-, and 120-minute sampling points. At the 15-minute sampling period, perfusion room settle plates contained 1.3 ± 0.52 CFU. This increased to 4.2 ± 4.2 CFU at the 30-minute sampling period.

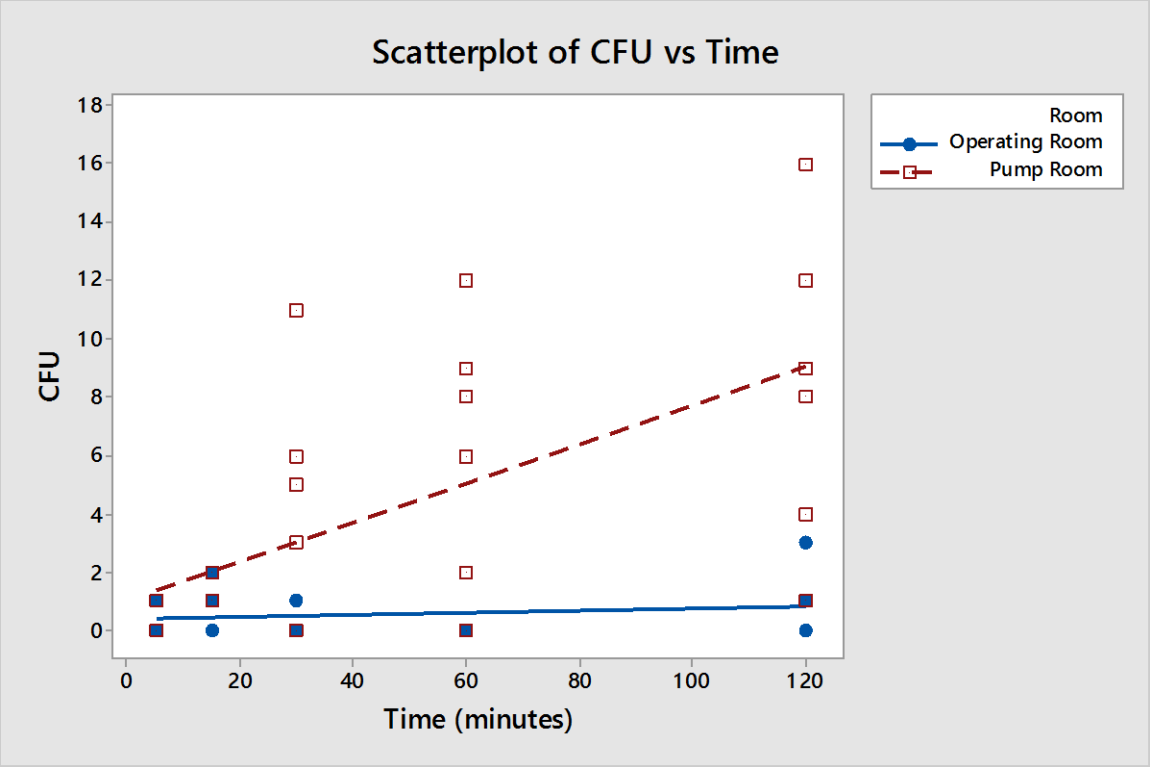

Figure 9: Scatterplot with Regression Line of CFU in the OR versus Perfusion Room.

6. Discussion

6.1 Sterility after 168 Hours of Standby

The goal of this study was to determine if a primed CPB circuit will be free of CFU after seven days of standby. The results of the circuit sterility study indicate that the recirculated pump fluid can maintain sterility for at least seven days in a setting mimicking clinical practice. These results indicate that no bacterial contamination was introduced to the circuit during the setup period, the priming period, or during the storage period. Additionally, the pump was not stored in an environment with positive pressure, indicating that the lack of positive pressure does not influence bacterial dissemination in a stored circuit. Finally, these data indicate that the venous reservoir and circuit are adequately sealed and protected from the environment, as the venous reservoir of a CPB circuit contains measureable contamination immediately after assembly, as indicated in Figure 7.

6.2 Sterile Gown and Reservoir Sampling

The results of the sterile gown sampling demonstrate that a sterile gown maintains sterility for less than 24 hours with re-use. All sterile gown samples from time 0 were free of bacterial contamination, indicating that the gown was introduced to contaminants after the initial sampling period. With the exception of samples taken at the 48-hour mark, all CFU results after 24 hours were within one standard deviation of subsequent samples. This indicates that after 24 hours, contamination of the gown does not continue to significantly increase in a time-dependent manner.

The results of sampling an uncovered venous reservoir denote that the increase in CFU after 24 hours is insignificant. Furthermore, after 24 hours, the average CFU/100 μL of a sterile gown was 20.4 ± 2.97, versus 2.9 ± 2.04 for an uncovered venous reservoir. Thus, the increase in CFU after 24 hours for the sterile gown is explained by factors beyond the air quality conditions of the perfusion room. Therefore, handling and re-use of sterile gowns must be addressed in the future.

6.3 Environmental Sampling

Previous studies had found that sterile trays left open in operating rooms become contaminated with increased duration of open exposure [12]. However, the results from operating room settle plates at ASLMC indicate that contamination does not significantly occur after two hours of exposure. This is in contrast with previous findings by Andersson *et al.* and Baird *et al.*, whose studies indicated that bacterial counts increased significantly with time and number of personnel in the operating room [9, 10]. Additionally, there was no significance found between contamination and different surgeons or date of sampling, which indicates that similar environments were provided for all surgical procedures.

However, the findings indicate that contamination does occur in a time-dependent manner in the perfusion room. At 30 minutes of exposure, over three times the amount of CFU (1.3 ± 0.52 vs 4.2 ± 4.2) was found in the perfusion room samples than in the operating room samples. This indicates that the operating room environment is less susceptible to contaminants than the perfusion room environment, and that additional precautions should be taken in the perfusion room to prevent circuit contamination.

Additionally, there is a large amount of variation in the perfusion room air samples. This can likely be attributed to room traffic of non-sterile personnel, the frequent opening and closing of doors, and the lack of positive pressure in the perfusion room. By attenuating these factors, the variability in air quality samples may decrease.

6.4 Testing Limitations

One limitation of this testing is the use of TSA as the growth medium. While TSA is used in testing for general microorganism contamination, it is limited in that it does not support the growth of fastidious bacteria, such as *Haemophilus* or *Neisseria* species, which have specific growth requirements [37]. Additionally, an incubation period of 48 hours at 37°C does not support the growth of slow-growing bacteria, such as *Mycobacterium*, which was implicated in recent heater-cooler device contamination incidents [32]. Growth of *Mycobacterium* varies by species, and requires several weeks to a month for colony detection [38]. Future studies could include testing on selective agars and use of variable incubation times. Another limitation is that this study is unable to establish a casual direct relationship between growth on TSA and SSI rates. While many bacteria have the ability to be opportunistic pathogens, other contaminating bacteria found in this study are not. For this reason, future studies could utilize an assay for endotoxin detection, or utilize identification tools such as PCR to identify colonies as opportunistic pathogens.

Another limitation of this study is that sampling of the venous reservoir was limited to 24 hours because of difficulty designating a circuit for non-use for beyond that period. Future studies could include extended periods of testing an uncovered CPB circuit

to ascertain if significance is found with increased exposure to the perfusion room environment. Additionally, settle plates in the OR and perfusion room had a maximum exposure time of two hours. Future studies could increase the exposure period to establish if there is significant contamination in the operating room at ASLMC for longer surgical procedures. Finally, settle plates in the OR were not sampled from the sterile field, but rather the periphery of the operating room. Thus, they were exposed to traffic of non-personnel entering and leaving the OR.

7. Conclusion and Recommendations

Postoperative infection is major risk in cardiac surgery. It is of utmost importance that operating room personnel, including the perfusionist, provide sterile surgical equipment to attenuate the risk of infection. The results in this study indicate that a primed and recirculated open CPB circuit can maintain sterility for at least seven days after priming. This information may be used to increase the flexibility of using primed CPB circuits in the clinical setting. Current policy at ASLMC designates the use of a primed circuit up to 96 hours, or 4 days. Here, it is indicated that with sterile assembly, priming, and storage, hospital policy could be updated to prolong safe storage to seven days.

Swabs of re-used sterile gowns in the perfusion room indicate that after 24 hours, the re-used gowns contain significant contamination. Furthermore, they contain more contamination as CFU/100 μL than a venous reservoir that is stored uncovered in the same environment. This suggests that sterile gowns should not be re-used. Given that a CPB circuit may remain assembled up to thirty days before use (local protocol), the circuit should be covered with a new, sterile gown immediately after assembly. Moreover, impermeable gowns may serve as a more effective cover for CPB circuits than semi-permeable gowns.

Finally, the analysis of settle plates in the operating room and perfusion room provides several recommendations for maintaining a sterile circuit both preoperatively and intraoperatively. First, after opening any syringe, needle, or other medical device in a sterile fashion, it should be used within 15 minutes of exposure to the perfusion room environment. After 15 minutes, the device should be disposed of, because of the threat of

contamination. Although this contamination may be statistically insignificant, there is a high variability of air contaminants resulting from lack of positive pressure and the movement of non-sterile personnel. Thus, pharmacological agents and pump additives should be added directly through a sterile manifold or other sterile route of entry. Second, after opening any medical device in a sterile fashion, it may remain sterile for at least two hours in the operating room. In the practice of perfusion, this indicates that uncovered manifolds, open syringes used to administer pharmacological agents, or any tubing with open ends may be used after two hours of exposure to the operating room environment.

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Appendix A: Raw Data

Table A-1: Sterility after 168 Hours of Standby.

Sampling Date	Hours Since Priming	100 µL CFU	1 mL CFU	Control
1/15/2018	0	0	0	0
1/15/2018	0	0	0	0
1/16/2018	24	0	0	0
1/16/2018	24	0	0	0
1/17/2018	48	0	0	0
1/17/2018	48	0	0	0
1/18/2018	72	0	0	0
1/18/2018	72	0	0	0
1/19/2018	96	0	0	0
1/19/2018	96	0	0	0
1/20/2018	120	0	0	0
1/20/2018	120	0	0	0
1/21/2018	144	0	0	0
1/21/2018	144	0	0	0
1/22/2018	168	0	0	0
1/22/2018	168	0	0	0

Table A-2: Sterile Gown and Reservoir Sampling.

Sampling Date	Hours Since Gown Opened	CFU/100 µL	Control
1/16	0	0	0
1/16	0	0	0
1/16	0	1	0
1/16	0	0	0
1/16	0	1	0
1/17	24	24	0
1/17	24	22	0
1/17	24	16	0
1/17	24	20	0
1/17	24	20	0
1/18	48	35	0
1/18	48	45	0
1/18	48	45	0
1/18	48	34	0
1/18	48	43	0
1/19	72	15	0

Table A-2: Sterile Gown and Reservoir Sampling (continued).

Sampling Date	Hours Since Gown Opened	CFU/100 µL	Control
1/19	72	19	0
1/19	72	11	0
1/19	72	25	0
1/19	72	23	0
1/20	96	12	0
1/20	96	24	0
1/20	96	24	0
1/20	96	22	0
1/20	96	28	0
1/21	120	19	0
1/21	120	29	0
1/21	120	19	0
1/21	120	24	0
1/21	120	22	0
1/22	144	15	0
1/22	144	31	0
1/22	144	27	0
1/22	144	27	0
1/22	144	24	0
1/23	168	25	0
1/23	168	36	0
1/23	168	28	0
1/23	168	29	0
1/23	168	27	0

Table A-2: Sterile Gown and Reservoir Sampling (continued).

Reservoir Sampling date	Hours Since Circuit Assembled	CFU/100 µL	Control
2/01/2018	0	2	0
2/01/2018	0	5	0
2/01/2018	0	0	0
2/01/2018	0	1	0
2/01/2018	0	2	0
2/01/2018	0	2	0
2/01/2018	0	4	0
2/02/2018	24	4	0
2/02/2018	24	2	0
2/02/2018	24	6	0

Table A-2: Sterile Gown and Reservoir Sampling (continued).

Reservoir Sampling date	Hours Since Circuit Assembled	CFU/100 µL	Control
2/02/2018	24	1	0
2/02/2018	24	0	0
2/02/2018	24	3	0
2/02/2018	24	4	0

Table A-3: Environmental Sampling.

Sample Date	Sample Location	Surgeon	Procedure	Time	CFU	Control
1/15/2018	OR 3	1	OPCAB	5 min	0	0
1/15/2018	OR 3	1	OPCAB	15 min	1	0
1/15/2018	OR 3	1	OPCAB	30 min	0	0
1/15/2018	OR 3	1	OPCAB	60 min	0	0
1/15/2018	OR 3	1	OPCAB	120 min	0	0
1/15/2018	OR 7	2	AVR	5 min	0	0
1/15/2018	OR 7	2	AVR	15 min	0	0
1/15/2018	OR 7	2	AVR	30 min	1	0
1/15/2018	OR 7	2	AVR	60 min	0	0
1/15/2018	OR 7	2	AVR	120 min	0	0
1/18/2018	OR 3	1	OPCAB	5 min	1	0
1/18/2018	OR 3	1	OPCAB	15 min	0	0
1/18/2018	OR 3	1	OPCAB	30 min	1	0
1/18/2018	OR 3	1	OPCAB	60 min	0	0
1/18/2018	OR 3	1	OPCAB	120 min	1	0
1/19/2018	OR4	1	OPCAB	5 min	0	0
1/19/2018	OR4	1	OPCAB	15 min	2	0
1/19/2018	OR4	1	OPCAB	30 min	1	0
1/19/2018	OR4	1	OPCAB	60 min	0	0
1/19/2018	OR4	1	OPCAB	120 min	3	0
1/16/2018	Pump Room East	N/A	N/A	5 min	0	0
1/16/2018	Pump Room East	N/A	N/A	15 min	2	0
1/16/2018	Pump Room East	N/A	N/A	30 min	11	0
1/16/2018	Pump Room East	N/A	N/A	60 min	9	0
1/16/2018	Pump Room East	N/A	N/A	120 min	12	0
1/16/2018	Pump Room West	N/A	N/A	5 min	1	0
1/16/2018	Pump Room West	N/A	N/A	15 min	1	0
1/16/2018	Pump Room West	N/A	N/A	30 min	0	0

Table A-3: Environmental Sampling (continued).

Sample Date	Sample Location	Surgeon	Procedure	Time	CFU	Control
1/16/2018	Pump Room West	N/A	N/A	60 min	8	0
1/16/2018	Pump Room West	N/A	N/A	120 min	8	0
1/18/2018	Pump Room East	N/A	N/A	5 min	0	0
1/18/2018	Pump Room East	N/A	N/A	15 min	1	0
1/18/2018	Pump Room East	N/A	N/A	30 min	3	0
1/18/2018	Pump Room East	N/A	N/A	60 min	12	0
1/18/2018	Pump Room East	N/A	N/A	120 min	16	0
1/18/2018	Pump Room West	N/A	N/A	5 min	1	0
1/18/2018	Pump Room West	N/A	N/A	15 min	1	0
1/18/2018	Pump Room West	N/A	N/A	30 min	5	0
1/18/2018	Pump Room West	N/A	N/A	60 min	6	0
1/18/2018	Pump Room West	N/A	N/A	120 min	9	0
1/19/2018	Pump Room East	N/A	N/A	5 min	0	0
1/19/2018	Pump Room East	N/A	N/A	15 min	2	0
1/19/2018	Pump Room East	N/A	N/A	30 min	0	0
1/19/2018	Pump Room East	N/A	N/A	60 min	2	0
1/19/2018	Pump Room East	N/A	N/A	120 min	4	0
1/19/2018	Pump Room West	N/A	N/A	5 min	1	0
1/19/2018	Pump Room West	N/A	N/A	15 min	1	0
1/19/2018	Pump Room West	N/A	N/A	30 min	6	0
1/19/2018	Pump Room West	N/A	N/A	60 min	0	0
1/19/2018	Pump Room West	N/A	N/A	120 min	1	0

Appendix B: Statistical Analysis

B-1: Sterile Gown and Reservoir Sampling.

Sterile Gown Analysis.

One-way ANOVA: CFU/100ul versus Hours Since Gown Opened

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were not assumed for the analysis.

Factor Information

Factor	Levels	Values
Hours Since Gown Opened	8	0, 24, 48, 72, 96, 120, 144, 168

Welch's Test

Source	DF Num	DF Den	F-Value	P-Value
Hours Since Gown Opened	7	12.3139	105.07	0.000

Model Summary

R-sq	R-sq(adj)	R-sq(pred)
85.97%	82.90%	78.07%

Means

Hours Since Gown Opened	N	Mean	StDev	95% CI
0	5	0.400	0.548	(-0.280, 1.080)
24	5	20.40	2.97	(16.72, 24.08)
48	5	40.40	5.46	(33.62, 47.18)
72	5	18.60	5.73	(11.49, 25.71)
96	5	22.00	6.00	(14.55, 29.45)
120	5	22.60	4.16	(17.44, 27.76)
144	5	24.80	6.02	(17.33, 32.27)
168	5	29.00	4.18	(23.81, 34.19)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Hours Since Gown Opened	N	Mean	Grouping
48	5	40.40	A
168	5	29.00	B
144	5	24.80	B C
120	5	22.60	B C
96	5	22.00	B C
24	5	20.40	B C
72	5	18.60	C
0	5	0.400	D

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
24 - 0	20.00	2.99	(10.32, 29.68)	6.69	0.000
48 - 0	40.00	2.99	(30.32, 49.68)	13.38	0.000
72 - 0	18.20	2.99	(8.52, 27.88)	6.09	0.000
96 - 0	21.60	2.99	(11.92, 31.28)	7.23	0.000
120 - 0	22.20	2.99	(12.52, 31.88)	7.43	0.000
144 - 0	24.40	2.99	(14.72, 34.08)	8.16	0.000
168 - 0	28.60	2.99	(18.92, 38.28)	9.57	0.000
48 - 24	20.00	2.99	(10.32, 29.68)	6.69	0.000
72 - 24	-1.80	2.99	(-11.48, 7.88)	-0.60	0.999
96 - 24	1.60	2.99	(-8.08, 11.28)	0.54	0.999
120 - 24	2.20	2.99	(-7.48, 11.88)	0.74	0.995
144 - 24	4.40	2.99	(-5.28, 14.08)	1.47	0.816
168 - 24	8.60	2.99	(-1.08, 18.28)	2.88	0.111
72 - 48	-21.80	2.99	(-31.48, -12.12)	-7.29	0.000
96 - 48	-18.40	2.99	(-28.08, -8.72)	-6.16	0.000
120 - 48	-17.80	2.99	(-27.48, -8.12)	-5.95	0.000
144 - 48	-15.60	2.99	(-25.28, -5.92)	-5.22	0.000
168 - 48	-11.40	2.99	(-21.08, -1.72)	-3.81	0.012
96 - 72	3.40	2.99	(-6.28, 13.08)	1.14	0.943
120 - 72	4.00	2.99	(-5.68, 13.68)	1.34	0.877
144 - 72	6.20	2.99	(-3.48, 15.88)	2.07	0.452
168 - 72	10.40	2.99	(0.72, 20.08)	3.48	0.028
120 - 96	0.60	2.99	(-9.08, 10.28)	0.20	1.000
144 - 96	2.80	2.99	(-6.88, 12.48)	0.94	0.980
168 - 96	7.00	2.99	(-2.68, 16.68)	2.34	0.303
144 - 120	2.20	2.99	(-7.48, 11.88)	0.74	0.995
168 - 120	6.40	2.99	(-3.28, 16.08)	2.14	0.412
168 - 144	4.20	2.99	(-5.48, 13.88)	1.41	0.848

Individual confidence level = 99.72%

Reservoir Analysis.

One-way ANOVA: CFU/100ul versus Hours Since Circuit Assembled

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were not assumed for the analysis.

Factor Information

Factor	Levels	Values
Hours Since Circuit Assembled	2	0, 24

Welch's Test

Source	DF Num	DF Den	F-Value	P-Value
Hours Since Circuit Assembled	1	11.6407	0.32	0.580

Model Summary

R-sq	R-sq(adj)	R-sq(pred)
2.63%	0.00%	0.00%

Means

Hours Since Circuit Assembled	N	Mean	StDev	95% CI
0	7	2.286	1.704	(0.709, 3.862)
24	7	2.857	2.035	(0.975, 4.740)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Hours Since Circuit Assembled	N	Mean	Grouping
24	7	2.857	A
0	7	2.286	A

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
24 - 0	0.57	1.00	(-1.61, 2.76)	0.57	0.580

Individual confidence level = 95.00%

B-2: Environmental Sampling.

Regression Analysis: CFU versus Time (minutes), Location

Method

Categorical predictor coding (1, 0)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	9	435.398	48.3775	6.02	0.000
Time (minutes)	1	0.011	0.0114	0.00	0.970
Location	4	2.734	0.6834	0.09	0.987
Time (minutes)*Location	4	112.418	28.1045	3.50	0.015
Error	40	321.482	8.0371		
Lack-of-Fit	15	33.482	2.2321	0.19	0.999
Pure Error	25	288.000	11.5200		
Total	49	756.880			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.83497	57.53%	47.97%	36.35%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.44	1.34	0.33	0.746	
Time (minutes)	-0.0008	0.0217	-0.04	0.970	5.00
Location					
OR 7	-0.15	2.32	-0.07	0.948	3.02
OR4	-0.01	2.32	-0.00	0.996	3.02
Pump Room East	0.36	1.73	0.21	0.836	3.91
Pump Room West	0.80	1.73	0.46	0.648	3.91
Time (minutes)*Location					
OR 7	-0.0011	0.0375	-0.03	0.978	3.17
OR4	0.0176	0.0375	0.47	0.641	3.17
Pump Room East	0.0907	0.0280	3.24	0.002	4.66
Pump Room West	0.0450	0.0280	1.61	0.115	4.66

Regression Equation

Location

OR 3 CFU = 0.44 - 0.0008 Time (minutes)

OR 7 CFU = 0.29 - 0.0019 Time (minutes)

OR4 CFU = 0.43 + 0.0168 Time (minutes)

Pump Room East CFU = 0.80 + 0.0899 Time (minutes)

Pump Room West CFU = 1.23 + 0.0442 Time (minutes)

Fits and Diagnostics for Unusual Observations

Obs	CFU	Fit	Resid	Std Resid

10	0.00	0.06	-0.06	-0.05		X
20	3.00	2.44	0.56	0.49		X
23	11.00	3.50	7.50	2.75	R	
34	12.00	6.19	5.81	2.13	R	
45	4.00	11.58	-7.58	-3.15	R	
50	1.00	6.54	-5.54	-2.30	R	

R Large residual
X Unusual X

One-way ANOVA: CFU versus Time (minutes) in Perfusion Room

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were not assumed for the analysis.

Factor Information

Factor	Levels	Values
Time (minutes)	5	5, 15, 30, 60, 120

Welch's Test

Source	DF Num	DF Den	F-Value	P-Value
Time (minutes)	4	11.4864	6.38	0.006

Model Summary

R-sq	R-sq(adj)	R-sq(pred)
43.34%	34.27%	18.41%

Means

Time (minutes)	N	Mean	StDev	95% CI
5	6	0.500	0.548	(-0.075, 1.075)
15	6	1.333	0.516	(0.791, 1.875)
30	6	4.17	4.17	(-0.21, 8.54)
60	6	6.17	4.49	(1.45, 10.88)
120	6	8.33	5.39	(2.68, 13.99)

Regression Analysis: CFU in Operating Room versus Time, Surgeon, Procedure, Day

```
The following terms cannot be estimated and were removed:
   Procedure


Method

Categorical predictor coding  (1, 0)


Analysis of Variance

Source      DF   Adj SS   Adj MS  F-Value  P-Value
Regression   4   3.7291  0.93228     1.52    0.247
  Time       1   0.3791  0.37911     0.62    0.444
  Surgeon    1   0.0000  0.00000     0.00    1.000
  Day        2   2.5333  1.26667     2.06    0.162
Error       15   9.2209  0.61473
Total       19  12.9500


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.784045  28.80%      9.81%       0.00%


Coefficients

Term          Coef   SE Coef  T-Value  P-Value   VIF
Constant     0.047     0.401     0.12    0.908
Time       0.00333   0.00423     0.79    0.444  1.00
Surgeon
  1       0.000000  0.000000        *        *     *
  2          0.000     0.496     0.00    1.000  1.50
Day
  15-Jan  0.000000  0.000000        *        *     *
  18-Jan     0.400     0.496     0.81    0.432  1.50
  19-Jan     1.000     0.496     2.02    0.062  1.50


Regression Equation

Surgeon  Day
1        15-Jan  CFU = 0.047 + 0.00333 Time

1        18-Jan  CFU = 0.447 + 0.00333 Time

1        19-Jan  CFU = 1.047 + 0.00333 Time

2        15-Jan  CFU = 0.047 + 0.00333 Time

2        18-Jan  CFU = 0.447 + 0.00333 Time

2        19-Jan  CFU = 1.047 + 0.00333 Time


Fits and Diagnostics for Unusual Observations
```

Obs	CFU	Fit	Resid	Std Resid	
20	3.000	1.446	1.554	2.48	R

R Large residual

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time (minutes)	N	Mean	Grouping
120	6	8.33	A
60	6	6.17	A B
30	6	4.17	A B
15	6	1.333	B
5	6	0.500	B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
15 - 5	0.83	2.12	(-5.38, 7.04)	0.39	0.995
30 - 5	3.67	2.12	(-2.54, 9.88)	1.73	0.433
60 - 5	5.67	2.12	(-0.54, 11.88)	2.68	0.086
120 - 5	7.83	2.12	(1.62, 14.04)	3.70	0.009
30 - 15	2.83	2.12	(-3.38, 9.04)	1.34	0.670
60 - 15	4.83	2.12	(-1.38, 11.04)	2.28	0.183
120 - 15	7.00	2.12	(0.79, 13.21)	3.31	0.022
60 - 30	2.00	2.12	(-4.21, 8.21)	0.95	0.876
120 - 30	4.17	2.12	(-2.04, 10.38)	1.97	0.309
120 - 60	2.17	2.12	(-4.04, 8.38)	1.02	0.842

Individual confidence level = 99.29%

Perfusion

Thesis Approval Form

Master of Science in Perfusion -- MSP

Milwaukee School of Engineering

This thesis, titled “Analysis of Sterile Practice in the Department of Cardiothoracic Surgery at Aurora St. Luke’s Medical Center, Milwaukee, Wisconsin,” submitted by the student Ryan Acker, has been approved by the following committee:

Faculty Chairperson: _____ Date: _____

Dr. Ron Gerrits

Faculty Member: _____ Date: _____

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