
Original Article***Investigations into the Sterility of Manually Assembled Extracorporeal Circuits with Vented Reservoirs***

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ABSTRACT

This study was designed to investigate the ability of an extracorporeal circuit (ECC) with a vented hard shell reservoir to remain sterile for a period of 72 h under dry conditions. The study was conducted in three phases. In Phase One: Two previously published methods for detecting contamination of the ECC were compared. A group of positive controls was collected by contaminating identical circuits with a known level of *Enterobacter cloacae* (ATTC: 13047) before initiating a regimen of "sample-dilute-sample" culturing. Negative controls for this phase were conducted by randomly sampling 1 L per manufacturer's lot of lactated ringers with each detection method. Culture results suggest that large volume filtration, but not small aliquot sampling, is sensitive to extremely low levels of contamination. No growth was detected in any negative control samples.

In Phase Two: 19 ECC consisting of a membrane oxygenator, vented hardshell reservoir, arterial filter, and PVC tubing were removed from their sterile packages, assembled, and left unprotected in the moderate traffic environment of a research laboratory. The circuits were then primed with Lactated Ringer's solution. The prime solution was sampled for aerobic contamination by large volume filtration. None of the 19 samples detected contamination.

In Phase Three: 43 ECC identical to the Phase Two circuits were assembled and left unprotected in the substerile pump room. The circuits were then primed, circulated, and cultured as in Phase Two. One of the 43 samples was discarded because of a recognized break in aseptic technique during sample collection. None of the remaining samples detected contamination. Mathematical calculations of binomial probabilities suggest that the chance of an open ECC developing a detectable level of contamination within 72 h of its dry assembly is insignificant.

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INTRODUCTION

In emergency situations, a delay in the initiation of cardiopulmonary bypass (CPB) may result in a greater incidence of morbidity or mortality for the patient. The initiation of CPB however, necessarily requires the assembly, priming, and debubbling of an extracorporeal circuit (ECC), which must be done aseptically and may require as long as 30 min. For this reason, many institutions have adopted the policy of having an ECC assembled prophylactically at all times. Unfortunately, there is no critical information available that establishes the length of time an ECC can be expected to remain sterile after it is removed from its packaging and assembled for use. Without this information, there is no foundation on which to base decisions regarding the length of time a circuit should be considered safe for clinical use and at what point the unused circuit should be discarded.

Disposal of unused ECCs is by itself a controversial issue. With the average hospital cost of the disposable components of an ECC at this institution being between \$400–500, the disposal of only a few circuits a year can amount to a large economic waste. In this era of health care reform there is a great deal of pressure to consider the quality of care in concert with the cost of services rendered. This study seeks to address both issues by investigating the ability of an ECC with a vented hard shell reservoir to remain sterile for a clinically relevant period of time (3 days) under dry conditions.

MATERIALS AND METHODS

This study was conducted in three phases. Phase one involved the collection of positive and negative controls. The positive controls (5 circuits, 35 samples) determined the lowest level of aerobic contamination that the two methods of detection used by previous investigators, the Bactec Plus 26 Aerobic Blood Culture Media^a and the Addi-Check II Quality Assurance Test Kit,^b are capable of reporting. Both methodologies are qualitative procedures for culture and recovery of microorganisms.

The Bactec method involves inoculation of a culture vial, which is then periodically inserted into the Bactec brand non-radiometric instrument, where gas in the head space of the vial is aspirated and assayed for carbon dioxide content. If a threshold level is exceeded, the vial is considered positive.

The Addi-Check method employs a 10 mL clear plastic canister containing a 0.45-micron cellulose filter. Fluid samples of any volume may be directed through the canister and across the filter. Microorganisms in the test solution will be harvested

onto the filter. With the addition of tryptic soy broth and incubation, the presence of aerobic microorganisms on the filter will lead to turbidity, a cloud appearance, in the broth solution, and indicate a positive culture.

Circuits included in the positive control group included a disposable oxygenator with an vented filtered reservoir,^c a 38-micron arterial filter,^d and an adult perfusion pack.^e These were removed from their sterile packaging and immediately assembled with the same concern for aseptic technique as if the ECC were being assembled for clinical use. Once assembled dry, the circuit was primed with 4 L of Lactated Ringer's solution (LR), and inoculated with a known concentration (1900–20,000 CFU) of *Enterobacter cloacae* (ATCC #13047). The prime solution was allowed to circulate for 3–5 min to ensure even distribution of the organism. The 3-step sample–dilute–sample regimen that follows was used to collect samples with both detection methods at seven different levels of contamination. First a sample (7 mL) was drawn from the arterial filter and injected into an aerobic Bactec incubation bottle. Next, 3 L were pumped through an Addi-Check canister and into a waste container. The canister was prepared for culture according to the manufacturer's recommendations. Then, 3 L of sterile LR were added to the ECC resulting in a 3:1 dilution of the remaining 1 L. This regimen of sampling and diluting resulted in a final contamination of the ECC of approximately 1 colony-forming unit (CFU)/1000 mL. The collected Bactec and Addi-Check samples were cataloged and incubated in the microbiology department for 7 and 14 days, respectively, to determine the lowest level of contamination each method is capable of detecting.

No attempt was made to provide positive control values for anaerobic organisms for two reasons. First, the dry, oxygenated environment of an unprimed ECC is not conducive to the growth of anaerobic microorganisms. Second, there is a considerable amount of variability regarding the conditions under which an anaerobic microorganism can survive. Therefore, any single group of positive control measurements would only be relevant for a few organisms.

The negative control group (N = 5) was conducted to document negative cultures of each manufacturer's lot of LR used in the positive control group. Immediately prior to priming each control circuit, a single 1 L bag of LR was randomly chosen as representative of the entire lot. A 7 mL sample was withdrawn from the bag and injected into a Bactec vial, and the remaining fluid was passed through the Addi-Check filter and processed as described above.

Phase two of this study was conducted on 19 circuits identical to those used in Phase one. The circuits were assembled in the suboptimal environment of a research laboratory respecting

a Becton Dickenson Diagnostic Instrument Systems, Sparks, MD

b Millipore Corporation, Bedford, MA

c Cobe Excel, Cobe Cardiovascular Inc., Arvada, CO

d Delta, Gish Biomedical Inc. Irvine, CA

e Gish Biomedical Inc. Irvine, CA

proper aseptic technique. Approximately 15 connections were necessary to assemble each circuit. The circuits were left un-protected on a countertop approximately 3.5 ft above the floor and 6 ft from a window for 72 h. During this time, the circuits were not draped, and no effort was made to limit the amount of traffic in the vicinity of the experimental circuits. Circuits were then primed with a volume of 4 L of LR which was allowed to circulate through all of the components for 3–5 min before sampling. While continuing to circulate fluid through all of circuit components, samples were taken by shunting 3 L of prime fluid out of the arterial line, through the cardioplegia circuit, through the Addi-Check canister (which was attached to the luer port of the bubble trap of the cardioplegia line) and into a waste bucket. Figure 1 illustrates how the Addi-Check was interfaced with the ECC.

Phase three of the study was conducted in the substerile “pump-room” adjacent to the operating suites. After obtaining approval from the Institutional Review Board for Protection of Human Subjects, 43 circuits consisting of a membrane oxygenator,^f a 38-micron arterial filter,^g and a preassembled adult

perfusion pack^h were assembled using aseptic technique and left in the pump room for 72 h. At the end of the challenge period, the circuits were primed with 4 L of LR, which were allowed to circulate through all circuit components for 3–5 min. 3 L of the prime were shunted through the Addi-Check canister as described in phase two. The circuit was then used for the day’s surgical case.

Statistical analysis: The data collected is binomial in nature; cultures are either positive or negative. Therefore, we based our analysis on a decision rule model as follows: We expected no contamination.

Of the 62 samples collected, one was discarded because of a recognized break in aseptic technique during sample collection. Examining the remaining 61 samples provided us a greater than 95% power to detect a 5% contamination rate. This means that if we assumed a hypothetical contamination rate of 5% or higher, this design has a 95% or greater chance of detecting one or more contaminated circuits.

RESULTS

PHASE ONE

Positive control data collection resulted in 35 samples for each detection method, collected across a wide range of contamination levels. Table 1 groups these data into five primary ranges, identifying the number of samples per range and the number of positive cultures for both the Bactec and Addi-Check Methods. The difference between the number of samples investigated (Row 1) and the number of positive cultures for each detection method (Rows 2, 3) represent the number of false-negative cultures for the Addi-Check and Bactec methods, respectively. The Addi-Check method reliably detects contamination of 1 CFU/1000 mL, and the Bactec method fails to reliably detect contamination below approximately 1 CFU/10 mL. Negative control samples from five separate lots of Lactated Ringers were collected. There were no positive cultures in the negative control group.

PHASES TWO AND THREE

There were no positive cultures in either group, therefore, the data collected from the circuits assembled in the laboratory and the pump-room were combined for analysis and are presented in Table 2. We conclude that the contamination rate of vented reservoir ECCs manually assembled and left unprimed for 72 h is significantly less than 5%.

DISCUSSION

Published investigations into the long-term sterility of the ECC when assembled without priming began in 1990 when

f Cobe Optima, Cobe Cardiovascular Inc., Arvada, CO

g Sentry, Cobe Cardiovascular Inc., Arvada CO

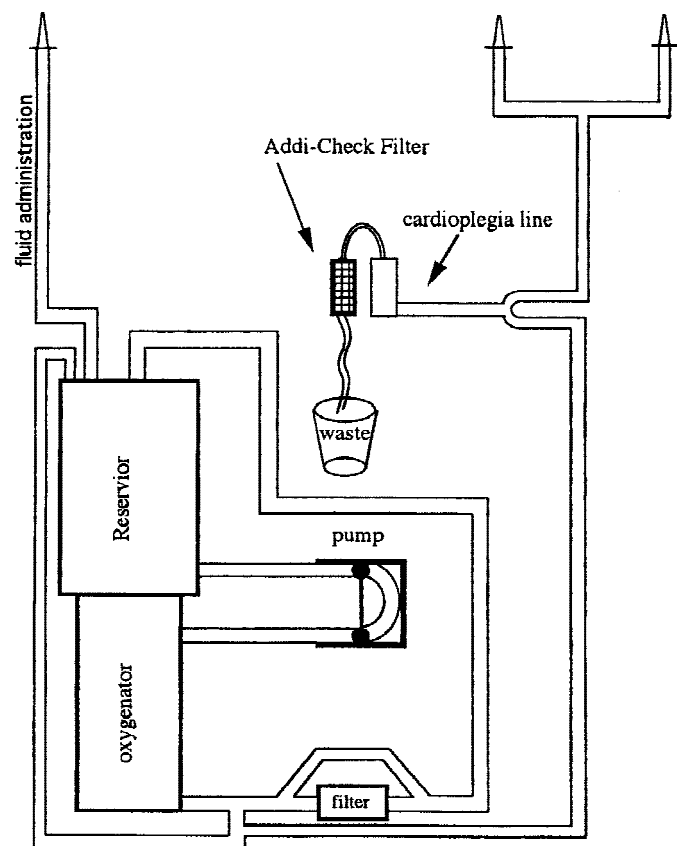


Figure 1: Diagram of the experimental ECC illustrations where the Addi-Check filter was interfaced with the circuit for sample collection.

h Cobe Cardiovascular Inc., Arvada, CO

Table 1: Positive control data illustrating the ability of two different methods of detecting aerobic contamination in a fluid sample of 4 L, organism used was *Enterobacter cloake*

Concentration of contaminant	1 CFU/ >1 mL	1 CFU/ 1-10 mL	1 CFU/ 10-50 mL	1 CFU/ 50-1000 mL	1 CFU/ >1000 mL
Number of samples investigated (total = 35)	4	9	10	6	6
Addi-Check (+) sample vol = 3 L	4	9	10	6	6
Bactec (+) sample vol = 7 mL	4	8	3	0	2

Chorak et al. (1) used the Bactec methodⁱ to investigate 26 ECCs that had been assembled without priming for a period of 48-60 h. Each of 26 circuits was then primed with an unidentified prime volume, and 3-4 mL of the prime were removed for culture of both aerobic and anaerobic organisms. One culture was positive for anaerobic growth, and all other cultures were negative. Interpretation of their data is limited by the small sample size used for culture and the absence of positive controls establishing that the method used was capable of harvesting and culturing any level of organismal contamination. No statistical analysis of the data was reported.

In 1993, Homishak et al. (2) published the second paper on this topic. This group investigated 17 ECCs that had been assembled without priming. In this study, detection of microbiological contamination was performed by filtering one-third (1L) of each circuit's prime volume through an Addi-Check canister system. Although this approach represents a considerable improvement over Chorak's method with regard to sample size versus prime volume, it falls short of utilizing the Addi-Check canister to its fullest capacity by not filtering a larger fraction of the prime volume. This study also neglected to report any data for positive controls. Statistical results were reported. However, the test seems to have been inappropriately applied. Furthermore, critical analysis of the circuit population reveals that, although circuits were investigated over a range spanning 13.5 to 60.5 h, the time period over which *all*

of 17 circuits were investigated was not clinically valuable (13.5 h).

Most recently, Young et al. (3) investigated circuit sterility 7 days after setup. Although their study did investigate a much longer period of time, meaningful conclusions from their data remain elusive. Only 12 circuits were investigated. Six were never primed, while the other six were primed immediately after set-up and remained stagnant throughout the study. Accordingly then, the number of circuits investigated after a dry challenge is zero (N = 0). The data collection methodology involved the use of cotton swabs to swab three separate surfaces, two of which were presumably dry even in the primed circuits. Considering that these cultures were prepared on Blood Agar plates, the volume of fluid sampled must have been less than the 7 mL samples shown in Phase One of our study to be ineffective at detecting clinically relevant levels of contamination. Despite purposely contaminating the circuits at the end of the data collection period and observing the development of turbidity in the prime solution, no attempt was made to culture the contaminated circuits. This is most unfortunate, because it would have provided some qualitative evidence regarding the sampling technique's ability to detect contamination. Again, no statistical analysis of the data was reported.

In summarizing the published research on this topic to date, it is clear that previous attempts to provide meaningful results have been hampered by a number of pitfalls:

1. small sample size;
2. no positive control data;
3. small number of circuits investigated; and
4. poor/no statistical evaluation.

Our study was designed to overcome these problems. Our sampling technique allowed the evaluation of 3 out of 4 L of prime solution. Our positive control investigations suggest that this technique is superior to smaller sample size techniques and is able to detect levels of contamination as low as 1 CFU/L. Investigation of greater than 60 ECCs provided valuable statistical results allowing us to conclude that at our institution

ⁱ Bactec NR6A, Becton Dickenson Diagnostic Instrument Systems, Sparks, MD 21152

Table 2: Experimental data: aerobic culture results of the prime fluid (3 of 4 L) of 61 ECCs that were assembled dry and remained unprotected in a nonsterile environment for a minimum of 72 hours before priming

Total number of circuits investigated	Negative cultures (-)	Positive cultures (+)
61	61	0

and with our staff, the probability that an ECC with a vented reservoir will have a detectable level of contamination, at priming and 3 days after assembly, is insignificant. These results have led to a change in our practice to allow more cost-effective use of assembled circuits.

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