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Microbial Burden of Hemodialysis Fluids: A Multi-Center Study

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Abstract

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By Nicole Ann Tocci

Standards for microbial contamination in hemodialysis fluids aim to protect patients from adverse events such as pyrogenic reactions, bacteremia, and endotoxemia. However, the international community has not achieved consensus on setting upper limits of contamination. At the time of data collection, the Association for the Advancement of Medical Instrumentation (AAMI) proposed new standards for upper limits and culturing methods for microbial contamination of hemodialysis fluids (AAMI RD52:2004), which have since been updated (ANSI/AAMI/ISO 11663:2009). The research performed a methods comparison for measuring microbial contamination in processed water (AAMI method: Trypticase soy agar (TSA) at 37°C for 48 hours vs. standard method: Reasoner's 2A agar (R2A) at 28°C for 7 days). Additionally, the research examined the association between microbial contamination and selected biomarkers provided by retrospective chart review. Nineteen metro-Atlanta hemodialysis facilities participated. Samples of processed water (n=223) and dialysate (n=223) were collected monthly (Jan-Mar, 1997) using aseptic technique, transported to CDC and immediately assayed. Six of the nineteen facilities provided patient data (n=454). Logistic regression was carried out to predict patient biomarkers that fell outside the acceptable target ranges determined by Centers for Medicare and Medicaid Services and National Kidney Foundation Clinical Practice Guidelines. Microbial recovery from processed water was significantly greater when using the standard method (R2A) compared to the AAMI method (TSA) (Wilcoxon's matched-pair's signed-rank test, $p<0.0001$). A larger percentage of non-compliance with AAMI standards would have been missed using the AAMI method with TSA (19.8%) than using the standard method with R2A (0.9%). Non-compliance with the AAMI standard for endotoxin in dialysate was a consistent predictor of negative patient outcomes as indicated by regression models for erythropoietin dose, serum albumin, and urea reduction ratio. Conversely, non-compliance with the microbial standard for colony counts of bacteria in water, as measured by TSA indicated a protective effect, possibly indicative of the method's lack of sensitivity. The study provides a connection between patient clinical data and hemodialysis fluid contamination. As the dialysis patient population grows, efforts to improve clinical outcomes and eliminate adverse events by minimizing microbial contamination will remain imperative.

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Background

Dialysis as a therapy for persons who have lost kidney function was introduced nearly 70 years ago (1). Hemodialysis is one of the life-saving, renal replacement therapies where waste products are externally removed from the blood when the kidneys have ceased to function. The waste products are transferred across a semi-permeable membrane that is contained in a dialyzer, also known as an artificial kidney, and is facilitated by dialysis fluid (2). The fluid is a flowing solution that is prepared by mixing treated tap water with a concentrated electrolyte solution, equivalent to the electrolyte concentration of plasma water, and is referred to as dialysate (3, 4).

Monitoring the chemical and microbiological quality of hemodialysis fluids is a chief component of hemodialysis quality control and patient safety. Compared to an otherwise healthy individual, hemodialysis increases patient exposure to water that is potentially contaminated. The average hemodialysis patient undergoing thrice weekly treatment is exposed to about 24,000 liters of dialysis fluid per year (2). This unique and prolonged exposure in combination with the fact that patients on hemodialysis have compromised immune systems and often have complex co-morbidities, puts them at increased risk for infection (5). There are generally worldwide agreements on the maximum levels of most chemical contaminants, however the level of acceptable and safe microbial contamination is still contested and changes often (6). The longer the patient is exposed to potentially contaminated dialysis fluid, the greater the risk of infection. Since its inception, advances in technology have led to reductions in hemodialysis session time. Thus, due to these advances and reductions in session time, the risks of septicemia and endotoxemia as a result of hemodialysis have subsequently been reduced (7).

The important control of hemodialysis microbiologic water quality is dependent on the maintenance of the entire water system(8). The microbial contamination problems in dialysis systems have been shown to be the product of many concurrent factors including insufficient water treatment systems, the types of dialyzers used, and the water and dialysate distribution

systems (9-11). Many hemodialysis centers use tap water for the preparation of dialysate (9). Knowing the microbiologic contamination of both the dialysis water and dialysate is imperative with regard to the control of healthcare-acquired infections.

Because dialysis centers draw their water from the municipal supply, dialysis center water treatment and distribution systems are constantly charged with the removal and/or inactivation of as much bacteria, endotoxin and chemicals from the water as possible in order to meet the therapeutic needs of the patients (5). Reverse osmosis (RO), which is used in the vast majority of dialysis centers in the US, can remove both bacteria and bacterial endotoxin from water (6). The RO process, during which water is forced under pressure through a semipermeable polyamide or cellulose membrane, removes more than 90% of inorganic and organic substance (pyrogens, bacteria and particulate matter) from the water (12). However, because the water treatment systems remove the antibacterial agents such as chlorine from the water, there is nothing to inhibit the regrowth and recontamination of the dialysis water by the bacteria (13).

Despite the use of water treatment systems for hemodialysis, investigations have reported persistent bacterial contamination in hemodialysis centers (14). Researchers have cited several contributing factors for the continued bacterial contamination; pyrogens in the deionization equipment, inadequate disinfection of the distribution system, passage of endotoxin from dialysate to plasma, microbial colonization of pipe surfaces stemming from suboptimal hydraulic design, presence of a storage tank, use of non-sterile rinse water, and an inadequate microbiological monitoring program (7, 15).

Highly water permeable synthetic dialyzer membranes were introduced in the late 1970s (16). These high-performance membranes have high water permeability (16). The high performance membranes are often favored due to their larger nominal pore size which enhances dialysis membrane performance (enhanced clearance of urea and other middle molecules). However, to prevent contamination the fluid must be highly purified (16).

Hemodialysis has three different treatment types: conventional and high efficiency, which have been grouped together as low-flux, and high-flux. Conventional dialyzer membranes have the smallest surface area and routinely use bicarbonate dialysate, although acetate was also used at the time of this study. Meanwhile, both high efficiency and high-flux dialysis use dialyzer membranes with larger surface areas. Of the three, high-flux has the highest ultrafiltration coefficient and conventional has the lowest (17). Unfortunately, the new types of membranes and dialysate resulted in increased bacteriologic contamination of the dialysate (18).

Despite the addition of synthetic membranes (polysulfone, polyethersulfone, polyacrylonitrile, acrylonitrile-sodium-methallyl sulfonate, polycarbonate, polyamide, and polymethylmethacrolate), the use of cellulosic membranes is still widespread. The pore size of dialyzer membranes may be less important than the membrane's ability to absorb bacterial products. As a result, conventional dialysis treatment with cellulose-based membranes may pose greater risk for bacterial contamination than high efficiency and high-flux dialysis with synthetic membranes (19).

Bicarbonate dialysate is a good growth medium for bacteria and supports rapid bacterial growth and endotoxin production (13, 20). This is particularly true when the dialysate contains metabolic products dialyzed from patient's blood (21). The dialysate buffer, glucose and temperature, which increases as the fluid enters the machine, all influence bacterial growth (22). Consequently, dialysate will often exceed the recommendations for microbial contamination of hemodialysis fluids set by the Association for the Advancement of Medical Instrumentation (AAMI) (20). Multi-center study results suggest that a significant portion of water and dialysate samples are not in compliance with the AAMI standards (23). In response to this, there is a growing body of evidence that points to association between the use of ultrapure dialysis fluid and a reduction in complications from hemodialysis (24). However, the clinical evidence for the use of ultrapure dialysate is still under discussion.

Microbial contaminants

Gram-negative water bacteria has been shown to multiply in hemodialysis-related fluids (25). This bacterial contamination has been related to outcomes such as pyrogenic reactions and bacteremia (3). As a result, there was a need to set appropriate limits for microbial contamination in dialysate and the water for dialysis when this association was made.

In dialysis fluids, certain types of Gram-negative bacteria are more common, such as *Pseudomonas, Xanthomonas (Stenotrophomonas),* and *Flavobacterium* (21). A variety of gram-negative water bacteria are considered non-pathogenic, however they can cause disease when introduced in large numbers to physiologically compromised patients as is the case with patients in a hemodialysis center.

High concentrations of bacterial contamination in dialysis fluids are associated with increased incidence of febrile reactions. Epidemiologic evidence has shown an increased risk for pyrogenic reactions and septicemia when the level of contamination exceeds 10,000 CFU/mL. However, there is no increased risk for pyrogenic reactions when the level of contamination is between 1,000-2,000 CFU/mL in the dialysate (25). And yet, studies show that microbial counts in water greater than 100-200 CFU/mL pose a risk due to the potential for microbial amplification in other parts of the dialysis system (25).

Repetitive subclinical inflammatory reactions in hemodialysis patients can be attributed to contaminated dialysate (26). Frequent and prolonged vascular access provides the opportunity for bacteria to enter bloodstream of the hemodialysis patient. Cytokine stimulation in the blood is dependent on both the concentration of endotoxin in the dialysate and on the permeability of dialysis membrane (22). There is an increased risk of bacteremia or endotoxemia when the level of bacteria and endotoxin in the dialysis fluid in high, thus increasing the probability that the bacteria or endotoxin will pass through the dialysis membrane or stimulate cytokine production (27).

In contaminated dialysis fluids, lipopolysaccharides (LPS, endotoxin) are released from the cell walls of the gram-negative water bacteria (28). Some studies have reported that endotoxin and endotoxin fragments can cross the dialyzer membranes while other studies have been inconclusive (20). The LPS (endotoxin) can cause pyrogenic reactions in hemodialysis patients (25). However, studies report no correlation between bacterial growth and endotoxin concentration in dialysis fluid samples (23). The relationship between waterborne bacteria and endotoxin concentrations in dialysate and the water must be understood (29). Further confirming the association between endotoxin and pyrogenic reaction, an Israeli study showed that hemodialyzed patients possessed significant endotoxin antibody titers compared to controls not undergoing hemodialysis (30).

In addition to concerns about bacteria and endotoxins, there is the emerging focus on biofilms. Biofilms, formations of microbes that have adhered to surfaces, are thought to be another source of chronic, subclinical inflammation (31). Unlike the ubiquitous water bacteria, once established, biofilms are nearly impossible to eradicate. Biofilms release pyrogens such as endotoxin and small bacterial DNA fragments that trigger Toll-like receptors on monocytes and induce cytokine production thus leading to an inflammatory response (31-33). These short bacterial-derived DNA fragments, oligodeoxynucleotides (ODN), are present in dialysate and are small enough to pass through the dialysis membrane (34). Notably, utlrafilters used in water treatment are unable to significantly remove ODN from dialysis fluid. Bacteria that grow in the water distribution line may release ODN and thus be transported to the dialysis machines (34). Like endotoxin, ODN cannot simply be cultured because they are not whole living bacteria, however they can still promote a chronic inflammatory response, which in dialysis patients is hazardous.

The Centers for Disease Control and Prevention (CDC) have investigated several outbreaks of pyrogenic reactions and bacteremia associated with both inadequate water treatment and contaminated dialysis fluid in hemodialysis facilities (35, 36). Outbreaks related to water

treatment were often associated with the insufficient disinfection of the water distribution systems and dialysis machines, which allowed for amplification of microbial products during fluid distribution and dialysis (37, 38). Additionally, there were cases of inadequate treatment of water at the municipal level, followed by insufficient treatment at the dialysis center which led to acute liver failure and even death (39). Contamination of dialysis fluids have led to several reported outbreaks of pyrogenic reactions. Levels of bacterial in dialysate above standard limits were responsible for outbreaks in at least three CDC outbreak investigations (40, 41).

Standards

In 1977, the Centers for Disease Control and Prevention (CDC) proposed guidelines for bacterial contamination in hemodialysis water (200 colony-forming units per milliliter (CFU/mL)) and dialysate (2,000 CFU/mL) (7). These limits were based on outbreak investigations where attack rates for pyrogenic reactions increased as the concentration of bacteria exceed 1,000 CFU/mL. In 1982, the Association for the Advancement of Medical Instrumentation (AAMI) issued the American National Standard to protect patients from adverse events such as pyrogenic reactions, for quality control surveillance of bacteriological contamination of hemodialysis fluids (42). These standards followed the 1977 CDC proposal. The 2004 AAMI recommendations aligned the standards with the more strict recommendations already in use in Europe (water 200 CFU/mL, dialysate 200 CFU/mL, endotoxin 2 endotoxin units per milliliter (EU/mL)) (43). The most recent International Standards Organization (ISO) recommendations were only slightly more strict than the previous AAMI standards, and were ultimately adopted by AAMI in 2009 (water 100 CFU/mL, standard dialysate 100 CFU/mL and 0.5 EU/mL, ultrapure dialysate 0.1 CFU/mL and 0.03 EU/mL) (44).

Although the United States has maintained lower standards for microbial contamination of dialysis fluids than much of Europe and parts of Asia, there are distinct differences in the implementation of the standards. While the standards are less strict in the US, hemodialysis centers are required to adhere to them in order to receive payment from the Federal Government

and are therefore monitored for compliance by inspections (24). Meanwhile, other nations have stronger recommendations but no requirements for compliance.

Compliance

In a multicenter study by Bambauer *et al.*, compliance with AAMI standards for hemodialysis treated water and dialysate were reported as 82.2% and 88.3% respectively (29). Laurence *et al.* reported overall compliance with AAMI standards for treated water at 70.0% (7). Meanwhile, Oie *et al.* reported compliance with AAMI treated water and dialysate standards at 94.5 and 57.5%, respectively (45). AAMI continues to progress towards the European's more strict microbiologic standards but the European Pharmacopeia still maintains a more conservative recommendation (3).

Biomarkers

One of the greatest concerns with microbiological contamination of hemodialysis fluids is the risk of pyrogenic reactions and septicemia. Pyrogenic reactions are most often characterized by shaking chills, fever, and hypotension (11). They are caused by the passing of pyrogens, most likely endotoxin, across the dialysis membrane. In addition to these more isolated adverse events, people with chronic kidney disease (CKD) have an increased risk of cardiovascular disease and all-cause mortality (46). While many risk factors contribute to this, one possible risk factor to consider is that of inflammation. Biomarkers for inflammation can be predictors of these adverse events. When specific biomarkers indicate increased inflammation in hemodialysis patients, measures can be taken to ensure that the hemodialysis process is not causing the inflammation through contaminated dialysis fluids or equipment.

C-reactive protein elevation. It has been suggested that CKD is not itself a cause of inflammation but rather that it is associated with risk factors for inflammation (47). Nevertheless, a chronic inflammatory state can be observed in those with CKD and becomes more pronounced in those undergoing treatment for end stage renal disease (ESRD) (48). The chronic inflammatory state is associated with adverse clinical outcomes such as anemia due to diminished

erythropoietin responsiveness (48). C-reactive protein (CRP) levels in the blood rise in response to inflammation and this biomarker is a direct measure for detecting inflammation in the patient. CRP is not routinely measured in either U.S. or metro-Atlanta hemodialysis patients.

Hypoalbuminemia. Another important inflammatory biomarker is serum albumin. In otherwise healthy individuals, low serum albumin is often associated with protein calorie malnutrition (49). However, in dialysis patients the decrease in albumin is largely a consequence of increased inflammation that results in decreased albumin synthesis (47, 49, 50). Studies have shown that hypoalbuminemia predicts death in hemodialysis patients (51). A 2009 study reported that patients with serum albumin below 3.0 g/dL have a higher rate of early mortality (50% in 6 months), and that this obscures later events predicted by escalated CRP (47). Consistently, in a study of inflammation and cardiovascular risk, low serum albumin was correlated with high CRP and cardiovascular mortality during follow-up (51). Additionally, in a study looking for predictors of mortality in the first two years of hemodialysis, low serum albumin was a significant predictor of mortality (52). A 2004 study noted a significant increase in serum albumin and reduction in CRP following the upgrade of the water treatment systems affecting the four metropolitan dialysis centers in the study (53), thus making the association between better water quality and decreased inflammation.

Urea reduction ratio. The urea reduction ratio (URR) is a measure to assess the delivered dose of dialysis. It is a function of the removal of the urea from the blood by the dialyzer (54). It is calculated as follows: $URR=100 \times (1-C_t/C_0)$, where C_t is the post dialysis blood urea nitrogen (BUN) and C_0 is the pre-dialysis BUN. Owen *et al.* reported that low urea reduction ratios during dialysis were associated with increased odds ratios for death (54). For patients on maintenance hemodialysis URRs should be >65% and the National Kidney Foundation (NKF) Clinical Practice Guidelines suggest a target of 70% (55, 56).

Erythropoietin hyporesponsiveness. Chronic inflammation can cause a reduced humeral response to erythropoietin, a hormone produced by the kidney which signals the bone

marrow production of red blood cells (57). Patients who are dialysis dependent are given erythrocyte stimulating agents (eg, recombinant human erythropoietin (rHu-EPO)) to control anemia. The use of ultrapure dialysate compared to conventional dialysate has been shown to reduce the amount of rHu-EPO required to maintain target hemoglobin levels in patients by lowering or removing the endotoxin from in the dialysate (58).

Microbiologic techniques

Although the upper limits of the microbiologic quality of hemodialysis fluids recommendations are based on colony-forming units, those counts are dependent on the type of media, culture methods used, and incubation conditions. In order to evaluate the microbiological quality of hemodialysis fluids, nutrient poor culture techniques are required (59). As of 2009, the AAMI recommends either Tryptone glucose extract agar (TGEA) or Reasoner's 2A (R2A) agar at 17-23°C (ambient temperature) for 168 hours as the standard (60). Endotoxin concentrations are measured by the *Limulus* amoebocyte lysate (LAL) assay (23).

At the time of this study (1997), the AAMI recommendation was for culturing on Trypticase soy agar (TSA) at 35-37°C (body temperature) for 48 hours. The AAMI limits of 200 CFU/mL for processed water and dialysate and 2000 CFU/mL for dialysis fluids were based on this method. However, studies have shown R2A to be more sensitive in demonstrating contamination of those water bacteria found in hemodialysis fluid (61) .

The standard TSA plate was hypothesized not to yield the full bacterial load for two main reasons. First, many of the bacteria do not grow at 37°C, as the organisms usually live in cooler temperatures than the human body (62, 63). Secondly, the organisms are unable to form detectable colonies during the 48-hour incubation period (63). R2A requires lower temperatures and longer incubation time, allowing for an increased recovery of bacteria which the method on TSA may fail to cultivate.

In a 1999 study of one dialysis center in the Netherlands, the TSA and R2A methods were compared using both the current European standard and the AAMI standard. Results of the

study demonstrated numerous samples with TSA compliance that failed to meet the standard on R2A media. This suggests that TSA underestimated the amount of bacteria in the fluids. The study showed that R2A missed fewer samples that were non-compliant with the standards(59). Having the most sensitive methods is critical to detect microbial contamination through routine environmental monitoring of hemodialysis fluids so that hemodialysis systems can be appropriately maintained.

Studies have shown that other techniques, including lower temperatures, longer incubation times, and using other media such as R2A yield higher bacterial counts (64). In addition to the media, temperature and incubation period, the plating technique, pour plate versus spread plate, can also produce significant differences among dialysis fluid samples (62). Thus, using the previously recommended AAMI method may have yielded an underestimation of the bacterial load in contaminated hemodialysis fluids.

In fact, studies have demonstrated that the method used with TSA produces two logs less CFU than samples cultured with the method used for R2A and TGEA (26). However, a 2009 study that compared waterborne bacterial counts using R2A media and TGEA, found that bacterial counts were significantly higher for R2A than for TGEA (65). The nutrient-poor R2A agar may still be the most promising medium for culturing water bacteria from hemodialysis fluids.

Although other methods for identifying and typing bacterial species in dialysis fluids have been suggested and tested, plating techniques are not obsolete. Techniques such as polymerase chain reaction (PCR) and solid-phase cytometry have provided the field with abundant data, however limitations exist. With regard to PCR, typing bacterial species is possible if a unique sequence has been obtained but some sequences are common across many species (32). Secondly, PCR will detect non-viable bacterial cells given that there are intact target nucleic acid sequences (66). Thus, it could overestimate bacterial burden or suggest that non-viable bacteria were factors in contamination when this may not be the case. Solid-phase cytometry is

another alternative to culture methods for assessing the microbiological quality of hemodialysis fluids. High correlation has been observed between the total viable count measured by solid-phase cytometry and that measured by the heterotrophic place count protocol (67). It provides more rapid assessment compared to culture methods (68), however its disadvantages include the high cost of instruments and the length of time required by laboratorian for processing (67). However, it is suggested that these methods, be used in tandem as they can help identify organisms that subsist in the 'viable but not culturable state' (69).

Adequate water treatment and well-organized quality control are necessary to improve patient outcomes. Cultures and endotoxin testing done within a hemodialysis center are intended to demonstrate that disinfection schedules are effective and to make adjustments where necessary to meet current standards (13). Microbiological purity of hemodialysis water is critical to the suppression of pyrogenic reactions and the prevention of the chronic inflammation associated with hemodialysis (70). Through these quality control measures, microbial contamination of dialysis fluids can be kept within the standards and patients can have improved health outcomes.

Methods

Study question

The primary aim of this multi-center study was a methods comparison for measuring microbial contamination in processed water and dialysate, comparing current assay methods for culturing water with the standard heterotrophic plate count used in routine water analysis. It was hypothesized that the standard heterotrophic plate count method would be more sensitive and that high colony counts would be associated with high endotoxin levels in both processed water and dialysate.

The secondary aim of the study was to determine the association between microbial contamination and selected biomarkers provided by retrospective chart review. The researcher sought to identify the risk factors for these adverse patient outcomes as defined by biomarkers which did not meet CMS goals and NKF Clinical Practice Guidelines. In this study, data on measurable parameters related to patient health were recorded that are routinely collected by facilities for Centers for Medicare and Medicaid Service (CMS). Because these clinical markers are readily available for nearly every patient in the United States, it lends the results of this analysis to be extrapolated to similar patient populations.

The study addressed the proposed microbial standard for hemodialysis fluids at the time of data collection (AAMI RD52:2004). However, the most recent microbial standards accepted by AAMI (ANSI/AAMI/ISO 11663:2009) are based upon chronic exposure to dialysis fluids containing bacteria and endotoxin. Due to these new recommendations, the analysis also took the lower threshold for microbial contamination of the dialysate and water into consideration.

Data was collected in a way that limited the likelihood of identifying any individual. No new information needed to be collected from patients. All data that was used had been collected through sample collection and testing, and retrospective chart review and was analyzed

retrospectively**.** The researcher applied to the Institutional Review Board (IRB), Emory University, and received exemption from IRB review.

Selection of facilities

A letter of invitation to participate in this study was mailed to all Metro-Atlanta Medicare certified hemodialysis facilities. Of these, nineteen agreed to participate in the study.

Sample collection

Over a three month period (January-March 1997) samples of water and dialysate were collected from each of the participating dialysis centers. The samples were collected in sterile, endotoxin free containers using aseptic technique and transported to the laboratory on the same day in coolers. Upon receipt in the laboratory, the samples were processed immediately.

Microbiologic methods

Water samples were split and cultured using the recommended method (at the time of the study) by the Association for the Advancement of Medical Instrumentation(71) and the standard heterotrophic plate count (HPC). In each case samples were serially diluted (10^0, 10^{-2}, 10^{-4}, 10^{-6}) in phosphate buffered rinse water cultured using the membrane filtration technique (27, 72). Membrane filters (45µ, 47mm cellulose acetate grid, Advantec Microfiltration Systems, Dublin, CA) and R_2A plates (BD-Microbiology Systems, Cockeysville, MD) and TSA plates (BD-Microbiology Systems). TSA plates were incubated for 48 hours at 36°C according to the recommendations of AAMI End Stage Renal Disease and Detoxification Committee's Standards and Recommended Practices (73) and R_2A plates were incubated at 28°C for 7 days as a generally accepted method for the HPC (72).

Dialysate samples were cultured as above by the recommended AAMI method only, i.e. membrane filtration technique using TSA as the medium and incubation for 48 hours at 36°C. All plates were counted using a binocular stereomicroscope (Bausch and Lomb).

Endotoxin assay

Endotoxin activity was measured using the kinetic turbidimetric *Limulus* amebocyte lysate test (LAL-5000 ver 2.0, Associates of Cape Cod, Falmouth, MA) and Pyros-software (Associates of Cape Cod). Results were recorded in endotoxin units (EU)/mL. Dialysate samples were diluted (1:10) with sterile endotoxin free water (Abbotts Labs, Chicago, IL).

Retrospective chart review

Of the 19 hemodialysis centers that participated in the dialysis fluids study, 6 centers (L, F, N, S, Q, K) contributed patient data through retrospective chart studies. These 6 centers were selected based on the microbial quality of the water as indicated by total cell counts (CFU/mL). Chart reviews contained patient data that was non-identifying including demographic information (age, gender, year started dialysis), clinical information (type of dialyzer membrane, dialysis treatment type, length of dialysis session, erythropoietin dose), underlying disease/co-morbidities (hypertension, diabetes, injecting drug use, HIV, hepatitis B, hepatitis C, glomerulonephritis, other), and monthly laboratory values (hematocrit (HCT), serum albumin (S_{alb}), urea reduction ratio (URR), and KT/V).

Variables

Microbial lab data was categorized by center (A-S). Colony counts for water were available for both TSA and R2A methods for 223 samples. Colony counts for dialysate were available for TSA for 223 samples. Endotoxin levels were available for all 446 samples of both water and dialysate. To normalize the colony count variables they were log transformed. Log transformed colony counts and endotoxin unit variables were continuous variables.

The median colony count per center was calculated. Dichotomous variables were created to reflect non-compliance with both the 2004 AAMI standard (AAMI RD52:2004) and the 2009 AAMI standard (ANSI/AAMI/ISO 11663:2009) based on the median colony counts by plating method and endotoxin units per center. These dichotomous variables were added to each patient record as determined by their dialysis center.

Each patient observation included age, length of treatment (hours), and year started hemodialysis, which were treated as continuous variables. Sex was treated as a dichotomous variable (1: male; 0: female). Categorical variables included dialysis center (0-5), dialysis membrane (0-4), and dialysis treatment type (0-2). Erythropoietin dose (units) was coded as a continuous variable, using the most recent dose as the patients only erythropoietin value. When included as a predictor in logistic regression models, a variable for erythropoietin dose/500 was created to show the effect of dosing. For the clinical lab variables (hematocrit, serum albumin, urea reduction ratio, and KT/V), one lab value per month (January to March, 1997) was provided. For each measure, these values were averaged to provide an individual patient average, thus providing each patient with one value for each clinical measure. Each of these values was dichotomized based on the CMS and NKF Clinical Practice Guidelines (Erythropoietin ≥ 5000; Hematocrit $\leq 37\%$; Serum albumin ≤ 3.5 g/dL; URR $\leq 65\%$; and KT/V ≤ 1.2). The dichotomized variables were used when the clinical labs were the outcome in the logistic regression model. Otherwise, the aforementioned continuous forms of the variables (calculated average) were used. The causes of end-stage renal disease (diabetes requiring treatment, hypertension requiring treatment, glomerulonephritis) were coded as dichotomous variables (1: having disease; 0: not having disease). The variables for comorbidities (known HIV infection, injecting drug use, hepatitis B surface antigen positive, hepatitis C antibody positive) were coded as dichotomous variable (1:yes; 0:no).

Statistical analysis

Data were analyzed using SAS 9.3 (Cary, NC). Assumptions of normality were assessed using plots and test of normality. Non-normal variables were transformed or categorized as required.

Means and standard errors were calculated for colony counts and endotoxin levels of processed water and dialysate data. To address the normality of the data, the colony units were log transformed. Because some colony counts and endotoxin levels were zero, one was added to

all values before taking the logarithm. Frequencies were used to determine compliance with the prescribed standards.

The laboratory data was tested for correlation (Pearson correlation coefficient) among the microbial contamination measures for each dialysis fluid on the log scale. Particularly, a correlation analysis of endotoxin levels and microbial burden was carried out. Tests for correlation were performed for each assay method with endotoxin levels to see which was more predictive.

Retrospective chart review data was compiled into a dataset with median microbial contamination for each fluid and method (processed water TSA, R2A, EU/mL; dialysate TSA and EU/mL) added to each patient entry as determined by their exposure (exposure: hemodialysis center). Assumptions of normality were assessed using plots and test of normality. Bivariate regression and correlation analyses were performed using all available data. A multivariable analysis of risk factors was carried out for each set of clinical lab values that did not meet the goals set by CMS or NKF Clinical Practice Guidelines. Due to the availability of standards for microbial burden, the microbial burdens for each outcome were dichotomized based on the proposed standard for microbial contamination of hemodialysis fluids at the time of sample collection (AAMI RD52:2004). A forward stepwise logistic regression process was used for the five models. Different regression models were evaluated for goodness of fit using the Hosmer and Lemeshow Goodness-of-Fit Test. Discriminatory performance was assessed using ROC and classification tables. These models and methods were repeated with the predictors dichotomized based on the most recent standard for microbial contamination of hemodialysis fluids (ANSI/AAMI/ISO 11663:2009).

Results

Microbial recovery

During 3 months of surveillance in the 19 metro-Atlanta hemodialysis facilities, 223 processed water and 223 dialysate samples were collected, analyzed, and colony counts and endotoxin levels were recorded.

The bacterial yields of TSA and R2A culture and endotoxin results are summarized in tables 1 and 2. The median colony count for processed water was 56 CFU/mL and for dialysate was 22 CFU/mL. Contamination of processed water and dialysate did not occur simultaneously, however some centers showed more contamination than others as is demonstrated by compliance with the microbial standard proposed at the time of data collection (AAMI RD52:2004) in table 2.

For processed water, colony counts were greater than the 2004 AAMI standard (<200 CFU/mL) for 15.7% of specimens using TSA agars and for 34.7% of specimens using R2A agars (table 2). In 19.8% (n=44) of specimens R2A yielded colony counts that exceeded the 2004 AAMI standard while TSA yielded colony counts within the standard. Conversely, in 0.9% (n=2) of specimens TSA yielded colony counts that exceeded the 2004 AAMI standard while R2A yielded colony counts within the standard. This illustrates that if TSA were the only method used, nearly 20% of non-compliance with the 2004 AAMI standard could be missed.

Microbial recovery from processed water was significantly greater when using R2A incubated for extended time at lower temperature. However this difference was greater for samples containing lower colony counts.

The correlation between the colony counts utilizing the two different culture methods appeared to be very strong ($r=0.79$, $P<0.0001$, Spearman's rank correlation test). The R2A media demonstrated significantly higher colony counts than TSA agar for processed water, regardless of whether 100 or 200 CFU/mL was chosen as the upper permitted bacterial limit (Wilcoxon's matched-pair's signed-rank test, $p <0.0001$).

Higher colony counts were also associated with higher endotoxin levels in processed water. The correlation between colony count and endotoxin was higher for R2A ($r=0.34$, $P<0.0001$) than for TSA ($r=0.25$, $P=0.0001$) (table 3). Additionally, there was a significant correlation between colony counts and endotoxin levels in the dialysate but the relationship was not as strong as in processed water.

Clinical effects

A total of 454 patients were studied who came from the six metro-Atlanta hemodialysis centers that were selected for more detailed analysis (F, K, L, N, Q, and S). The baseline characteristics of the patient data are shown in table 4. The mean age of the population was 56.81 years (median: 58, range: 15-87, 25th percentile: 46, 75th percentile: 69) and 51.56% of the patients were reported female. Males and females varied significantly only with respect to three of these characteristics, hypertension requiring treatment, injecting drug use, and glomerulonephritis. Hypertension requiring treatment was present in 93.5%, diabetes requiring treatment was present in 46.7%, injecting drug use in 2.12% and HIV infection in 4.1%. The median time on dialysis was 3.0 years (range: 0-18 years). The centers differed significantly (not shown) from one another with respect to erythropoietin dose, average serum albumin, average urea reduction ratio, average KT/V, length of hemodialysis treatment, hypertension requiring treatment, glomerulonephritis, type of dialysis membrane and dialysis treatment type (convention, high-efficiency, and high-flux).

Correlations were run between clinical labs and predictors of non-compliance. Logistic regression models were constructed to predict clinical labs that did not meet CMS goals or NKF Clinical Practice Guidelines. Models were constructed based on the available clinical data and lab data for microbial contamination of fluids during the study period.

Erythropoietin

Pearson correlation coefficients were computed to assess the relationships between the erythropoietin dose (units) that was administered to a patient and several predictor variables (table 5). There were no significant correlations between erythropoietin dose and non-compliance with the 2004 AAMI standards for contamination of dialysis fluids (AAMI RD52:2004, >200 CFU, >2 EU/mL). However, there were weak but significant correlations between a patient's erythropoietin dose and non-compliance with the current AAMI standards for microbial contamination of dialysis fluids (ANSI/AAMI/ISO 11663:2009, >100 CFU, >0.5 EU/mL). There was significant, positive correlation between erythropoietin dose and non-compliance with the current AAMI standard for endotoxin units in dialysate ($r= 0.371$, $n= 431$, $p= <0.0001$), colony counts on TSA for water ($r= 0.228$, $n= 431$, $p= <0.0001$), and colony counts on TSA for dialysate ($r= 0.216$, $n=431$, $p= <0.0001$). There was significant, negative correlation between erythropoietin dose and non-compliance with the current AAMI standard regarding colony counts on TSA for water ($r= -0.274$, $n=431$, $p= <0.0001$). Overall, three of four predictors show a weak but positive correlation between erythropoietin dose and non-compliance with microbial standards. Positive correlation indicates that increases in erythropoietin dose were weakly correlated with non-compliance with microbial standards.

For the purpose of logistic regression, erythropoietin has been dichotomized based on the upper tertile of the study population, at 5000 units. Any patient with an erythropoietin dose greater than or equal to 5,000 units was considered to have a high erythropoietin dose.

2004 AAMI Standard. Based on the selection criteria, the model for high erythropoietin dose included non-compliance with the 2004 AAMI microbial standard for endotoxin units in dialysate and average hematocrit (table 6).

According to the model, the log odds of a high erythropoietin dose was positively and significantly related to non-compliance with microbial standards for endotoxin units in dialysate ($p= 0.0307$), whereas being negatively related to average hematocrit ($p<0.0001$). Given

that all other factors were the same, the odds of having an erythropoietin dose in the upper tertile were 1.64 (95% CI: 1.050-2.728) times greater for a patient who received dialysis in a center with a median endotoxin level in dialysate that was not in compliance with the 2004 AAMI standard compared to a patient who received dialysis at a center with a median endotoxin level that was in compliance with the standard.

The area under the ROC curve demonstrated very good distinguish ability (AOC= 0.7104), demonstrating the models good performance. Additionally, the overall model evaluation indicated a statistically significant model (Likelihood ratio test, Score test, Wald test $p<0.0001$). The Hosmer-Lemeshow test yielded a $\chi^2(8)$ of 5.964 and was insignificant ($p > 0.05$), suggesting that the model was fit to the data well.

2009 AAMI Standard. Based on the selection criteria, the model for high erythropoietin dose included the same predictors as the model using variables defined by the 2004 AAMI standard, non-compliance with the microbial standard for endotoxin units in dialysate and average hematocrit (table 7). However, the main difference between the two models is the magnitude of the odds ratio for non-compliance with the microbial standard for endotoxin units in dialysate. Given that all other factors were the same, the odds of having an erythropoietin dose in the upper tertile were 58.56 (95%CI: 23.032-148.896) times greater for a patient who received dialysis in a center with a median endotoxin level in dialysate that was not in compliance with the 2009 ANSI/AAMI/ISO standard compared to a patient who received dialysis at a center with a median endotoxin level that was in compliance with the standard.

The area under the ROC curve demonstrated very good distinguish ability (AOC= 0.8894), demonstrating the models good performance. Like the previous model, the overall model evaluation indicated a statistically significant model (Likelihood ratio test, Score test, Wald test $p<0.0001$). The Hosmer-Lemeshow test yielded a $\chi^2(8)$ of 12.278 and was insignificant ($p > 0.05$), suggesting that the model was fit to the data well.

Hematocrit

Pearson correlation coefficients were computed to assess the relationships between a patient's average hematocrit level and several predictor variables. There was weak but significant correlation between average hematocrit level and three predictors (Table 8). The strongest correlation was with another clinically provided value, erythropoietin dose (r= -0.482, n=430, p= <0.0001). Additionally, there was a weak positive correlation between average hematocrit level and average serum albumin (r= 0.267, n= 434, p= <0.0001). Patient average hematocrit level showed a weak, positive correlation with non-compliance to the 2004 AAMI microbial standards for endotoxin units in dialysate (r= 0.104, n= 453, p= 0.0267). Positive correlation indicates that low hematocrit is weakly correlated with a center's non-compliant median endotoxin level in dialysate by 2004 AAMI standards. Patient average hematocrit level showed no correlation with variables for non-compliance with current microbial standards.

For the logistic regression, average hematocrit has been dichotomized based on the of reference level of 37. Any patient with a hematocrit level less than or equal to 37 was considered to have a low average hematocrit.

2004 AAMI Standard. Based on the selection criteria, the model for low hematocrit included non-compliance with the 2004 AAMI microbial standard for colony count in water using R2A as well as with erythropoietin dose (per 500 units) (table 9).

The log odds of a low hematocrit were positively related to non-compliance with the 2004 AAMI microbial standard for water using R2A (p= 0.0287) and erythropoietin (p= 0.0137). Therefore, given that all other factors were the same, the odds of a low hematocrit was 3.25 (95% CI: 1.130-9.363) greater for those patients in a center with median colony counts on R2A for water that were non-compliant with the 2004 standard compared to those patients from a center where the median colony counts on R2A for water were in compliance with the standard.

The models good performance was demonstrated by the area under the ROC curve (AOC= 0.7593) and very good distinguish ability. Additionally, the overall model evaluation

indicated a statistically significant model (Likelihood ratio test p= 0.0002, Score test p= 0.0009, Wald test p= 0.0022). The Hosmer-Lemeshow test yielded a $\chi^2(8)$ of 9.832 and was insignificant ($p > 0.05$), suggesting that the model was fit to the data well.

2009 AAMI Standard. Based on the selection criteria, the model for low hematocrit was identical to the above model that included the variables defined by the 2004 AAMI standard versus variables defined by the 2009 AAMI standard (table 10).

Serum albumin

Pearson correlation coefficients were computed to assess the relationships between a patient's average serum albumin measurement and several predictor variables (table 11). There were weak but significant correlations between serum albumin and two methods of measuring compliance with the microbial standards proposed at the time of the study (AAMI RD52:2004). There was positive correlation between average serum albumin and non-compliance with the microbial standards for colony count using R2A for water samples (r=0.098, n= 434, p= 0.0422). Additionally, there was negative correlation between average serum albumin and non-compliance with the microbial standards for endotoxin units for dialysate samples (r= -0.103, n= 434, p= 0.0319). Patient average serum albumin also had significant correlation with two other patient clinical values, erythropoietin dose and average hematocrit levels as mentioned above.

There were also weak but significant correlations between a patient's average serum albumin and non-compliance with the current AAMI standards for microbial contamination of dialysis fluids (ANSI/AAMI/ISO 11663:2009). There was significant, negative correlation between average serum albumin and AAMI standard non-compliance for water endotoxin units (r= -0.096, n= 434, p= 0.0453) and dialysate endotoxin units (r= -0.104, n=434, p= 0.0297). There was significant, positive correlation between average serum albumin and current AAMI standard non-compliance for colony counts on R2A for water (r= 0.098, n=434, p= 0.0422). Overall, two of three predictors show a weak but negative correlation between average

serum albumin and non-compliance with current microbial standards. Negative correlation indicates decreases in average serum albumin were weakly associated with non-compliance with microbial standards.

For the purpose of logistic regression, average serum albumin has been dichotomized based on the goal level determined by the Centers for Medicare and Medicaid Services (CMS), 3.5 g/dL. Any patient with a serum albumin level less than or equal to 3.5 g/dL was considered to have a low average serum albumin.

2004 AAMI Standard. Based on the selection criteria, the model for low serum albumin included non-compliance with the 2004 AAMI microbial standard for colony count in water using TSA, dialysate endotoxin units, and average hematocrit (table 12).

The log odds of a low serum albumin were positively related to non-compliance with the 2004 AAMI microbial standard for the standard for endotoxin units in dialysate ($p= 0.0009$) and negatively related to non-compliance with the standard for water colony counts using TSA ($p= 0.0188$) and average hematocrit ($p<0.0001$). Given that all other factors were the same, the odds of a low serum albumin was 0.30 (95% CI: 0.108—0.817) times as great for those patients in a center with median colony counts on TSA for water that were non-compliant with the 2004 standard compared to those patients from a center where the median colony counts on TSA for water were in compliance with the standard. However, given that all other factors were the same, the odds of a low serum albumin was 3.59 (95% CI: 1.691-7.628) times for those patients in a center where median endotoxin units in dialysate that were non-compliant with the 2004 standard compared to those patients from a center where the median endotoxin units in dialysate were in compliance with the standard.

The area under the ROC curve demonstrated very good distinguish ability (AOC= 0.7301), demonstrating the models good performance. Additionally, the overall model evaluation indicated a statistically significant model (Likelihood ratio test, Score test, Wald test $p<0.0001$).

The Hosmer-Lemeshow test yielded a $\chi^2(8)$ of 6.698 and was insignificant ($p > 0.05$), suggesting that the model was fit to the data well.

2009 AAMI Standard. Based on the selection criteria, the model for low serum albumin included non-compliance with the 2009 AAMI microbial standard for colony count in water using R2A, water endotoxin units, and average hematocrit (table 13).

The log odds of a low serum albumin were negatively related to non-compliance with the 2009 AAMI microbial standard for water colony counts using R2A ($p= 0.0055$) and average hematocrit ($p<0.0001$) and positively related to non-compliance with the standard for endotoxin units in water ($p= 0.0013$). Given that all other factors were the same, the odds of a low serum albumin was 0.40 (95% CI: 0.210-0.764) times as great for those patients in a center with median colony counts on R2A for water that were non-compliant with the 2009 standard compared to those patients from a center where the water was in compliance with the standard. However, given that all other factors were the same, the odds of a low serum albumin was 2.96 (95% CI: 1.527-5.725) times as great for those patients in a center where median endotoxin units in water that were non-compliant with the 2009 standard compared to those patients from a center where the water was in compliance with the standard.

The area under the ROC curve demonstrated very good distinguish ability (AOC= 0.7307), demonstrating the models good performance. Additionally, the overall model evaluation indicated a statistically significant model (Likelihood ratio test, Score test, Wald test $p<0.0001$). The Hosmer-Lemeshow test yielded a $\chi^2(8)$ of 4.621 and was insignificant ($p > 0.05$), suggesting that the model was fit to the data well.

Urea reduction ratio

Pearson correlation coefficients were computed to assess the relationships between a patient's average urea reduction ratio (URR) and several predictor variables (table 14). There were weak but significant correlations between URR and two methods of measuring compliance

with the microbial standards proposed at the time of this study (AAMI RD52:2004, <200 CFU, <2 EU/mL). There was negative correlation between average URR and non-compliance with the microbial standards for colony count set by AAMI using R2A for water samples (r= -0.115, n= 438, p= 0.0158), and for dialysate endotoxin units (r= -0.131, n= 438, p= 0.0059). Negative correlation indicates that URR decreases with non-compliance.

However, there were slightly stronger correlations between a patient's average URR and non-compliance with the current AAMI standard for microbial contamination of dialysis fluids based on the patient's center (ANSI/AAMI/ISO 11663:2009, <100 CFU, <.5 EU/mL)). There was significant, negative correlation between average URR and current AAMI standard non-compliance for water endotoxin units (r= -0.200, n= 438, p= <0.0001), dialysate endotoxin units (r= -0.134, n= 438, p= 0.0050), and colony counts on R2A for water (r= -0.115, n= 438, p= 0.0158). There was significant, positive correlation between average URR and AAMI standard non-compliance for colony counts on TSA for water (r= 0.226, n=438, p= <0.0001). Overall, three of four predictors show a weak but negative correlation between average URR and non-compliance with microbial standards. This negative correlation indicates decreases in average URR were weakly correlated with non-compliance with microbial standards.

For the logistic regression, average URR has been dichotomized based on the goal level determined by the Centers for Medicare and Medicaid Services (CMS), 65%. Any patient with an average URR less than or equal to 65% was considered to have a low average URR.

2004 AAMI Standard. Based on the selection criteria, the model for low URR included non-compliance with the 2004 AAMI microbial standard for colony count in water using TSA and R2A as well as dialysate endotoxin units (table 15).

The log odds of a low URR were positively related to non-compliance with the 2004 AAMI microbial standards for endotoxin units in dialysate (p<0.0001) and colony counts in water using R2A (p<0.0001) and negatively related to non-compliance with the standard colony counts in water using TSA (p<0.0001). Given that all other factors were the same, the odds of a

low URR was 0.10 (95% CI: 0.041-0.263) times as great for those patients in a center with median colony counts on TSA for water that were non-compliant with the 2004 standard compared to those patients from a center where the water was in compliance with the standard. However, given that all other factors were the same, the odds of a low URR was 3.63 (95% CI: 2.019-6.523) times as great for those patients in a center with median colony counts on R2A for water that were non-compliant with the 2004 standard compared to those patients from a center where the water was in compliance with the standard. Likewise, given that all other factors were the same, the odds of a low URR was 6.57 (95% CI: 3.192-13.516) times as great for those patients in a center where median endotoxin units in dialysate that were non-compliant with the 2004 standard compared to those patients from a center where the dialysate was in compliance with the standard.

The area under the ROC curve demonstrated low distinguish ability (AOC= 0.6638), demonstrating the models questionable performance. However, the overall model evaluation indicated a statistically significant model (Likelihood ratio test, Score test, Wald test $p<0.0001$).

2009 AAMI Standard. Based on the selection criteria, the model for low URR included non-compliance with the microbial standard for colony count in water using TSA and dialysate endotoxin units (table 16).

The log odds of a low URR were negatively related to non-compliance with the 2009 AAMI microbial standard for water colony counts using TSA ($p= 0.0001$) and positively related to non-compliance with the standard for endotoxin units in water ($p= 0.0004$). Given that all other factors were the same, the odds of a low URR was 0.41 (95% CI: 0.255-0.645) times as great for those patients in a center with median colony counts on TSA for water that were non-compliant with the 2009 standard compared to those patients from a center where the water was in compliance with the standard. However, given that all other factors were the same, the odds of a low URR was 2.19 (95% CI: 1.419-3.365) times as great for those patients in a center where

median endotoxin units in water that were non-compliant with the 2009 standard compared to those patients from a center where the water was in compliance with the standard.

The area under the ROC curve demonstrated low distinguish ability (AOC= 0.6480), demonstrating the models weak performance. However, the overall model evaluation indicated a statistically significant model (Likelihood ratio test, Score test, Wald test $p<0.0001$).

KT/V

Pearson correlation coefficients were computed to assess the relationships between a patient's average KT/V and several predictor variables (table 17). There were weak, positive correlations between KT/V and three methods of measuring non-compliance with the microbial standards proposed at the time of this 1997 study (AAMI RD52:2004). There was positive correlation between average KT/V and non-compliance with the microbial standards set by AAMI for colony count using TSA for water samples ($r= 0.127$, $n= 441$, $p= 0.0078$), endotoxin units in water ($r= 0.127$, $n= 441$, $p= 0.0078$), and colony count using TSA for dialysate samples ($r= 0.127$, $n= 441$, $p= 0.0078$).

There were also correlations between a patient's average KT/V and non-compliance with the current AAMI standard for microbial contamination of dialysis fluids (ANSI/AAMI/ISO 11663:2009). There was positive correlation between average KT/V and non-compliance with the AAMI standard for colony counts for water using TSA ($r= 0.160$, $n= 441$, $p= 0.0008$) and endotoxin units in dialysate ($r= 0.119$, $n= 441$, $p= 0.0125$).

Overall, regardless of the year of the standard, there appears to be a weak but positive correlation between average KT/V and non-compliance with microbial standards. Increases in average KT/V were weakly correlated with non-compliance with microbial standards.

For the logistic regression, average KT/V has been dichotomized based goal level determined by the Centers for Medicare and Medicaid Services (CMS), 1.2. Any patient with a KT/V level less than or equal to 1.2 was considered to have a low average KT/V.

2004 AAMI Standard. Based on the selection criteria, the model for low KT/V only included average hematocrit (table 18).

The log odds of a low KT/V were negatively related to a patient's average hematocrit (p= 0.0229). Given that all other factors were the same, the odds of a low KT/V was 0.93 (95% CI: 0.864-0.989) times greater for each unit increase in average hematocrit.

The area under the ROC curve demonstrated low distinguish ability (AOC= 0.6381), demonstrating the models weak performance. However, the overall model evaluation indicated a statistically significant model (Likelihood ratio test p= 0.0027, Score test p= 0.0024, Wald test p= 0.0031). The Hosmer-Lemeshow test yielded a $\chi^2(8)$ of 7.591 and was insignificant ($p > 0.05$), suggesting that the model was fit to the data well.

2009 AAMI Standard. Based on the selection criteria, the model for low KT/V included non-compliance with the 2009 AAMI microbial standard for colony count in water using TSA and dialysate endotoxin units (table 19).

The log odds of a low KT/V were negatively related to non-compliance with the microbial standard for colony count in water using TSA (p= 0.0044) and dialysate endotoxin units (p= 0.0496). Given that all other factors were the same, the odds of a low KT/V were 0.42 (95% CI: 0.228-0.761) times as great for those patients in a center with median colony counts on TSA for water that were non-compliant with the 2009 standard compared to those patients from a center where the water was in compliance with the standard. Similarly, the odds of a low KT/V were 0.58 (95% CI: 0.341-0.999) times as great for those patients in a center with median dialysate endotoxin units that were non-compliant with the 2009 standard compared to those patients from a center where the water was in compliance with the standard.

The area under the ROC curve demonstrated low distinguish ability (AOC= 0.6513), demonstrating the models weak performance. However, the overall model evaluation indicated a statistically significant model (Likelihood ratio test p= 0.0041, Score test p= 0.0050, Wald test p= 0.0060).

Discussion

Despite technological advances in the area of hemodialysis, adverse events are still common (36). Furthermore, many of these can be attributed to microbial contamination of hemodialysis fluids (14). For this reason facilities that perform hemodialysis have a program in place to monitor the microbial quality of their dialysis fluids (processed water and dialysate).

The upper limits of microbial contamination set by both AAMI and ISO standards are not the same in practice and they employ different methods to culture samples of dialysis fluids (8). If a particular method underestimates the bacterial load of fluid samples, the patient population that is exposed to those fluids could be at risk. This is because the test could be interpreted as being within the standard when in fact a more sensitive culture method reveals the fluid has exceeded the microbial contamination limits of the standard. Thus, it is important that techniques and media for bacterial cultivation are compared to ascertain their sensitivity and effectiveness for indicating the bacterial concentration of a given sample.

The highest frequency and level of contamination in this study was found in the dialysate and was true for both colony counts and endotoxin concentration. In this case, dialysate was cultured using the AAMI recommended method that employed TSA incubated at 36°C with a 48 hour incubation period (AAMI, 2004).

The use of media that is poor in nutrients seems the most appropriate for organisms that have adapted to the oligotrophic habitats including water (63). Hemodialysis fluids can also be considered to contain a similar environment. This study supported this assumption; the total colonies on the nutrient-poor R2A were significantly higher overall when compared to medium-nutrient TSA.

The bacterial colony counts detected in this study indicate that the microbiological quality of the water and dialysate analyzed was frequently above the standard limits proposed by AAMI at the time of data collection (AAMI RD52:2004). Colony counts on TSA were out of

compliance with the standard in 15.7% of samples and colony counts on R2A were out of compliance with the standard in 34.7% of samples. If the study was to expect compliance with the current standard (ANSI/AAMI/ISO 11663:2009), the levels of non-compliance (the percent of samples above the maximum contamination limit) would increase to 27.8% for TSA and 52.7% for R2A. The updated standards highlight the lack of sensitivity of the TSA method in determining total bacterial counts.

A single medium or method cannot be expected to detect all viable bacteria in a particular sample. However, it is important to enhance microbial recovery with the most sensitive method available. Additionally, one could combine culture methods with other microbiology methods such as PCR or solid-phase cytometry to illustrate the broader scope of microbial contamination.

In addition to the suggested monthly microbial testing of the water treatment system and dialysis fluids, certain biomarkers as indicators may also identify microbial contamination levels above AAMI/ISO standards and may be used as performance indicators. Indicators associated with chronic inflammation would then be particularly indicative of a negative response to exposure to contaminated dialysis fluids.

In the present study, logistic regression models were developed to predict clinical labs that did not meet CMS targets or NKF Clinical Practice Guidelines in maintenance hemodialysis patients. Microbial contamination of dialysis fluids and readily available clinical laboratory values were used as predictors.

The demographics of the study population were comparable to the general population in the United States currently on hemodialysis. This lends the study to extrapolation to a broader population.

The prediction models used untransformed variables, although when used as a predictor erythropoietin dose was represented per 500 units to better illustrate the strength of the association. Analysis of other variables, particularly C-reactive protein, which are not routinely

measured in maintenance hemodialysis facilities because Medicare does not routinely reimburse for this test, may demonstrate further predictive power of our models.

These results indicated that microbial contamination is significant to patient outcomes regarding high erythropoietin dose, low hematocrit level, low serum albumin, and low urea reduction ratio.

Non-compliance with the AAMI standard for microbial contamination of hemodialysis fluids for endotoxin in dialysate appeared in four out of 10 models as a positive predictor for the given outcome, more than any other predictor of contamination. Non-compliance with the proposed standard for endotoxin (>2 EU/mL) in dialysate at the time of data collection was significant for high erythropoietin, low serum albumin, and low URR.

Given these results, endotoxin in dialysate appears to be the strongest and most consistent predictor of negative outcomes in this study. In contrast, colony counts on TSA for water samples consistently gave odds ratios below 1, thus appearing protective against negative outcomes. It could be hypothesized that because TSA provides a less sensitive measure of microbial contamination, it does not act as an accurate predictor of clinical outcomes for patients in maintenance hemodialysis clinics. Conversely, non-compliance with the standard using the more sensitive method of testing on R2A was more in accordance with non-compliance for endotoxin units. Though the correlation was weak, the method using R2A demonstrated a significant and stronger correlation to endotoxin units than did method using TSA.

Studies have shown that hypoalbuminemia and low URR predict death in hemodialysis patients (51, 54); therefore it is imperative to know whether certain levels of microbial contamination increase a patient's likelihood of having a low serum albumin or URR.

This study indicates that low serum albumin may be related to non-compliance with standards for endotoxin in both dialysate and water. However, the models also indicate that bacteria above the standard level as indicated by both TSA and R2A could be protective. While this could be counterintuitive, it could indicate that endotoxin is more important to patient

outcomes than the presence of bacteria. This could be because endotoxin and other small fragments can more easily pass through the membrane and cause the chronic, subclinical inflammation that is believed to encourage the hypoalbuminemia.

The study indicates that low URR may be related to non-compliance with the AAMI standards for colony counts in water (using R2A) and endotoxin units in both water and dialysate. However, in both models colony counts in water on TSA appeared protective. As suggested in the context of serum albumin, this could suggest that endotoxin is more important to patient outcomes than bacteria or that the method used with TSA is not sensitive enough to identify the true level of bacteria in the sample.

Chronic inflammation can cause reduced humeral response to erythropoietin or erythropoietin resistance, thus causing a dialysis patient to require increased amounts erythrocyte-stimulating agents to overcome anemia. High doses of this recombinant human erythropoietin can therefore be an indication of inflammation. Non-compliance with the AAMI standard for microbial contamination of dialysis fluids for endotoxin units in dialysate was a strong predictor of negative patient outcome measured by high erythropoietin dose.

The logistic regression models for KT/V revealed the most conflicting data. The only predictors of standard non-compliance indicated that high levels of bacteria or endotoxin were protective against low KT/V. This may suggest that KT/V is not a good indicator of inflammation or patient outcomes due to contaminated dialysis fluid even though it is a measure of dialysis treatment adequacy.

High erythropoietin dose, low serum albumin, and low urea reduction ratio appear to be positively related to microbial contamination by endotoxin. As these factors all impact mortality, it is important that the effects of microbial contamination on these factors be understood and monitored. Dialysate endotoxin appears to be most consistent predictor of negative patient outcomes.

Future directions

The regression did not take into account change over time. However, values for patient biomarkers, as well as for microbial contamination of dialysis fluids were available during a period of three months (January – March, 1997). Further analysis could be done to elaborate on the effect of contamination on the biomarkers based on distinctions between months. However, because of the study size (454 patients) and the fact that patients were from six different centers (thus defining their exposure to fluids), the volume of missing biomarker data made the chosen method of modeling the preferred method. It would be even more indicative to see within the same center, longitudinal measurements of C-reactive protein, serum albumin and erythropoietin dose and the associated microbial contamination of dialysis fluids in order to infer whether decreased contamination leads to a decrease in patient inflammatory status.

Additionally, future studies could include prospective studies that seek to link chronic microinflammatory state and further complications with microbial contamination of dialysis fluids. Some prospective studies have already been done in the area, such as HEMO, however the HEMO study did not specifically focus on microbial contamination and patient outcomes (74, 75).

Limitations and strengths

Participation by hemodialysis centers was voluntary; as such, minor selection bias may have been introduced into the results of this study. All water and dialysate samples were collected in sterile, endotoxin free containers using aseptic technique to prevent contamination. However, it is plausible that a proportion of the non-compliant samples in this study were false positives due to contamination during the sampling procedure, transport, or processing. Therefore, perceptible differences in water quality therefore could possibly be attributed to variations in procedures among centers. In this study, however, sampling procedures were not evaluated.

The limulus amebocyte lysate assay does not detect all levels of endotoxin, and the minimum limit of detection that can be achieved is 0.001 endotoxin units for the turbidimetric kinetic assay system (LAL-5000, Associates of Cape Cod). In this study the reproducible limit of detection was 0.03 EU/mL, which consequently is the AAMI standard level for ultrapure dialysate (22, 24). This assay method only detects bacterial endotoxin and will miss other inflammatory microbial metabolites that may cross the dialyzer membrane as well. Therefore, it could be assumed that standards for microbial purity are based on the limits of the test and not clinical and microbiological needs for therapeutic quality of dialysis fluids.

In terms of adverse events, the study did look for pyrogenic reactions. Only one patient experienced a pyrogenic reaction from Center L during the three-month study period. In order to expand on this area of research, a future study could expand the study period or population, or add bacteremia as an additional adverse outcome.

The multicenter study design provided a robust amount of data and allowed for the creation of significant logistic regression models with good fit. The ability to compare not only two plating methods but also correlate the bacterial concentration with endotoxin levels strengthened the study with a constant by which to judge performance.

In this chart review, data on measurable parameters related to patient health were reported that are routinely collected by facilities for Centers for Medicare and Medicaid Service (CMS). Using quality control data for the microbiological quality of the dialysis fluids to predict patient outcomes provides hemodialysis centers with a potential way to protect patients from harmful adverse outcomes from contaminated fluids using data that is readily available in any US hemodialysis facility.

Conclusion

In conclusion, logistic regression models were developed to predict clinical labs that did not meet CMS goals or NKF Clinical Practice Guidelines in maintenance hemodialysis

patients. They demonstrated connections between clinical observations that did not meet these goals and non-compliance with AAMI standard for microbial contamination, particularly endotoxin units in dialysate and water. Future model iterations may include other outcome variables that are stronger predictors of inflammation, such as C-reactive protein. Additionally, further study could look prospectively at patient clinical labs and changing microbial quality of water. The clinical utility that this study provides is a connection between clinical information that is readily available for any hemodialysis patient and contamination of dialysis fluids. There is predicted to be a growing population of people in need of hemodialysis and it is in the interest of field of nephrology, and the dialysis centers to improve clinical outcomes and limit adverse events.

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Tables

Table 1. Colony counts and endotoxin levels in both water and dialysate at 19 dialysis centers, Atlanta, GA

Specimen Source	# of Specimens	Median Colonies TSA Agar	Range Colonies TSA Agar	Median Colonies R2A Agar	Range Colonies R2A Agar	Median Endotoxin EU/mL	Range Endotoxin EU/mL
Processed Water	223	21	0-17,000	115	0-27,400	0.3	0-103
Dialysate	223	22	0-36,200	ND	ND	0.7	0-124

Note. ND: Not determined; TSA: Trypticase soy agar; R2A: Reasoner's 2A agar; EU/mL: Endotoxin units per milliliter.

Table 2. Colony counts and endotoxin levels for processed water at 19 metro-Atlanta hemodialysis centers

Dialysis Center	# of Specimens	Median Colony Counts		Median	% in Compliance with standard* Counts <200	
		TSA	R2A	EU/mL	TSA	R2A
CENTERS INCLUDED IN CLINICAL EFFECTS STUDY						
F	10	82	214	0.24	80.0	40.0
K	10	2	145	1.68	100.0	70.0
L	10	142	135	0.42	60.0	60.0
N	11	1	70	0.03	100.0	90.9
Q	10	84	313	0.57	100.0	30.0
S	15	281	3800	5.91	40.0	13.3
ALL OTHER HEMODIALYSIS CENTERS IN STUDY						
A	16	0	1	0.02	93.8	93.8
B	14	4	33	0.07	92.9	92.9
C	12	0	17	0.03	100.0	100.0
D	8	244	391	1.71	37.5	12.5
E	14	20	70	0.05	92.9	92.9
G	6	31	139	0.12	100.0	83.3
H	9	93	190	1.09	88.9	66.7
I	15	51	67	0.15	66.7	66.7
J	5	7	126	0.23	100.0	75.0
M	12	13	11	0.18	91.7	81.8
O	16	96	221	0.79	68.8	43.8
P	15	26	157	0.58	100.0	53.3
R	15	12	42	0.39	100.0	73.3
Combined	223	21	117	0.30	84.3	65.3

Note. *Standard: AAMI RD52:2004; TSA: Trypticase soy agar; R2A: Reasoner's 2A agar; EU/mL: Endotoxin units per milliliter.

Table 3. Correlation between colony counts and endotoxin levels of water and dialysate

Group	Specimen		Correlation Coefficient	P Value
Processed Water	CFU-TSA	CFU-R2A	0.79	<0.0001
	CFU-TSA	EU/mL	0.25	0.0001
	CFU-R2A	EU/mL	0.34	<0.0001
Dialysate	CFU-TSA	EU/mL	0.17	0.0122

Note. CFU: Colony forming units; EU/mL: Endotoxin units per milliliter; TSA: Trypticase soy agar; R2A: Reasoner's 2A agar. Correlation coefficient measured by Spearman's Rank test.

Table 4. Patient characteristics from the six maintenance hemodialysis facilities used to evaluate effects of dialysis fluids on facility outcome data

Variable, mean(sd)	**Total**	(n=454)	**Center F**	(n=99)	**Center K**	(n=64)	**Center L**	(n=93)	**Center N**	(n=54)	**Center Q**	(n=66)	**Center S**	(n=78)
Age, years	56.81	(15.01)	53.81	(13.22)	54.19	(15.94)	61.56	(14.14)	53.12	(14.53)	57.27	(14.94)	59.01	(16.21)
Length of dialysis treatment, hours	3.34	(0.41)	3.16	(0.35)	3.44	(0.39)	3.28	(0.50)	3.32	(0.32)	3.58	(0.27)	3.38	(0.41)
Erythropoietin dose, units	5177.61	(3420.13)	3906.59	(737.53)	5495.54	(3462.14)	3486.81	(1831.59)	7182.35	(4876.79)	8087.88	(3543.34)	4617.11	(3294.01)
Clinical labs														
Average hematocrit	31.78	(4.09)	30.89	(4.63)	32.46	(5.10)	32.10	(3.71)	31.84	(3.87)	31.25	(3.66)	32.37	(3.14)
Average serum albumin, g/dL	3.86	(0.39)	3.93	(0.31)	3.71	(0.45)	3.88	(0.34)	3.84	(0.41)	3.86	(0.45)	3.88	(0.38)
Average urea reduction ratio, %	67.31	(7.13)	66.30	(6.68)	63.56	(5.77)	70.82	(5.69)	69.30	(5.18)	65.47	(10.27)	67.81	(5.76)
Average KT/V	1.44	(0.28)	1.33	(0.24)	1.35	(0.23)	1.48	(0.26)	1.58	(0.20)	1.43	(0.40)	1.52	(0.23)
Causes of ESRD, n(%)														
Diabetes requiring treatment	202	(45.70)	37	(37.76)	33	(52.38)	40	(43.01)	17	(36.96)	32	(49.23)	43	(55.84)
Hypertension requiring treatment	415	(93.47)	92	(92.93)	59	(93.65)	80	(86.02)	44	(93.62)	64	(98.46)	76	(98.70)
Glomerulonephritis	32	(7.34)	13	(13.27)	2	(3.17)	11	(11.83)	1	(2.44)	3	(4.69)	2	(2.60)
Comorbidities, n(%)														
Known HIV infection	18	(4.09)	1	(1.03)	4	(6.35)	3	(3.23)	4	(8.89)	5	(7.69)	1	(1.30)
Injecting drug use	9	(2.12)	1	(1.10)	0	(0.00)	1	(1.08)	2	(4.65)	3	(4.92)	2	(2.70)
Hepatitis B surface antigen positive	9	(2.13)	1	(1.25)	3	(4.69)	0	(0.00)	2	(4.55)	3	(4.62)	0	(0.00)
Hepatitis C antibody positive	13	(2.86)	1	(1.01)	3	(4.69)	1	(1.08)	9	(16.67)	0	(0.00)	0	(0.00)

Table 5. Correlation between erythropoietin dose and predictors

Variable	Predictor	Correlation Coefficient	P Value
Erythropoietin dose, units	New AAMI Standard, ANSI/AAMI/ISO 11663:2009		
	Non-compliance: Dialysate endotoxin	0.3706	<0.0001
	Non-compliance: Water colony count, TSA	-0.2739	<0.0001
	Non-compliance: Water endotoxin	0.2280	<0.0001
	Non-compliance: Dialysate colony count, TSA	0.2160	<0.0001
	Clinical Measures		
	Average hematocrit	-0.4819	<0.0001
	Average serum albumin	-0.2115	<0.0001
	Average URR	-0.0973	0.0472

Table 6. Logistic regression analysis of 454 patient records for high erythropoietin dose (≥ 5000 units)

Predictor	Estimate	SE	P value	OR	95% CI for OR
Non-compliance: Dialysate endotoxin standard	0.526	0.244	0.0307	1.64	1.050-2.728
Average hematocrit	-0.205	0.034	<.0001	0.81	0.763-0.870

Test	Chi square	df	P value
Overall model evaluation	45.2286	2	<.0001
Likelihood ratio test	44.301	2	<.0001
Score test	38.158	2	<.0001
Wald test			
Goodness-of-fit test			
Hosmer & Lemeshow	5.964	8	0.6513

Note. Non-compliance with microbial contamination standards are in reference to allowable levels set by AAMI RD52:2004, bacteria <200 CFU/mL and endotoxin <2 EU/mL. Colony counts measured on either Trypticase Soy Agar (TSA) or Reasoner's 2 Agar (R2A). (1=Non-compliant, 0=Compliant).

Table 7. Logistic regression analysis of 454 patient records for high erythropoietin dose (≥ 5000 units)

Predictor	Estimate	SE	P value	OR	95% CI for OR
Non-compliance: Dialysate endotoxin standard	4.070	0.476	<.0001	58.56	23.032-148.896
Average hematocrit	-0.366	0.050	<.0001	0.69	0.629-0.765

Test	Chi square	df	P value
Overall model evaluation	191.9279	2	<.0001
Likelihood ratio test	150.236	2	<.0001
Score test	83.242	2	<.0001
Wald test			
Goodness-of-fit test			
Hosmer & Lemeshow	12.278	8	0.1392

Note. Non-compliance with microbial contamination standards are in reference to allowable levels set by ANSI/AAMI/ISO 11663:2009, bacteria <100 CFU/mL and endotoxin <0.5 EU/mL. Colony counts measured on either Trypticase Soy Agar (TSA) or Reasoner's 2 Agar (R2A). (1=Non-compliant, 0=Compliant).

Table 8. Correlation between average hematocrit and predictors

Variable	Predictor	Correlation Coefficient	P Value
Average hematocrit, %	Previous AAMI Standard, AAMI RD52:2004		
	Non-compliance: Dialysate endotoxin	0.1041	0.0267
	Clinical Measures		
	Erythropoietin dose	-0.4819	<0.0001
	Average serum albumin	0.2668	<0.0001

Table 9. Logistic regression analysis of 454 patient records for low hematocrit levels (≤ 37%)

Predictor	Estimate	SE	P value	OR	95% CI for OR
Non-compliance: Water colony count standard, R2A	1.180	0.539	0.0287	3.25	1.130-9.363
Erythropoietin dose, per 500 units	0.002	0.001	0.0137	1.00	1.000-1.003

Test	Chi square	df	P value
Overall model evaluation	17.307	2	0.0002
Likelihood ratio test	13.921	2	0.0009
Score test	12.199	2	0.0022
Wald test			
Goodness-of-fit test			
Hosmer & Lemeshow	9.832	8	0.2770

Note. Non-compliance with microbial contamination standards are in reference to allowable levels set by AAMI RD52:2004, bacteria <200 CFU/mL and endotoxin <2 EU/mL. Colony counts measured on either Trypticase Soy Agar (TSA) or Reasoner's 2 Agar (R2A). (1=Non-compliant, 0=Compliant).

Table 10. Logistic regression analysis of 454 patient records for low hematocrit levels (≤ 37%)

Predictor	Estimate	SE	P value	OR	95% CI for OR
Non-compliance: Water colony count standard, R2A	1.180	0.539	0.0287	3.25	1.130-9.363
Erythropoietin dose, per 500 units	0.002	0.001	0.0137	1.00	1.000-1.003

Test	Chi square	df	P value
Overall model evaluation	17.307	2	0.0002
Likelihood ratio test	13.921	2	0.0009
Score test	12.199	2	0.0022
Wald test			
Goodness-of-fit test			
Hosmer & Lemeshow	9.832	8	0.2770

Note. Non-compliance with microbial contamination standards are in reference to allowable levels set by ANSI/AAMI/ISO 11663:2009, bacteria <100 CFU/mL and endotoxin <0.5 EU/mL. Colony counts measured on either Trypticase Soy Agar (TSA) or Reasoner's 2 Agar (R2A). (1=Non-compliant, 0=Compliant).

Table 11. Correlation between average serum albumin and predictors

Variable	Predictor	Correlation Coefficient	P Value
Average serum albumin, g/dL	Previous AAMI Standard, AAMI RD52:2004		
	Non-compliance: Dialysate endotoxin	-0.1030	0.0319
	Non-compliance: Water colony count, R2A	0.0976	0.0422
	New AAMI Standard, ANSI/AAMI/ISO 11663:2009		
	Non-compliance: Dialysate endotoxin	-0.1044	0.0297
	Non-compliance: Water colony count, R2A	0.0976	0.0422
	Non-compliance: Water endotoxin	-0.0962	0.0453
	Clinical Measures		
	Average hematocrit	0.2668	<0.0001
	Erythropoietin dose	-0.2115	<0.0001

Table 12. Logistic regression analysis of 454 patient records for low serum albumin levels (≤ 3.5 g/dL)

Predictor	Estimate	SE	P value	OR	95% CI for OR
Non-compliance: Water colony count standard, TSA	-1.215	0.517	0.0188	0.30	0.108-0.817
Non-compliance: Dialysate endotoxin standard	1.279	0.384	0.0009	3.59	1.691-7.628
Average hematocrit	-0.178	0.039	<.0001	0.84	0.775-0.904

Test	Chi square	df	P value
Overall model evaluation	25.281	2	<.0001
Likelihood ratio test	27.068	2	<.0001
Score test	23.739	2	<.0001
Wald test			
Goodness-of-fit test			
Hosmer & Lemeshow	6.698	8	0.5695

Note. Non-compliance with microbial contamination standards are in reference to allowable levels set by AAMI RD52:2004, bacteria <200 CFU/mL and endotoxin <2 EU/mL. Colony counts measured on either Trypticase Soy Agar (TSA) or Reasoner's 2 Agar (R2A). (1=Non-compliant, 0=Compliant).

Table 13. Logistic regression analysis of 454 patient records for low serum albumin levels (≤ 3.5 g/dL)

Predictor	Estimate	SE	P value	OR	95% CI for OR
Non-compliance: Water colony count standard, R2A	-0.916	0.330	0.0055	0.40	0.210-0.764
Non-compliance: Water endotoxin standard	1.084	0.337	0.0013	2.96	1.527-5.725
Average hematocrit	-0.196	0.040	<.0001	0.82	0.761-0.889

Test	Chi square	df	P value
Overall model evaluation	35.1834	3	<.0001
Likelihood ratio test	37.664	3	<.0001
Score test	31.166	3	<.0001
Wald test			
Goodness-of-fit test			
Hosmer & Lemeshow	4.621	8	0.7972

Note. Non-compliance with microbial contamination standards are in reference to allowable levels set by ANSI/AAMI/ISO 11663:2009, bacteria <100 CFU/mL and endotoxin <0.5 EU/mL. Colony counts measured on either Trypticase Soy Agar (TSA) or Reasoner's 2 Agar (R2A). (1=Non-compliant, 0=Compliant).

Table 14. Correlation between average urea reduction ratio (URR) and predictors

Variable	Predictor	Correlation Coefficient	P Value
Average URR, %	Previous AAMI Standard, AAMI RD52:2004		
	Non-compliance: Dialysate endotoxin	-0.1315	0.0059
	Non-compliance: Water colony count, R2A	-0.1152	0.0158
	New AAMI Standard, ANSI/AAMI/ISO 11663:2009		
	Non-compliance: Water colony count, TSA	0.2262	<0.0001
	Non-compliance: Water endotoxin	-0.2002	<0.0001
	Non-compliance: Dialysate endotoxin	-0.1339	0.0050
	Non-compliance: Water colony count, R2A	-0.1152	0.0158
	Clinical Measures		
	Erythropoietin dose	-0.0973	0.0472

Table 15. Logistic regression analysis of 454 patient records for low urea reduction ratios (≤ 65%)

Predictor	Estimate	SE	P value	OR	95% CI for OR
Non-compliance: Water colony count standard, TSA	-2.268	0.475	<.0001	0.10	0.041-0.263
Non-compliance: Water colony count standard, R2A	1.289	0.299	<.0001	3.63	2.019-6.523
Non-complianc: Dialysate endotoxin standard	1.882	0.368	<.0001	6.57	3.192-13.516

Test	Chi square	df	P value
Overall model evaluation	33.592	3	<.0001
Likelihood ratio test	32.151	3	<.0001
Score test	29.581	3	<.0001
Wald test			

Note. Non-compliance with microbial contamination standards are in reference to allowable levels set by AAMI RD52:2004, bacteria <200 CFU/mL and endotoxin <2 EU/mL. Colony counts measured on either Trypticase Soy Agar (TSA) or Reasoner's 2 Agar (R2A). (1=Non-compliant, 0=Compliant).

Table 16. Logistic regression analysis of 454 patient records for low urea reduction ratios (≤ 65%)

Predictor	Estimate	SE	P value	OR	95% CI for OR
Non-compliance: Water colony count standard, TSA	-0.903	0.237	0.0001	0.41	0.255-0.645
Non-compliance: Water endotoxin standard	0.782	0.220	0.0004	2.19	1.419-3.365

Test	Chi square	df	P value
Overall model evaluation	28.4392	2	<.0001
Likelihood ratio test	27.383	2	<.0001
Score test	25.920	2	<.0001
Wald test			

Note. Non-compliance with microbial contamination standards are in reference to allowable levels set by ANSI/AAMI/ISO 11663:2009, bacteria <100 CFU/mL and endotoxin <0.5 EU/mL. Colony counts measured on either Trypticase Soy Agar (TSA) or Reasoner's 2 Agar (R2A). (1=Non-compliant, 0=Compliant).

Table 17. Correlation between average KT/V and predictors

Variable	Predictor	Correlation Coefficient	P Value
Average KT/V	Previous AAMI Standard, AAMI RD52:2004		
	Non-compliance: Water colony count, TSA	0.1266	0.0078
	Non-compliance: Water endotoxin	0.1266	0.0078
	Non-compliance: Dialysate colony count, TSA	0.1266	0.0078
	New AAMI Standard, ANSI/AAMI/ISO 11663:2009		
	Non-compliance: Water colony count, TSA	0.1596	0.0008
	Non-compliance: Dialysate endotoxin	0.1189	0.0125

Table 18. Logistic regression analysis of 454 patient records for low KT/V (≤ 1.2)

Predictor	Estimate	SE	P value	OR	95% CI for OR
Average hematocrit	-0.078	0.034	0.0229	0.93	0.864-0.989

Test	Chi square	df	P value
Overall model evaluation	11.796	2	0.0027
Likelihood ratio test	12.024	2	0.0024
Score test	11.522	2	0.0031
Wald test			
Goodness-of-fit test			
Hosmer & Lemeshow	7.591	8	0.4745

Note. Non-compliance with microbial contamination standards are in reference to allowable levels set by AAMI RD52:2004, bacteria <200 CFU/mL and endotoxin <2 EU/mL. Colony counts measured on either Trypticase Soy Agar (TSA) or Reasoner's 2 Agar (R2A). (1=Non-compliant, 0=Compliant).

Table 19. Logistic regression analysis of 454 patient records for low KT/V (≤ 1.2)

Predictor	Estimate	SE	P value	OR	95% CI for OR
Non-compliance: Water colony count standard, TSA	-0.876	0.308	0.0044	0.42	0.228-0.761
Non-compliance: Dialysate endotoxin standard	-0.539	0.274	0.0496	0.58	0.341-0.999

Test	Chi square	df	P value
Overall model evaluation	10.986	2	0.0041
Likelihood ratio test	10.585	2	0.0050
Score test	10.233	2	0.0060
Wald test			
Goodness-of-fit test			
Hosmer & Lemeshow	1.490	2	0.4748

Note. Non-compliance with microbial contamination standards are in reference to allowable levels set by ANSI/AAMI/ISO 11663:2009, bacteria <100 CFU/mL and endotoxin <0.5 EU/mL. Colony counts measured on either Trypticase Soy Agar (TSA) or Reasoner's 2 Agar (R2A). (1=Non-compliant, 0=Compliant).

EMORY UNIVERSITY
Institutional Review Board

September 27, 2011

Nicole Tocci, BA
Public Health

RE: Determination: No IRB Review Required
eIRB Number 53746- Title: *Microbial Burden of Hemodialysis Fluids: A Multi-Center Study*
PI: Nicole Tocci, BA

Dear Ms. Tocci:

Thank you for requesting a determination from our office about the above-referenced project. Based on our review of the materials you provided, we have determined that it does not require IRB review because it does not meet the definition of "research" involving "human subjects" or the definition of "clinical investigation" as set forth in Emory policies and procedures and federal rules, if applicable.

Specifically, in this project, you will be working with de-identified data, performing data analysis and interpretation as well as consulting on study design. As there will be no interaction with human subjects and no identifiable data will be used, this project does not fall under the purview of the IRB.

This determination could be affected by substantive changes in the study design, subject populations, or identifiability of data. If the project changes in any substantive way, please contact our office for clarification.

Thank you for consulting the IRB.

Sincerely,

Zoa Hepburn, BA
Biomedical Analyst Assistant

This letter has been digitally signed