Comparison of techniques for culture of dialysis water and fluid

Jo-Ann B. MALTAIS,1 Klemens B. MEYER,2 Meredith C. FOSTER2

1Maltais Consulting, Arvada, Colorado, USA; 2William B. Schwartz Division of Nephrology, Tufts Medical Center, Boston, Massachusetts, USA

Abstract

Introduction: Microbiological culture of dialysis water and fluid is a routine safety measure. In the United States (U.S.), laboratories perform these cultures on trypticase soy agar at 35–37°C for 48 h (TSA-48h), not on the tryptone glucose extract agar or Reasoner’s 2A agar at 17–23°C for 7 days (TGEA-7d and R2A-7d, respectively) recommended by international standards. We compared culture methods to identify samples exceeding the accepted action level of 50 CFU/mL.

Methods: Dialysis water and fluid samples collected from 41 U.S. dialysis programs between 2011 and 2014 were cultured at two U.S. laboratories. Each sample was cultured using (1) either TGEA-7d or R2A-7d and (2) TSA-48h. We compared proportions exceeding the action level by different methods and test characteristics of TSA-48h to those of TGEA-7d and R2A-7d.

Findings: The proportion of water samples yielding colony counts ≥50 CFU/mL by TGEA-7d was significantly different from the proportion by TSA-48h (P = 0.001; difference in proportion 4.3% [95%CI 1.3–7.3%]). The proportions of dialysis fluid samples ≥50 CFU/mL by TGEA-7d and TSA-48h were not significantly different; there were no significant differences for comparisons of R2A-7d to TSA-48h.

Discussion: In dialysis fluid, TSA-48h was comparable to TGEA-7d and R2A-7d in identifying samples as having bacterial counts ≥50 CFU/mL. In dialysis water, TSA-48h was comparable to R2A-7d in identifying samples ≥50 CFU/mL, but TGEA-7d did yield significantly more results above 50 CFU/mL. Nonetheless, the negative predictive value of a TSA-48h result of <50 CFU/mL in dialysis water exceeded 95%.

Key words: hemodialysis, microbiological culture, standards, dialysis water, dialysis fluid

INTRODUCTION

Microbiological culture of dialysis water and dialysis fluid (“dialysate” in the U.S.) is a routine measure to ensure quality of dialysis treatment and is considered important to assure safe dialysis. International standards for dialysis water and dialysis fluid quality are established by the International Organization for Standardization (ISO). Multiple national standards organizations are members of ISO, including the Association for the Advancement of Medical Instrumentation (AAMI) which acts as secretariat for ANSI, the official U.S. standards organization member of ISO. National standards organizations determine...
whether to adopt international standards within individual countries. AAMI is a non-governmental organization, and AAMI and ISO standards have the status only of recommendations in the U.S., unless explicitly endorsed in regulation.

The applicable international standards for microbiological culture of dialysis water and dialysis fluid (ISO-13959, ISO-11663, ISO-23500, and ISO-26722) recommend culturing on tryptone glucose extract agar (TGEA) or Reasoner’s 2A agar (R2A) at 17–23°C for 7 days (d) “or the equivalent,” and define 50 colony-forming units/milliliter (CFU/mL) as the action level at or above which facility staff should promptly take measures to reduce the microbial load, such as disinfection of the equipment by which dialysis water and dialysis fluid are prepared, stored and delivered to the patient. In the presence of 100 CFU/mL or more, the standards dictate dialysis treatment interruption. The current AAMI standards (ANSI-AAMI 13958, ANSI-AAMI 13959, ANSI-AAMI 11663, ANSI-AAMI 23500, ANSI-AAMI 26722) for dialysis treatment in the U.S. endorse the ISO standards both with respect to colony count action levels and limits, and

| Author/Year Published | Sample Type (Number of Samples) | Medium | Incubation Conditions |
|-----------------------|---------------------------------|--------|-----------------------|
| Reasoner & Geldreich, 1985 | Potable water (varied, 3 or 10) | PCA (PP, SP), m-SPC (MF), R2A (SP) (MF), R3A(SP) (MF) | 35°C, 168h; Read at 48, 72, 168h |
| Nystrand and Nurbo, 1996 | Bicarbonate concentrate (5 clinics, 67 samples) | TGEA, TGEA + 2.48% NaHCO3 | 17–23°C, 7d |
| Pass et al, 1996 | Dialysis water (RO) (68) | TSA (PP), SMA (PP), R2A (PP) | TSA-37°C, 48h, then 23°C for 5d SMA-23°C, 48, 72, 168h |
| | Dialysis fluid (55) | TSA (PP), SMA (PP), R2A (PP) | TSA-37°C, 48h, then 23°C for 5d SMA-23°C, 48, 72, 168h |
| | Lab prepared bicarbonate concentrate (120) | TSA (PP), SMA (PP), R2A (PP) | TSA-37°C, 48h, then 23°C for 5d SMA-23°C, 48, 72, 168h |
| Ledebo and Nystrand, 1999 | Dialysis water (7 clinics) | TGEA | 20°C, 5d |
| | Dialysis fluid (NA) | TSA, TGEA, Blood agar | TSA, Blood Agar-37°C, 2d |
| | Bicarbonate concentrate (7 clinics) | TGEA, TGEA + 2.48% NaHCO3 | TGEA-37°C, 2d or 20°C, 5d |
| van der Linde et al, 1999 | Tap water (13) | TSA (SP), R2A (SP) | 25+/−2°C, 10d |
| | Pre-treated water (33) | TSA (SP), R2A (SP) | |
| | Dialysis water (RO) (59) | TSA (SP), R2A (SP) | |
| | Dialysis fluid (124) | TSA (SP, MF), R2A (SP, MF) | 20°C, 7d |
| Punakabutra et al, 2004 | RO water (143) | TSA (SP, R2A (SP) | TSA-20°C & 37°C, 48h & 7d |
| | RO water (175) | TSA (SP, MF), R2A (SP, MF) | R2A-20°C & 37°C, 48h & 7d |

Abbreviations: MF = membrane filtration technique; m-SPC = membrane filtration-standard plate count agar; NaHCO3 = sodium bicarbonate; PCA = plate count agar; PP = pour plate technique; R2A = Reasoner’s 2A medium; RO = reverse osmosis; SMA = standard methods agar; SP = spread plate technique; TGEA = tryptone glucose extract agar; TSA = trypticase (trypsic) soy agar; NA = not available.

Table 1 Summary of current literature comparing culture methods
with respect to culture technique. They also allow for use of trypticase soy agar, standard methods agar and plate count agar incubated at 35–37°C for 48 h.5–9 Dialysis practice in the United States, however, is effectively determined not only by the current AAMI standards but also by the 2004 AAMI standard (RD52),10 because the 2004 standard was incorporated into the Centers for Medicare and Medicaid (CMS) 2008 End-Stage Renal Disease Conditions for Coverage (CMS 2008 ESRD CfC).11 RD52 recommended culture of dialysis water and dialysis fluid using TSA at 35–37°C for 48 h (TSA-48h). This method had been recommended by AAMI dialysis water and fluid standards since the 1981 AAMI RD5 standard.12 Both approaches have microbiologic rationales: TGEA and R2A methods are performed at a temperature and nutrient level close to that of dialysis water and its resident microorganisms, and have been shown to yield higher colony counts in several studies.10,13–16 TSA-48h cultures are performed at the temperature at which the patient is exposed to dialysis fluid in the dialyzer.17 In addition, TSA-48h has the operational advantage that results are available more quickly, allowing earlier corrective action, and allows a laboratory to operate more efficiently.

Most previous studies examining the use of various methods to culture water and dialysis fluid (Table 1)18 concluded that lower nutrient medium, lower incubation temperatures and longer incubation times, similar to those specifically recommended by the ISO standards, result in higher total counts and better recovery of pigmented Gram negative organisms than do methods using higher nutrient content, shorter incubation times and higher incubation temperatures,13–16,19 similar to the methods recommended by AAMI RD52,10 incorporated into the CMS 2008 ESRD CfC.11 Although the current international standards cite these studies to justify their recommendations, the studies are small, use samples of little relevance to the dialysis environment (such as potable water), use reference points higher than 50 CFU/mL, and are inconsistent in incubation conditions and in the pairing of culture methods compared. One study did directly compare the international methods using the current action level as a reference point, but it examined only samples of dialysis water, and not dialysis fluid.5 Our study aim was to determine whether culture of dialysis water and fluid using TSA-48h could reasonably be considered “equivalent to" culture using TGEA-7d or R2A-7d for purposes of providing safe dialysis treatment. We used a large number of routinely collected samples to compare methods for the detection of bacterial colony counts meeting or exceeding the current action level of 50 CFU/mL in dialysis water and dialysis fluid.

### MATERIALS AND METHODS

#### Study sample and culture methods

Between 2011 and 2014, 681 samples of water prepared for use in dialysis treatment and 593 samples of dialysis fluid were collected. Each sample was divided into four aliquots for the following culture methods:

- **TSA-48h**: bacterial culture on trypticase soy agar (TSA) at 35–37°C for 48 h (2 days);
- **TGEA-7d**: bacterial culture on tryptone glucose extract agar (TGEA) at 17–23°C for 7 days;
- **R2A-7d**: bacterial culture on Reasoner’s 2A agar (R2A) at 17–23°C for 7 days.

### Table 2  Paired comparison of number of samples of (A) water and (B) dialysis fluid with bacterial culture colony counts above the ISO-13959 and ISO-11663 action level of ≥50 colony-forming units/milliliter (CFU/mL) by TSA-48h versus TGEA-7d; (C) water and (D) dialysis fluid by TSA-48h versus R2A-7d

| (A) Water (N = 234) | TGEA-7d | (B) Dialysis fluid (n = 186) | TGEA-7d | (C) Water (N = 447) | R2A-7d | (D) Dialysis fluid (n = 407) | R2A-7d |
|---------------------|---------|-----------------------------|---------|---------------------|--------|-----------------------------|--------|
| ≥50 CFU/mL          |         | ≤50 CFU/mL                  |         | ≥50 CFU/mL          |         | ≤50 CFU/mL                  |         |
| TSA-48h             | 6       | 0                           | 6       | TSA-48h             | 15     | 2                           | 17     |
| <50 CFU/mL          | 10      | 218                         | 228     | <50 CFU/mL          | 4      | 165                         | 169    |
| Column Total        | 16      | 218                         | 234     | Column Total        | 19     | 167                         | 186    |

P for difference in paired proportion = 0.002

| (C) Water (N = 447) | R2A-7d | (D) Dialysis fluid (n = 407) | R2A-7d |
|---------------------|--------|-----------------------------|--------|
| ≥50 CFU/mL          |         | ≤50 CFU/mL                  |         |
| TSA-48h             | 3       | 3                           | 6       | TSA-48h             | 12     | 1                           | 13     |
| <50 CFU/mL          | 4       | 437                         | 441     | <50 CFU/mL          | 0      | 394                         | 394    |
| Column Total        | 7       | 440                         | 447     | Column Total        | 12     | 395                         | 407    |

P for difference in paired proportion = 1.0

TSA-48h = bacterial culture on trypticase soy agar (TSA) at 35–37°C for 48 h (2 days); TGEA-7d = bacterial culture on tryptone glucose extract agar (TGEA) at 17–23°C for 7 days. R2A-7d = bacterial culture on Reasoner’s 2A agar (R2A) at 17–23°C for 7 days.
fluid from 41 dialysis programs covering multiple geographic areas were cultured at two U.S. laboratories that provide routine analysis of dialysis water and dialysis fluoride. These samples were submitted to the laboratories by dialysis facilities in the course of monitoring of routine operations. Each laboratory received unique samples with no comparison of findings by different laboratories on the same samples. Each sample was cultured using 1) TGEA or R2A at 17–23°C for 7d and TSA at 35–37°C for 48h (2d) or 2) R2A at 17–23°C for 7d and TSA at 35–37°C for 48h. Spread plate technique for microbial culture was used for all samples.

Statistical methods

The following comparisons were made of the proportions of samples yielding bacterial colony counts ≥50 CFU/mL, the action level defined by ISO-13959, ISO-11663, ISO-23500, ISO-26722, AAMI RD52, and CMS 2008 ESRD CIC: (1) TSA-48h versus TGEA-7d; (2) TSA-48h versus R2A-7d. We used the McNemar Test to compare differences in paired proportions. We evaluated method performance by calculating sensitivity, specificity, positive predictive value, and negative predictive values. In primary analyses, either the TGEA-7d or R2A-7d method was considered the reference method for comparisons with TSA-48h as the index test. However, because neither the 48 h method nor the 7d method is truly a reference standard, we also performed secondary analyses with TSA-48h considered the reference method with either the TGEA-7d or R2A-7d method considered the index test. Statistical analyses were performed using Stata SE, version 12.1. A 2-sided P value <0.05 was considered statistically significant.

RESULTS

Bacterial cultures were performed on 681 dialysis water samples (234 TSA-48h and TGEA-7d; 447 TSA-48h and R2A-7d) and 593 dialysis fluid samples (186 TSA-48h and TGEA-7d; 407 TSA-48h and R2A-7d). Zero bacterial growth in dialysis water was observed in 58.3% (397/681) of samples based on the TSA-48h method, in 0% (0/234) of samples based on the TGEA-7d method, and in 88.6% (396/447) based on the R2A-7d method. For dialysis fluid, zero bacterial growth was observed in 64.4% (382/593) of samples based on the TSA-48h method, in 0% (0/186) of samples based on the TGEA-7d method, and in 95.1% (387/407) based on the R2A-7d method.

Table 2 shows paired comparison of the number of samples of (A) dialysis water and (B) dialysis fluid with total colony counts at or above the action level of ≥50 CFU/mL by TSA-48h versus TGEA-7d and (C) dialysis water and (D) dialysis fluid by TSA-48h versus R2A-7d. In samples cultured using both TSA-48h and TGEA-7d (Table 2A,B) the action limit of ≥50 CFU/mL was exceeded in 2.6% (95% confidence interval [CI] 0.5–
4.6%) and 6.8% (95% CI 3.6–10.1%) of dialysis water samples and in 9.1% (95% CI 5.0–13.3%) and 10.2% (95% CI 5.8–14.6%) of dialysis fluid samples by the TSA-48h and TGEA-7d methods, respectively. The proportion of dialysis water samples yielding microbiological culture colony counts \( \geq 50 \, \text{CFU/mL} \) by TGEA-7d was significantly different from the proportion by TSA-48h \( (p = 0.002; \text{difference in proportion of } 4.3\% \, [95\% \text{CI} 1.3–7.3\%]) \). The proportion of dialysis fluid samples exceeding the action level by TGEA-7d was not significantly different than by the TSA-48 h method \( (P = 0.69) \). In samples cultured using both TSA-48h and R2A-7d (Table 2C,D) the action level of \( \geq 50 \, \text{CFU/mL} \) was exceeded in 1.3% (95% confidence interval [CI] 0.3% to 2.4%) and 1.6% (95% CI 0.4–2.7%) of dialysis water samples and in 3.2% (95% CI 1.5–4.9%) and 2.9% (95% CI 1.3–4.6%) of dialysis fluid samples by the TSA-48h and R2A-7d methods, respectively. There were no significant differences in proportions for comparisons of R2A-7d to TSA-48h.

Table 3 compares the culture methods’ test characteristics when either TGEA-7d (Table 3A) or R2A-7d (Table 3B) was used as the reference method. The sensitivity and specificity of TSA-48h as an index test for identifying viable bacteria in dialysis water samples above the action level compared to TGEA-7d as the reference method were 37.5% and 100%, respectively (Table 3A). The sensitivity of 37.5% can also be considered as the proportion of samples considered positive by TGEA-7d for which TSA-48h would provide warning 5 days earlier. Although TSA-48h sensitivity in dialysis water was low, the negative predictive value was 95.6%, which means that the probability that a dialysis water sample from the systems tested that yielded a negative result by TSA-48h would also yield a negative result by the 7d method exceeded 95%. The sensitivity and specificity of TSA-48h as a test for identifying dialysis water samples at or above the action level compared to R2A-7d as the reference method were 42.9% and 99.3%, respectively (Table 3B). In dialysis fluid samples, the sensitivity and specificity of TSA-48h compared to TGEA-7d as reference method were 78.9% and 98.8%, respectively. As with comparisons in dialysis water samples, the positive predictive value was >95% (98.8%, Table 3A). The sensitivity and specificity of TSA-48h as a test for identifying dialysis fluid samples above the action level compared to R2A-7d as the reference method were 100.0% and 99.7%, respectively (Table 3B).

In secondary analyses, we evaluated test performance of either TGEA-7d or R2A-7d as an index test compared to TSA-48h as the reference method (Supporting information Table S1). The sensitivity and specificity of TGEA-7d as a test for identifying viable bacteria in dialysis water samples at or above the action level compared to TSA-48h as the reference method were 100% and 95.6%, with a low predictive value for a positive test (37.5%); in dialysis fluid the sensitivity and specificity was 88.2% and 97.6%, respectively. The sensitivity and specificity of R2A-7d as a screening test for identifying viable bacteria in dialysis water samples above the action level compared to TSA-48h as the reference method were 50.0% and 99.1%; in dialysis fluid the sensitivity and specificity were 92.3% and 100.0%, respectively.

**DISCUSSION**

Microbiological standards for the quality of dialysis water and fluid have changed over the past seventy years, and continue to evolve. The observation in the 1970s of correlations between colony counts in dialysis fluid and the incidence of pyrogenic reactions led to the 1981 ANSI-AAMI RD5 limits for dialysis fluid \((<2000 \, \text{CFU/mL})\) and dialysis water \((<200 \, \text{CFU/mL})\).20 In 2004, the dialysis fluid limit was reduced to \(<200 \, \text{CFU/mL}\), and in 2009, both dialysis water and fluid limits were reduced to \(<100 \, \text{CFU/mL}\).5 A guiding principle of AAMI is that “there should be ‘at most one globally applied standard and one globally accepted test, with conformity assessment processes appropriate to the needs of the parties, for each characteristic of a product, process or service’.”21 However, it was not possible to harmonize microbiological culture methods with the most recent ISO standards due to non-inclusion in the ISO standards of the TSA-48h method commonly used in the U.S. for bacterial total viable count analysis of dialysis water and fluid. Given that, by regulation, the 2004 requirements as specified in ANSI-AAMI RD52 must be followed by U.S. dialysis facilities,11 in 2014 ANSI-AAMI published the dialysis related standards of ISO with a U.S. deviation to include the TSA-48h method.

We found that in dialysis fluid, the proportion of samples identified as having total bacterial counts exceeding the currently recommended action level of 50 CFU/mL by TSA-48h was comparable to the proportion identified either by TGEA-7d or by R2A-7d. Use of a method requiring incubation for seven days was not more likely to yield results above the action level than use of the method requiring incubation for 48 h. In dialysis water the proportion of samples identified as reaching or exceeding the action level by TSA-48h was comparable to the proportion identified by R2A-7d, but TGEA-7d did yield significantly more results above the action level than...
did TSA-48h. Nonetheless, in the population of dialysis water samples submitted in the course of routine facility monitoring in the United States, the negative predictive value of a TSA-48h result of fewer than 50 CFU/mL exceeded 95%.

These findings cannot be interpreted as showing the superiority of TGEA-7d over R2A-7d in total bacterial count analysis of dialysis water, because the two methods were applied to different samples. Nor, for the same reason, can they be interpreted as demonstrating the equivalence of TGEA-7d and R2A-7d for the analysis of dialysis fluid. They do suggest that in dialysis fluid, TSA-48h identifies samples as reaching or exceeding the ≥50 CFU/mL action level at the same rate as does either TGEA-7d or R2A-7d, and that in dialysis water, TSA-48h identifies samples as exceeding the ≥50 CFU/mL action level at the same rate as does R2A-7d. In dialysis water, TSA-48h does not identify samples reaching or exceeding the 50 CFU/mL action level at as high a rate as does TGEA-7d. However, in a population of dialysis water samples submitted for routine monitoring, in which the prevalence of bacterial contamination as measured by the reference standard was low, TSA-48h results below the action level still conveyed a less than 5% chance that TGEA-7d results would be above the action level 5 days later.

Thorough analyses of sources of bacterial contamination in a dialysis facility are important. For routine monitoring, the ability to identify dialysis fluid samples at or exceeding the microbiological action level is arguably more important than the ability to identify actionable water samples, because it is dialysis fluid that reaches the patient. The discrepancy observed here may indeed reflect bacterial killing by salts present in dialysis fluid or its pH. For the purpose of maintaining safe dialysis fluid, TSA-48h would seem to have an advantage over either TGEA-7d or R2A-7d: it is as likely to identify results at or above the action level, and can do so five days earlier. TGEA-7d appears to have a role in the definitive bacteriologic investigation of dialysis water samples. This suggests that microbiological cultures using TGEA-7d might be useful in follow-up to disinfection of a contaminated water system, since low colony counts or slow growing waterborne bacteria associated with residual biofilm might not be detected by TSA after 48 h.

Recommendations regarding the methods considered optimal for bacterial culture of those fluids, including media types, incubation times and temperatures and plating techniques have varied in emphasis. One authority says that “the primary purpose of the assays is to determine the degree of magnitude of microbial contamination and not to precisely quantify or identify bacteria in these fluids.” Others say that “For a realistic viable count, the original conditions of the sample should be mimicked as much as possible” and that “the aim is to reproduce the natural growth conditions for relevant strains as closely as possible.” Arduino and colleagues noted that “gram-negative organisms responsible for producing significant amounts of endotoxin will grow to be visible on a culture plate [TSA] within 24 to 48 h” and that “the ready availability of TSA in clinical and commercial laboratories makes this the medium of choice in the culturing of dialysis fluids.” A number of studies report that lower nutrient levels and temperature promote recovery of more bacteria from water samples than are recovered at higher nutrient levels and temperatures. It has been observed that dialysis fluid does not always yield the same taxa that are recovered from the water used to prepare the fluid that bacteria likely to be pathogenic and those acquired nosocomially are better recovered on high nutrient media at higher temperatures, and that “A particular hazard is introduced when the water temperature is raised to 37°C within the dialysis machine, since this temperature supports growth of the more dangerous mesotrophic bacteria, for example, P. aeruginosa or S. aureus.”

Current ISO standards recommend use of TGEA-7d, R2A-7d, or a validated “equivalent” method to culture dialysis water and fluid, but they do not define the criteria for considering a method to be equivalent. We propose that the criterion for determining equivalence of culture methods should not be microbiological, but operational: does one method provide information that allows dialysis facility staff to provide treatments to their patients that are as safe as the treatments they would provide on the basis of information from the other method? Establishing equivalence would first require consensus as to the minimum difference between the results of two culture methods that would be considered materially to affect the safety of dialysis treatment, followed by rigorous prospective equivalence trials comparing new microbiological culture methods to those recommended by the international standards. A complete analysis would, further, take into account the trade-off between sensitivity and timeliness entailed in using lower or higher nutrient media.

In the absence of such analyses, our findings suggest that the culture of dialysis fluid using TSA-48h can be used to identify samples above the action level with similar results when compared to TGEA-7d or R2A-7d. We did not find evidence that R2A-7d, one of the methods recommended by the ISO standards, would identify samples either of dialysis water or of dialysis fluid exceeding the action level more frequently than would TSA-48h;
because any TSA-48h results would arrive sooner than an R2A-7d result, TSA-48h should be considered to allow dialysis at least as safe as R2A-7d. Almost 2/3 (62.5%) of dialysis water samples in which TGEA-7d identified bacterial growth above the action level would be missed by TSA-48h. On the other hand, in more than 1/3 (37.5%) of water samples exceeding the action limit by TGEA-7d, TSA-48h would be associated with the same finding, and would reduce the delay in intervention from 7 to 2 days. For dialysis fluid, which reaches the patient, because there was no difference in the proportion of samples at or exceeding 50 CFU/mL, and because TSA-48h results would allow intervention 5 days earlier, culture using TSA-48h would allow safer dialysis than culture using TGEA-7d or R2A-7d. Finally, holding water samples from 6479 dialysis facilities, 27 8,507 homes28 and the several thousand hospitals in which hemodialysis is performed in the U.S. for an additional five days would have operational as well as safety consequences.

Strengths of this study include the large number of samples obtained from routine monitoring and the use of microbiological techniques that reflect routine practice and current standards in the United States. Limitations include that the samples were not cultured using both TGEA-7d and R2A-7d, which would have allowed for a direct comparison of the two methods recommended in the international standards.

We propose that culture of dialysis water and fluid using TSA-48h be considered equivalent to culture using TGEA-7d and R2A-7d for purposes of assessing compliance to the ANSI-AAMI and ISO standards as well as CMS regulation. This determination will make current microbiologic monitoring of dialysis throughout the U.S. compliant with both national and international standards and U.S. regulation. If culture using TSA-48h is not considered equivalent to culture using TGEA-7d and R2A-7d, a large number of dialysis facilities will ultimately be forced to change practice without evidence that this change in practice is beneficial to their patients.

The U.S. Pharmacopeia states that

"Whether or not a particular system needs to be monitored using high- or low-nutrient media with higher or lower incubation temperatures or shorter or longer incubation times should be determined during or prior to system validation and periodically reassessed as the microbial flora of a new water system gradually establishes a steady state relative to its routine maintenance and sanitization procedures. The establishment of a “steady-state” can take months or even years and can be perturbed by a change in use patterns, a change in routine and preventative maintenance or sanitization procedures, and frequencies, or any type of system intrusion, such as for component replacement, removal, or addition. The decision to use longer incubation periods should be made after balancing the need for timely information and the type of corrective actions required when an alert or action level is exceeded, with the ability to recover the microorganisms of interest. The advantages gained by incubating for longer times, namely recovery of injured microorganisms, slow growers, or more fastidious microorganisms, should be balanced against the need to have a timely investigation and to take corrective action, as well as the ability of these microorganisms to detrimentally affect process products or processes."29

We suggest that the benefits of timely corrective action identified by the U.S. Pharmacopeia are very important in the dialysis treatment setting, in which patients are constantly exposed to large volumes of dialysis fluid. We suggest that culturing dialysis water and dialysis fluid on trypticase soy agar with incubation at 35–37°C for 48h should be considered the equivalent, for purposes of defining compliance to standards, to culture on tryptone glucose extract agar (TGEA) or Reasoner’s 2A agar (R2A) at 17–23°C for 7 days. Further investigation should be undertaken to determine the combination of culture techniques and culture frequency, in combination with indirect measures of microbiological contamination of dialysis water and fluid, such as endotoxin, that will promote the safest dialysis treatment.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article at the publisher’s Web site:

**Table S1:** Test performance characteristics for bacterial culture colony counts above the ISO-13959 and ISO-11663 action level of ≥50 colony-forming units/milliliter for (A) TGEA-7d (screening test) vs. TSA-48h (reference test) and (B) R2A-7d (screening test) vs. TSA-48h (reference test).