Improved bacteriological surveillance of haemodialysis fluids: a comparison between Tryptic soy agar and Reasoner’s 2A media

Klaas van der Linde1, Bing T. Lim2, Jan M. M. Rondeel3, Lea P. M. T. Antonissen2 and Gijs M. Th. de Jong1

1Department of Internal Medicine, Albert Schweitzer Hospital, Dordrecht, 2Regional Laboratory of Medical Microbiology (RLM), Dordrecht/Gorinchem, and 3Department of Clinical Chemistry, Albert Schweitzer Hospital, Dordrecht, The Netherlands

Abstract

Background. Accurate microbiological surveillance in haemodialysis centres is important as end-stage renal patients can suffer from pyrogenic reactions due to bacterial contamination of dialysis fluids. To evaluate the microbiological quality of haemodialysis fluids, special nutrient-poor culture techniques are necessary. Although the Association for the Advancement of Medical Instrumentation (AAMI) recommends Tryptic soy agar (TSA) as the standard agar, several studies have resulted in a general preference for Reasoner’s 2A (R2A) agar, as it appeared to be more sensitive in demonstrating contamination of typical haemodialysis associated bacteria. In the Netherlands TSA is still used for culturing dialysate, while dialysis water is cultured on R2A. Therefore, the aims of our study were to evaluate bacterial yields of dialysis fluids on both media, and to qualify their use in routine microbiological monitoring within our haemodialysis centre.

Methods. Between April 1995 and March 1996, 229 samples of pre-treated and final purified dialysis water, and samples of dialysates were collected. The specimens were aseptically taken from the tap, various points of the reverse osmosis (RO) water-treatment system, and the effluent tubes of 32 bicarbonate haemodialysis machines. Samples of 0.1 ml were inoculated in duplicate on spread plates with TSA and R2A agars. After 10 days of incubation at 25 ± 2°C, the numbers of colonies were quantified. The ranges of spread were taken 0–100 and 0–200 colony-forming units per milliliter (c.f.u./ml).

Results. The R2A agar had significantly higher colony counts than TSA agar for both dialysis water and dialysates. Considering 100 c.f.u./ml as the upper allowable bacterial limit for all dialysis fluids, microbiological non-compliance (bacterial growth) would be missed in 16% when using only TSA media (TSA ≤100 c.f.u./ml and R2A >100 c.f.u./ml), while this was 3% when using only R2A (TSA >100 c.f.u./ml and R2A ≤100 c.f.u./ml, P<0.0001). Considering 200 c.f.u./ml as the upper limit, non-compliance would have been missed in 10% when using only TSA (TSA ≤200 c.f.u./ml and R2A >200 c.f.u./ml), and 2% when using R2A (TSA >200 c.f.u./ml and R2A ≤200 c.f.u./ml, P=0.0011).

Conclusions. Microbiological surveillance of haemodialysis fluids, including pre-treated dialysis water samples collected from RO treatment systems, can be performed more precisely with R2A media than TSA, when incubated at 25 ± 2°C for 10 days.

Key words: bacterial contamination; culture media; haemodialysis; pyrogen; water purification

Introduction

Bacterial contamination of haemodialysis treatment systems is a major problem in renal replacement therapy. As some bacteria have adapted to nutrient-poor and low temperature environments, dialysis fluids are potential breeding sources [1,2]. Moreover, bacterial contamination is clearly related to pyrogenic reactions in haemodialysis patients [1,3–6]. Therefore, fluids used in haemodialysis need to meet certain microbiological quality criteria, and should be monitored on a regular basis.

In 1982, the Association for the Advancement of Medical Instrumentation (AAMI) released guidelines regarding the quality control surveillance of bacteriological contamination of haemodialysis fluids, to protect patients with end-stage renal disease from pyrogenic adverse reactions. The maximum allowable limit of viable bacteria was set at 200 colony-forming units per millilitre (c.f.u./ml) for water used to prepare dialysate (salt-poor), and 2000 c.f.u./ml for the final dialysate (salt-rich) [7]. In the Netherlands the microbiological criteria for haemodialysis are based on the European
Pharmacopoeia [8]. The upper accepted bacterial limit for dialysis water is more strict at 100 c.f.u./ml. Criteria for dialysate are not mentioned. However, 100 c.f.u./ml is recommended as the upper limit also [9].

The bacterial colony counts of dialysis water and dialysates depends of which agars and culture conditions are used. The AAMI recommends to culture haemodialysis fluids on Tryptic soy agar (TSA) at 37°C for 48 h. However, special nutrient-poor culture techniques, including low temperature (25±2°C), and extended incubation time (>48 h) have yielded higher bacterial counts. Especially on Reasoner’s 2A (R2A) media, several typical haemodialysis-associated water-borne bacteria appear to grow better, or sometimes even more selectively when using such culture conditions [2,10,11]. So by using AAMI-approved culture methods the actual bacterial load of haemodialysis fluids may be underestimated or even missed.

In the Netherlands, the Dutch Dialysis Group currently advises culture of samples of dialysis water on R2A, and samples of dialysates on TSA at 25±2°C for 10 days [12]. Based upon the above-mentioned studies, this guideline is probably partially inadequate. Therefore, the aims of this study were to evaluate bacterial yields of dialysis water and dialysates on both agars, and to qualify their use in present routine microbiological surveillance within our own haemodialysis centre.

Subjects and methods

In our haemodialysis department we regularly perform bacteriological surveillance, following a national protocol of the Dutch Dialysis Group [12]. Between April 1995 and March 1996, all the samples collected from dialysis water and prepared dialysate were used for this study.

Salt-poor dialysis water was sampled from the tap (municipal water), and various key points of two different reverse osmosis (RO) water-treatment systems, including Christ (Christ Holland B.V., Zoetermeer, the Netherlands) and Culco RCS5 (Culco Nederland, Alkmaar, the Netherlands). Table 1 summarizes the number of samples and the different collection points.

Table 1. Samples of haemodialysis fluids

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse osmosis water treatment systems</td>
<td></td>
</tr>
<tr>
<td>incoming water (tap)</td>
<td>13</td>
</tr>
<tr>
<td>after activated charcoal</td>
<td>11</td>
</tr>
<tr>
<td>after break tank</td>
<td>11</td>
</tr>
<tr>
<td>after softener</td>
<td>11</td>
</tr>
<tr>
<td>after reverse osmosis</td>
<td>29</td>
</tr>
<tr>
<td>after storage tank</td>
<td>10</td>
</tr>
<tr>
<td>before ultraviolet</td>
<td>10</td>
</tr>
<tr>
<td>after ultraviolet</td>
<td>10</td>
</tr>
<tr>
<td>Effluent tubes of bicarbonate dialysis machines</td>
<td></td>
</tr>
<tr>
<td>Cobe Centry 3</td>
<td>93</td>
</tr>
<tr>
<td>Gambro AK10</td>
<td>12</td>
</tr>
<tr>
<td>Hospal Integra</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>229</td>
</tr>
</tbody>
</table>

Samples of salt-rich dialysate were collected from the effluent tubes of 32 bicarbonate haemodialysis machines, including Cobe Centry 3 (n=22, Cobe Laboratories, Veenendaal, the Netherlands), Gambro AK10 (n=5, Gambro B.V., Breda, the Netherlands), and Hospal Integra (n=5, Hospal B.V., Uden, the Netherlands). All haemodialysis machines used Bicarts (Gambro B.V., Breda, the Netherlands).

The specimens were aseptically obtained at random intervals in pyrogen-free plastic tubes, temporally stored at 4°C within 30 min, and incubated within 5 h. At the Regional Laboratory of Medical Microbiology (RLM) 0.1 ml of the undiluted samples was pipetted, and inoculated in duplicate on spread plates with TSA [13] and R2A agars [14]. Both media were commercially obtained (Difco, Inc., Detroit, MI, USA), prepared according to the manufacturer’s instructions, and sterilized at 121°C. The cultures were incubated at 25±2°C for 10 days. This method is based on recent published studies [2,10,11], and the Dutch standard for water cultures [15].

Colonies were determined from 0 to 20 per plate, and multiplied by 10 giving the amount of c.f.u./ml. The bacterial yields of 18 samples (tap water: n=2, dialysis water: n=6, dialysates: n=10) were calculated only until 10 per plate. The ranges of spread were 0–200 c.f.u./ml and 0–100 c.f.u./ml per plate respectively. Plates with more colonies were not counted further, and reported as >200 c.f.u./ml and >100 c.f.u./ml respectively. In this way the bacterial yields of dialysis water and dialysates were monitored on TSA and R2A. Also the effect of using only one medium (TSA or R2A) was evaluated on the microbiological compliance of haemodialysis fluids.

For statistical analysis an SPSS package (release 6.0 for Windows) was employed, using Spearman’s rank correlation test, Wilcoxon’s matched-pairs signed-rank test, and chi-square test.

Results

During 1 year of bacteriological surveillance in our haemodialysis department, 229 samples were collected from the water tap, several points of the RO water-treatment system, and the effluent tubes of bicarbonate dialysis machines (Table 1). Within 5 h of sampling, all specimens were cultured on spread plates with TSA and R2A agars at a temperature of 25±2°C for 10 days. After this period the number colonies of each plate was quantified.

The bacterial yields of both media are summarized in Tables 2 and 3. The correlation between the cultures in terms of colony counts on the media appeared to be excellent (r=0.67, P<0.0001, Spearman’s rank correlation test). The R2A agar showed significantly higher colony counts than TSA agar for both dialysis water and dialysates, irrespective of whether 100 or 200 c.f.u./ml was chosen as the upper permitted bacterial limit (Wilcoxon’s matched-pairs signed-rank test).

Additionally, we evaluated the impact of using only TSA or R2A on a hypothetical microbiological compliance. The European guidelines include 100 c.f.u./ml as the upper allowable bacterial limit. If all the samples (n=229) had had to comply with this criterion, then
**Table 2.** Tryptic soy agar versus Reasoner’s 2A medium, cut-off reading 100 c.f.u./ml

<table>
<thead>
<tr>
<th>Medium</th>
<th>Salt-poor water (dialysis and tap water)</th>
<th>Salt-rich water (dialysate)</th>
<th>All samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tryptic soy agar</strong></td>
<td>105</td>
<td>124</td>
<td>229</td>
</tr>
<tr>
<td>median c.f.u./ml</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>LQ-UQ c.f.u./ml*</td>
<td>5–100*</td>
<td>5–68</td>
<td>5–100*</td>
</tr>
<tr>
<td><strong>Reasoner’s 2A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>medium c.f.u./ml</td>
<td>&gt;100*</td>
<td>40*</td>
<td>55*</td>
</tr>
<tr>
<td>LQ-UQ c.f.u./ml</td>
<td>15–100*</td>
<td>10–100</td>
<td>10–100</td>
</tr>
</tbody>
</table>

*Colony-forming units per millilitre.

*Lower quartile–upper quartile.

*P* < 0.00001 vs Tryptic soy agar (Wilcoxon matched-pairs signed-rank test).

31% of dialysis water and 18% of dialysate cultures would have been out of compliance when the TSA media only was used. In contrast, 52 and 24% would have been out of compliance when the samples were cultured on R2A only respectively. In 51% (*n* = 116) R2A yielded higher colonies than TSA, whereas in 16% (*n* = 37) TSA had higher counts than R2A. Microbiological non-compliance (bacterial growth) would be missed in 16% (*n* = 37) when using only TSA media (TSA ≤ 100 c.f.u./ml and R2A > 100 c.f.u./ml), while this was 3% (*n* = 7) when using only R2A (TSA > 100 c.f.u./ml and R2A ≤ 100 c.f.u./ml, *P* < 0.0001, chi-square test).

In a similar evaluation, considering 200 c.f.u./ml as the upper allowable bacterial limit (AAMI guidelines) for all dialysis fluids (*n* = 211), 18% of dialysis water and 10% of dialysate cultures on TSA media would have been out of compliance, compared to 34% and 11% of samples cultured on R2A respectively. In 57% (*n* = 121) the bacterial yields of R2A media were higher than TSA; and in 18% (*n* = 39) TSA had higher colony counts than R2A. In these cases non-compliance would have been missed in 10% (*n* = 22) when using only TSA (TSA ≤ 200 c.f.u./ml and R2A > 200 c.f.u./ml), and 2% (*n* = 5) when using R2A instead (TSA >200 c.f.u./ml and R2A ≤ 200 c.f.u./ml, *P* = 0.0011, chi-square test).

**Discussion**

Pyrogenic reactions in haemodialysis patients are complications of renal replacement therapy. The frequency of dialysis-related fevers is about 0.7 times per 1000 haemodialysis treatments [16]. They are associated with contamination of the haemodialysis treatment system, especially Gram-negative organisms like Pseudomonas [1,4–6,16–22]. Moreover, some authors believed that febrile reactions were directly proportional to the number of bacteria found in haemodialysis fluids [1,17]. Therefore, several authors recommended keeping the levels of microbiological contaminants as low as possible [23,24]. Others even promoted the use of sterile haemodialysis fluids [25,26]. A recent study, however, showed a low percentage of pyrogenic reactions per haemodialysis treatment, despite high bacterial concentrations in dialysis water and bicarbonate dialysates. The authors suggested that modern and even high-flux dialyser membranes may be an effective barrier to micro-organisms [1,61].

Considering these observations, and the fact that haemodialysis patients are exposed to enormous amounts of water, usually more than 100 litres per treatment, a stringent microbiological surveillance of haemodialysis systems is necessary. The AAMI recommends the culture of dialysis fluids on TSA agar (or
an equivalent medium) at a temperature of 37°C for 48 h, after which bacterial growth can be quantified [7]. In contrast with these guidelines, several authors have shown that typical haemodialysis-associated water-borne bacteria appear to grow better, or sometimes even more selectively on the nutrient-poor medium R2A, using an extended incubation time (>48 h), and a relatively low temperature (25°C) [2,10,11]. Unfortunately such culture methods are not common practice as different guidelines are used worldwide [9]. Even a recent study published in this journal evaluated the microbiological quality of haemodialysis fluids by using only AAMI-approved standards, including a high culture temperature (37°C) and a short incubation time (48 ± 3 h) [27].

Although in Dutch haemodialysis centres nutrient-poor culture techniques are accepted, TSA media are still used for monitoring bacterial contamination of dialysis water, while the R2A medium is utilized for dialysates [12]. The goal of our study was to evaluate the value of both media in routine microbiological surveillance in our haemodialysis centre. Based upon former studies [2,10,11], and the Dutch standard for water cultures [15], all dialysis fluids samples were incubated at 25 ± 2°C for 10 days. For optimal comparison of microbiological monitoring techniques, we also included samples from various stages of the RO water-treatment system before the final purified product. These samples usually have high microbiological growth (e.g. after the charcoal and softener) [26], permitting comparison of higher bacterial counts.

The results of the present study showed that R2A produces significantly higher bacterial yields than TSA media for both (pre-treated) dialysis water and dialysates. Moreover, when the maximum limit of viable bacteria for all samples was considered as 100 c.f.u./ml (European criterion) or 200 c.f.u./ml (AAMI criterion), numerous TSA cultures would have been within bacteriological compliance (16 and 10% respectively), whereas the same samples cultured on R2A media showed colony counts higher than the upper permitted limits. In such cases TSA underestimates the actual bacterial contamination, and would falsely suggest a compliance with the microbiological regulations. In cases of R2A cultures, non-compliance would be missed in only a very low percentage (3 and 2% respectively).

In conclusion, to evaluate bacterial contamination of haemodialysis fluids accurately, special nutrient-poor culture techniques are necessary, including R2A agar. This medium appeared to determine bacterial growth better than TSA for both dialysis water and final bicarbonate dialysates, when incubated at 25 ± 2°C during 10 days. It is also effective in monitoring pretreated dialysis water at different stages of the RO water treatment system. By using AAMI-approved culture standards, the actual bacterial load of haemodialysis fluids may be underestimated or even missed. So in terms of quality control and patient risk, we recommend using R2A agars combined with a relative low culture temperature, and an extended incubation time. However, as the spectrum of bacterial contamination is not the same for different haemodialysis centres and countries, multicentre studies using different media and culture parameters are mandatory in order to confirm this.

References

14. Reasoner DJ, Geldreich EE. A new medium for the enumeration and subculture of bacteria from potable water. Annual Meeting of the American Society of Microbiology, 1979; 180

Received for publication: 13.5.98
Accepted in revised form: 4.6.99