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Original Research Article

Comparative Study of Membrane Filtration and Spread Plate Technique for Dialysis Water Analysis

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Abstract

Introduction*:** The purity of the hemodialysis fluids is crucial for hemodialysis patients who are inevitably exposed to a large volume of water during hemodialysis. During this process the semi-permeable artificial membrane comes into direct contact with the bloodstream. Therefore it is important to monitor the purity of dialysis water. ***Aim and objectives*:** To compare two different methods for dialysis water analysis. ***Material and methods: 50 samples of dialysis water were collected from Saveetha Medical College and Hospital for this study. The study was conducted from 2018 December to 2019 March after getting Institutional Human Ethical Committee (IHEC) Clearance. By using the criteria of the AAMI, the present study is taken up to analyse the sensitivity of two different cultures technique i.e., spread plate and membrane filteration technique. ***Results:*** The standardization shows that spread plate technique was 80% effective and membrane filtration technique was 70% effective in identifying 100 CFU/mL of bacteria tested. Out of 50 unknown samples tested, 2 were ultra-pure, 21 were between 0.1-50 CFU/mL, 16 were between 50-100 CFU/mL and 11 were >100 CFU/mL by Spread plate technique. Likewise, 6 were ultra-pure, 30 were between 0.1-50 CFU/mL, 10 were between 50-100 CFU/mL and 4 were >100 CFU/mL by Membrane Filtration technique. ***Conclusion:*** From this study, spread plate technique proves to be equally sensitive with membrane filtration technique for analyzing dialysis water but when ultrapure water needs to be analyzed, spread plate technique gives much better bacterial recovery. i.e., only 2 samples were proved to be ultra-pure by Spread plate technique.

Keywords: Dialysis water, hemodialysis, spread plate technique, membrane filtration technique, ultra-pure, the Association for the Advancement of Medical Instrumentation (AAMI).

INTRODUCTION

The purity of the hemodialysis fluids is crucial for hemodialysis patients who are inevitably exposed to a large volume of water during hemodialysis. Bacteria-contaminated hemodialysis fluids could induce bacteremia as well as endotoxin-mediated pyrogenic reaction [1, 2]. For standard hemodialysis, the Association for the Advancement of Medical Instrumentation (AAMI) has recommended that the viable colony count of bacteria should be less than 100 CFU/ml (active level >50CFU/ml) and the endotoxin level should be less than 0.25 EU/ml (active level less than or equal to 0.125 EU/ml) [3]. The AAMI has recommended that the hemo-dialysis fluids should be cultured on either Tryptic Soy Agar (TSA) or standard method agar (SMA) and be incubated at 37°C for 48 hours [4]. Membrane filteration technique is an effective, accepted technique for testing fluid samples for microbiological contamination. Spread plate technique is used for viable plate counts, in which the total number of colony forming units on a single plate is enumerated [5]. By using the criteria of the AAMI, the present study is taken up to analyse the sensitivity of two different cultures technique i.e., spread plate and membrane filteration technique.

MATERIAL AND METHODS

Sample size

50 samples of dialysis water were collected from Saveetha Medical College and Hospital for this study. The study was conducted from 2018 December to 2019 March after getting Institutional Human Ethical Committee (IHEC) Clearance. IHEC number is SMC/IEC/2018/11/532.

Membrane filtration technique

In this technique, sample is passed through the membrane using a filter funnel and vacuum system.

Any organisms in the sample are concentrated on the surface of the membrane. The membrane is then placed in a special plate containing a pad saturated with the appropriate medium with the trapped bacteria. The passage of nutrients through the filter during incubation facilitates the growth of organisms in the form of colonies, on the upper surface of the membrane [6]. The colonies thus formed can be transferred to confirmation media. The following steps were carried out.

- The sample was collected and necessary dilutions were made.
- The appropriate nutrient or culture medium was selected. The broth was Dispense into a sterile Petri dish, evenly saturating the absorbent pad.
- The forceps were shown in flame, and the membrane from the sterile package was removed.
- The membrane filter was placed into the funnel assembly.
- The pouring lip of the sample container was heated and the sample was poured into the funnel.
- Then the vacuum was turned on and the sample was allowed to draw completely through the filter.
- The funnel was rinsed with sterile buffered water. The vacuum was turned on and allows the liquid to draw completely through the filter.
- Flame the forceps and remove the membrane filter from the funnel.
- The membrane filter is placed into the prepared Petri dish.
- At the proper temperature and for the appropriate time period incubate it.
- The colonies were Counted and confirmed.

Spread plate technique

Spread plate technique is a method employed to plate a liquid sample for the purpose of isolating or counting the bacteria present in a mixed culture and distributing it evenly [7]. A perfect spread plate technique will contain results with visible and isolated colonies of bacteria that are evenly distributed in the plate and are countable. This technique is commonly applied for microbial testing of foods or any other samples or to isolate and identify variety of microbial flora [5]. This study is taken up to compare both of these techniques to find out which is the best control to be used in dialysis water analysis.

Serial dilution

- A series of 6 test tubes containing 9 ml of sterile distilled water was prepared.
- By using a sterile pipette, add 1ml of sample in the first tube of the set. This is labelled as 10^{-1}
- Mix the contents well by swirling the tube upside down few times.
- From the first tube, 1ml of the sample is taken and transfer to second tube. This is labelled as $10^{-2.}$
- The procedure is repeated with all the remaining tubes labeling them until 10^{-6}.

Plating

- Pipette out 0.1 ml from the appropriate desired dilution series onto the center of the surface of an agar plate.
- The L-shaped glass spreader (hockey stick) is dipped into alcohol.
- The glass spreader is flamed over a bunsen burner.
- The sample is evenly spread over the surface of agar using the sterile glass spreader, carefully rotating the Petri dish underneath at an angle of 45o at the same time.
- The plate is incubated at 37°C for 24 hours.
- The colony forming units (CFU) value of the sample is calculated. Once the colonies are counted, multiply by the appropriate dilution factor to determine the number of CFU/mL in the original sample.

RESULT

In this study a total number of 50 samples of dialysis water were collected from Saveetha Medical College and Hospital, Thandalam, Chennai from December 2018 to March 2019.

In this study, two culture techniques (spread plate and membrane filter) were compared to find out the sensitivity and specificity of the methods in analyzing the dialysis water for bacteriological examination. Before processing unknown 50 samples, five samples of known CFU/mL i.e., 100 CFU/mL were tested by the above-mentioned methods. This was done for standardization of the methodology. This standardization shows that spread plate technique was 80% effective and membrane filtration technique was 70% effective in identifying 100 CFU/mL of bacteria tested. The result of standardization is depicted in Table 1. Out of 50 unknown samples tested, 2 were ultra-pure, 21 were between 0.1-50 CFU/mL, 16 were between 50-100 CFU/mL and 11 were >100 CFU/mL by Spread plate technique. Likewise, 6 were ultra-pure, 30 were between 0.1-50 CFU/mL, 10 were between 50-100 CFU/mL and 4 were >100 CFU/mL by Membrane Filtration technique. This is represented in Table 2 and Figure1, 2 and 3 respectively. Figure 4 –A shows positive control of spread plate technique, B shows negative control of spread plate technique, C shows the positive control of membrane filtration technique and D shows negative control of membrane filtration technique. Figure 5 shows the bacterial growth in spread plate technique. Figure 6 shows the bacterial growth in membrane filtration technique.

Table-1: Comparison of Spread plate and membrane filtration technique in analyzing 100CFU/mL of Standard

		Growth showing results as standard	
		Positive	**Negative**
100 CFU/mL	Spread plate Technique	4(80%)	1(20%)
	Membrane Filtration Technique	3(60%)	2(40%)

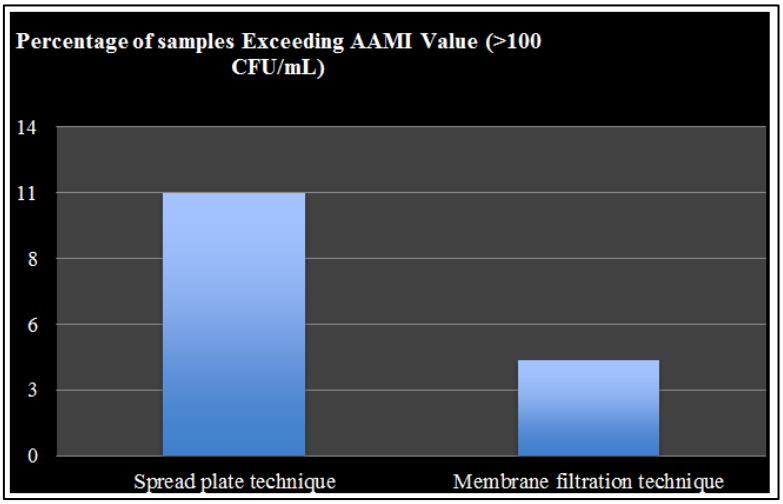

Fig-1: Comparison of Samples exceeding AAMI Value by Spread plate technique and Membrane filtration technique

Table-2: Comparing Spread plate exceeding AAMI and Ultra-pure with Membrane filtration exceeding AAMI and Ultra-pure values, Chi square =7.1172, P = 0.0076. The result is significant at P<0.05. There is no significant difference between both the methods

		Spread plate technique			Total samples tested
		Exceeding AAMI Value	**Exceeding ultra-pure value**	**Ultra-pure samples**	
Membrane filtration technique	Exceeding AAMI Value	2	2	0	4
	Exceeding ultra-pure value	9	30	1	40
	Ultra-pure samples	0	5	1	6
Total		11	37	2	50

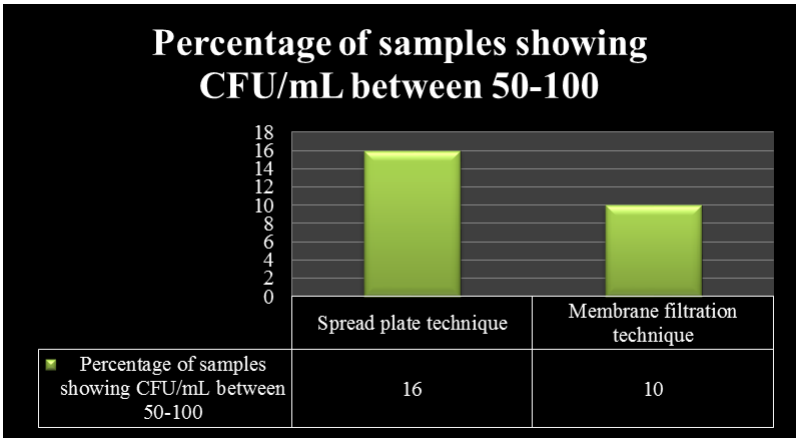

Fig-2: Comparison of Samples Values between 50-100 CFU/mL by Spread plate technique and Membrane filtration technique

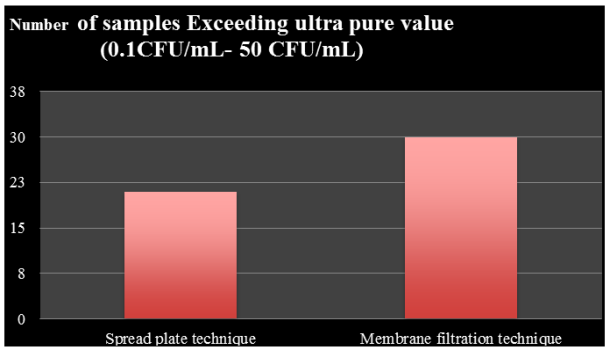

Fig-3: Comparison of Samples exceeding ultra-pure value by Spread plate technique and Membrane filtration technique

Statistical analysis: Wilcoxon signed rank test was performed for Figure 1,2 and 3data. Z= -3.91, P=<0.0001 the result is significant at P<0.05. There is no significant difference in identifying CFU/mL values between 0.1-50 by both the methods.

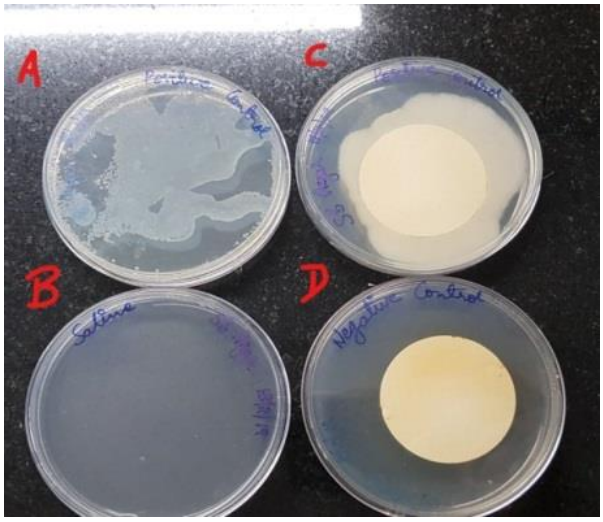

Fig-4: A shows positive control of spread plate technique, B shows negative control of spread plate technique, C shows the positive control of membrane filtration technique and D shows negative control of membrane filtration technique

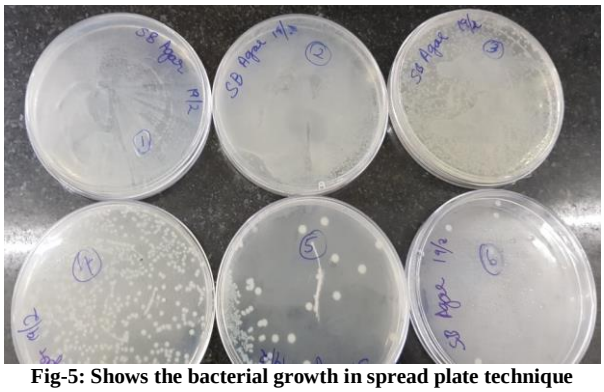

Fig-5: Shows the bacterial growth in spread plate technique

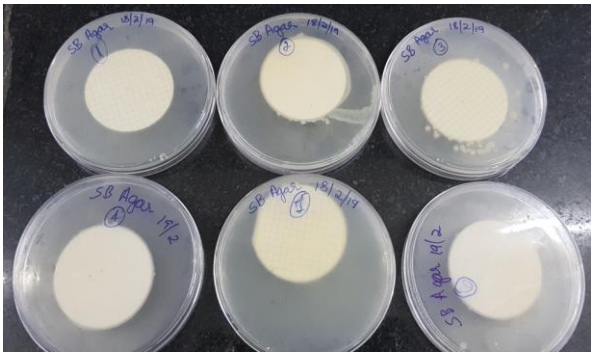

Fig-6: Shows the bacterial growth in membrane filtration technique

DISCUSSION

Microbiological standards for the quality of dialysis water and fluid have changed over the past seventy years, and continue to evolve. Ensuring the necessary quality of dialysate is a vital aspect of this type of treatment considering the repeated, large volumes each patient is subjected to. Specifically, chemical, bacterial, and associated endotoxin contamination can threaten a dialysis patient's health. Dialysis patients often have additional co-morbidities (e.g., diabetes, hypertension, cardiovascular disease, etc.) that can make them more vulnerable to adverse outcomes. Aging, obesity, and hypertension rates are also increasing in the U.S. population, which are associated with ESRD and chronic kidney disease [8].

In this study, there is no significant difference between two methods. The spread plate technique showed equal sensitivity as like membrane filter technique for analyzing dialysis water. In a study of comparison of different culture methods on bacterial recovery in hemodialysis fluids reported by Punakabutra *et al*. the percentages of positive culture and percentages of CFU values exceeding the ultrapure value by the membrane filtration method were significantly higher than the spread plate method (91.4 vs 60.0%, $p < 0.01$and 87.4 vs 60.0%, $p < 0.01$)[9-12]. In contrast, the percentages of the CFU values exceeding the AAMI value by the membrane filtration method were significantly lower than by the spread plate method (4.0 vs 26.3%, $p < 0.01$. In the study of bacterial Recovery in Hemodialysis Fluids by Mokhtar *et al*. the percentages of positive culture and percentages of CFU values exceeding the ultra-pure value on R2A by the membrane filtration method were significantly higher than the spread plate method (91.4 vs 60.0%, $p < 0.01$and 87.4 vs 60.0%, $p < 0.01$). In contrast, the percentages of the CFU values exceeding the AAMI value on R2A by the membrane filtration method were significantly lower than by the spread plate method (4.0 vs 26.3%, $p < 0.01$)[13-15].

From this study, spread plate technique proves to be equally sensitive with membrane filtration technique for analyzing dialysis water but when ultrapure water needs to be analyzed, spread

plate technique gives much better bacterial recovery. i.e, only 2 samples were proved to be ultra-pure by Spread plate technique. However, 6 samples were shown as ultra-pure by Membrane filtration technique. This may be because of the more volume of water used in the membrane filtration method which failed to pick out the minimal number of bacteria. It should be always noted that the counting area on the medium of the membrane filtration method will be smaller when compared to the spread plate method in identifying >100 CFU/mL (Exceeding AAMI Value).

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