Comparative Evaluation of Different Bacterial Media for Total Aerobic Bacterial Count in Deionised Water

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Deionised water is used in the production of active pharmaceutical ingredients and pharmaceutical applications such as cleaning of equipments. It must meet the prescribed chemical and microbiological requirements. Total aerobic bacterial count is estimated to evaluate the microbiological quality of deionised water. Deionised water samples were tested for total aerobic bacterial count on three different media, plate count agar, soyabean casein digest agar and R2A agar using pour plate method. The results obtained were compared and it was observed that R2A agar gave slightly higher results as compared to plate count agar media while the count on soyabean casein digest agar were significantly low. Therefore, on the basis of the comparative validation of media, it can be decided whether low nutrient media like R2A agar is to be included or excluded in the routine testing, thus saving resources.

USP-24[1] pour plate (serial dilution) method was used for total aerobic bacterial count (TBC) testing of DI water. Sampling was done by draining the water for 5 min and then collecting water sample in a 1-l sterile water bottle. Membrane filtration method was also employed for low count samples. All the media used in the study were obtained from Himedia Labs, Mumbai. The incubation temperature of 30–35° for 72 h was employed for soyabean casein digest agar (SCDA) and plate count agar (PCA), while for R2A agar (R2A), 20–25° for 5 d was used. All the determinations were done in duplicate and average was taken for comparative evaluation.

Table 1 shows the composition of three media used in this study[1-5]. In Table 2, results of TBC obtained on three media have been included. In general, TBC is determined on PCA or SCDA on routine basis and R2A medium is used during validations studies[1]. In general, it has been reported[5-7] that R2A medium give higher counts as compared to other media. In this study also, it was observed that R2A medium gave slightly higher counts but comparable to PCA. Colonies on the R2A agar were minute and pigmented in comparison to PCA or SCDA where the size was bigger. A total of 500

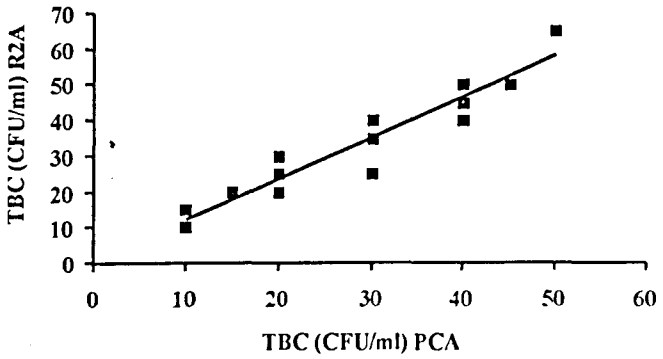

Fig. 1: Comparison of total aerobic bacterial count (TBC) per ml on plate count agar and R2A agar media.

Results of TBC obtained on R2A agar (R2A) and plate count agar (PCA) are plotted on X and Y-axis respectively to see the correlation. The correlation (R^2) CFU/ml on two media was found to be 0.947 as per the linear regression analysis (Y=1.1522X+1.3092).

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TABLE 1: COMPOSITION OF BACTERIOLOGICAL MEDIA USED

Soyabean Casein Digest Agar (SCDA)	g/l	Plate Count Agar (PCA)	g/l	R2A Agar	g/l
Casein enzymatic hydrolysate	15	Casein enzymatic hydrolysate	5	Casein acid hydrolysate	0.5
Papaic digest of soyabean meal	5	Yeast extract	5	Yeast extract	0.5
Sodium chloride	5	Dextrose	1	Protease peptone	0.5
Agar	5	Agar	15	Dextrose	0.5
pH	7.3± 0.2	pH	7.0±0.2	Starch (soluble)	0.5
				Dipotassium phosphate	0.3
				Magnesium sulphate	0.024
				Sodium pyruvate	0.3
				Agar	15
				pH	7.2±0.2

TABLE 2: TOTAL VIABLE AEROBIC BACTERIAL COUNT OBTAINED IN DEIONISED WATER SAMPLES USING DIFFERENT MEDIA

TBC (CFU/ML) PCA (30-35°/72 h)*	TBC (CFU/ML) R2A (20-25°/5 d)*	TBC (CFU/ML) SCDA (30-35°/72 h)*	TBC (CFU/ML) PCA (30-35°/72 h)*	TBC (CFU/ML) R2A (20-25°/5 d)*	TBC (CFU/ML) SCDA (30-35°/72 h)*
15	20	15	20	30	15
10	10	10	10	15	15
40	45	35	10	10	<10
45	50	35	10	15	10
20	30	15	20	20	10
10	10	<10	10	15	10
20	20	10	30	35	25
40	50	30	40	50	30
30	35	30	30	40	20
15	20	10	20	20	15
50	65	40	30	25	20
40	40	25	20	25	15
			10	10	<10

Results of total viable aerobic bacterial count (TBC) obtained on soyabean casein digest agar (SCDA), plate count agar (PCA) and R2A agar (R2A). Each value is the average of two determinations. *Denotes temperature and period of incubation.

samples were analyzed and data of 25 has been presented in Table 2. We observed that PCA (30–35° for 72 h) and R2A (20–25° for 5 d) gave comparable results, with R2A giving slightly higher values in some cases. This equivalency in count is attributed to the initial microbial load of water sample, which may be having low or negligible count of injured or slow growing bacteria. R2A being a low nutrient medium supports the growth of slow growing bacteria or those injured due to variety of reasons that include chlorine treatment and heat treatment[1].

Further, in order to know more about the correlation, results obtained on two media were plotted following the procedure recommended by Altman and Bland[8], as presented in fig. 1 and the results show good correlation between the two. SCDA medium gave low counts, which is in agreement with earlier findings[6].

It is concluded from the study that R2A agar, once compared to PCA can be included or excluded during routine monitoring depending upon the results obtained during comparative validations. If both the media yield equivalent counts, then R2A medium can be excluded during routine monitoring or *vice-versa*. But for this purpose, adequate data has to be generated. It is also concluded that count on R2A medium will depend upon the nature of the microbial load present in the sample as explained above.

Moreover USP[1] clearly states that low nutrient medium like R2A and high nutrient medium like PCA are to be compared during validation of water system and on that basis it has to be decided whether particular system needs to be monitored additionally using low nutrient medium (R2A) on regular basis or the normal medium like PCA is sufficient.

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Spectrophotometric Estimation of Venlafaxine with Folin Ciocalteu Reagent

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A simple, rapid, precise, accurate and highly specific spectrophotometric method has been developed for the determination of venlafaxine in its pharmaceutical dosage form. The method is based on the formation of blue colored chromogen due to the reaction of venlafaxine with Folin Ciocalteu reagent in presence of alkali, which exhibits λ_{max} at 730 nm. Beer's law obeyed in the concentration range of 2.5-25 μg/ml. The blue color obtained was stable for more than 24 hours. The method was successfully applied for the quantitative determination of venlafaxine and its pharmaceutical dosage form. The accuracy and reproducibility of the proposed method was statistically validated by recovery studies.

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