J. Milk Food Technol. Vol. 39, No. 12, Pages 852-853 (December, 1976)

A Research Note

Contribution of KH_2PO_4 to Toxicity in Phosphate Buffered Dilution Water Systems

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(Received for publication April 19, 1976)

ABSTRACT

It appears that different sources of KH_2PO_4 used to prepare phosphate buffered dilution water have various effects on the ability of the dilution water to maintain a stable bacterial population. When the water used to prepare the dilution water is of either very good or very poor quality, the KH_2PO_4 source is of no consequence. However, if water of an intermediate quality is used, the KH_2PO_4 source becomes of consequence in the over-all quality of the system.

There have been various degrees of concern over the years about the best dilution system to use for various bacteriological applications. Interest has been primarily in the areas of which buffering and/or protective agents to use (*2, 5, 7*) or the quality of the distilled water (*3, 6*).

During a study to develop a simplified method to determine distilled water suitability (*4*), it became apparent that yet another factor may play a role in determining the ability of a dilution system to maintain a given level of a bacterial population over time. That factor is variability between lots, brands, and conditions of the dihydrogen potassium phosphate (KH_2PO_4) used to prepare the stock phosphate buffer solution. This research note reports our preliminary data on the subject.

TABLE 1. *Identification of various sources of KH_2PO_4 studied*

Study code	Supplier	Lot no.	Size	Comments
G	Mallinckrodt Chemical Works	XJX	1 lb	sealed bottle
H	Matheson Coleman and Bell	25	1 lb	unsealed bottle
I	Fisher Scientific Company	740075	¼lb	unsealed bottle uneven crystals
J	Fisher Scientific Company	735003	1 lb	sealed bottle powdery & lumpy
K	Mallinckrodt Chemical Works	NDT	1 lb	unsealed bottle
L	Mallinckrodt Chemical Works	MXX	1 lb	unsealed bottle used for prescriptions
M	Mallinckrodt Chemical Works	XTP	1 lb	unsealed bottle
N	Mallinckrodt Chemical Works	TCH	1 lb	unsealed bottle
X	University of Iowa Pharmaceutical Services	—	—	Stock buffer solution

MATERIALS AND METHODS

Various lots and brands of KH_2PO_4 were obtained and used to prepare a number of stock buffer solutions as described in *Standard Methods for the Examination of Dairy Products* (*1*). The sources of the KH_2PO_4 are presented in Table 1.

Stock solutions were used to prepare standard 99-ml dilution blanks in combination with three different qualities of distilled water, and any differences in toxicity were evaluted by the Interval Plating Procedure as previously described (*4*). The three qualities of distilled water used are indicated in Table 2.

RESULTS

Using the suggestion in the Interval Plating Procedure

TABLE 2. *Characteristics of distilled water studied*

No.	Specific resistance (ohms)	Treatment
1	1.2×10^6	Water From Central Distillation Unit passed through Millipore Super Q[a]
2	0.63×10^6	Water From Central Distillation Unit passed through mixed-bed resin
3	0.32×10^6	Water From Central Distillation Unit

[a]Millipore Corp., Bedford, Mass.

TABLE 3. *Effect of interaction of different quality waters and various sources of KH_2PO_4 on interval plating survival*[a]

KH_2PO_4 Source	Percent change in bacterial population		
	Water 1	Water 2	Water 3
G	+ 2.3	−49.8	−53.5
H	+ 8.5	− 1.3	−78.3
I	− 1.2	−21.4	−44.0
J	− 1.7	−44.3	−68.3
K	−12.6	−16.9	−81.9
L	− 5.7	− 6.3	−83.8
M	− 9.4	−33.3	−49.4
N	+13.1	−14.4	−45.9
X	− 7.2	−26.2	−64.6

[a]% change in *E. coli* (ATCC 25922) population when allowed to remain in dilution system for 60 min.

(4) that a change in bacterial population after standing 60 min in buffered diluent should not be greater than± 15% it will be noted from Table 3 that none of the diluents exceeded this range when water of 1.2×10^6 ohms-cm^2 specific resistance was used. However, only three of the diluents were within the specified range with water of 0.63×10^6 ohms specific resistance and none qualified with the lowest quality distilled water.

These results suggest that when water of a high quality is used the quality of the KH_2PO_4 source appears to be of no consequence in contributing to toxic effects. When a poor quality distilled water is used to prepare the dilution system, the system appears to be toxic, also regardless of the KH_2PO_4 source. Therefore, very good or very poor qualities of distilled water seem to be the dominant factor in determining the toxicity, or lack of it, in phosphate buffered dilution systems with the quality of phosphate source not being of consequence. However, when water of an intermediate quality is used, the KH_2PO_4 source produces differences, with some of the systems being acceptable by interval plating, and others not.

DISCUSSION

At this preliminary stage it is difficult to speculate on the significance of these results. It seems that distilled waters at the two extremes of quality, very good or very poor, dominate the situation, and mask any effect of an inferior KH_2PO_4 source. Ideally, the answer would be to always have available a distilled water source of high and consistent quality whereby the need to be concerned about toxic factors in the dihydrogen potassium phosphate (KH_2PO_4) would be eliminated or at least considerably reduced. However, most small laboratories are fortunate if their distilled water is in the intermediate quality range (500,000 - 600,000 ohms specific resistance), thus making it entirely possible that the buffering agent is adversely affecting the quality of the buffer system.

Of the nine sources examined, only three, in combination with the intermediate quality water, (sources H, L, N) were able to meet the criteria established in the Interval Plating Procedure. Sources I and J, which were questionable by visual inspection, did indeed produce an unacceptable dilution system, but the remaining sources produced results which cannot be correlated with any of the descriptive information.

This report, as mentioned previously, is preliminary. Additional studies must be undertaken before the situation inferred in this note is seriously evaluated. However, this research note should alert those involved in laboratory quality control that another factor, previously taken for granted, may well be a significant variable in enumeration systems.

ACKNOWLEDGMENT

The authors would like to thank Mrs. Vera Remmes for her technical assistance.

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