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A Research Note

Group Comparative Study of VRB-2 Agar in the Recovery of Coliforms from Raw Milk, Ice Cream and Cottage Cheese

R. T. MARSHALL[1]*, P. A. HARTMAN[2], R. Y. CANNON[3], L. LAMBETH[4], G. H. RICHARDSON[5], K. R. SPURGEON[6], D. B. WEDDLE[7], M. WINGFIELD[6] and C. H. WHITE[8]

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ABSTRACT

The method of Hartman et al. for recovering stressed coliform bacteria was evaluated in a group study. The experimental medium, VRB-2, was more productive than VRB medium by 31% for raw milk, 70% for ice cream and 61% for cottage cheese. Differences in colony counts of coliforms caused by incubating for 48 vs. 24 h and by boiling vs. autoclaving were not statistically significant. Among 40 samples tested in two laboratories, counts obtained at 30 vs. 32 C were not significantly different.

It has been well documented that injured coliforms, which would otherwise not grow in selective media, will repair in nonselective media (*4*). Hartman et al. (*1*) developed a modified procedure for making coliform counts on solid media and reported that yields of coliforms on their Violet Red Bile-2 (VRB-2) medium were increased compared with standard Violet Red Bile (VRB) medium (*2*). Speck et al. (*5*) published a similar method at the same time.

In an effort to confirm the comparative performances of VRB and VRB-2 we undertook this group study. In addition to evaluating the medium, we tested incubation time and temperature and method of medium preparation.

MATERIALS AND METHODS

VRB and VRB-2 media (Difco) were hydrated according to Standard Methods (*2*) and aliquots were heat-treated by boiling for 2 min and by autoclaving at 121 C for 5 min. Dilutions of raw milk, ice cream and cottage cheese were prepared and plated according to Standard Methods (*2*) except as noted below. Some samples of ice cream and cottage cheese were inoculated with raw milk or with cultures of coliform bacteria. When this was done, cottage cheese was stored at 4 C and ice cream at − 20 C for 24 h before plating to allow organisms to be stressed. Ten locally-obtained samples per product were examined per laboratory, but not all products were examined in each laboratory, usually because samples with sufficient coliforms were not available (see Table 1).

Eight plates of each dilution were prepared per sample and duplicates were poured with VRB agar-boiled, VRB agar-autoclaved, VRB agar-2 boiled and VRB agar-2 autoclaved. VRB agar was poured in the usual way. The sample was mixed into a basal layer of 8 to 10 ml of Standard Methods agar in VRB-2 plates. On solidification, 8-10 ml of VRB-2 agar was poured onto the basal layer. Plates were counted after being incubated at 32 ± 1 C for 24 and 48 h. In two laboratories plates were also incubated at 30 ± 1 C. Data presented are for only 32 C except where otherwise noted. Average counts of duplicate plates were used in the statistical analyses.

Data (logarithms of counts) were analyzed by Analysis of Variance with main variables being laboratory, medium, treatment of medium and temperature of incubation. All possible interactions were tested. Means were differentiated by Least Significant Difference tests (*3*).

RESULTS AND DISCUSSION

The experiment was designed to determine whether medium, time of incubation or method of preparing the medium were significant variables in determining the coliform count of raw milk, ice cream and cottage cheese.

Geometric means of coliform counts by product, laboratory and medium appear in Table 1. The main effect of medium, averaged over other variables, was significant ($P < 0.05$) with each product. VRB-2 medium was more productive by 31% with raw milk, 70% with ice cream and 61% with cottage cheese. However, further breakdown of the data showed that within laboratory differences between the two media were only significant with raw milk in laboratory 3, with ice cream in laboratories 2 and 3, and with cottage cheese in laboratory 3. Laboratories in which significant differences were recorded were generally those in which counts were highest. This observation is not surprising since there is high variability among low counts such as are observed in counting coliform bacteria. Coefficients of variation were

[1]*Department of Food Science and Nutrition, University of Missouri-Columbia, Columbia, MO 65201.*
[2]*Department of Bacteriology, Iowa State University, Ames, IA.*
[3]*Department of Animal and Dairy Sciences, Auburn University, Auburn, AL.*
[4]*Health Department, Springfield, MO.*
[5]*Department of Nutrition and Food Sciences, Utah State University, Logan, UT.*
[6]*Dairy Science Department, South Dakota State University, Brookings, SD.*
[7]*Mid-America Farms, Springfield, MO.*
[8]*Dean Foods Company, Rockford, IL.*
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TABLE 1. *Geometric mean counts by product, laboratory and medium; percentages of difference and significance.*

Product	Lab[a]	Mean counts/ml VRB	VRB-2	Percentage difference[b]
Raw milk	1	10.4	12.0	15NS
	2	15.3	16.7	9NS
	3	14.9	35.4	138*
	4	29.4	41.5	41NS
	5	24.8	22.6	−9NS
	6	13.6	18.3	35NS
	Means	16.9	22.2	31*
Ice cream	2	6.4	10.9	70*
	3	8.9	18.8	111*
	4	7.3	10.0	37NS
	Means	7.5	12.7	70*
Cottage cheese[c]	1	9.1	12.2	34NS
	2	7.7	7.9	3NS
	3	9.4	41.2	338*
	7	28.0	31.3	12NS
	Means	11.7	18.8	61*

[a]n = 10 (samples per product per laboratory).
[b][(Count on VRB-2 - count on VRB ÷ count on VRB] × 100.
[c]Data in this section of table do not include counts made after 48-h incubation because of an accident in one laboratory.
NSNonsignificant.
* Significant at 5% level (individual percentages difference by LSD and means by ANOV).

.27, .47, and .46 for the milk, ice cream and cottage cheese analyses, respectively.

Counts (averaged over laboratories within products) made after 48 h of incubation were not significantly higher than those made after 24 h (Table 2). Even though differences did not prove statistically significant, counts among products were 11 to 19% higher after 48 h incubation. An insufficient number of colonies was confirmed as coliforms to determine whether the incidence of false-positive colonies differed with incubation time.

There was no significant main effect of boiling vs. autoclaving of the media (Table 2), and in no individual laboratory with any product was there a significant difference in response to heat treatment between the two media for the various products tested.

Only laboratories 1 and 4 determined counts at both 30 and 32 C; laboratory 1 for raw milk and cottage cheese, laboratory 2 for raw milk and ice cream. In none of the experiments was there a significant effect of temperature on coliform count. Mean counts at 30 vs. 32 C, respectively, averaged for milk 23.0 and 23.3, for ice cream 8.5 and 8.7, and for cottage cheese 9.5 and 10.3/ml.

TABLE 2. *Geometric mean counts averaged over laboratories within products as affected by time of incubation and treatment of media.*

Product	n	Coliforms/ml Time of incubation 24 h	48 h	Treament of medium Boiled	Autoclaved
Raw milk	60	18.4	20.5	19.4	19.4
Cottage cheese	40	11.7	13.1	12.5	12.3
Ice cream	30	8.9	10.6	9.3	10.1
Average[a]		13.0	14.7	13.7	13.9

[a]Arithmetric averages of the means derived by logarithmic statistical analyses. Differences between individual pairs of geometric means were all insignificant ($P < 0.5$).

CONCLUSIONS

The procedure in which VRB-2 medium was used was considerably more productive of coliform-type colonies than was the similar procedure in which VRB medium was used. Neither boiling nor autoclaving at 121 C for 5 min influenced productivity of either medium.

Extending the time of incubation from 24 to 48 h may be beneficial with ice cream. However, this was a minor variable, and there is little practical reason to consider increasing the incubation time. There is reason, however, to maintain a standard for time of incubation because of the tendency for counts to increase after 24-h of incubation. Decreasing the incubation temperature from 32 to 30 C should not change the value of the coliform count method when it is used with products examined in this study.

REFERENCES

1. Hartman, P. A., P. S. Hartman, and W. W. Lanz. 1975. Violet Red Bile 2 agar for stressed coliforms. Appl. Microbiol. 29:537-539.
2. Hausler, W. J., ed. 1972. Standard methods for the examination of dairy products. 13th ed. American Public Health Association, Inc., Washington, DC. pp. 64, 92-93.
3. Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods. 6th ed. The Iowa State University Press, Ames, IA.
4. Speck, M. L., and B. Ray. 1977. Effects of freezing and storage on microorganisms in frozen foods: a review. J. Food Prot. 40:333-336.
5. Speck, M. L., B. Ray, and R. B. Read, Jr. 1975. Repair and enumeration of injured coliforms by a plating procedure. Appl. Microbiol. 29:549-550.