

# Comparative Recovery of Coliforms from Meat and Milk Using m-Colibblue24 and Standard Methods

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### Abstract

A new membrane filtration medium, m-ColiBlue24 (mCB), simultaneously detects Total Coliforms (TC) and *Escherichia coli* (EC) in a single 24-hour incubation. Samples of ground beef, ground pork, and milk were analyzed by mCB and compared to standard methods. When TC from beef and pork samples were compared, there were no significant difference between mCB and pour plating on Violet Red Bile (VRB) agar. In a most probable number (MPN) analysis, nine counts from mCB were within the 95% confidence interval (CI) and 13 were outside the CI. VRB yielded six counts within and 16 outside the CI. The raw milk samples contained confirmed TC counts as high as  $9.00 \times 10^2$  on mCB and  $2.20 \times 10^3$  on VRB. Furthermore, the raw milk had up to  $1.73 \times 10^3$  confirmed EC population. The BBL Crystal system was used to identify representative isolates from mCB and VRB. Of 59 mCB isolates, 64.4% were classical TC. Of the 51 VRB isolates, 62.7% were identified as classical TC.

### Introduction

Advances in culturing techniques, demographic changes, and changes in public acceptance of preservatives in foods has led to a steady progression of newly emerging food-borne pathogens. These constant changes have allowed indicator organisms to remain an important concept in permitting accurate microbial analysis of foods.

Currently, research is ongoing to improve methods for monitoring indicator organisms. One newly developed medium is mCB. mCB uses a membrane filtration (MF) format that simultaneously detects EC and TC in a single 24-hour incubation (2). This simultaneous detection is possible because TC appears red and EC appears blue after a 24-hour incubation period. Studies were conducted to compare mCB to standard methods to evaluate its effectiveness.

### Materials and methods

#### Samples

The examined beef and pork samples were purchased from a local retail store. Raw milk samples were obtained from the Iowa State University Dairy Facility.

#### Experimental protocols

The beef and pork samples were analyzed in three ways: filtered and placed on mCB (Hach, Loveland, CO), pour plated on VRB (Difco) with overlay, and five tube MPNs. The latter two analyses followed the Compendium of Methods for the Microbiological Examination of Foods (1). For the filtration of the meats, a  $10^{-1}$  slurry was made. Then, 1 ml. of the slurry was added to 10 ml. of buffered peptone water and filtered through a 0.45  $\mu$ m. filter. The filter was placed on an absorbent pad saturated in mCB. The mCB and filters were incubated at 35°C for 24 ± 4 hours.

The raw milk samples were analyzed by pour plating on VRB with overlay and placed on mCB after filtering. The procedure for the pour plating followed the Compendium of Methods for the Microbiological Examination of Foods (1). The procedure for filtering the milk was similar to the method mentioned above.

Representative colonies from VRB and mCB were isolated and identified by BBL Crystal System.

### Results and Discussion

Comparative counts of TC populations in raw milk on mCB and VRB are shown in Table 1. The presumptive TC populations were verified in brilliant green lactose broth (BGLB). Counts ranged from  $4.67 \times 10^1$  to  $9.00 \times 10^2$  colony forming units (CFU)/ml. on mCB. Counts on VRB ranged from  $1.67 \times 10^1$  to  $2.20 \times 10^3$  CFU/ml. The table also presents the presumptive counts of *E. coli* on mCB that were verified by EC + MUG. The counts ranged from <10 to  $1.73 \times 10^2$  CFU/ml.

Statistical analysis was done comparing the TC counts from meat samples on mCB and VRB. There were 76 meat samples tested, and the least squares mean for mCB was 4.62 and 4.38 for VRB. These counts showed no significant difference when  $P > 0.10$ .

While testing the 76 meat samples, colonies were randomly isolated from their respective media and identified. There were 59 isolates from the mCB and 51 from VRB. The mCB had 64.4% classical TC (*Escherichia* spp., *Klebsiella* spp., *Citrobacter* spp., and *Enterobacter* spp.), 89.8% *Enterobacteriaceae*, 8.5% *Vibrionaceae*, and 1.7% *Acinetobacter* sp. VRB had 62.7% classical TC, 88.2% *Enterobacteriaceae*, and 11.8% *Vibrionaceae*. Of the 59 mCB isolates, 19 were blue colonies (presumptive *E. coli*). All 19 blue colonies were confirmed as *E. coli*.

A scatter plot of the data shown in Graph 1 compared TC recoveries from 76 meat samples. The line on the graph showed a strong positive correlation between mCB and VRB with a correlation coefficient of 0.89. When TC recoveries were compared between mCB and MPN from 35 meat samples, there was a weak correlation coefficient

of 0.37. This correlation coefficient shows the difficult nature of trying to compare two dissimilar methods. mCB had nine counts within and 13 counts outside the CI. However, VRB had six counts within and 16 outside the CI. Therefore, a graph comparing VRB and MPN would have shown a poorer correlation than mCB vs MPN.

A comparison of presumptive *E. coli* populations in meats by mCB and MPN was performed on 35 samples. There were 17 counts within and nine counts outside the CI for MPNs. The other nine counts were non-estimable.

#### **References**

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2. Erdmann, J., Dickson, J.S., Grant M.A. A New Membrane Filtration Medium for Simultaneous Detection of *E. coli* and Total Coliforms. pp. 194-200. In: 1995 Swine Research Report. Iowa State University Press, Ames, IA.

**Table 1.**  
**Presumptive and Verified Total Coliform**  
**Populations in Raw Milk**

Sample	m-ColiBlue24 CFU/ml			VRB CFU/ml		
	initial	BGLB+	verified	initial	BGLB+	verified
1	$4.67 \times 10^1$	55%	$2.59 \times 10^1$	$2.00 \times 10^1$	83%	$1.67 \times 10^1$
2	$5.33 \times 10^2$	40%	$2.13 \times 10^2$	$9.67 \times 10^1$	100%	$9.67 \times 10^1$
3	$9.00 \times 10^2$	100%	$9.00 \times 10^2$	$2.20 \times 10^3$	100%	$2.20 \times 10^3$
4	$1.73 \times 10^2$	100%	$1.73 \times 10^2$	$3.37 \times 10^2$	100%	$3.37 \times 10^2$

**Presumptive and Verified *E. coli* Populations in**  
**Raw Milk**

	initial	ECM+	verified
1	$1.67 \times 10^1$	100%	$1.67 \times 10^1$
2	presumptive <i>E. coli</i>	<10/ml	
3	$3.33 \times 10^1$	100%	$3.33 \times 10^1$
4	$1.70 \times 10^2$	100%	$1.73 \times 10^2$

