An Evaluation of a Conductance Method for the Enumeration of *Enterobacteriaceae* in Milk

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**ABSTRACT**

A method for the quantitative detection of *Enterobacteriaceae* in raw milk utilizing automated, conductance monitoring of a selective medium was studied. The levels of pure *Enterobacteriaceae* cultures as well as *Enterobacteriaceae* levels in naturally contaminated raw milk were accurately measured using the conductance method when statistically compared to results obtained by an agar plating method. The correlation coefficient (r-value) for naturally contaminated raw milk samples was 0.92. Using a pass/fail limit of 100 CFU/ml, additional raw milk samples obtained by an agar plating method. The correlation coefficient was studied. The levels of pure *Enterobacteriaceae* cultures as well as the processing environment, is the *Enterobacteriaceae* plate count method as described elsewhere (1). Many new techniques have been proposed for the rapid, automated detection of microbial numbers. One method which has been studied extensively and put into practical applications is conductance monitoring by automated instrumentation. Conductance changes which occur in suitable nutrient medium have been shown to relate linearly to the Log$_{10}$ number of microorganisms, and have been found to be an accurate method for quantifying microbial levels (2,5). Conductance changes occur in a suitable nutrient medium due to the catabolic breakdown of nutrient compounds in the medium to compounds of higher charge, which affects the conductance. There have been many methods developed using this technique to monitor microbial levels and activity (2). A conductance method for the quantitative detection of coliforms has been developed and found to correlate well with results determined by an agar plating method (4). An indicator test which is widely recognized and used in Europe as a means of monitoring quality of food and beverage products as well as the processing environment, is the *Enterobacteriaceae* plate count test. This analysis is very similar to the coliform test, with the exception that non-lactose fermenting *Enterobacteriaceae* are detected (such as *Salmonella*, *Shigella*, and *Yersinia*). This study was undertaken to evaluate a conductance monitoring method for the enumeration of *Enterobacteriaceae*.

**MATERIALS AND METHODS**

**Conductance method**

The Malthus 2000 Microbiological Analyzer (Radiometer America Inc., Westlake, Ohio) was used to monitor conductance changes occurring in the medium. The principles of this instrument have been described elsewhere (1). Samples were either vortexed or hand shaken and one ml was transferred to a test tube containing 5.0 ml of Malthus *Enterobacteriaceae* Medium (MEM) (Radiometer America Inc.). A platinum electrode was then inserted into the medium, and the entire electrode cell transferred to the Malthus 2000 water bath incubator set at 35°C (+/- 0.006°C). Incubation temperature stability is critical to obtaining accurate results (2) due to the proportional relationship between conductance and temperature to the medium being analyzed. The conductance of the inoculated medium was measured (in microsiemens) every 6 min and stored in the computer. When a significant change in conductance occurs in the medium, a Detection Time (DT) is recorded by the computer. The DT's obtained were then compared statistically with plate count results (Log$_{10}$ CFU) by linear regression analysis.

**Enterobacteriaceae plate count method**

Enumeration of *Enterobacteriaceae* levels was accomplished using the *Enterobacteriaceae* Plate Count Method as described in The ICMSF publication Microorganisms in Foods 1 (3). This method is very similar to the coliform plating method, with the exception that 10 g/l glucose (Difco Lab. Inc., Detroit, MI) is added to Violet Red Bile Agar (VRBA) (Difco). Confirmation of typical colonies (purple) is based on ability to ferment glucose and the oxidase test. Typical colonies that are glucose-positive and oxidase-negative are reported as *Enterobacteriaceae*.

**Pure and naturally contaminated samples**

Pure cultures of *Enterobacter cloacae* ATCC 23355, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 13883, and a non-*Enterobacteriaceae* culture *Pseudomonas aeruginosa* ATCC 27853, were grown overnight in BHI (Difco) at 35°C before analysis. The overnight culture was diluted 1:1000 in phosphate buffer. This dilution was used as the initial sample in the following protocol. Samples were serially diluted (1:10) four times and each dilution subjected to the conductance and agar plating tests.

To monitor naturally present levels of *Enterobacteriaceae*, 15 raw milk samples (labeled A to O) were obtained and stored...
overnight on ice. These samples were tested by the conductance and agar plating methods after 0, 2, 3.25, 5, and 6.75 h incubation at 35°C. This was necessary to ensure that levels of Enterobacteriaceae would span at least 3 log cycles to perform a linear regression analysis on the data. After this phase of the study was completed, raw milk samples (N=50) were again subjected to Enterobacteriaceae analyses by both methods, this time without the temperature abuse step.

RESULTS AND DISCUSSION

Pure cultures

The first step of this study was to determine if the conductance monitoring method did indeed detect quantitatively the levels of pure Enterobacteriaceae cultures. Figure 1 is the conductance curves for the five dilutions of the E. coli cultures. This graph illustrates the relationship between microbial numbers and detection times. The conductance curve showing the earliest DT (represented by a “+” sign on the curve) of 3.4 h was the undiluted sample, containing $2 \times 10^5$ CFU/ml. The other curves in order of increasing DT are the diluted samples containing $2 \times 10^4$, $2 \times 10^3$, $2 \times 10^2$, and $2 \times 10^1$ CFU/ml of E. coli. The other Enterobacteriaceae strains analyzed produced similar results. A correlation coefficient (r-value) of -0.92 was obtained when DT and Log$_{10}$ CFU results of all four Enterobacteriaceae cultures were compared using linear regression analysis. All of the Enterobacteriaceae cultures analyzed produced a total change in conductance of greater than 200 microsiemens. The non-Enterobacteriaceae strain, P. aeruginosa (Fig. 2), produced delayed responses in comparison to the Enterobacteriaceae strains and the total magnitude of conductance change was less than 100 microsiemens. Therefore, it appeared from the pure culture results that total magnitude of conductance change could be used as a criterion for discriminating between samples containing Enterobacteriaceae and samples containing non-Enterobacteriaceae microorganisms capable of growth in the selective medium evaluated.

Naturally contaminated samples

Since it was observed during the first phase of this study that Enterobacteriaceae strains produced strong conductance responses and DT’s which were related to initial numbers, we then evaluated samples naturally contaminated with Enterobacteriaceae to verify this response in the presence of competing microorganisms. Figure 3 is a conductance graph showing conductance curves for several of the raw milk samples analyzed. The cluster of similar curves with DT’s approximately 8 h was samples that were found to contain Enterobacteriaceae by the agar plate method. Also shown is the medium blank (no DT), as well as two samples which produced delayed DT’s and total conductance changes of less than 200 microsiemens. The corresponding plate count of these two samples were <10 Enterobacteriaceae CFU/ml; however, the agar plates
did contain a number of atypical colonies (not purple). Similar curves (<200 microsiemen change) were recorded on several other samples which also contained <10 Enterobacteriaceae CFU/ml. It is likely that the growth occurring in those samples was due to gram-negative non-Enterobacteriaceae, which are commonly isolated in raw milk.

An interesting observation from this phase of our study was one particular milk sample (sample I) which produced typical Enterobacteriaceae curves (Fig. 4). The Enterobacteriaceae plate count on this sample was quite high, but it was noticed that the colonies were very small. Confirmation results of this sample found it to be oxidase-negative and glucose-negative. This isolate was identified as Yersinia enterocolitica by an API-20E identification kit (Analytab Products, Plainview, New York). This Yersinia culture, a member of the Enterobacteriaceae Family, would have been determined non-Enterobacteriaceae by the traditional plate count method due to the negative glucose fermentation.

The Enterobacteriaceae conductance and plate count results obtained on the raw milk samples were compared using linear regression analysis. A scattergraph of the data is shown in Fig. 5 with plate count results on the X-axis and conductance DT on the Y-axis. The linear regression line is also shown on the graph (solid line). A strong linear relationship between Log_{10} CFU and DT can be observed from this graph as well as from the high correlation coefficient (r = 0.92). For the next phase of our study, a level of 100 Enterobacteriaceae CFU/ml was selected to be our cutoff limit for classifying a new batch of raw milk samples into pass/fail groups. Using the regression equation, it was determined that 100 CFU/ml corresponded with a DT of 7.2 h (dotted line in Fig. 5). Therefore, samples which produced a DT of >7.2 h or no growth would be classified as pass samples, and those with a DT of ≤ 7.2 h would be classified as fail samples.

Results of the 50 additional raw milk samples analyzed by Enterobacteriaceae plate count and conductance methods are shown in Fig. 6. The cutoff line, which was determined in the previous phase of this study and shown in Fig. 5 is superimposed on this scattergraph. Using the 7.2 h cutoff limit, 34 of the 50 samples passed by the conductance method and by the plate count method, 11 samples failed by both methods, 5 samples passed by conductance and failed by the plate count limit, and no samples were found to fail the conductance and pass by the plate count limit. Overall, 90% of the results were in total agreement. The five data points that produced conflicting results are circled on the graph. Three of the points

![Figure 4. Conductance curves for three samples found to contain high levels of Yersinia enterocolitica.](image)

![Figure 5. Relationship between conductance DT and Log_{10} VRBG counts for naturally contaminated, temperature abused raw milk samples. Solid line is linear regression line. Correlation coefficient = 0.92.)](image)

![Figure 6. Relationship between conductance DT and Log_{10} VRBG counts for naturally contaminated raw milk samples.](image)
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are located very close to the cutoff line. Raw milk samples normally contain low levels of Enterobacteriaceae and this was observed during our analysis of raw milk. This observation resulted in a clustering of data points in the low plate count and high DT area. On the graph in Fig. 6 the lowest plate counts observed (10 or <10 CFU/ml) correspond with a large amount of variation on the DT axis (7.8 - 15 h). Some of the samples which produced these delayed DT’s may actually be stressed or non-Enterobacteriaceae microorganisms. This does not effect the accuracy of the results since the DT’s produced by these cultures which do produce a DT in the selective medium evaluated produce DT’s which are significantly delayed. Results of this study show that levels of Enterobacteriaceae can be automatically detected in raw milk with results obtainable within 6 - 12 h at levels of 500 - <10 CFU/ml, respectively.

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