IDEXX
Literature Cover Sheet

IDEXX #: 3D

Title: Ability of the Colilert Method to Recover Oxidant -Stressed E. *coli*

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Topic: Stressed E. *coli*

Highlights:
This study demonstrates the equivalency of Colilert to the reference EC+MUG method in its ability to recover low numbers (<4/100 ml) of stressed E. *coli*.

Test of differences between the two methods were analyzed by two - way analysis of variance and by Friedman's non parametric two way test. Both statistical methods demonstrated that there were no statistical evidence of difference between the two methods.

Subculture of fluorescent Colilert and EC+MUG tubes yielded E. *coli* from more than 97%.

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Ability of the Colilert method to recover oxidant-stressed *Escherichia coli*

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Methods for the microbiological analysis of drinking water must be able to detect *Escherichia coli* that may be injured by treatment. The Colilert method, which simulaneously detects total coliforms and *E. coli* in water samples by the observation of direct colour changes produced by defined substrates in the media, was found to be equivalent to the reference EC MUG method in its ability to recover low numbers (< 4/100 ml) of oxidant-stressed *E. coli*.

Escherichia coli is present at concentrations of 10^8–10^9/g faeces and is specific to the mammalian colon (Dufour 1976). Accordingly, *E. coli* has been utilized by public health and medical microbiologists as a specific indicator of faecal pollution of drinking water (Sarhan & Foster 1991). The methods for the detection of *E. coli* from drinking water involve several sequential steps, which often require several days and are not easily performed in the field (American Public Health Association 1989). The Colilert method simultaneously enumerates total coliforms and *E. coli* directly from a water sample and is based on the direct observation of colour production (Edberg *et al.* 1988). It involves the addition of a water sample to the Colilert powder, mixing to dissolve, incubation and observation of the colour end-points. Although the method was tested and found to be equivalent to *Standard Methods* multiple tube-fermentation and membrane filtration methods for total coliforms (Covert *et al.* 1989; Edberg *et al.* 1988, 1989, 1990) and accepted procedures for *E. coli* from drinking water (Katamay 1990; Kromoredjo & Fujioka 1991) and the environment (Rice *et al.* 1990, 1991), a question was raised in a limited study concerning its ability to detect oxidant-stressed *E. coli* (Clark *et al.* 1991). Evaluated in this study was the ability of the Colilert method to detect oxidant-stressed *E. coli*. This method was compared with the EC MUG procedure recently approved in the USA and in use in Europe for some time (Moberg 1985). A well-established oxidant injury protocol was employed (Berman & Hoff 1984; Berman *et al.* 1988). Specimens from patients with acute diarrhoea were chosen as the starting material because these represent the greatest challenge to the ability of the Colilert method to recover *E. coli*. Furthermore, stool entering drinking water from infected patients would represent the greatest health threat.

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Materials and Methods

SAMPLES

Faecal specimens from patients suspected of having infectious diarrhoea were cultured to recover bacterial pathogens (Balows *et al.* 1991). A portion of the specimen was refrigerated (4–8°C) for 24–48 h until a diagnosis was established. When a specific agent was identified the stool sample was utilized in the oxidant stress protocol.

OXIDANT STRESS PROTOCOL

In order to ensure oxidant stress while retaining a sufficient number of viable injured *E. coli* for comparative analysis the method of Berman and Hoff (1984) as modified for bacteria (Berman *et al.* 1988) was utilized.

OXIDANT STRESS

A 5 g stool sample was added to 1 l sterile water and mixed well with continuous magnetic stirring. To this mixture sodium hypochlorous acid was added to yield a final measurable chlorine level of approximately 5 mg/l as measured by the DPD method (American Public Health Association 1989). After 10 min at room temperature with vigorous stirring the activity of chlorine was halted with sodium thiosulphate. The number of *E. coli* viable after chlorination was determined by assaying viable colonies with the m-TEC method (Dufour *et al.* 1981). While awaiting the results of this analysis the sample was refrigerated at 4–8°C for 24 h. It was expected that this procedure would reduce viable *E. coli* by approximately 10^3–10^6 and that the surviving species would be oxidant-stressed.

RECOVERY OF *E. coli*

Based on the density of *E. coli* observed on m-TEC, the sample was diluted in sterile, laboratory grade water to yeild a density of between 2 and 10 cfu/100 ml. From this dilution two rows of 10 tubes each were inoculated with 10 ml sample into double-strength lauryl tryptose broth (LTB). In parallel, two rows of 10 tubes each containing Colilert powder received 10 ml sample from the same vessel. The vessel contents were continuously mixed with a magnetic stirrer.

At 48 h the LTB tubes were examined for the production of gas. Aliquots from all gas-positive tubes were transferred to EC MUG at 44·5°C. At 24 h the tubes were examined for fluorescence at 366 nm, which indicates the presence of *E. coli*. To confirm the specificity of the observed fluorescence-positive EC MUG tubes, these were subcultured onto MacConkey's agar. Lactose-fermenting colonies were identified to species using the Sensititre microdilution system (Radiometer, Denmark). The number of *E. coli* recovered from each row of 10 tubes was expressed as a MPN value (American Public Health Association 1989).

The Colilert analyses were interpreted as follows: after 24 h incubaton at 35°C the tubes were examined for the presence of a yellow colour (total coliform-positive). All tubes showing this colour were exposed to long-wave u.v. light (366 nm). Fluorescence indicated the presence of *E. coli*. Fluorescent Colilert tubes were then processed as described for EC MUG tubes to establish the presence of *E. coli*. The number of *E. coli* recovered from each row of 10 tubes was expressed as a MPN value (American Public Health Association 1989).

COMPARISON OF THE METHODS

For each row of 10 tubes a MPN value was used for comparison. Each sample contained two duplicate 10 tube analyses. The data were analysed by three statistical means: tests of differences between methods (analysis of variance and Friedman's non-parametric two-way); correlation (Pearson and Spearman); and two-way analysis of variance (Fleiss 1981).

Results and Discussion

The comparison between *E. coli* recovery by the EC MUG and the Colilert methods is shown in Table 1. The oxidant stress protocol yielded *E. coli* concentrations generally in the target range of 2–10/100 ml although, as would be expected at such low densities of heterogeneously dispersed particles, there was variation. However, *E. coli* densities fell in a range between 1·1 and 23·0/100 ml, which were suitable for statistical comparisons in all cases except two. More than 90% of the values fell within 95% confidence limits of variation, indicating that the experimental conditions were under control.

Tests of differences between the two methods were analysed by two-way analysis of variance and by Friedman's non-parametric two-way test. The analysis of variance is used when comparing new procedures while the Friedman test makes distributional assumptions concerning the within-sample rank of the data, and is considered to be more robust. Both statistical methods demonstrated that there was no statistical evidence of differences between the two methods. The F statistic for the two-way

Table 1. Comparison of recoveries of oxidant-stressed *Escherichia coli* by the Colilert and EC MUG methods

Sample no.	Disease	MPN EC MUG row 1/row 2*	MPN Colilert row 1/row 2*
1	Viral	1·1/ 5·1	2·2 1·1
2	Shigella	9·2/ 3·6	6·9 6·9
3	Viral	5·1/ 1·1	6·9 5·1
4	Viral	12·0/ 5·1	3·6 6·9
5	Shigella	< 1·1/ 2·2	1·1/ 2·2
6	Viral	3·6/ 12·0	5·1/ 9·2
7	Viral	1·1/ 3·6	2·2/ 3·6
8	Viral	2·2/ 3·6	1·1/ 1·1
9	*E. coli*, toxigenic	5·1/ 12·0	3·6/16·1
10	Viral	12·0/ > 23·0	9·2/12·0

* A total of two rows, or duplicates, of 10 ml in 10 tubes per row was inoculated from each diluted sample. The MPN numbers represent the *E. coli* density from the first row of 10 tubes/*E. coli* density from the second row of 10 tubes.

analysis of variance was $F = 0{\cdot}98$ and had a corresponding P value of 0·35. The Friedman statistic was $F = 0{\cdot}52$ and $P = 0{\cdot}48$. Correlation coefficients were determined by the Spearman and Pearson tests. The correlation coefficients were calulated by matching data by replicate (i.e. sample 2 by EC MUG repetition 2 was matched with sample 2, Colilert repetition 2). The Pearson correlation was 0·73 and Spearman correlation 0·71. These correlations are in the moderately high range, with 0·75 or higher generally considered high (Fleiss 1981).

Subculture of fluorescent EC MUG and Colilert tubes yielded *E. coli* from more than 97%. Both EC MUG and Colilert fluorescent tubes contained enteric bacteria other than *E. coli*. It is likely that in the small number of cases in which *E. coli* was not identified it was present in a mixed culture with other enteric bacteria and was not retrieved. Furthermore, there are approximately 5% of misidentifications at the species level of environmental Enterobacteriaceae (e.g. naming *E. coli* as *E. hermanii* or vica versa) (D'Amato *et al.* 1981). Therefore, fluorescence by either EC MUG or Colilert was specific for *E. coli* by the two methods.

The Colilert method offers public health and medical microbiologists a tool to analyse water for the two bacterial indicators—total coliforms and *E. coli*—in an extremely simple format. It is only necessary to add water to the powder, incubate for a maximum of 24 h and observe for colour. The method has been approved by the United States Environmental Protection Agency for total coliforms, and approval for *E. coli* is pending. The contamination of drinking water with faecal material is a major threat to public health. Of most danger would be faecal material from patients suffering from infectious diarrhoea entering the system. The recovery of *E. coli* from the latter would be the greatest challenge to any analytical system. Accordingly, the ability of the Colilert method to recover oxidant-stressed *E. coli* was demonstrated in this study. This method was equivalent to the reference EC MUG method and both detected injured *E. coli* in numbers as low as 1·1/100 ml. The Colilert method offers an accurate choice for the microbiological analysis of total coliforms and *E. coli* from drinking water.

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