Evaluation of the ß-Glucuronidase Test with the multiple tube technique for specific determination of *Escherichia coli*

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The use of *Escherichia coli* as an indicator of the sanitary quality of water and seafood products has been classically recommended by different official organizations (APHA, 1985; Greenberg & Hunt, 1985; WHO, 1977), since this microorganism provides a measure of fecal contamination. According to the APHA (1985) two methods are currently available for enumerating *E. coli* from those samples: the Most Probable Number (MPN) procedure and the membrane filtration (MF) technique. Both present advantages and disadvantages, but the former requires the need of presumptive and confirmatory tests, which are costly, time-consuming and arduous.

Recent reports of fluorogenic assay procedures have shown the promise for a rapid and specific detection of *E. coli* in seawater (Mates, 1987; Gauthier *et al.*, 1988), seafood (Rippey *et al.*, 1987) and foods (Robinson, 1984). This new method relies on the almost exclusively presence of the enzyme ß-glucuronidase in *E. coli*. This enzyme cleaves a fluorogenic substrate, 4-methylumbeliferyl-ß-D-glucuronide (MUG) to yield an end product, 4-methylumbeliferone, which is fluorescent under UV light.

In the present study, a comparative study of MPN technique using MacConkey purple supplemented with MUG as presumptive broth and nutrient agar with MUG as confirmatory test from seawater, sediment and shellfish samples was carried out.

All the strains isolated with positive fluorescence in nutrient agar-MUG from the gas-positive and fluorescence-positive tubes were identified as *E. coli*. On the other hand, the isolates positive in gas and fluorescence from the tubes and MUG-negatives on nutrient agar were identified as: *Klebsiella pneumoniae* (66.7%), *E. coli* (16.7%) and *Enterobacter agglomerans* (16.7%) from seawater samples; *K. pneumoniae* (100%) from sediment samples; and *K. pneumoniae* (33.3%), *E. coli* (33.3%), *E. cloacae* (14.8%), *E. sakazakii* (4.8%), *Citrobacter freundii* (4.8%), *Serratia liquefaciens* (4.8%), and *Buttiaux agrestis* (4.8%) from shellfish samples. The high frequency of *E. coli* MUG-negative detected in these samples from tubes presumptively positive may be explained by the negative effect exerted by the selective agents contained in the MacConkey-purple broth, which could affect the permease of the ß-glucuronidase activity, or may be a competence effect exerted by the background flora present in the shellfish, or a self-activity of ß-glucuronidase by the shellfish tissues (Koburger & Miller, 1985; Holt *et al.*, 1989).

A high proportion of the strains MUG-negative on nutrient agar isolated from the gas-positive tubes were identified as *K. pneumoniae*. This result agrees with those reported by Rippey *et al.* (1987) and Damoglou *et al.* (1988), being considered the mainly false-positive species in the coliform analysis.

In Table 1 the characteristics of MPN tubes in which *E. coli* was isolated are shown. *E. coli* was verified in about 90% of fluorescent tubes. Only one *E. coli* strain as isolated from fluorescence-negative and gas-positive tubes, and MUG-positive on nutrient agar. For this reason, this methodology is considered to increase the accuracy of the MPN technique to isolate *E. coli* from samples of different sources.

TABLE 1.- Percentages of *Escherichia coli* isolated from MPN tubes (MacConkey-purple broth).

SAMPLE	MUG Tubes +	MUG Tubes -	Gas Tubes +	Gas Tubes -	mFC Colony Typical	mFC Colony NonTypical	AN-MUG +	AN-MUG -
Seawater	91	17	64.7	ND	76.5	20	100	14.3
Sediment	100	17	78.6	0	93.8	0	100	22.6
Shellfish	84	0	70.8	0	71.4	21	100	0
Total	90	12	70.9	0	78.7	14.6	100	15,5

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