THE MUG TEST FOR A RAPID EVALUATION OF *ESCHERICHIA COLI* IN SEAWATERS

ZACCONE R., MONTICELLI L., CARUSO G.

Istituto Sperimentale Talassografico CNR, Sp. S. Raineri, 86-98122 Messina, Italia

Monitoring seawater pollution requires prompt and reliable answers, enabling researchers to carry out prompt and adequate measures. When compared with the enumeration methods of fecal and total coliforms, researches focusing on the use of *E. coli* as indicator cells result in a more accurate indicator-pathogen ratio (FREIER and HARTMAN, 1987; ZACCONE *et al.*, in press). Some media containing fluorogenic substrates have recently been used for the direct identification of *E. coli* as well as pathogenic bacteria, *Shigella, Salmonella.*

The 4-methylumbelliferyl-β-D glucuronide (MUG) is a fluorogenic substrate, specific for *E. coli* : it possesses the enzyme β glucuronidase, which is involved in the lactose metabolism. For a rapid determination of *E. coli*, we have studied the accuracy and sensitivity of a medium containing MUG substrate and compared it to the m-FC medium. Seawater samples have been collected from several coastal areas of Sicily, filtered through 0.45µm Nuclepore membranes, and placed on the following media : m-FC Broth (Difco) + 1,2% Agar (Difco) without rosolic acid, incubated for 24 hours at 44.5°C (STANDARD METHODS, 1992); EDC Agar MUG (Biolife), incubated for 18 hours at 37°C and 44.5°C. The suggested method provided for a pre-incubation on Tryptic Soy Agar at 37°C for 4 hours. We avoided this step in order to study the possible application of such method during coastal survey in a ship laboratory. The presumptive target colonies classified as presumed *E. coli* were identified by the API 20E system. The data have been processed to obtain accuracy coefficient (A.C. = n. of confirmed *E. coli* target colonies/presumptive target colonies) and selectivity coefficient (S.C. = presumptive target colonies/ presumptive target colonies + presumptive non target colonies) (Fig.1) and an analysis of media performance has been carried out (Tab. 2) (SANTIAGO-MERCADO and HAZEN, 1987; PAGEL *et al.*, 1982).

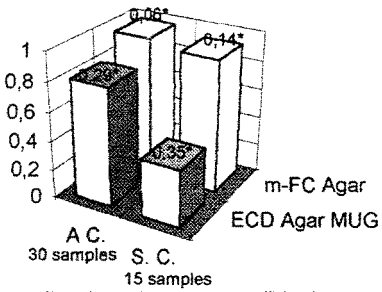

Fig 1: Comparison of recovery efficiencies.
* Standard deviation

	ECD Agar n	%	m-FC Agar n	%
E. coli	166	84,69	69	94,52
K. rhinoscleromatis	5	2,55		
K. oxytoca	1	0,51		
C. freundii	1	0,51	1	1,36
P. vulgaris	1	0,51		
Vibrio spp.	7	3,57		
Pseudomonas spp.	3	1,53	2	2,73
Gram -, ox -, non identified bacteria	2	1,02		
Yellow pigment	10	5,10	1	1,36
Total	196	99,99	73	99,97

Tab.1. Identification of presumptive target colonies grown on ECD Agar MUG and m-FC Agar

The m-FC Agar medium presents a higher A.C. when compared with the ECD Agar MUG; this is due to the higher percentage of confirmed *E. coli* (lower number of false-positive colonies) in the presumptive target colonies isolated from both media (Fig.1, Tab.1). The growth of non target background organisms was higher for ECD Agar MUG than m-FC; this is mainly due to the different composition of media as well as to the different qualitative composition of sample microflora. This is also shown by C.S. values (Fig.1). The m-FC Agar presents a higher selectivity towards the other bacterial species.

Tab. 2 shows the effect of both incubation temperatures on the samples inoculated on ECD Agar MUG. When a temperature of 44.5°C was utilized, the bacterial counts decreased (only 7% of samples has higher recovery on ECD Agar MUG). Incubation temperature of 37°C allows the recovery of a higher amount of presumptive target colonies (58% of samples). The possibility of *E. coli* direct enumeration at 37°C may support the recovery of stressed cells, avoiding the introduction of a resuscitation procedure. However, the higher performance of ECD Agar is inconsistent with a lower accuracy and selectivity. The existence of a direct proportionality between two media was proved by calculating the correlation coefficient (Tab.2).

For preliminary environmental surveys of *E. coli* the use of ECD Agar incubated at 37°C may be suggested.

Media (temperature of incubation)	n	%	Performance
ECD Agar(44,5°C)/m-FC Agar (44,5°C)	21	75	mFC Agar(100)>ECD Agar(37,02)* (4,32)**
	2	7	ECD Agar(100)>mFC Agar(40,00)* (4,47)**
	5	18	m-FC Agar = ECD Agar
r=0,97 P<0,01			
ECD Agar(37°C)/ m-FC Agar (44,5°C)	11	58	ECD Agar(100)>mFC Agar(48,15)* (4,42)**
	3	16	mFC Agar(100)>ECD Agar(66,76)* (0,07)**
	5	26	m-FC Agar = ECD Agar
r=0,91 P<0,01			

Tab. 2 : Growth of presumptive target organisms (performance of media)
* percentage mean ** standard error

REFERENCES

FREIER T.A. and HARTMAN P.A.,1987. Improved membrane filtration media for enumeration of total coliforms and *Escherichia coli* from sewage and surface waters. *Appl. Environ. Microbiol.*, 53 : 1246-1250.

PAGEL J.E., QURESHI A.A., YOUNG D.M., VLASSOFF T.L., 1982. Comparison of four membrane filter methods for fecal coliform enumeration. *Appl. Environ. Microbiol.*, 43 : 787-793.

SANTIAGO-MERCADO J. and HAZEN T.C., 1987. Comparison of four membrane filter methods for fecal coliforms enumeration in tropical waters. *Appl. Environ. Microbiol.*, 53 : 2922-2928.

STANDARD METHODS FOR EXAMINATION OF WATER AND WASTEWATER, 1992. 18th. edition, APHA Washington.

ZACCONE R., CRISAFI E., CARUSO G., in press. Evaluation of fecal pollution in coastal waters by immunofluorescence. *Mar. Microb. Food Webs.*