A COMPARISON OF TWO METHODS FOR QUANTITATIVE DETECTION AND ENUMERATION OF *ESCHERICHIA COLI* FROM BIOLOGICAL WASTE

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Material and methods

A total of 120 naturally contaminated samples of sludge, biowastes, food waste, and liquid manure were detected with the following two methods:

-Micromethod (MPN-technique) by inoculation in liquid medium

-Macromethod (MPN-technique) in liquid medium

The presence of *Escherichia coli* in micromethod requires the following stages:

a) Sample preparation: suspension of biological waste in tryptone salt diluent
b) Inoculation of the diluted sample in a row of microtitre plate wells containing dehydrated culture medium
c) Examination of the microtitre plates under ultraviolet light at 366 nm in the dark after an incubation period of 36 h minimum and 72 h maximum at 44°C ± 1°C. The presence of *Escherichia coli* is indicated by a blue fluorescence resulting from hydrolysis of MUG (4-methylumbelliferyl-ß-D-glucuronide). The *Escherichia coli* count corresponding to the number of fluorescent wells is provided by a statistical analysis based on Poisson's law.

The macromethod is based on that described on Schindler (1991) and requires following stages:

a) Suspension of the sample in 0,9 % m/V sodium chloride
b) Serial dilutions of this suspension in the same diluent
c) Transfer of 1 ml of these diluted suspensions into 3 tubes containing 9 ml MUG fluorocult lauryl sulfate broth
d) Incubation at 44 °C ± 1 °C for 40 h ± 4 h
e) The presence of *Escherichia coli* in the macromethod is demonstrated by detection of gas production, beta glucuronidase activity and Indol production in the tubes.

Results and conclusion

A summary of the complete results is in the following table 1.

Tab. 1: Recapitulatory results of the comparative investigations to the detection of *Escherichia coli* from four different substrates with two different MUG fluorescence methods

Origin of the examined samples	Calculated values- in CFU/ml	Macromethod	Micromethod
Biowastes (n=30)	Minimum	9.2×10^0	3.7×10^1
	Maximum	2.3×10^6	5.6×10^5
	Median	4.3×10^4	3.3×10^3
Sludge (n=30)	Minimum	2.3×10^2	2.0×10^2
	Maximum	2.3×10^4	2.1×10^3
	Median	1.5×10^3	5.2×10^2
Liquid manure (n=30)	Minimum	2.3×10^4	1.2×10^4
	Maximum	1.5×10^6	5.8×10^4
	Median	4.3×10^4	3.4×10^4
Food waste (n=30)	Minimum	1.5×10^3	8.0×10^2
	Maximum	4.3×10^4	1.6×10^4
	Median	2.2×10^4	2.3×10^4

n: Number of the examined samples
CFU/ml: Colony forming units

For the following reasons the macromethod has showed the most advantages of all the present method:

- The results of the macromethod and micromethod which were calculated in two different statistic table show in all analysed substrates (except for the liquid manure) that the median values of the macromethod are in average between a half and one power of higher than those of micromethod (see illustration 1). In the 30 analysed liquid manure samples the median values were in the same range of 10^4 CFU/ml.

The highest gained values of the macromethod were also one power of ten higher than the results obtained with the micromethod. The results of the analysed food wastes make an exception: the calculated number of *Escherichia coli* (10^4 CFU/ml) was the same in both methods. There is no big difference of minimum values of the analysed substrates in both tested methods. The lowest concentration was within the range of 10^1 CFU/ml.

- A second advantage of the macromethod beside the micromethod consist in two biochemical characteristic which cannot be confirmed through the miniature procedure: lactose splitting at 44°C and indol formation. In addition, all “gas-positive” samples were tested on fluorescence

and indol formation. In this point of view the *Escherichia coli* can be differentiated from the other coliform bacteria through a fluorescence optical procedure under a temperature of 44°C, but on that occasion it makes sense to include the indol formation in the detection procedure.

In the following illustration 1 all the obtained median values of the analysed substrates are graphically represented.

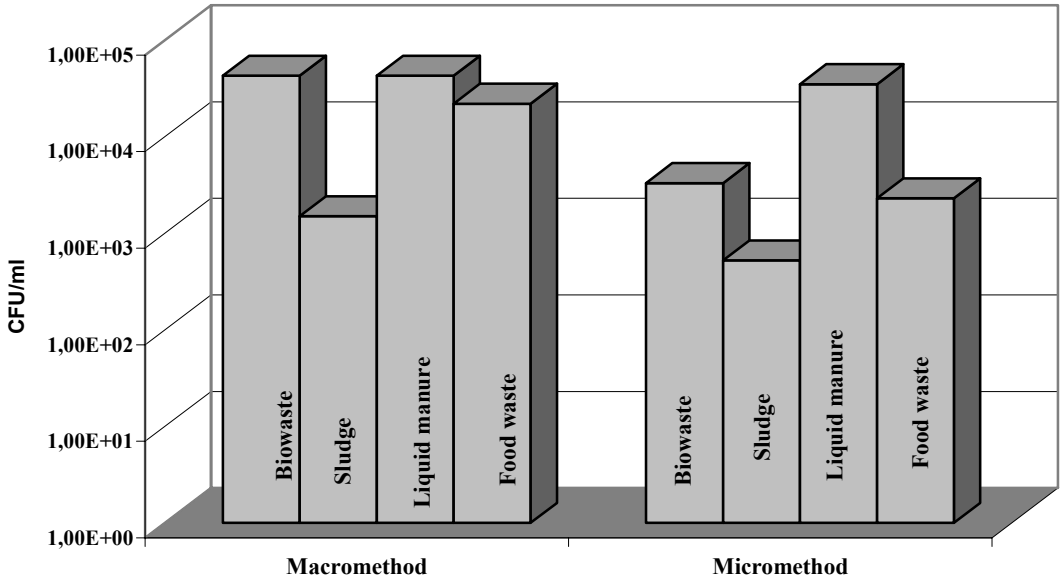

Illustration 1: Detection of *Escherichia coli* in different substrates with two MUG-fluorescence methods.

According to our results, the macromethod was found to give a higher number of *Escherichia coli* positive samples. The ability to detect *Escherichia coli* with the macromethod, and the amount of labor required for each analysis is reduced compared to the micromethod. In contrast to the macromethod the micromethod gives different results, whereby this difference was not big and does not contradict the use of this method. In conclusion, the macromethod can be recommended to detect and enumerate *Escherichia coli* from biological waste samples.

References.

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3. *Anonym (2004): Detection and enumeration of Escherichia coli in sludges, soils, soil improves, growing media and biowastes. Part 3: Macromethod (Most Probable Number) in liquid medium*