



General guidance for enumeration of presumptive *Escherichia coli*: Most probable number technique - AS 5013.15-2006

This standard is identical with ISO 7251:2005, Microbiology - General guidance for enumeration of presumptive *Escherichia coli* – Most probable number technique.

SCOPE

Products intended for human consumption or feeding of animals (including raw meats and carcase swabs or rinses) and food environmental samples.

PRINCIPLES

Enumeration of *E. coli* is divided into the following steps:

- **Sample preparation and inoculation**
For non-liquid samples dilute 1:10 using peptone salt solution or buffered peptone water or other diluent as specified for the method being used. Carcass sponges can be enumerated without further dilution. Liquid samples can be enumerated without dilution or by using appropriate decimal dilutions of the original sample. In all cases the specified method for the sample being analysed is followed. An MPN series is prepared by adding a specified amount of sample and decimal dilutions of sample to tubes of Lauryl sulfate tryptose broth¹ (selective enrichment medium) containing Durham tubes. Double strength LS broth should be used to prepare the initial MPN dilution.
- **Incubation**
Lauryl sulfate tryptose broth is incubated at $37 \pm 1^\circ\text{C}$ for 24 ± 2 h. Tubes without gas production are incubated for up to 48 ± 2 h.
- **Presumptive *E. coli***
Sample from tubes with gas production is inoculated into liquid selective media (EC broth) containing Durham tubes. EC broth is incubated at $44 \pm 1^\circ\text{C}$ for 24 ± 2 h. Tubes without gas production are incubated for 48 ± 2 h. Tubes with gas are inoculated into peptone water² preheated to 44°C and incubated at $44 \pm 1^\circ\text{C}$ for 48 ± 2 h. All tubes are examined for indole production.
- **Calculation of MPN**
The MPN is determined from the number of tubes that gave rise to gas formation and in which indole was produced. Either MPN tables or computer programs can be used to estimate the number of *E. coli* per g or mL of initial sample³.

¹ Single strength lauryl sulfate tryptose broth consists of enzymatic digest of plant and animal tissue (20g); lactose (5g); K_2HPO_4 (2.75g); KH_2PO_4 (2.75g); sodium chloride (5g); sodium lauryl sulphate (0.1g); water (1000ml).

² Enzymatic digest of casein (10g); sodium chloride (5g); water (1000ml)

³ The MPN count is dependent on the amount of sample initially added ie if 0.1g is added to the first set of tubes then the count obtained from the MPN tables needs to be multiplied by 10 to give the MPN per g. The count obtained is for presumptive *E. coli*.

CHECKLIST

Sample preparation & inoculation	Is the amount of sample added to the initial MPN tubes appropriate for the sample being analysed?	_____
	Is 10 ml of sample/initial dilution used to inoculate double strength lauryl sulfate tryptose broth?	_____
	Is a minimum of 1 ml (of appropriate dilution) used to inoculate all subsequent tubes of single strength lauryl sulfate tryptose broth?	_____
Incubation	Is lauryl sulfate tryptose broth incubated at the specified temperature (i.e. 37°C)?	_____
	Is gas production recorded properly (i.e. tubes tapped to overcome super-saturation of gas)?	_____
	Are tubes examined for gas production at 24 and if necessary 48 h?	_____
Presumptive <i>E. coli</i>	Is EC broth incubated at 44°C?	_____
	Is gas production recorded properly (i.e. tubes tapped to overcome super-saturation of gas)?	_____
	Are tubes examined at 24 and if necessary 48 h?	_____
	Is indole production carried out at 44°C in peptone water?	_____
Calculation of MPN	Is the MPN calculated taking into account the initial dilution of the sample?	_____
	Are improbable MPN values excluded or investigated?	_____
	Is the count reported as CFU/cm ² for swab and surface samples?	_____