



TEMPO EC Method for automated enumeration of *Escherichia coli* in food – AOAC 2009.02

SCOPE

This method is applicable for enumeration of *E. coli* in meat and meat products and carcass swabs.

PRINCIPLES

TEMPO EC (*Escherichia coli*) is an automated test for the enumeration of *E. coli* in 24 hours in food products. The TEMPO EC test consists of a vial of culture medium and a 'card', which are specific to this test. *E. coli* if present reduce the substrate in the culture medium resulting in a fluorescent signal, which is detected by the TEMPO Reader. The TEMPO system estimates the number of *E. coli* present in the original sample according to a calculation based on the Most Probable Number (MPN) method.

The enumeration of *E. coli* is broken down into stages as follows:

- **Sample preparation**
Samples are diluted 1:10 in Butterfield's buffered peptone water and are homogenize in the TEMPO bag. For carcass swabs samples can be taken directly from the swab without further dilution. TEMPO Automated System is used to reconstitute culture media (using sterile distilled water). Media and sample mixture (1 mL filtered sample in 3 mL media) are transferred into the TEMPO card (consult the manufacturer's instructions).
- **Incubation**
TEMPO card with media is incubated at $35 \pm 1^\circ\text{C}$ for 22 – 27 hours.
- **Interpretation**
Reading is performed by the TEMPO system by counting the number of positive wells obtained in relation to the volume of the wells and the dilution of the sample. Results are expressed in CFU per g, mL or cm² of original sample, using MPN tables.

CHECKLIST

Sample Preparation	Is Butterfield's buffered peptone water used for preparation of samples and dilutions?	_____
	Is sterile distilled water used for reconstitution of the TEMPO media?	_____
	Is TEMPO media stored at 2–25°C in a dark place?	_____
	Is the dilution used capable of detecting <i>E. coli</i> at the level required?	_____
	Is a positive control run with each batch of samples analysed (or daily)?	_____
Incubation	Are TEMPO cards incubated for 22-27 h at 35 ± 1°C?	_____
Interpretation	Are the manufacturer's instructions available and are they reproduced in the laboratory manual?	_____
	Are technicians familiar with the operation of TEMPO Automated System?	_____
	Is the count correctly reported ie CFU/cm ² for swab and surface samples and CFU/g for meat samples?	_____