Australian Government
Department of Agriculture

SimPlate Colour Indicator: Detection and Quantitation of Coliforms and *E. coli* in Foods- AOAC 2005.03

SCOPE

This method is applicable for the detection and quantification of confirmed total coliforms and *E. coli* in poultry meats, red meats, sea foods, dairy foods, egg products, processed meats and all other foods. The method is applicable for use with carcase swabs.

PRINCIPLES

When grow in CEc-CI medium coliform bacteria produce a colour changes due to acid production while *E. coli* produce a blue fluorescent colour due to the reaction of β-glucuronidase with reagent in the media. Thus, coloured wells without fluorescence under UV light indicate the presence of coliforms, while coloured wells with fluorescence indicate the presence of *E. coli*. The total coliforms and *E. coli* counts are determined by using the SimPlate Conversion Table (see manufacturer's instructions).

The detection and enumeration of coliforms/*E coli* can be broken down into the following stages:

- **Sample preparation**
  Samples are diluted 1:10 in appropriate diluent ie (Butterfield's phosphate buffer, buffered peptone water or peptone salt solution). If necessary, prepare 10-fold serial dilutions appropriate for the anticipated population in the product. Add 1 mL or 0.1 mL of appropriate dilutions to 9.0 or 9.9 mL of pre-prepared CEc-CI medium (medium is prepared by resuspending powdered medium with sterile deionized water) either directly or in the SimPlate. Media and sample are gently mixed in the SimPlate device and excess removed as per the manufacture's instructions. For raw meat, raw seafood and raw produce 0.1 mL of supplement R must be added to the hydrated medium. For carcass swabs 1 mL of the initial suspension (ie 25 mL) can be used without further dilution. Note the starting colour of the wells.

- **Incubation**
  Incubate the SimPlate device with medium at 35-37 ^{o}C for 24-28 h in the dark. Do not invert the device.

- **Interpretation**
  Coloured wells indicate the presence of coliforms. Wells that show fluorescence under UV (366 nm) light are interpreted as being positive for E. coli. The count is estimated[1] using the SimPlate Conversion Table.

[1] To calculate the number of microorganisms per g, mL or cm^2 of sample, multiply the count (using the conversion table supplied) by the appropriate dilution factor.

CHECKLIST

Sample Preparation	Is an appropriate diluent used for preparation of samples and serial dilutions?	
	Is the expiry date of opened SimPlate packs controlled?	
	Are SimPlate devices stored at 2–30°C in a dark place?	
	Is reconstituted medium used within 12 h of preparation?	
	Is supplement R added for meat swabs and raw meat samples?	
	Is the dilution used appropriate for the expected population of the sample being analysed?	
	How is excess sample removed from the SimPlate?	
Incubation	Is SimPlate device incubated at 36 ± 1 °C for 26 ±2 h?	
Interpretation	Are the manufacturer's instructions available and are they summarised in the laboratory manual?	
	Are technicians familiar with the detection of blue fluorescence under UV light?	
	Is 366 nm wavelength UV used to detect blue fluorescence colour?	
	Which wells correspond to the presence of coliforms?	
	Which wells correspond to the presence of *E. coli*?	
	Is a conversion table used to determine the total number of organisms?	
	Are detailed examples provided for determining the count in samples?	