Neogen® Petrifilm Rapid *E. coli*/Coliform Count Plate - AOAC 2018.13

SCOPE

These methods are applicable to testing of raw meat, carcase swabs and other foods.

Note that Neogen Petrifilm is not supplied by a NATA or ISO 17025 certified media supplier and therefore new batches of media must undergo quality control prior to use. A checklist for Neogen Petrifilm QC is provided for guidance.

PRINCIPLES

The Neogen® Petrifilm Rapid *E. coli*/Coliform Count Plate[1] is a sample-ready culture medium that contains 5-bromo-4-chloro-3-indolyl-D-glucuronide, an indicator of glucuronidase activity (forms blue precipitate)[2] and tetrazolium indicator that facilitates colony enumeration. Plates are hydrated with sample and gelling agents cause the media to solidify. Gas is formed as a result of the fermentation of lactose by coliform bacteria (including *E. coli*). Glucuronidase negative bacteria form red colonies as a result of the reduction of tetrazolium chloride.

The enumeration is broken down into stages as follows:

- **Inoculation**
  Sample is diluted, as specified in the relevant standards or methods, in Butterfield's Phosphate Buffer Diluent[3] or Peptone Salt Solution[4] (diluents containing citrate, bisulfate, or thiosulfate must not be used) and 1 mL plated onto Neogen Petrifilm. Plates are incubated in stacks (maximum of 20 units per stack). Carcase sponges should be hydrated with 25 mL of diluent and can be enumerated without further dilution. Serial dilution must be prepared using Butterfield's Phosphate Buffer Diluent or Peptone Salt Solution.
- **Incubation**
  Petrifilm plates are incubated at 36 ± 2°C for *E. coli* and coliforms; and 42 ± 1°C for *E. coli* for 18 - 24 h.
- **Interpretation**
  All blue to blue-green colonies with or without gas irrespective of size or intensity of colour are counted as *E. coli*. Red colonies with gas are non-*E coli* coliforms. The total coliform count is the sum of red with gas and blue colonies with or without gas. Plate counts containing more than 100 CFU are to be estimated. When the number of colonies is too numerous to count, repeat the test with higher dilutions.

  For swab samples, counts should be expressed as CFU/cm^2.

  For meat and meat products, counts should be expressed as CFU/g.

[1] Neogen Food Safety – CAT# 6436/6437

[2] Most *E. coli* O157:H7 are glucuronidase negative and will not form blue colonies on *E. coli* Petrifilm.

[3] 0.0425g/L KH_2PO_4 adjusted to pH 7.2

[4] Peptone 1 g, sodium chloride 8.5 g and water 1 L. Autoclave at 121 ±1°C for 15 min, pH after sterilization 6.9 ± 0.2, store in the dark at 0-5°C for one month.

CHECKLIST

Inoculation	Are Neogen Rapid Petrifilm plates warmed to room temperature before use?	_____
	Are the correct diluents used for preparation of samples and dilutions?	_____
	Is a positive control run with each batch of samples analysed?	_____
Incubation	What is the expiry date of opened packs?	_____
	How is the expiration date of opened packs of Petrifilm controlled?	_____
	How are open packs stored?	_____
	What are the incubation conditions and period?	_____
	Are Petrifilm incubated in stacks of <20?	_____
	What is the maximum number of colonies counted on Neogen Rapid Petrifilm plates?	_____
	How are counts outside the countable range reported?	_____
Interpretation	What colonies are identified and counted as *E. coli*?	_____
	What colonies are identified and counted as coliforms?	_____
	Is the count reported as CFU/cm^2 for swabs and surface samples?	_____

PETRIFILM QC CHECKLIST

	Is media QC carried out on all new batches of Neogen *E. coli*/Coliforms Rapid Petrifilm?	_____
	Are new batches clearly identified and held in quarantine until QC results are known?	_____
	Are morphology checks for positive and negative controls recorded for new batches of Petrifilm?	_____
	Is recovery of *E. coli* on new batches of Neogen Rapid Petrifilm compared to that on non-selective agar?	_____
	Is an appropriate performance standard used to pass new batches of Petrifilm, i.e. 50%?	_____