E. coli Petrifilm - AOAC 991.14 and AOAC 998.08

AOAC 991.14 and 998.08 differ only in the time of incubation at 35°C. AOAC 998.08 is a validation study for incubation of Petrifilm at 35°C for 24 ± 1 h and applies only to raw meats, poultry and seafood (including carcass sponge samples). Other foods have not been validated and plates must be incubated as per AOAC 991.14, i.e. 35° ± 1°C for 48 ± 4 h. 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.

SCOPE

These methods are applicable to:

AOAC 991.14 - all foods

AOAC 998.08 - poultry, seafood, raw and cooked meat

PRINCIPLES

Neogen® Petrifilm (modified violet-red bile media) contains 2,3,5-triphenyltetrazolium chloride and glucuronidase indicator which forms a blue precipitate around any *E. coli* colonies that may be present[1]. 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 2,3,5-triphenyltetrazolium chloride.

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

- **Inoculation**
  Sample is diluted, as specified in the relevant standards or methods, in Butterfield's buffered phosphate diluent[2], Buffered Peptone Water[3] or 0.1% Peptone Salt Solution[4] and one mL plated onto 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 buffered phosphate diluent or Buffered Peptone Water.

- **Incubation**
  E. coli Neogen Petrifilm plates are incubated at 35°C for 24 ± 1 h (AOAC 998.08, meat, poultry and seafood samples) or 48 ± 4 h (AOAC 991.14, all other food samples; count coliforms after 24 ± 2 h and incubate additional 24 ± 2 h for *E. coli* count). Count all colonies in the countable range (1-150). Estimate count on plates with >150 colonies. If an estimation is not possible due to overgrowth, repeat the test with a higher dilution.

- **Interpretation**
  All blue colonies associated with gas are counted as *E. coli*. Red colonies with gas are non-*E. coli* coliforms. The total coliform count is the sum of red and blue colonies (with gas).

[1] Most *E. coli* O157:H7 are glucuronidase negative and will not form blue colonies on *E. coli* Petrifilm.
[2] 0.0425g/L KH_2PO_4 adjusted to pH 7.2
[3] Enzymatic digest of casein (10g); NaCl (5g); $Na_2HPO_4.12H_2O$ (9g); KH_2PO_4 (1.5g); water 1,000 ml, as per AS 5013.10
[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 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 Petrifilm plates (150)?	
	Are colony counts above 150 estimated?	
	Is a higher dilution used when count is too numerous to count?	
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 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 Petrifilm compare to that on non-selective agar?	
	Is an appropriate performance standard used to pass new batches of Petrifilm, i.e. 50%?	