Aerobic Plate Count in Foods (Neogen® Petrifilm Method) - AOAC 990.12

SCOPE

All 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.

PRINCIPLES

Aerobic Plate Count (APC)[1] Neogen® Petrifilm contains nutrients and 2,3,5-triphenyltetrazolium chloride as an indicator of bacterial growth. Reduction of triphenyltetrazolium by bacteria results in red coloured colonies[2]. Plates are hydrated with sample and gelling agents cause the media to solidify.

The enumeration of APC is broken down into stages as follows:

- **Inoculation**
  Generally, samples are diluted 1:10 in Butterfield's buffered phosphate diluent[3] or Buffered Peptone Water[4] (or other diluent as recommended by the manufacturer) and one-ml plated onto Petrifilm. Plates are incubated in stacks (maximum of 20 units per stack). Carcass sponges should be hydrated with 25 ml of diluent. Serial dilutions must be prepared using appropriate diluent.
- **Incubation**
  APC Neogen® Petrifilm plates are incubated at 35 ± 1°C for 48 ± 3 h. Count all colonies in the countable range (1-300). Estimate count on plates with >300 colonies. If an estimation is not possible due to overgrowth, repeat the test with a higher dilution. For carcass sponge/swabs analyse the neat sample if applicable to get a countable range.
- **Interpretation**
  All red colonies are counted. For swab samples counts should be expressed in CFU/cm^2.

[1] Sometimes refer to as TVC (Total Viable Counts)

[2] Not all bacteria are able to reduce 2,3,5-triphenyltetrazolium in 48 h. This can be a particular problem in dairy samples. Neogen Petrifilm is generally considered to provide a good estimate of the number of bacteria present in meat sample.

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

[4] 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

CHECKLIST

Inoculation	Is the diluent used recommended by the manufacturer?	
	Are appropriate dilutions used to ensure a counting range?	
	What is the maximum number of colonies counted on Petrifilm plates (300)?	
	Are colony counts above 300 estimated?	
	Is a higher dilution used when count is too numerous to count?	
Incubation	How are open packs stored?	
	What is the shelf life of opened Petrifilm?	
	What are the incubation conditions and period?	
	Are Neogen Petrifilm stored in stacks of <20?	
Interpretation	What colonies are identified and counted?	
	How are counts outside the countable range reported?	

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 on new batches of Petrifilm compared to that on non-selective agar?	
	Is an appropriate performance standard used to pass new batches of Petrifilm, i.e. 70%?	