On-Farm Testing using Neogen Petrifilms

Equipment Needed

Below is a list of equipment needed with links to suitable items, but other suppliers and suitable alternatives are available.

The incubator, illuminated magnifying glass and clinical waste bin are one-off purchases, the rest are consumables that will need replacing. The quantities in the examples below will be enough for 50 TVC and 50 Coliform tests.

Item	Example supplier link
Incubator capable of holding temperature at 35°C	IVYX Scientific 5L Incubator (Amazon)
Illuminated Magnifying Glass	eSynic Magnifying Lamp (Amazon)
Rapid Aerobic Count (RAC) Plates (Neogen Ref 6478)	Petrifilm™ Rapid Aerobic Count (RAC) Plates (Gem Scientific)
E.Coli/Coliform Count (EC) Plates (Neogen Ref 6404)	Petrifilm™ E. coli/Coliform Count (EC) Plates (Gem Scientific)
Mini Flip Top Vial 9ml Peptone Broth	Buffered Peptone Water Broth (BPW) Flip top vial 9ml pk 100 (Trafalgar Scientific), Also available from Gem Scientific but not on their website (ref 3MBPPFV9BPW)
1ml Syringes	Terumo MDSS01SE 1 ml Syringe (Amazon)
Sterile Sample Pots	MILISTEN 50 Pcs Scientific Specimen Container (Amazon)
Clinical Waste Bin	Yellow 12 Litre Medical Clinical Waste Bin (Amazon)
Clinical Waste Bags	CX50/CWMD2 Clinical Waste Bag (Amazon)
Marker Pens	Sharpie Permanent Markers – Fine Point (Amazon)

Note: Even if using an incubator with an integrated thermometer, this should be calibrated regularly or checked against a second calibrated thermometer to ensure continued accuracy.

Petrifilm plates should be refrigerated until use, other equipment can be stored at ambient temperature in a clean environment. The petrifilms should be supplied with the appropriate spreader plate.

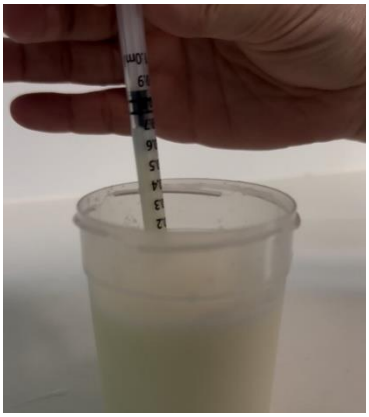
Petrifilms contain a growth medium, so we are advised to dispose of used petrifilms in a clinical waste bin to reduce the risk of contact with humans and food preparation areas after use. The plates dry out and can be stored until the bin is full, when it can be collected by a waste company.

The On-Farm Lab

On-farm testing takes very little space, but to prevent contamination, tests should be performed in a hygienic area, on a cleanable surface, free of pests and wind that may carry dust. As the testing involves culturing bacteria, it should be separated from stored food and food processing. An electric supply is needed to run the incubator, and hand washing facilities should be available nearby.

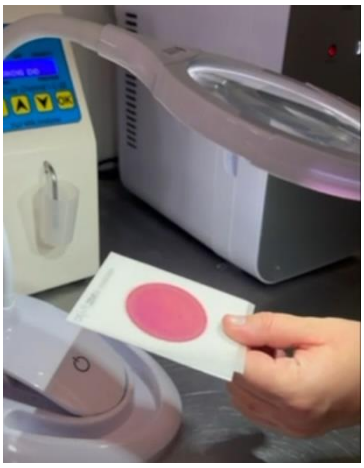
Step by Step Guide to Performing the Tests

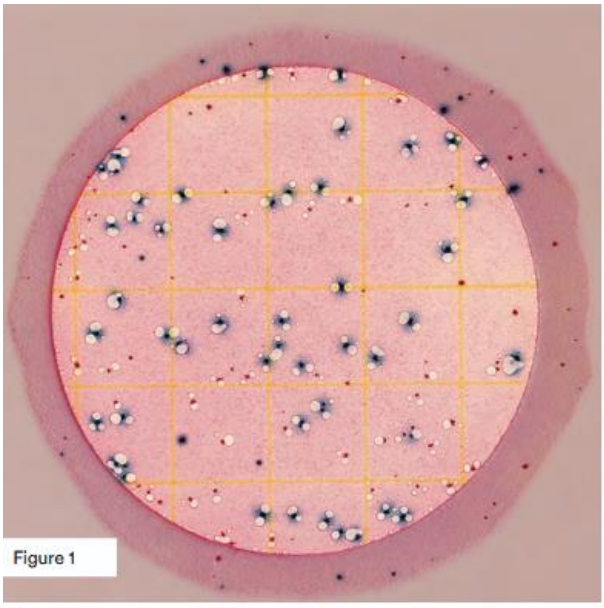
Before beginning tests or handling equipment, ensure the environment, surface and hands are thoroughly cleaned.



Coliform Test

1. Collect a milk sample aseptically in a sterile sample container.
2. Take an E.Coli/Coliform Petrifilm from the fridge and mark with the sample location and date.
3. Use a fresh sterile syringe to draw up 1ml of milk from the sample.
4. Peel back the film of the EC plate and decant the milk from the syringe into the centre of the plate.
5. Carefully roll the film back over the sample. Press the spreader lightly on the petrifilm's circle.
6. Place petrifilm in the incubator at 35°C.
7. After 24 hours remove the petrifilm from the incubator and place under an illuminated magnifying glass.
8. Count the spots that are surrounded by air bubbles only. These are the coliforms. As the sample was not diluted the result is the same number, so a plate with 10 spots surrounded by air bubbles would be recorded as 10 cfu/ml.





***E. coli* count = 49 (blue colonies with gas)**
Total coliform count = 87 (red and blue colonies with gas)

Interpretation Notes

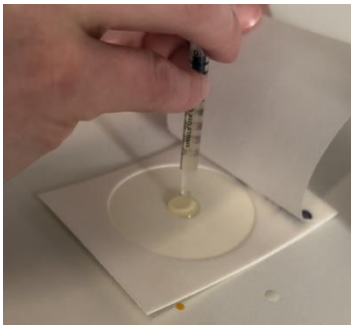
Gas bubbles may form in the centre of a colony so that the colony surrounds it. Gas bubbles not associated with a colony are not counted, nor are any colonies on the foam barrier at the edge of the circle.

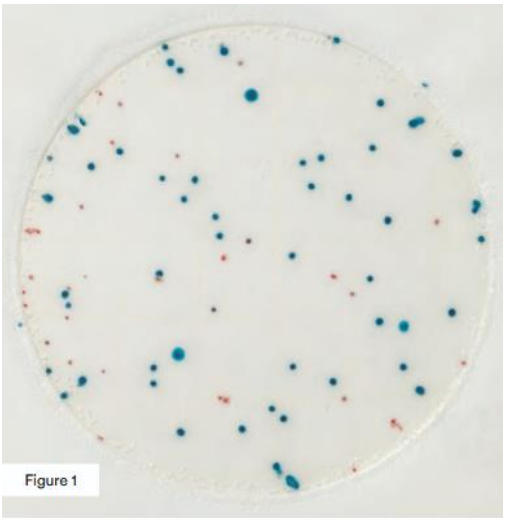
The recommended counting limit on EC plates is 150 colonies. If there are many small, indistinct colonies and/or a deepening of the gel colour to a purple-blue they are considered too numerous to count. A high concentration of non-coliform organisms may turn the gel yellow.

See Neogen's guide to interpreting EC Plate results here.

Total Viable Count

1. Collect a milk sample aseptically in a sterile sample container.
2. Take a Rapid Aerobic Count Petrifilm from the fridge and mark with the sample location and date.
3. Use a fresh sterile syringe to draw up 1ml of milk from the sample.
4. Empty the syringe into a 9ml vial of Peptone Buffer Water. Cap the vial and shake it for 2-3 seconds.
5. Draw up 1ml of the diluted sample in the first vial and empty into a second 9ml vial of Peptone Buffer Water. Cap and shake this vial for 2-3 seconds.
6. Use the syringe to draw up 1ml of the 2nd dilution.
7. Peel back the film cover of the RAC plate and decant the dilution from the syringe carefully into the middle of the circle.
8. Gently roll back the film. Press the spreader lightly onto the pretrifilm. Note that the circle on this plate is flat without a lip that prevents the sample spreading beyond the edge of the circle, so the spreader should be used with the side with a rim facing down to contain the spread of the sample.
9. Place petrifilm in the incubator at 35°C.
10. After 24 hours remove the petrifilm from the incubator and place under an illuminated magnifying glass.
11. Count the spots of any size on the petrifilm. Each spot is a bacterial colony. The TVC result will be the number of spots multiplied by 100 (due to the dilutions), so a plate with 10 spots would be recorded as 1,000 cfu/ml.





Aerobic bacteria count = 88

Interpretation Notes

If colonies spread and create a halo, the points within the spread zone can usually be counted.

The recommended counting limit on RAC Plates is 300 colonies. High concentrations of colonies may cause the gel to turn blue or red, or lack visible colonies in the centre with many small ones at the edges.

See Neogen's guide to interpreting RAC plates here.