1.0 Purpose

The purpose of this procedure is to define the test methods for selective and non-selective finished product and raw material microbial limit testing using Petrifilm. This procedure defines processes for sample preparation, incubation conditions, and interpretation of the results. The methods defined in this procedure, for enumeration testing, are not applicable to products containing viable microorganisms as active ingredients. Recovery studies for all Petrifilm methods were performed under protocols MV-LAB-13-002.

2.0 Scope

This procedure applies to all finished product and raw materials with D-715 listed as the test method.

3.0 Responsibility

3.1 It is the responsibility of QC Analysts to follow this procedure.

3.2 It is the responsibility of QC Laboratory Management to implement this procedure and to ensure that the procedure is being followed.

3.3 It is the responsibility of QC Laboratory Management to keep this procedure aligned with current practices and to oversee validations and recovery studies.

4.0 Definitions

4.1 **Diluent** – A sterile solution used to dissolve and dilute samples (e.g., Butterfield's Phosphate Buffer)

4.2 **Inoculum –** The prepared sample that is placed on the Petrifilm® plate via pipet

4.3 **IPA –** Isopropyl Alcohol

4.4 **QC –** Quality Control

4.5 **OOS –** Out of Specification

4.6 **TNTC** – Too Numerous to Count

4.7 **Est –** Estimation

4.8 **TAPC** – Total Aerobic Plate Count

4.9 **AC –** Aerobic Plate Count

4.10 **EC –** E. coli / Coliform Count Plates

4.11 **EB –** Enterobacteriaceae Count

4.12 **STX –** Staph Express Count

4.13 **RYM –** Rapid Yeast and Mold Count

4.14 **PQV** – Process Quality Verification

4.15 **cGMP** – Current Good Manufacturing Practices

5.0 References

5.1 6475/6477, Neogen® Petrifilm® Rapid Yeast and Mold Count Plate Product Instructions

5.2 6400/6406/6442/6403, Neogen® Petrifilm® Aerobic Count Plate Product Instructions

5.3 6404/6414/6444, Neogen® Petrifilm® E. Coli/Coliform Count Plate Product Instructions

5.4 6420/6421, Neogen® Petrifilm® Enterobacteriaceae Count Plate Product Instructions

5.5 6446/6490/6491/6492/6493, Neogen® Petrifilm® Staph Express Count System Product Instructions

5.6 2003.07, AOAC Official Method, Staphylococcus aureus Count in processed foods

5.7 990.12, AOAC Official Method, Aerobic Plate Count in Foods

5.8 991.14, AOAC Official Method, Coliform and *Escherichia coli* Counts in Foods

5.9 2003.01, AOAC Official Method, Enumeration of *Enterobacteriaceae* in Selected Foods

5.10 997.02, AOAC Official Method, Yeast and Mold Counts in Foods

5.11 Neogen® Petrifilm® Plate Guide to Dilution Preparations

5.12 NSF/ANSI 173 -2012- Standards for Dietary Supplements, August 7th, 2012

5.13 USP <2023>, Monograph, Microbiological Attributes of Nonsterile Nutritional and Dietary Supplements

5.14 U.S. Food and Drug Administration (2001) Bacteriological Analytical Manual, Chapter 3, Edition 8, Revision A, 1998

5.15 D-715-F1, Form, Microbial Limit Test Ticket

5.16 D-715-F2, Microbial Sample Log

5.17 D-715-F3, Conductivity and pH Test Ticket

5.18 D-809, SOP, Use and Calibration of Conductivity Meter

5.19 D-706, SOP, Use and Calibration of pH Meters

5.20 D-715.0, SOP, Microbial Limit Testing using Agar Plates

5.21 D-902, SOP, Establishment of Specifications

5.22 D-125, SOP, Microbiological Method Suitability

5.23 C-201, SOP, Deviation and Investigation Procedure

5.24 QS-108, SOP, Corrective and Preventative Action (CAPA)

5.25 D-824, SOP, Operation and Cleaning of the Autoclaves in the QC Laboratory

5.26 D-101, SOP, Laboratory Housekeeping

5.27 A-106, SOP, Documentation Guidelines for cGMP Records

5.28 C-501, SOP, Document Control Procedure

5.29 C-502, SOP, Record Storage, Retention, and Destruction

5.30 RPT-21-0028, Report, D-715 Estimation of Uncertainty

6.0 Required Supplies, Media and Equipment

6.1 Butterfield's Phospate Buffer-99mL or 90mL

6.2 0.1% Peptone Salt-90mL

6.3 Sterile Millipore Water

6.4 Isopropyl Alcohol

6.5 Sterile 120mL containers with lid

6.6 200ul and 1mL variable pipette with sterile tips

6.7 100, 250, 500 and 1L wide mouth storage bottles with screw cap top.

6.8 Balance

6.9 pH meter

6.10 Sterile 1N NaOH solution

6.11 Sterile 1N HCl solution

6.12 Neogen® Petrifilm® Rapid Yeast and Mold Count Plates

6.13 Neogen® Petrifilm® Aerobic Count Plates

6.14 Neogen® Petrifilm® Enterobacteriaceae Count Plates

6.15 Neogen® Petrifilm® E. coli / Coliform Count Plates

6.16 Neogen® Petrifilm® Staph Express Count Plates

6.17 Neogen® Petrifilm® Staph Express Disks

6.18 Neogen® Petrifilm® spreaders for Aerobic, Rapid Yeast and Mold, E. coli /Coliform, Enterobacteriaceae and Staph Express Count plates.

6.19 Compound Microscope with LCD Display

6.20 Biological Safety Cabinet

6.21 20 °C to 25°C and 30 °C to 35°C Incubators (adjustable)

6.22 Autoclave

6.23 Darkfield Colony Counter

7.0 Procedure

7.1 When a sample is received into the QC Laboratory that requires Microbial Testing, check the final product profile specification to ascertain if Petrifilm is an allowed method.

7.2 Before initiating testing, record sample information onto form D-715-F2 Microbial Sample Log as follow:

7.2.1 Product Name

7.2.2 Batch#

7.2.3 Sample preparation number

7.2.3.1 Sample preparation number will be assigned to the sample based on the next consecutive number listed

7.2.4 Automatic Pipette#

7.2.5 Date testing started

7.2.6 Analyst initial

7.3 Alternatively, any use log associated with the micro lab may be used as the sample logbook. All samples tested must be captured in a logbook at time of testing.

7.4 Diluent Preparation

7.4.1 Butterfield's Phosphate Buffer. First, prepare a Potassium Dihydrogen Phosphate Stock Solution by mixing 34.0 g of Monobasic Potassium Phosphate in 500 ml of Millipore water. Mix to dissolve. Calibrate the pH meter at pH 7.00 and 10.01 then adjust pH of stock solution to 7.2 ± 0.1 using 1N Sodium Hydroxide. Complete stock solution to 1 L with Millipore water. Prepare bottle and autoclave at 121°C for 15 minutes. Store in refrigerator after sterilization. Prepare Butterfield's Phosphate dilution blanks by adding 1.25 ml of the Potassium Dihydrogen Phosphate Stock Solution to 1 L of Millipore water. Prepare bottle and autoclave at 121°C for 15 minutes. Butterfields can also be bought thru a commercial vendor at 90mL or 99mL.

7.4.2 0.1% Peptone Salt (85% Sodium Chloride and 0.1% Peptone). Add 8.5 g of Sodium Chloride and 1.0 g of Peptone with 1L of Millipore water. Mix and heat to dissolve. Autoclave at 121°C for 15 minutes. Refrigerate until use. 0.1 % Peptone Salt can also be bought through commercial vendor at 90mL.

7.5 Sample labeling and Preparation

7.5.1 Sterilize all sample preparation equipment and media/diluents prior to use as per SOP D-824 Operation and Cleaning of the Autoclaves in the QC Laboratory. Single use, sterile disposables may also be used.

7.5.2 Label sample containers with a minimum of sample preparation number, and any extra testing over the following: TAPC, Y&M, EC, SAL

7.5.3 Create a parent sample of no less than 10g by transferring NLT 10g to a sterile/sanitized mortar (sanitize by wiping thoroughly with 70% IPA and allow the mortar and pestle time to dry before use). Aseptically pulverize tablets using the mortar and pestle or open capsules to create a blend of fill and capsules. Alternatively, parent sample may also be allowed to sit and disintegrate in diluent for approximately 15 minutes.

7.5.4 Typical sample dilutions are 1:10 and 1:100. 1:10 samples can be prepared in 120mL sterile capped cups using approximately 10g of sample and 90 mL of diluent or 11g of sample in 99mL of diluent. 1:100 samples are typically prepared by adding approximately 1g of material to a 120mL sterile capped cup and diluting up to 100mL with diluent. Other dilution ratios can be used if they have been validated or are higher than the validated dilution (i.e. validate dilution is 1/10, plate either 1:10. 1:100 etc). Document volume of diluent used for dilution ratios that require a diluent volume different from 90 or 99 ml (e.g. 1:20, 1:50). Refer to Finished Product Summary for Validated Dilution and Diluent Attachment 6.

7.5.5 If the sample has not been validated for petrifilm method, prepare a sample dilution as described above, using Butterfields's Buffer or Peptone Salt as the diluent. Reserve the original sample for validation testing (if applicable). Alternatively, agar method (D-715.0) can be used as reference test method at any time, even if it is not listed as the preferred method. Refer to SOP D-715.0 Microbial Limit Testing using Agar Plates.

7.5.6 Before inoculation, sample pH should be adjusted to neutral pH with 1N NaOH for acids products and 1N HCl for alkaline products per Neogen instructions. If pH indicator strips are used, sample pH should be adjusted to 7.0.

7.5.7 The sampling of certain raw materials such as RMS001583 can be challenging. To ensure an appropriate aseptic technique, the QC analyst must adhere to the steps defined below:

7.5.7.1 Inspect the 5-Gallon Water Jug submitted for testing to look for cracks, perforations or broken seal. Reject the 5-Gallon Water Jug if has visible damage or seal integrity has being compromised.

7.5.7.2 Spray with 70% IPA to saturate all the exterior surface of the water jug, before opening.

7.5.7.3 Allow to air dry for couple of minutes.

7.5.7.4 Use new gloves to release the seal and prepare materials for sample collection.

7.5.7.5 Carefully take off the lid with pre-saturated 70% IPA wipes to avoid contamination of the exposed ridge.

7.5.7.6 To prevent prolonged exposure, water sample should be immediately collected after opening.

7.5.8 Document the sample and test information required on form D-715-F1 Microbial Limit Test Ticket before start plating. For raw materials such as RMS001583 that require testing other than microbial, document additional testing on D-715-F3 Conductivity and pH Test Ticket. Refer to SOP D-706 and SOP D-809 for pH and conductivity measurements.

7.6 Inoculation of Petrifilm® Plates- General

7.6.1 Sanitize the interior of the biological safety cabinet with IPA as per D-101 Laboratory Housekeeping before plating. Sterile sleeve covers and gloves should be used from plate preparation to sample plating.

7.6.2 Obtain the Petrifilm® plates and confirm that they are not expired. Label the Petrifilm with a minimum of sample preparation number and dilution plated (if multiple dilutions are being plated)

7.6.3 For routine screening, inoculate two plates per testing required as follow:

7.6.3.1 AC petrifilm for TAPC Test

7.6.3.2 RYM petrifilm for Yeast and Mold Test

7.6.3.3 EC petrifilm for E.coli and Coliforms Test

7.6.3.4 EB petrifilm for Salmonella and Enterobacteriacea Test

7.6.3.5 STX petrifilm for Staphylococcus aureus Test

7.7 Plating sample on Neogen® Petrifilm® Aerobic Count (AC) Plates:

7.7.1 Place AC Petrifilm® plate on a level surface in a biological safety cabinet. Lift top film.

7.7.2 With pipette perpendicular to Petrifilm® plate, place 1 mL of sample onto

center of bottom film.

7.7.3 Release top film and allow it to drop. Do not roll top film down.

7.7.4 With ridge side down, place spreader on top film over inoculum.

7.7.5 Gently apply pressure on spreader to distribute inoculum over circular area. Do not twist or slide the spreader.

7.7.6 Lift spreader. Wait at least one minute for gel to form.

7.8 Plating sample on Neogen® Petrifilm® Rapid Yeast and Mold Count Plates:

7.8.1 Place Petrifilm® plate on a level surface in a biological safety cabinet. Lift top film.

7.8.2 With pipette perpendicular to Petrifilm® plate, place 1 mL of sample onto center of bottom film.

7.8.3 Release top film and allow it to drop.

7.8.4 Place the Petrifilm Rapid Yeast and Mold spreader on the center of the plate.

7.8.5 Distribute the sample with a gentle downward pressure on the center of the spreader. Do not twist or slide the spreader.

7.8.6 Lift spreader. Wait at least one minute for gel to form.

7.9 Plating sample on Neogen® Petrifilm® Enterobacteriaceae Count Plates (EB) and E. coli / Coliform Count (EC) Plates:

7.9.1 Place Petrifilm® plate on a level surface in a biological safety cabinet. Lift top film.

7.9.2 With pipette perpendicular to Petrifilm® plate, place 1 mL of sample to onto

center of bottom film.

7.9.3 Carefully roll top film down to avoid entrapping air bubbles. Do not let top film drop.

7.9.4 Wait at least one minute for gel to form.

7.10 Plating sample on Neogen® Petrifilm® Staph Express Count Plates:

7.10.1 Place Petrifilm® plate on a level surface in a biological safety cabinet. Lift top film.

7.10.2 With pipette perpendicular to Petrifilm® plate, place 1 mL of sample to onto center of bottom film.

7.10.3 Carefully roll top film down to avoid entrapping air bubbles. Do not let top film drop.

7.10.4 Spread the inoculant using a Staph Express spreader.

7.10.5 Gently apply pressure on spreader to distribute inoculum over circular area. Do not twist or slide the spreader.

7.10.6 Lift spreader. Wait at least one minute for gel to form.

7.11 Remove all materials from the biological safety cabinet and sanitize per D-101 Laboratory Housekeeping.

7.12 Incubation of Petrifilm® (AC, EB, EC, and STX) Plates:

7.12.1 Place AC, EB, EC, and STX plates in the incubator (typically 33°C ± 2°C) with the clear side up in stacks of no more than 20 plates. Document the sample start date on form D-715-F1 Microbial Limit Test Ticket.

7.12.2 Incubate the Aerobic Count plates for 48 ± 2 hours to 72 hours before reading.

The smallest colonies should be at least 1mm diameter before reading. Document the results and length of incubation on form D-715-F1 Microbial Limit Test Ticket. Justification is required for reads less than 46 hours.

7.12.3 Incubate the Enterobacteriaceae Count plates for 24 ± 2 hours to 72 hours before reading. Document the results and length of incubation on form D-715-F1 Microbial Limit Test Ticket. Justification is required for reads less than 22 hours.

7.12.4 Incubate the E. coli / Coliform Count plates for 48 ± 2 hours to 72 hours before reading. Document the results and length of incubation on form D-715-F1 Microbial Limit Test Ticket. Justification is required for reads less than 46 hours.

7.12.5 Incubate the Staph Express Count plates for 24 ± 2 hours to 72 hours. For plates that have colonies other than red-violet at 24 hours, insert a Staph Express disk between the plate and cover and incubate at 37°C ± 2°C for an additional 60 to 180 minutes (blue-green colonies do not require this step). If colonies exist that, do not have pink zones around them the plate must be incubated for the full 180 minutes before reading (Reference Attachment 5). Document the results and length of incubation on form D-715-F1 Microbial Limit Test Ticket. Justification is required for reads less than 22 hours.

7.13 Incubation of Petrifilm® Rapid Yeast and Mold Count Plates:

7.13.1 Place the plates in the incubator (typically 23°C ± 2°C) with the clear side up in stacks of no more than 20 plates. Document the sample start date on form D-715-F1 Microbial Limit Test Ticket.

7.13.2 Rapid Yeast and Mold Count Plates are incubated for 72 hours before reading. Document the results and length of incubation on form D-715-F1 Microbial Limit Test Ticket.

7.14 In the event of plates drying in the incubator ovens, place a 500mL beaker of sterile water in the incubator to achieve approximately 30 – 70% RH to minimize moisture loss and ensure the plates do not dry out.

7.15 Interpretation of Results

7.15.1 Visually determine the raw count or if the colonies are difficult to see, use the Darkfield Colony Counter to determine the raw count. Document and calculate the final result on form D-715-F1 Microbial Limit Test Ticket. Refer to the excerpts from the Neogen Interpretation Guides (Attachments 1 – 4) for counting instructions. The raw count is totaled as follows:

7.15.1.1 The raw count is the actual number of colonies on the plate.

7.15.1.2 Calculate the cfu / (g or mL) and document the result on form D-715-F1 Microbial Limit Test Ticket. The reported count is calculated as follows:

(CFUs (plate 1 + plate 2) / 2) (x Total DF)
divided by the volume plated (mL)

Dilution factor (DF) = Final Volume (mL)
divided by the sample (g) or (mL)

Example: DF = (90 mL + 10.30 g) / 10.30 g

Note: If multiple dilutions are plated, multiply each dilution factor up to the readable dilution.

7.15.2 For accurate calculations, raw counts should be between 25 to 250 cfu per plate.

7.15.3 If no colonies appear on the plate, the result should be reported as less than 1 times the corresponding lowest dilution used. Calculated count from raw counts outside the acceptable range should be reported as an estimated result (est).

7.15.4 For AC plates, all red colonies are counted. Petrifilm AC Plates containing greater than 250 colonies can either be estimated or recorded as TNTC. If the colonies are high in density but separated enough to count, estimation can be done by counting the number of colonies in one or more representative squares and determining the average number per square. The average number can be multiplied by 20 to determine the estimated count per plate. If a more accurate count is required, the sample will need to be retested at higher dilutions.

7.15.4.1 If spreaders or liquefiers are present (i.e., bacteria species than can liquefy the gel in the AC petrifilm), count only colonies in a few unaffected squares. Determine average and estimate counts per plate (See Attachment 1).

7.15.5 For RYM plates, all colonies are counted. Petrifilm RYM Plates containing greater than 250 colonies can either be estimated or recorded as TNTC. Estimation can be done by counting the number of colonies in one or more representative squares and determining the average number per square. The average number can be multiplied by 30 to determine the estimated count per plate. If a more accurate count is required, the sample will need to be retested at higher dilutions.

7.15.5.1 Yeast are small, defined edges, pink-tan to blue-green in color, usually raised, and uniform color.

7.15.5.2 Mold are large colonies, diffuse edged, variable in color, appear flat, look fuzzy, may have dark centers.

7.15.6 The EB plates detect salmonella as well as other Enterobacteriaceae. Enterobacteriaceae can be identified on the plate by red colonies with gas bubbles with yellow zones or yellow colored environment around the colony. These colonies are recorded as total Enterobacteriaceae count. Red colonies

without gas are not to be counted. Upon the presence of presumptive salmonella growth, selective and differential media can be used as supporting media for confirmatory test. Follow section 7.15.10 to transfer suspected salmonella colonies to a selective and differential agar. Refer to SOP D-715.0 Microbial Limit Testing using Agar Plates. Additionally, the excerpts from HardyCHROM™ instructions guides (Attachment #6) can be used as reference for differential agar.

7.15.7 The EC plates detect E. coli as well as other coliforms colonies. E. coli can be identified on the plate by gas bubbles surrounding blue to blue-red colonies. Non-E. coli Coliforms can be identified on the plate by gas bubbles surrounding red and blue colonies and are recorded as total coliforms. E. Coli specific colonies are blue colonies with gas. Upon the presence of presumptive E. coli growth, selective and differential media can be used as supporting media for confirmatory test. Follow section 7.15.10 to transfer suspected E. coli colonies to a selective and differential agar. Refer to SOP D-715.0 Microbial Limit Testing using Agar Plates. Additionally, the excerpts from HardyCHROM™ instructions guides (Attachment #6) can be used as reference for differential agar.

7.15.8 The STX plates detect Staphylococcus aureus as well as other Staphylococcus species. Staphylococcus aureus can be identified by red-violet colonies and colonies with pink zones when Staph Express Disks are used (Reference Attachment 5 for interpretation of positive results). If no colonies or only red-violet colonies are present after 24 ± 2 hours, red-violet colonies are recorded and the test is completed. If confirmation step is required, count only pink highlighted colonies. Alternatively, selective and differential media can be used as supporting media to confirm the presence Staphylococcus aureus growth. Follow section 7.15.10 to transfer suspected Staphylococcus aureus colonies to a selective and differential agar. Refer to SOP D-715.0 Microbial Limit Testing

using Agar Plates. Additionally, the excerpts from HardyCHROM™ instructions guides (Attachment #6) can be used as reference for differential agar.

7.15.9 For selective plates such as EC, EB, and STX showing no growth, the result is reported as "Negative" or "Absent" per product profile specifications.

7.15.10 Where necessary, colonies may be isolated for further identification. Lift the top film using proper aseptic technique; pick the colony from the gel and subculture on agar. Reference SOP D-1016 Microbial Identification via Biolog Microstation for further steps.

7.15.11 In the event of inconclusive readings, noted results as "Inconclusive" and proceed with retest. Serial dilutions of the original sample (if available) should be performed. New sample can be requested for testing and D-715.0 method can be use as reference method if considered most suitable for sample testing.

7.15.12 If any material does not meet acceptance criteria for any microbial testing, then the original sample may be immediately re-plated for confirmation. If the value observed after the re-plate is still outside of acceptance criteria then a Deviation should be initiated as detailed in C-201 Deviation and Investigation Procedure. Alternatively, initiation of a CAPA following SOP QS-108 Corrective and Preventative Action (CAPA) may be performed.

8.0 Specifications

8.1 The maximum specification thresholds for microbial activity in dietary supplements are described NSF/ANSI 173- August 7, 2012. Raw material and finished product specifications for use in HBI Ion Labs dietary supplements comply with the specifications set forth in NSF/ANSI 173. Specifications for a raw material are listed on the corresponding raw material test ticket. Specifications for a finished product are listed in the product profile. Additional specification details are located in SOP D-902 Establishment of

Specifications. Unless a deviation in testing is required, the method listed on Product Profile is the method to use even if it has been validated on other methods. If both methods are on the Product Profile, D-715 Microbial Limit Testing using the Neogen® Petrifilm® System is preferred.

8.2 Raw Material Categories and Definitions

8.2.1 **Vitamin and/or mineral ingredient** – Purified chemical or mineral.

8.2.2 **Botanical ingredient (non-extract)** – Crude botanical material (whole, cut or powdered herb.)

8.2.3 **Botanical ingredient (Extract / other dietary supplement ingredient) –** The complex, multicomponent mixture obtained after using a solvent to dissolve components of the biomass. Extracts may be in dry, liquid, or semi-solid form. Excipients may be added to extracts to adjust the concentration, enhance stability, limit microbial growth, and to improve drying, flow, or other manufacturing characteristics.

8.3 Acceptable limits for microbial contaminants in raw materials.

Table 1 *Specifications listed as colony forming units per gram (CFU/g)

Raw Material Ingredient	Aerobic	Yeast/Mold	Enterobacteriaceae	Salmonella spp.	E. coli[a]	S. aureus
Vitamin and/or mineral ingredient	1×10^3	1×10^2	1×10^2	None Detected	None Detected	None Detected
Botanical ingredient – non-extract	1×10^7	1×10^5	1×10^4	None Detected	[b]1×10^2	None Detected
Botanical Ingredient-extract / other dietary supplement Ingredient	1×10^4	1×10^3	1×10^2	None Detected	None Detected	None Detected

(a) No guidance is available on acceptable limits for non-E. Coli coliforms.

(b) Upon the presence of E. coli type testing must be performed for enterovirulent E. coli. There is a zero tolerance for the presence of enterovirulent E. coli.

8.4 Finished product Categories and definitions

8.4.1 **Category I** – Finished products containing only vitamin and minerals.

8.4.2 **Category II** – Finished products containing Botanical ingredient – extract / other dietary supplement ingredient.

8.4.3 **Category III** – Finished products containing botanical ingredients – non extract.

8.5 Maximum acceptable limits for microbial contaminants in Finished Products.

Table 2

*Specifications listed as colony forming units per gram (CFU/g)

Finished Product	Aerobic	Yeast/Mold	Enterobacteriaceae	Salmonella spp.	E. coli[1]	S. aureus
Category I	$1 X 10^3$	$1 X 10^2$	$1 X 10^2$	None Detected	None Detected	None Detected
Category II	$1 X 10^4$	$1 X 10^3$	$1 X 10^2$	None Detected	None Detected	None Detected
Category III	$1 X 10^7$	$1 X 10^5$	$1 X 10^4$	None Detected	[(d)]$1 X 10^2$	None Detected

(c) No guidance is available on acceptable limits for non- E. Coli coliforms.

8.6 Upon the presence of E. coli type testing must be performed for enterovirulent E. coli. There is a zero tolerance for the presence of enterovirulent E. coli USP Microbial Limits per <2023>.

Table 3 *Specifications listed as colony forming units per gram (CFU/g)

Material	Aerobic	Yeast/Mold	E. coli / Salmonella
Powdered Botanicals	$1 X 10^5$	$1 X 10^3$	None Detected
Powdered Botanical Extracts	$1 X 10^4$	$1 X 10^3$	None Detected
Nutritional supplements with botanicals	$1 X 10^4$	$1 X 10^3$	None Detected
Nutritional supplements with highly refined ingredients	$1 X 10^3$	$1 X 10^2$	None Detected

9.0 Reporting

9.1 Reporting results for TAPC

9.1.1 The expanded uncertainty for the method when quantifying TAPC is 28% with a coverage factor of 2

9.2 Reporting results for Yeast and Mold

9.2.1 The expanded uncertainty for the method when quantifying Yeast and Mold is 7% with a coverage factor of 2

10.0 Documentation Requirements

10.1 A PQV check must be performed for each completed page of form D-715-F2 Microbial Sample Log as outlined in SOP A-106 Documentation Guidelines for cGMP Records.

10.2 All documentation will be distributed and maintained as outlined in SOP C-501 Document Control and SOP C-502 Record Storage, Retention, and Destruction.

11.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	01/23/12	New	-	-
1	05/09/13	Changed the SOP format, revised and updated SOP to be consistent with validated test method, updated D-715-F2 Test Ticket to reflect new format, added D-715-F1 microbial sample log.	-	-
2	11/11/13	Added rapid yeast and mold count plates to Section 5.6.2.2	13-1027	B. Johns
3	11/06/14	Added Staph Express System Instructions	14-0888	B. Johns
4	01/29/15	New title. Added NSF/ANSI 173-2012 microbial limit specifications and reference. Added USP <2023> Specifications and reference. Added new sections 7.0 Specifications and 8.0 Safety Testing. Added D-101 Laboratory Housekeeping reference.	15-0118	B. Johns
5	10/11/16	New Title. New D-715-F2 format to include use of agar plates. Reference recovery study protocols. Expanded incubation times based on subvalidations. Expanded incubation temperature ranges to reflect current practices.	16-0914	R. Casabianca
6	03/08/17	Update test tickets for clarity and to better define start / stop incubation points.	17-0257	B. Johns
7	04/11/18	Edited D-715-F1 sample log to include pH data. Removed *P. aeruginosa* testing from test ticket; testing included in D-715.0. Updated and clarified D-715-F2a. Moved D-715-F2b to D-715.0-F2. Added Darkfield Colony Counter to equipment list.	18-0125	E. Johnson
8	03/04/20	Changed growth recovery calculation for clarity. Edited form D-715.0 to combine testing methods D-715 and D-715.0. Added diluent prep according to 3M recommendations.	19-0733	L. McWade
9	05/19/21	Added what colonies are to look like for all petri-film plates. Added Butterfield's Buffer required media. Corrected final result calculation. Corrected all dilutions for each of use	CC-21-0204	G. Shaw
10	11/23/21	Modified SOP to actual process used. Added ISO requirements/measurement of uncertainty. Changed form numbers for test ticket and log.	CC-21-0434	G. Shaw
11	07/12/23	Added information related to Validated dilution and diluents for Final Products. Updated format. Added documentation requirements.	CC-23-0339	G. Shaw

12	11/04/23	Added documentation requirements. Added additional SOP references. Revised test ticket and log form to include logbook number. Added alternative options for sample logbook.	CC-23-0545	K. Burris
13	09/23/24	Updated incubation times, reporting terminology, addition of form D-715-F3. Clarified sample preparation, growth recovery calculation and interpretation.	CC-24-0470	A. Perez
14	05/27/25	Added sampling procedure for 5 Gallon Water Jug. Updated SOP to reflect current practices, including the use and interpretation of alternative methods. Added information related to Validated dilution and diluents for Final Products. Edited brand name for petrifilm plates.	CC-25-0230	A. Perez

12.0 Attachments

10.1 Attachment 1 – Aerobic Count Plate Interpretation Guide Excerpt

10.2 Attachment 2 – Yeast and Mold Count Plate Interpretation Guide Excerpt

10.3 Attachment 3 – Enterobacteriaceae Count Plate Interpretation Guide Excerpt

10.4 Attachment 4 – E. coli / Coliform Count Plate Interpretation Guide Excerpt

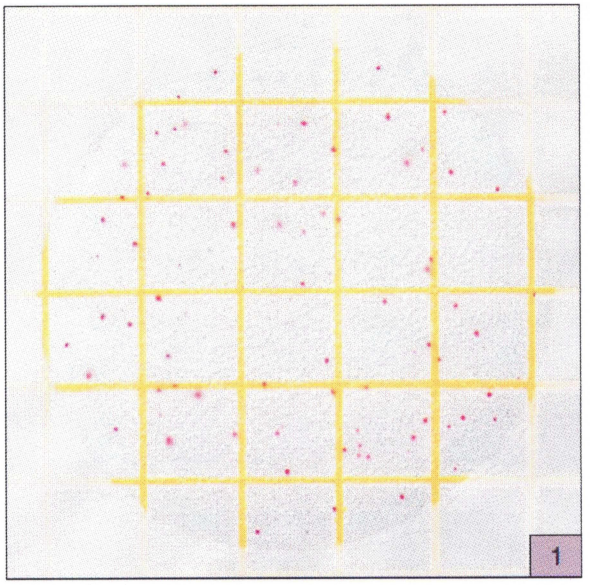
10.5 Attachment 5 – Staph Express Count Plate Preparation and Interpretation Guide

10.6 Attachment 6 – Chromogenic Medium Interpretation Guide Excerpt

10.7 Attachment 7 – Finished Product Summary for Validated Dilution and Diluent

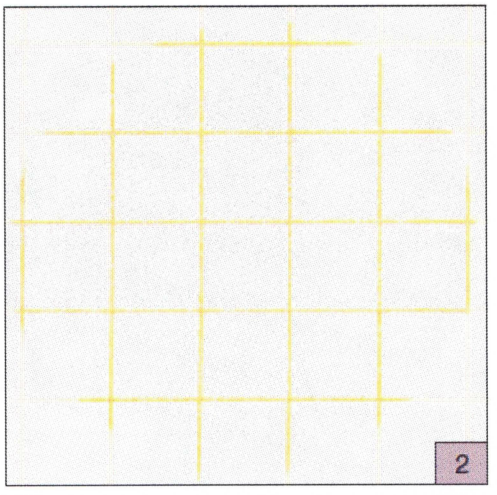
Attachment 1 – Aerobic Count Plate Interpretation Guide Excerpt

The Petrifilm Aerobic Count (AC) plate is a ready-made culture medium system that contains Standard Methods nutrients, a cold-water-soluble gelling agent, and an indicator that facilitates colony enumeration. Petrifilm AC plates are used for the enumeration of aerobic bacteria.



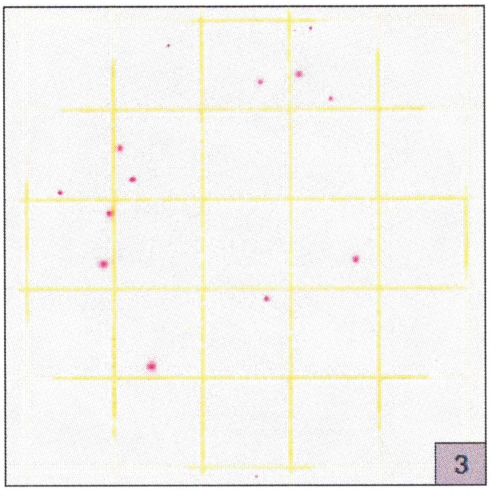
Aerobic Bacteria Count = 152

A red indicator dye in the plate colors the colonies. Count all red colonies regardless of their size or color intensity.



Count = 0

It is easy to interpret the Petrifilm AC plate. Figure 2 shows a Petrifilm AC plate without colonies.

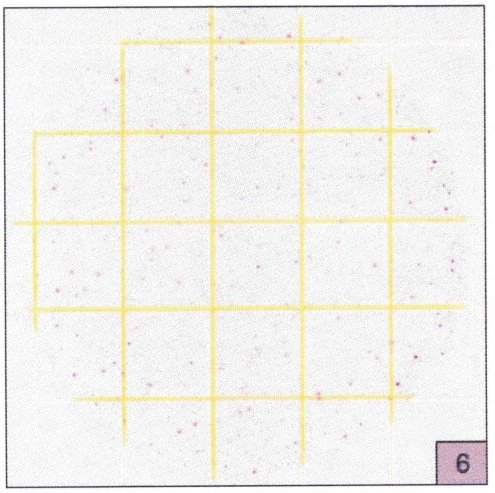


Count = 16

Figure 3 shows a Petrifilm AC plate with a few bacterial colonies.

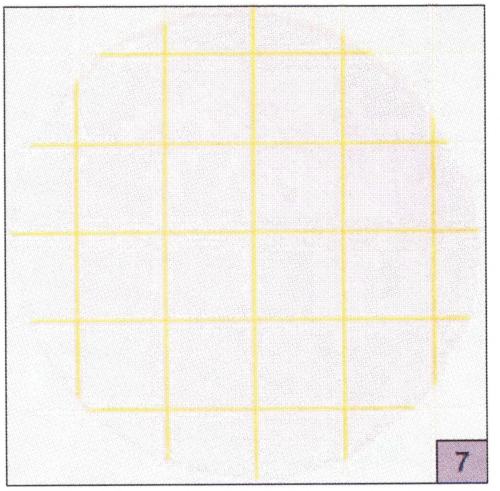
Attachment 1 – Aerobic Count Plate Interpretation Guide Excerpt (Cont'd)

TNTC (Too Numerous to Count) To obtain a more accurate count, dilute the sample further



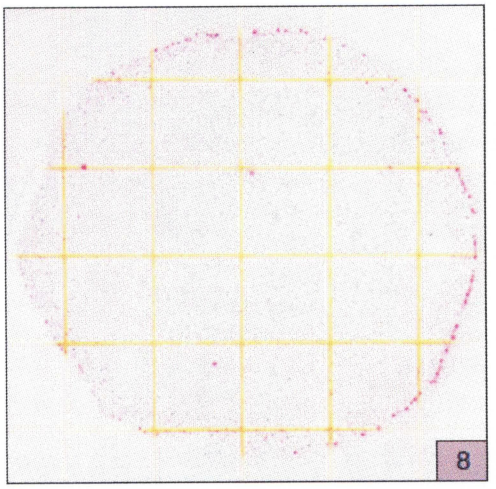
Count = TNTC **(Estimated count = 10^3)**

Figure 6 shows a Petrifilm AC plate with colonies that are too numerous to count (TNTC).



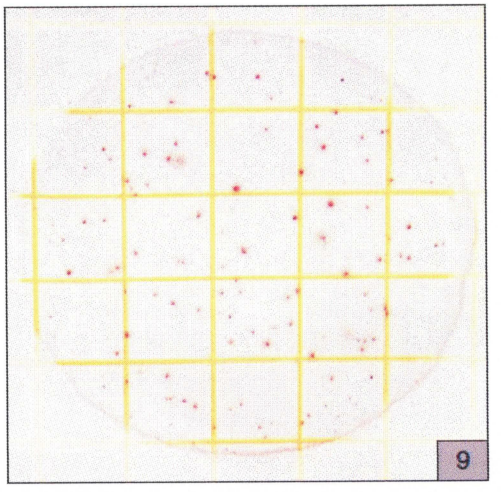
Count = TNTC **(Estimated count = 10^5)**

With very high counts, the entire growth area may turn pink, as shown in figure 7. You might observe individual colonies only at the edge of the growth area. Record this as a TNTC result.



Count = TNTC **(Estimated count = 10^3)**

Occasionally, distribution of colonies appears uneven, as shown in figure 8. This is also an indication of a TNTC result.

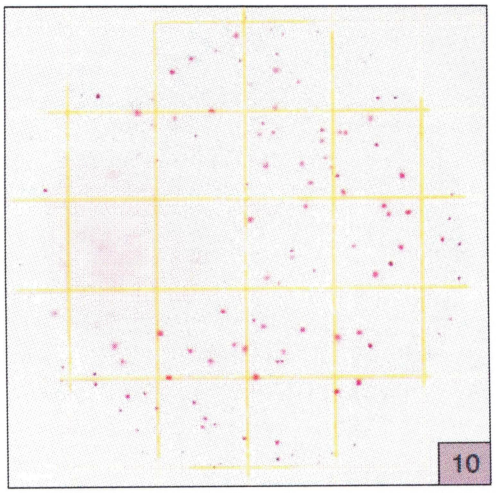


Count = TNTC **(Estimated count = 10^7)**

The colonies on the Petrifilm AC plate in figure 9 appear countable at first glance. However, when you look closely at the edge of the growth area, you can see a high concentration of colonies. Record this as a TNTC result.

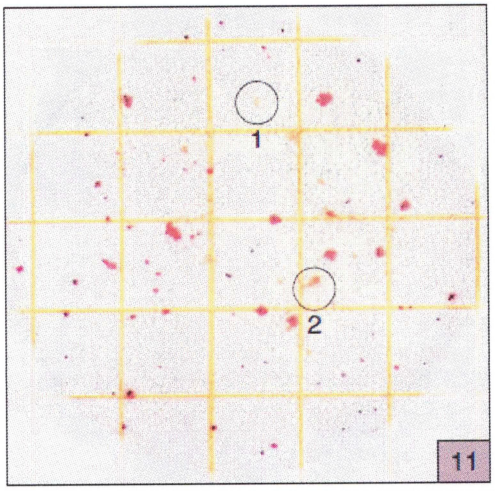
Attachment 1 – Aerobic Count Plate Interpretation Guide Excerpt (Cont'd)

Gel Liquefication and Food Particles



Estimated Count = 160

A few species of bacteria liquify the gel in the Petrifilm AC plate, as shown in figure 10. When this occurs, determine the average count in a few unaffected squares and then multiply it by 20 to obtain the estimated count. Do not count red spots within the liquified area.

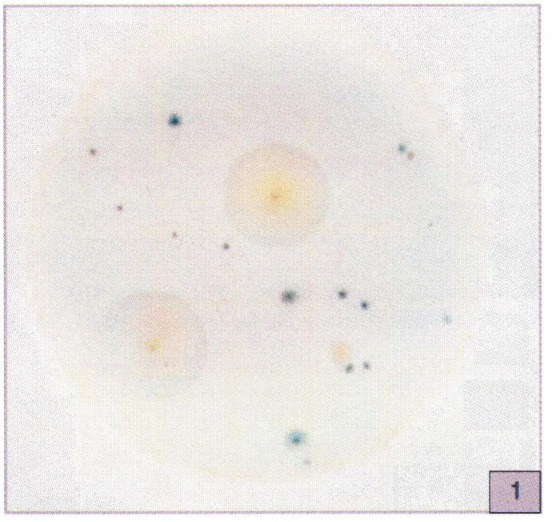


Count = 83

Because colonies on Petrifilm AC plates are red, you can distinguish them from opaque, irregularly shaped food particles (see circles 1 and 2).

Attachment 2 – Yeast and Mold Count Plate Interpretation Guide Excerpt

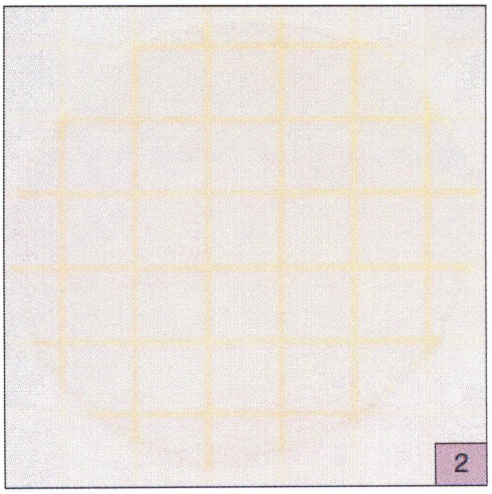
The Petrifilm™ Yeast and Mold (YM) Count Plate is a ready-made culture medium that contains a cold-water soluble gelling agent, nutrients and an indicator dye to provide contrast and facilitate counting.



Total Count = 20
Yeast Count = 16
Mold Count = 4

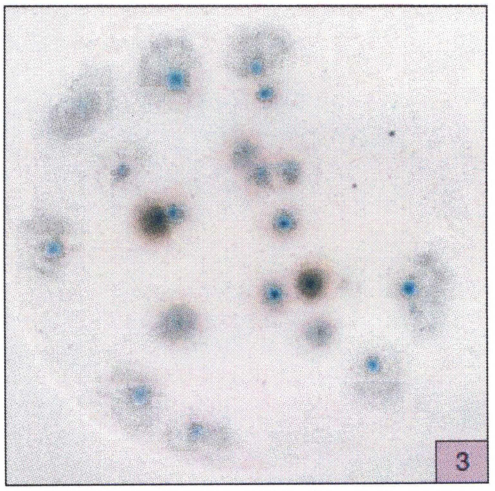
This Petrifilm YM Plate contains both yeast colonies and mold colonies.

Attachment 2 – Yeast and Mold Count Plate Interpretation Guide Excerpt (Cont'd)



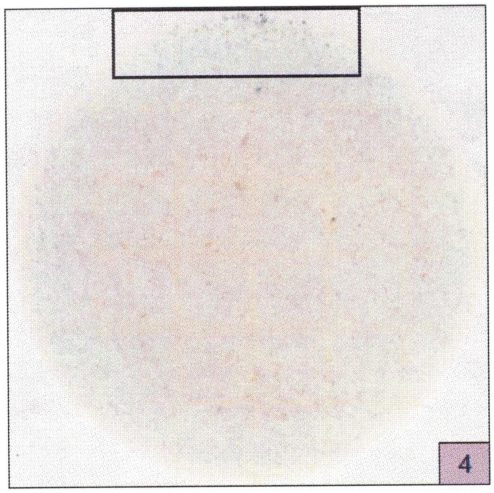
Yeast and Mold Count = 0

Figure 2 shows a Petrifilm YM Plate without yeast or molds.



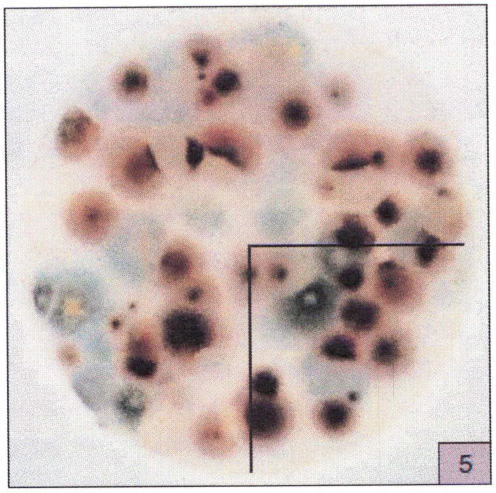
Estimated Total Count ~ 500
Estimated Yeast Count ~ 480
Mold Count = 21

When colonies number more than 150, estimate the count. Determine the average number of colonies in one square (1 cm^2) and multiply it by 30 to obtain the total count per plate. The inoculated area is approximately 30 cm^2. Yeast colonies may range in color from tan (as in this example) to pink to blue-green.



Estimated Yeast Count ~ TNTC (actual count > 10^4)

The Petrifilm YM Plate in figure 4 contains yeast colonies too numerous to count (TNTC). The small, blue colonies at the edge of the plate (highlighted in the box) are present throughout the entire plate although less visible.

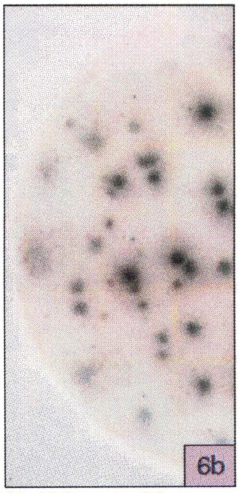


Estimated Mold Count ~ 64

The mold colonies in figure 5 are beginning to crowd and overlap each other on the plate. Count each colony margin or focus. The plate can be divided into sections to assist in counting. In this example, approximately 1/4 of the plate was counted, then the number of colonies counted was multiplied by 4 to get the estimated count on the plate. The section shown has 16 molds.

Attachment 2 – Yeast and Mold Count Plate Interpretation Guide Excerpt (Cont'd)

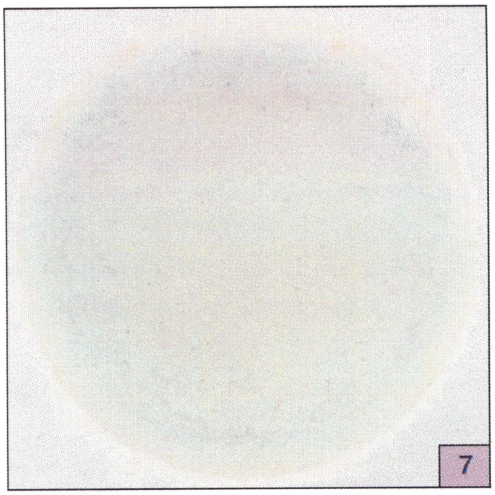
Mold Count ~ TNTC



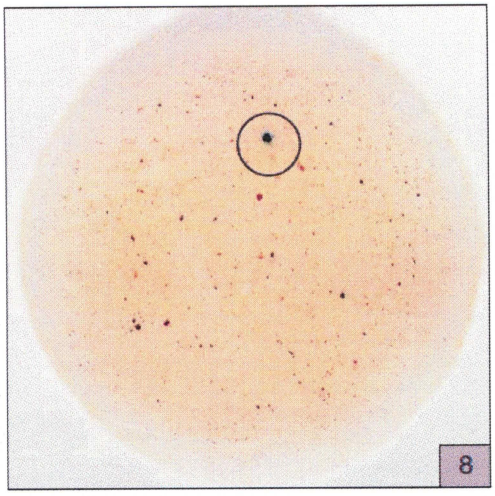
Mold Count = 64

Plates in figures 6a and 6b are the same sample. Figure 6a is a 1:10 dilution and has colonies that are small, faint and numerous, making it difficult to count. Figure 6b is a 1:100 dilution and shows how diluting product to obtain a colony count of less than 150 colonies makes counting easier. As with most growth media, in a highly competitive environment (such as figure 6a), typical colony growth will be inhibited. For heavily contaminated samples such as these, higher dilutions are recommended for a more accurate count and more typical colony growth (as in figure 6b).

PHOSPHATASE REACTION



Yeast and Mold Count = 0



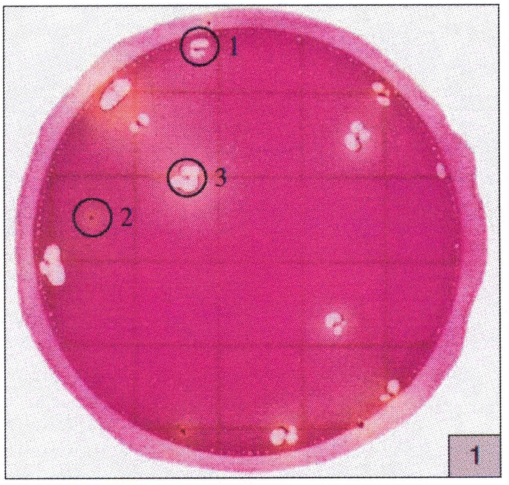
Yeast and Mold Count = 0

Petrifilm YM Plates utilize a phosphatase indicator dye. Therefore, some food products that contain phosphatase may cause a blue color reaction to occur on the Petrifilm YM Plate. Two types of color reactions are sometimes seen: a uniform blue background color or intense, blue spots. Figure 7 shows uniform blue background color and figure 8 shows intense blue spots which are often seen with spices or granulated products. Figure 8 also shows food particles that yielded phosphatase.

To reduce a phosphatase reaction, follow one or more of these techniques:

1. **Dilute Sample**: Further sample dilution will minimize blue background color or reduce the number of intense blue spots.
2. **Sample Preparation**: Mix sample and let settle for 3–5 minutes before plating. Draw sample from center portion of sample container or use filtered homogenizer bag to avoid plating large particles.
3. **Check and Note**: Observe plates within 24-36 hours of incubation and make note of any color change to aid in final interpretation.

Attachment 3 – Enterobacteriaceae Count Plate Interpretation Guide Excerpt

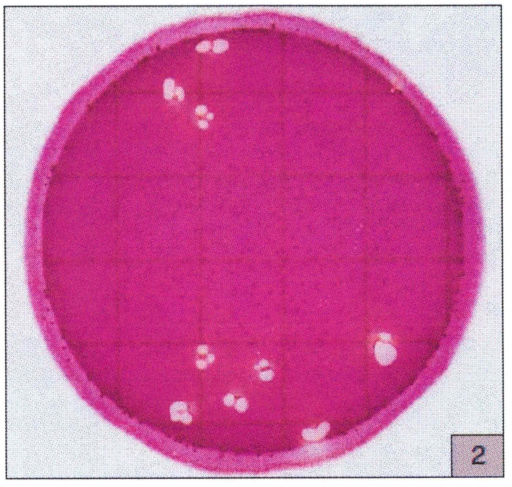


***Enterobacteriaceae* count = 13**

An indicator in the Petrifilm Enterobacteriaceae Count plate colors all colonies red. The top film traps gas produced by some bacteria. Acid-producing bacteria are seen as red colonies surrounded by yellow zones.

Bacteria producing gas and/or acid are considered to be presumptive *Enterobacteriaceae* and will have one of the following characteristics on the Petrifilm Enterobacteriaceae Count plate: colonies associated with gas bubbles and no acid zones (see figure 1, circle 1), colonies with yellow acid zones but no gas production (see figure 1, circle 2), or colonies producing both gas and acid (see figure 1, circle 3).

Figure 1 also illustrates how bubble patterns can vary. Sometimes gas disrupts the colony so that the colony "outlines" the gas bubble as in figure 1, circle 3.

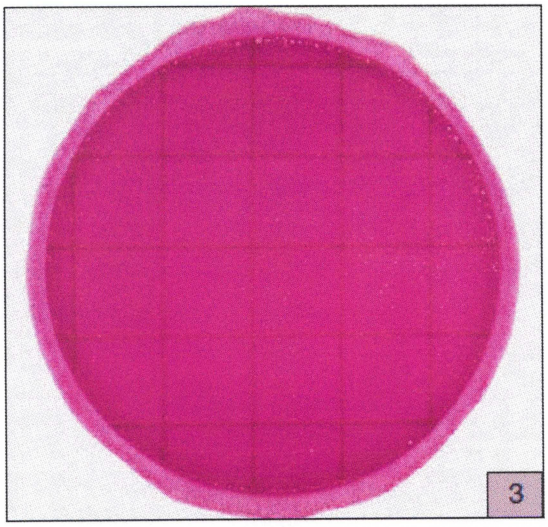


***Enterobacteriaceae* count = 9**

Figure 2 shows a Petrifilm Enterobacteriaceae Count plate with a few *Enterobacteriaceae* colonies and a high number of non-*Enterobacteriaceae*, gram-negative colonies.

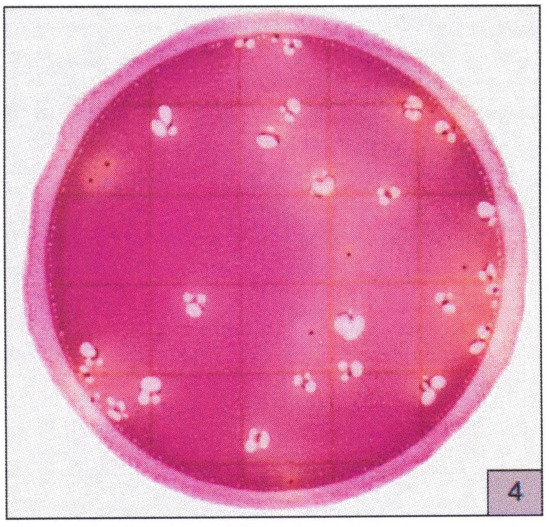
Do not count colonies on the foam dam since they are removed from the selective influence of the medium.

Attachment 3 – Enterobacteriaceae Count Plate Interpretation Guide Excerpt (Cont'd)

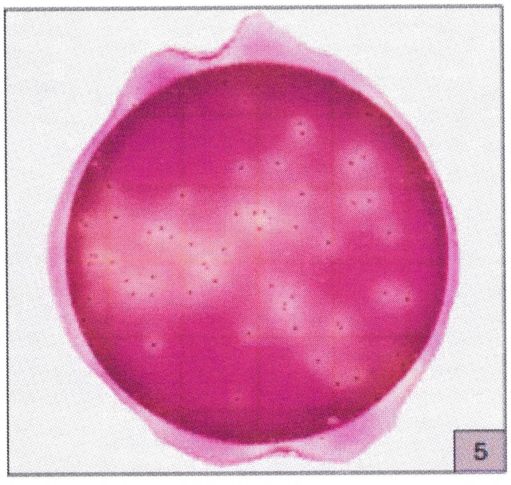


***Enterobacteriaceae* count = 0**

Notice the change in gel color in figures 3 through 8. As the *Enterobacteriaceae* count increases, the color of the gel lightens from purple to yellow or cream colored.



***Enterobacteriaceae* count = 35**

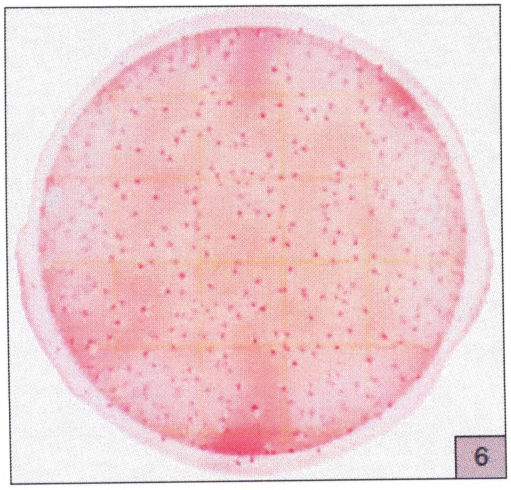


***Enterobacteriaceae* count = 77**

The recommended counting range on Petrifilm Enterobacteriaceae Count plates is 15–100 colonies. Samples having counts greater than 100 *Enterobacteriaceae* per plate may be estimated. The circular growth area is approximately 20 cm^2. Estimates can be made by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number of colonies per square by 20 to determine the estimated count per plate.

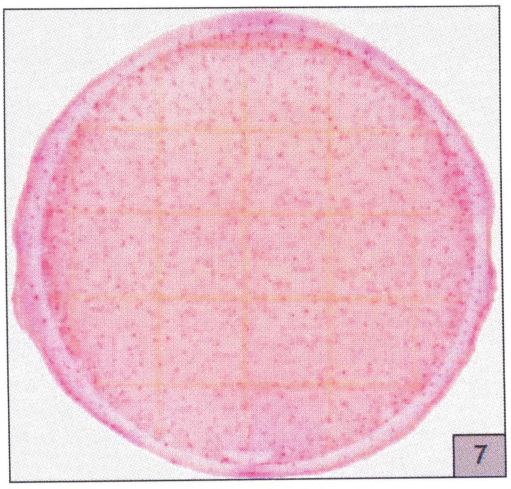
Attachment 3 – Enterobacteriaceae Count Plate Interpretation Guide Excerpt (Cont'd)

TNTC (Too Numerous to Count) To obtain a more accurate count, dilute the sample further.



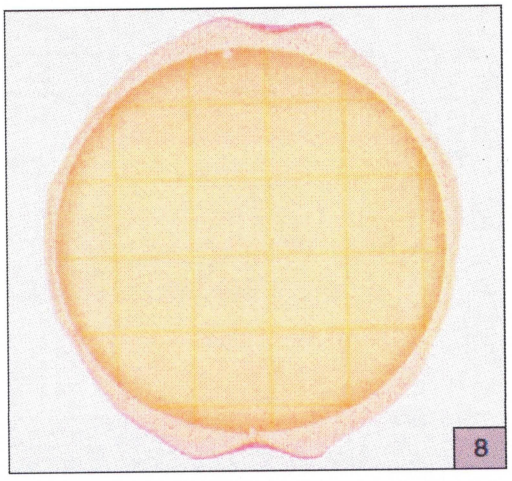
***Enterobacteriaceae* count = TNTC**

Petrifilm Enterobacteriaceae Count plates with more than 100 colonies are considered too numerous to count (TNTC) and have a light background color along with at least one of the following characteristics: many small colonies or many gas bubbles. See figure 6.



***Enterobacteriaceae* count = TNTC**

In figure 7, the count is so high that acid zones and gas bubbles are not easily seen. A lightening of the gel color indicates that the result is TNTC.

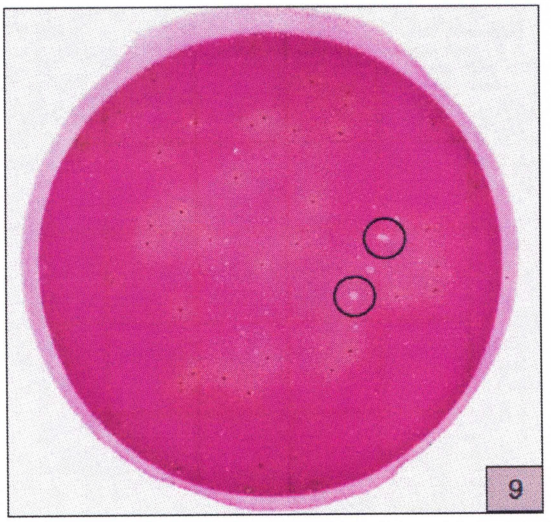


***Enterobacteriaceae* count = TNTC**

The Petrifilm Enterobacteriaceae Count plate in figure 8 has two characteristics indicating TNTC colonies: lightening of the gel color and many small colonies.

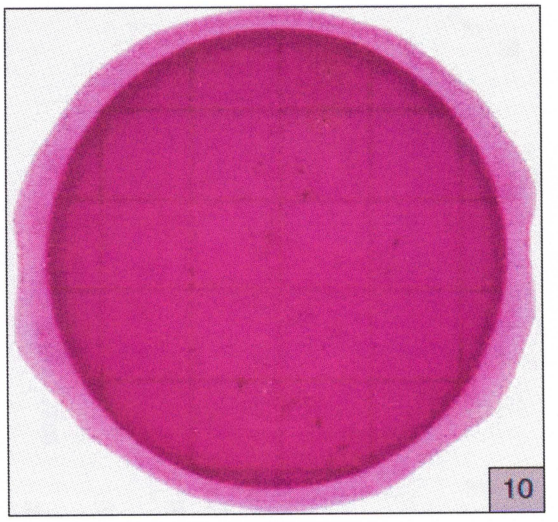
Attachment 3 – Enterobacteriaceae Count Plate Interpretation Guide Excerpt (Cont'd)

Artifact Bubbles and Food Particles Do Not Enumerate



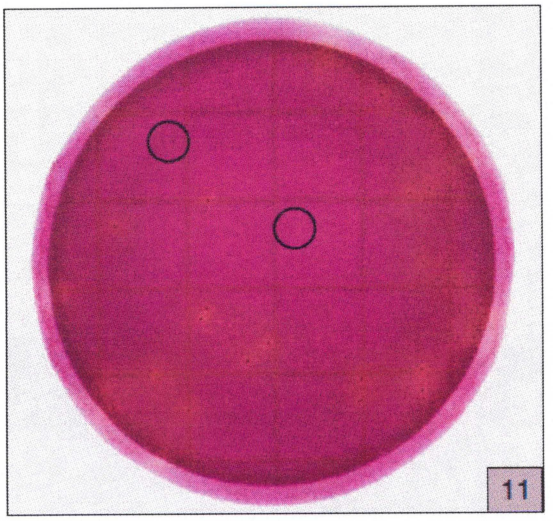
***Enterobacteriaceae* count = 44**

Artifact bubbles may result from improper inoculation of the Petrifilm Enterobacteriaceae Count plate. They are irregularly shaped and not associated with a red colony. See figure 9.



***Enterobacteriaceae* count = 2**

Food particles are often irregularly shaped or filamentous and are not associated with gas bubbles or acid zones. See figure 10.



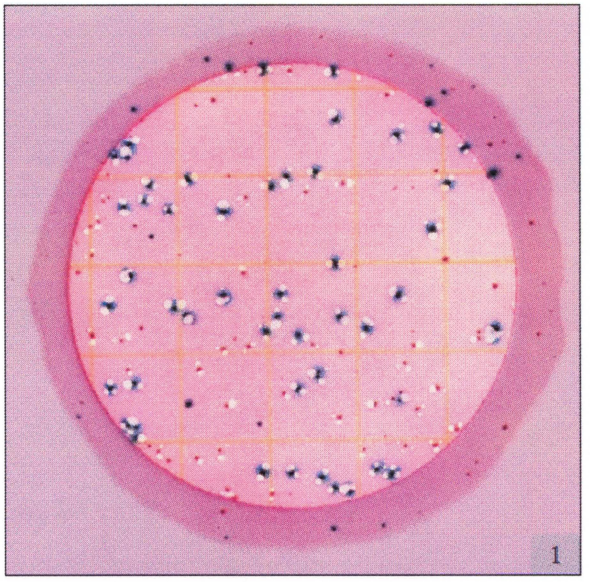
***Enterobacteriaceae* count = 29**

Food particles also can be seen as dark spots but are not associated with gas bubbles or acid zones. See figure 11.

Attachment 4 – E. coli / Coliform Count Plate Interpretation Guide Excerpt

Petrifilm E. coli/Coliform Count (EC) plates contain Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity, and an indicator that facilitates colony enumeration. Most *E. coli* (about 97%) produce beta-glucuronidase which produces a blue precipitate associated with the colony. The top film traps gas produced by the lactose fermenting coliforms and *E. coli*. About 95% of *E. coli* produce gas, indicated by blue to red-blue colonies associated with entrapped gas on the Petrifilm EC plate (within approximately one colony diameter).

AOAC INTERNATIONAL and U.S. FDA Bacteriological Analytical Manual (BAM) define coliforms as gram-negative rods which produce acid and gas from lactose during metabolic fermentation. Coliform colonies growing on the Petrifilm EC plate produce acid which causes the pH indicator to make the gel color darker red. Gas trapped around red coliform colonies indicates confirmed coliforms.

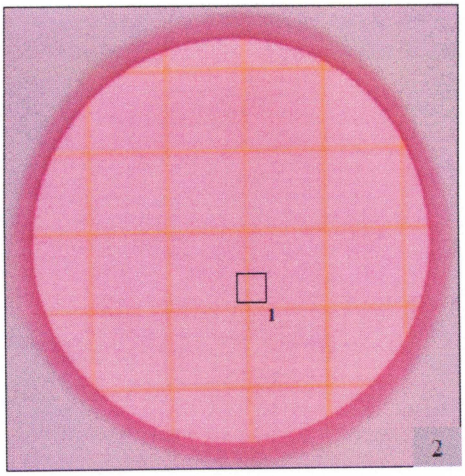


The identification of *E. coli* may vary by country (see Reminders for Use section for incubation times and temperatures):

AOAC INTERNATIONAL validated method
E. coli = **49** (blue colonies with gas)
Total coliform = 87 (red and blue colonies with gas)

Do not use this plate alone for the detection of *E. coli* O157. Like most other *E. coli*/coliform media, this plate will not specifically indicate whether any O157 strain is present.

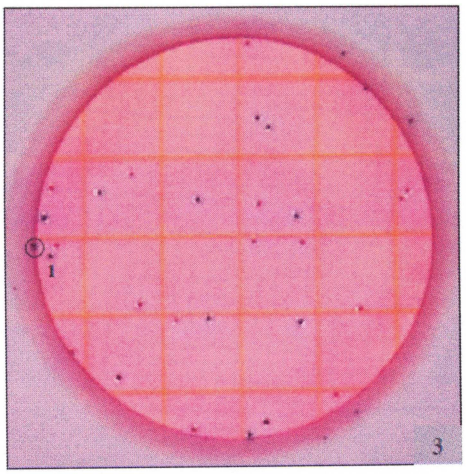
Attachment 4 – E. coli / Coliform Count Plate Interpretation Guide Excerpt (Cont'd)



No growth = 0

Notice the changes in gel color in figures 2 through 8. As the *E. coli* or coliform count increases, the color of the gel turns to dark red or purple-blue.

Background bubbles are a characteristic of the gel and are not a result of *E. coli* or coliform growth. See square 1.

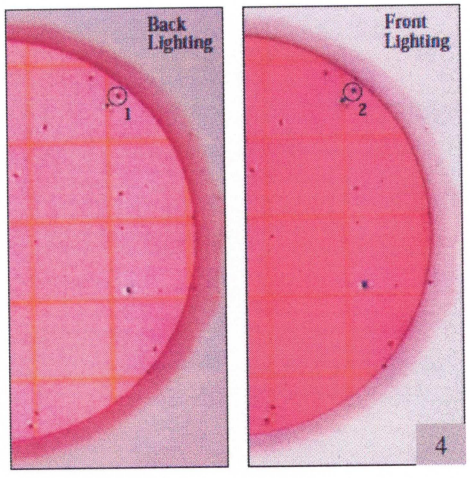


***E. coli* count = 13**

Total coliform count = 28

The counting range for the total population on Petrifilm EC plates is 15–150.

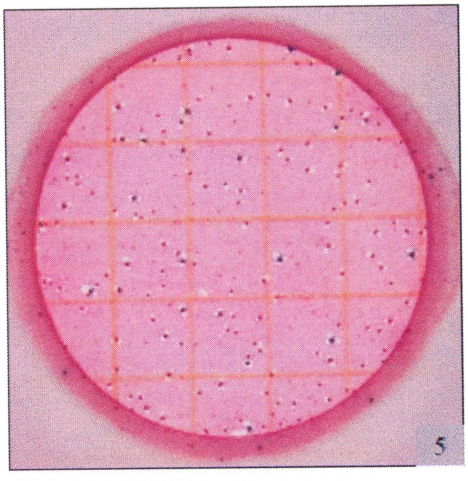
Do not count colonies that appear on the foam barrier because they are removed from the selective influence of the medium. See circle 1.



***E. coli* count = 3**

Any blue in a colony (blue to red-blue) indicates the presence of *E. coli*. Front lighting will enhance the detection of blue precipitate formed by a colony.

Circle 1 shows a red-blue colony counted using back lighting. Circle 2 shows the same colony with front lighting. The blue precipitate is more evident in circle 2.



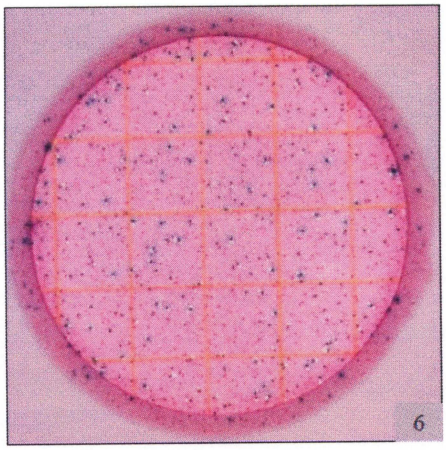
***E. coli* count = 17**

Estimated total coliform count = 150

The circular growth area is approximately 20 cm^2. Estimates can be made on plates containing greater than 150 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine the estimated count per plate.

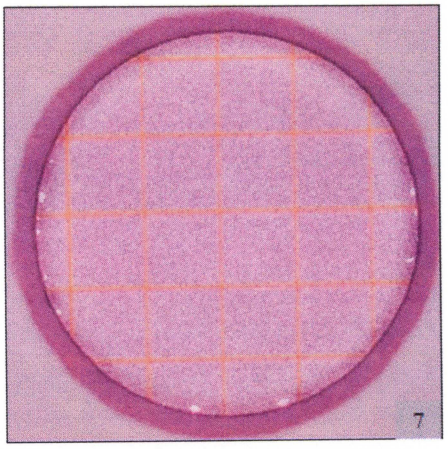
Attachment 4 – E. coli / Coliform Count Plate Interpretation Guide Excerpt (Cont'd)

TNTC (Too Numerous to Count) *To obtain a more accurate count, dilute the sample further:*



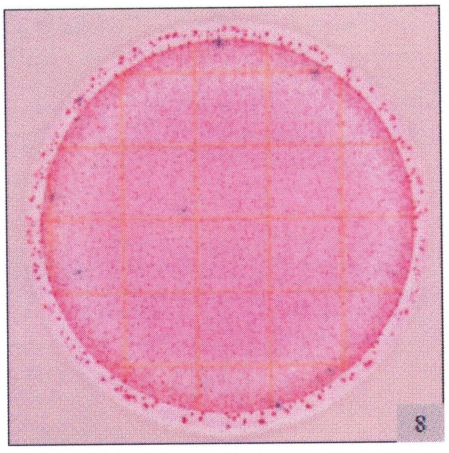
Actual count ~ 10^6

Petrifilm EC plates with colonies that are TNTC have one or more of the following characteristics: many small colonies, many gas bubbles, and a deepening of the gel color from red to purple-blue.



Actual count ~ 10^8

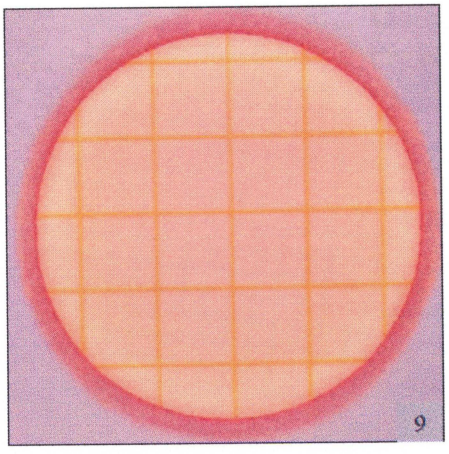
High concentrations of *E. coli* may cause the growth area to turn purple-blue.



Presumptive *E. coli* count ~ 8

Estimated total coliform count ~ 10^8

When high levels of coliforms are present (>10^5), some strains of *E. coli* may produce less gas and blue colonies may be less definitive. Count all blue colonies without gas and/or blue zones as presumptive *E. coli*. Pick blue colonies without gas and confirm if necessary.

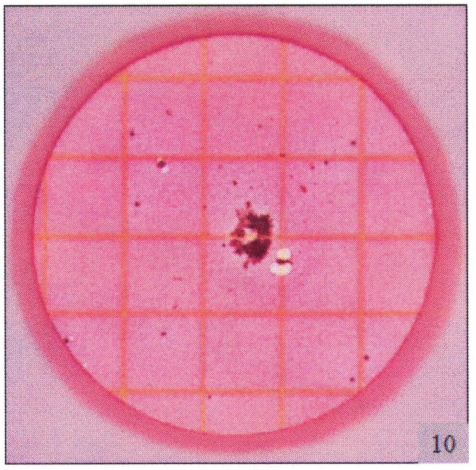


Actual count ~ 10^8

When high numbers of non-coliform organisms such as *Pseudomonas* are present on Petrifilm EC plates, the gel may turn yellow.

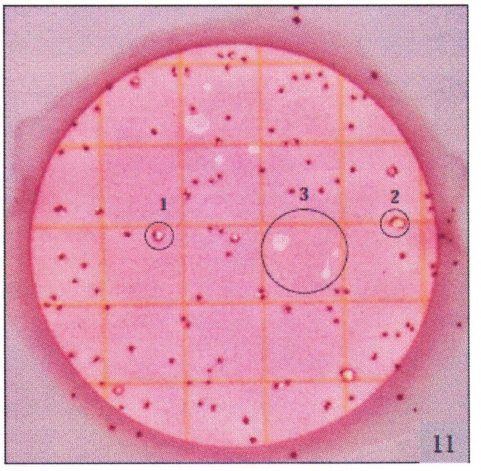
Attachment 4 – E. coli / Coliform Count Plate Interpretation Guide Excerpt (Cont'd)

Bubbles



Total coliform count = 3

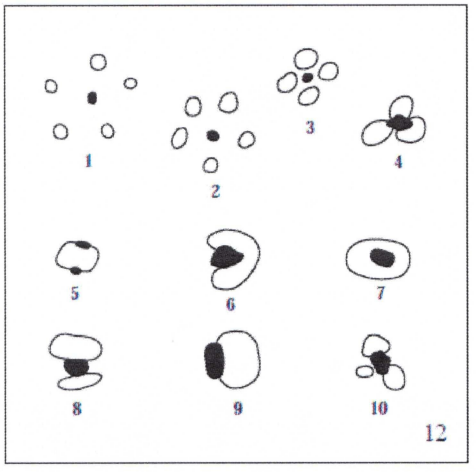
Food particles are irregularly shaped and are not associated with gas bubbles.



Total coliform count = 78

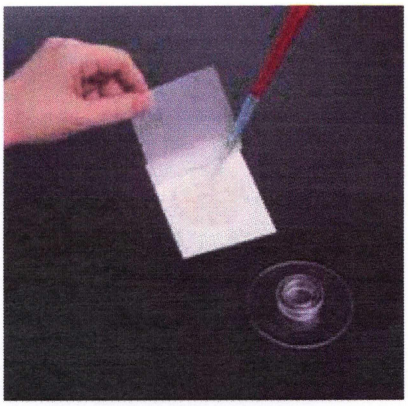
Bubble patterns may vary. Gas may disrupt the colony so that the colony "outlines" the bubble. See circles 1 and 2.

Artifact bubbles may result from improper inoculation or from trapped air within the sample. They are irregularly shaped and are not associated with a colony. See circle 3.



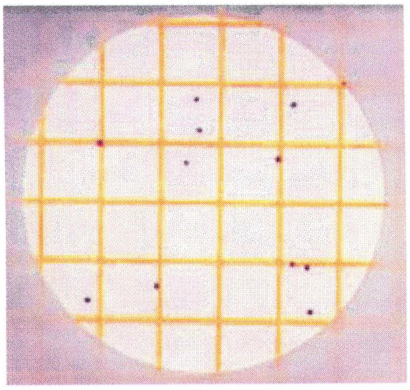
Examples 1–10 show various bubble patterns associated with gas producing colonies. All should be enumerated.

Attachment 5 – Staph Express Count Plate Preparation and Interpretation Guide Excerpt

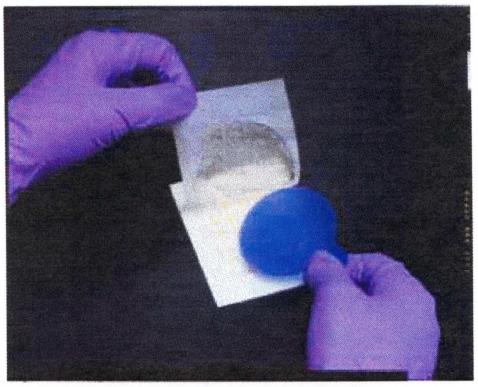


A) Inoculate Plate

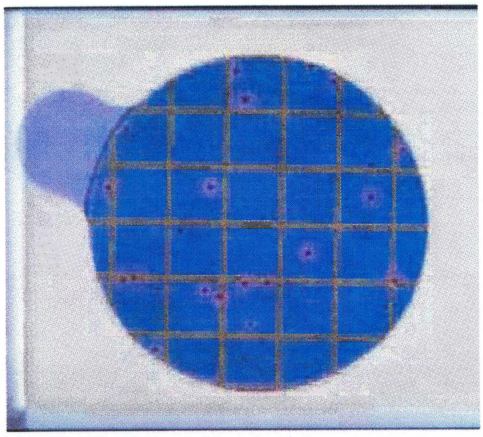
B) Stamp plate



C) Incubate Plate



D) Add Staph Express Disk



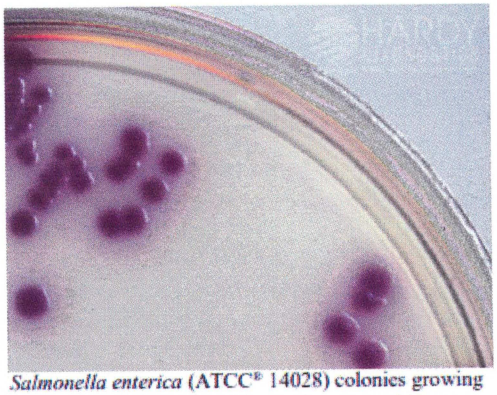
E) Read Plate- Staphylococcus aureus distinguished from other aerobic cultures by pink highlighted colonies.

Attachment 6 – Chromogenic Medium Interpretation Guide Excerpt

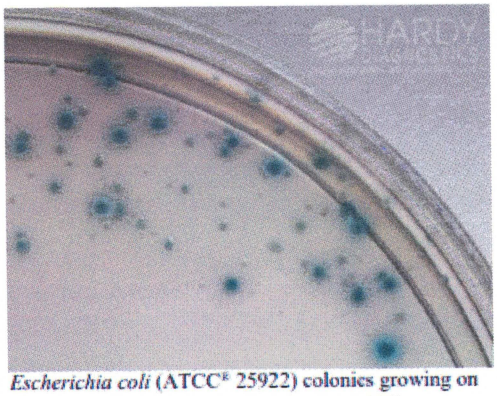
Method of Use: Allow the plates to warm to room temperature. The agar surface should be dry prior to inoculating. Streak for isolation with a sterile loop. Incubate plates in an inverted position, protected from the light, aerobically at 35-37 degrees C. for 24 hours. Examine plates for colonies showing typical morphology and color.

HardyCHROM™ Salmonella

Salmonella spp., including S. typhi and S. paratyphi A, produce magenta colored colonies. Other members of the Enterobacteriaceae (if present) produce blue, blue-green, white, or colorless colonies. Gram-positive bacteria and nonglucose fermenting bacteria will be inhibited.



Salmonella enterica (ATCC® 14028) colonies growing on HardyCHROM™ Salmonella (Cat. no. G309). Incubated aerobically for 24 hours at 35 deg. C.

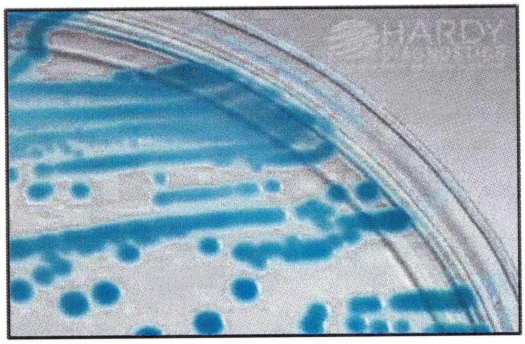


Escherichia coli (ATCC® 25922) colonies growing on HardyCHROM™ Salmonella (Cat. no. G309). Incubated aerobically for 24 hours at 35 deg. C.

HardyCHROM™ ECC

Pink to violet colored colonies are a positive test for the presence of E. coli. Turquoise colonies are a positive test for the presence of coliform bacteria other than E. coli. Other gram-negative bacteria appear as white or colorless colonies. Gram-positive bacteria are inhibited

Escherichia coli (ATCC&re6g; 25922) colonies growing on HardyCHROM™ ECC (Cat. no. G303). Incubated aerobically for 24 hours at 35°C.

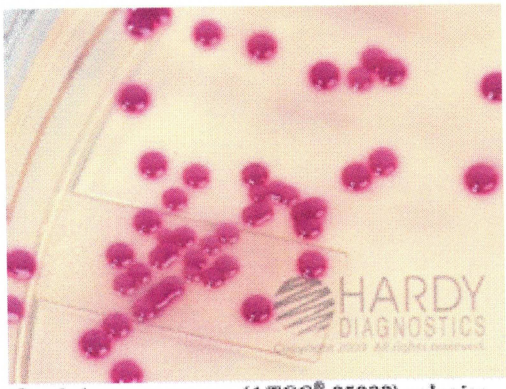


Klebsiella pneumoniae (ATCC® 13883) colonies growing on HardyCHROM™ ECC (Cat. no. G303). Incubated aerobically for 24 hours at 35°C.

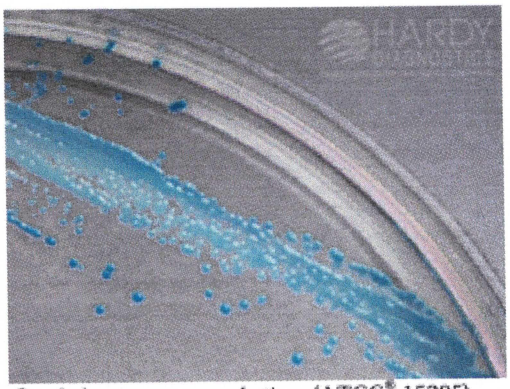
Attachment 6 – Chromogenic Medium Preparation and Interpretation Guide Excerpt (Cont'd)

HardyCHROM™ Staphylococcus aureus

Staphylococcus aureus will appear as smooth, deep pink to fuchsia colored colonies. Most other organisms, including Staphylococcus epidermidis will be partially to completely inhibit. Other organisms that may grow on HardyCHROM™ Staph aureus may appear as cream, blue, or colorless colonies. Staphylococcus saprophyticus will appear as turquoise colored colonies. Some gram-positive organisms other than S. aureus may appear as blue colonies.
Some non-S. aureus colonies may develop a light pink color after 48 hours. Do not incubate plates more than 24 to 28 hours.



Staphylococcus aureus (ATCC® 25923) colonies growing on HardyCHROM™ Staph aureus (Cat. no. G311). Incubated aerobically for 24 hours at 35°C.



Staphylococcus saprophyticus (ATCC® 15305) colonies growing on HardyCHROM™ Staph aureus (Cat. no. G311). Incubated aerobically for 24 hours at 35°C.

Attachment 7 – Finished Product Summary for Petrifilm Validated Dilution and Diluent

Product Profile Number	Product (g)	Dilution	Diluent
PGM00021	10 g	1:10	Peptone Salt-90 mL
PGM00022	10 g	1:10	Butterfields buffer- 90 mL
PLS00007	10 g	1:10	Peptone Salt-90 mL
PPW00005	10 g	1:10	Butterfields buffer- 90 mL
PPW00011	10 g	1:10	Peptone Salt- 90 mL
PPW00020	10 g	1:10	Butterfields buffer- 90 mL
PTB00001	10 g	1:10	Butterfields buffer-90 mL
PTB00012	10 g	1:10	Butterfields buffer-90 mL
PTB00014	10 g	1:10	Butterfields buffer-90 mL
PTB00019	10 g	1:10	Butterfields buffer-90 mL
PTB00020	10 g	1:10	Butterfields buffer-90 mL
PTB00023	10 g	1:10	Butterfields buffer-90 mL
PTB00026	10 g	1:10	Butterfields buffer-90 mL
SCT00004	10 g	1:10	Butterfields buffer-90 mL
SCT00024	10 g	1:10	Peptone Salt-90 mL
SCT00058	10 g	1:10	Peptone Salt-90 mL
SCT00059	10 g	1:10	Peptone Salt-90 mL
SCT00085	10 g	1:10	Peptone Salt-90 mL
SCT00113	10 g	1:10	Peptone Salt-90 mL
SCT00128	10 g	1:10	Peptone Salt-90 mL
SCT00179	10 g	1:10	Butterfields buffer- 90 mL
SCT00257	10 g	1:10	Peptone Salt-90 mL
SCT00297	10 g	1:10	Butterfields buffer- 90 mL
SCT00361	10 g	1:10	Butterfields buffer- 90 mL
SCT00364	10 g	1:10	Peptone Salt-90 mL
SCT00384	10 g	1:10	Peptone Salt-90 mL
SCT00392	10 g	1:10	Peptone Salt-90 mL
SCT00393	10 g	1:10	Butterfields buffer- 90 mL
SCT00439	10 g	1:50	Butterfields buffer
SCT00452	10 g	1:10	Peptone Salt – 90 mL
SCT00467	10 g	1:10	Butterfields buffer- 90 mL
SEC00011	10 g	1:10	Butterfields buffer- 90 mL
SEC00013	10 g	1:10	Butterfields buffer- 90 mL
SEC00025	10 g	1:10	Butterfields buffer- 90 mL
SEC00030	10 g	1:10	Butterfields buffer- 90 mL
SEC00037	10 g	1:10	Butterfields buffer- 90 mL
SEC00038	10 g	1:10	Butterfields buffer- 90 mL
SEC00040	10 g	1:10	Butterfields buffer- 90 mL

Attachment 7 – Finished Product Summary for Petrifilm Validated Dilution and Diluent (continued)

Product Profile Number	Product (g)	Dilution	Diluent
SEC00052	10 g	1:10	Butterfields buffer- 90 mL
SEC00079	10 g	1:10	Peptone Salt-90 mL
SEC00133	10 g	1:10	Peptone Salt-90 mL
SEC00139	10 g	1:10	Butterfields buffer- 90 mL
SEC00144	10 g	1:10	Butterfields buffer- 90 mL
SEC00176	10 g	1:10	Peptone Salt-90 mL
SEC00199	10 g	1:10	Butterfields buffer-90 mL
SEC00200	10 g	1:10	Peptone Salt-90 mL
SEC00205	10 g	1:10	Peptone Salt-90 mL
SEC00208	10 g	1:10	Peptone Salt-90 mL
SEC00220	10 g	1:10	Peptone Salt-90 mL
SEC00237	10 g	1:10	Peptone Salt-90 mL
SEC00239	10 g	1:10	Butterfields buffer-90 mL
SEC00243	10 g	1:10	Peptone Salt-90 mL
SEC00264	10 g	1:10	Peptone Salt-90 mL
SEC00269	10 g	1:10	Butterfields buffer-90 mL
SEC00270	10 g	1:10	Peptone Salt-90 mL
SEC00290	10 g	1:10	Peptone Salt-90 mL
SEC00291	10 g	1:10	Peptone Salt-90 mL
SEC00295	10 g	1:10	Peptone Salt-90 mL
SEC00308	10 g	1:10	Butterfields buffer-90 mL
SEC00320	10 g	1:10	Butterfields buffer-90 mL
SEC00325	10 g	1:10	Butterfields buffer-90 mL
SEC00327	10 g	1:10	Peptone Salt-90 mL
SEC00332	10 g	1:10	Butterfields buffer-90 mL
SEC00333	10 g	1:10	Butterfields buffer-90 mL
SEC00341	10 g	1:10	Butterfields buffer-90 mL
SEC00362	10 g	1:10	Butterfields buffer- 90 mL
SEC00368	10 g	1:10	Butterfields buffer-90 mL
SEC00372	10 g	1:10	Butterfields buffer- 90 mL
SEC00373	10 g	1:10	Butterfields buffer- 90 mL
SEC00398	10 g	1:10	Peptone Salt-90 mL
SEC00401	10 g	1:10	Butterfields buffer-90 mL
SEC00403	10 g	1:10	Peptone Salt-90 mL
SEC00421*	10 g	1:10	Butterfields buffer-90 mL
SEC00423	10 g	1:10	Peptone Salt- 90 mL
SEC00426	10 g	1:10	Peptone Salt-90 mL

* Refer Method Suitability test details

Attachment 7 – Finished Product Summary for Petrifilm Validated Dilution and Diluent (continued)

Product Profile Number	Product (g)	Dilution	Diluent
SEC00433	10 g	1:10	Peptone Salt-90 mL
SEC00438	10 g	1:10	Peptone Salt- 90 mL
SEC00442	10 g	1:10	Peptone Salt- 90 mL
SEC00498	10 g	1:50	Butterfields buffer + 14 mL of 1N HCl
SEC00593*	10 g	1:10	Butterfields buffer-90 mL
SEC00601	10 g	1:50	Butterfields buffer
SEC00602	10 g	1:100	Butterfields buffer + 17 mL of 1N NaOH
SEC00609	10 g	1:10	Butterfields buffer-90 mL
SEC00610	10 g	1:50	Butterfields buffer + 3 mL of 1N HCl
SEC00617	10 g	1:50	Butterfields buffer + 25 mL of 1N HCl
SGM00281	10 g	1:10	Peptone Salt-90 mL
SGM00331	10 g	1:10	Peptone Salt-90 mL
SGM00336	10 g	1:10	Peptone Salt-90 mL
SGM00337	10 g	1:10	Peptone Salt-90 mL
SGM00349	10 g	1:10	Peptone Salt-90 mL
SGM00351	10 g	1:10	Peptone Salt-90 mL
SGM00357	10 g	1:10	Butterfields buffer-90 mL + 5 mL of 1N NaOH
SGM00358	10 g	1:10	Peptone Salt-90 mL
SGM00359	10 g	1:10	Peptone Salt-90 mL
SGM00360	10 g	1:10	Peptone Salt-90 mL
SGM00379	10 g	1:10	Peptone Salt-90 mL
SGM00391	10 g	1:10	Peptone Salt-90 mL
SGM00418	10 g	1:10	Peptone Salt- 90 mL
SGM00428	10 g	1:10	Peptone Salt-90 mL
SGM00475	10 g	1:10	Butterfields buffer-90 mL
SGM00507	10 g	1:20	Butterfields buffer + 4 mL of 1N NaOH
SGM00549	10 g	1:20	Butterfields buffer + 5 mL of 1N NaOH
SGM00550*	10 g	1:10	Butterfields buffer-90 mL
SGM00552	10 g	1:10	Butterfields buffer-90 mL
SGM00553 CA	10 g	1:10	Butterfields buffer-90 mL + 1 mL of 1N NaOH
SGM00558	10 g	1:10	Butterfields buffer-90 mL
SGM00562	10 g	1:10	Butterfields buffer-90 mL
SGM00567	10 g	1:20	Butterfields buffer
SGM00585	10 g	1:10	Butterfields buffer-90 mL + 2 mL of 1N NaOH
SGM00598	10 g	1:10	Butterfields buffer-90 mL + 2 mL of 1N NaOH
SLC00039	10 g	1:10	Peptone Salt-90 mL
SLC00048	10 g	1:10	Peptone Salt-90 mL
SLC00049	10 g	1:10	Butterfields buffer-90 mL
SLC00056	10 g	1:10	Butterfields buffer-90 mL

* Refer Method Suitability test details

Attachment 7 – Finished Product Summary for Petrifilm Validated Dilution and Diluent (continued)

Product Profile Number	Product (g)	Dilution	Diluent
SLC00084	10 g	1:10	Butterfields buffer-90 mL
SLC00173	10 g	1:10	Butterfields buffer-90 mL
SLC00174	10 g	1:10	Butterfields buffer-90 mL
SLC00183	10 g	1:10	Peptone Salt-90 mL
SLC00215	10 g	1:10	Butterfields buffer-90 mL
SLC00218	10 g	1:10	Butterfields buffer-90 mL
SLC00232	10 g	1:10	Butterfields buffer-90 mL
SLC00234	10 g	1:10	Butterfields buffer-90 mL
SLC00321	10 g	1:10	Butterfields buffer-90 mL
SLC00334	10 g	1:10	Butterfields buffer-90 mL
SLC00343	10 g	1:10	Butterfields buffer-90 mL
SLC00356	10 g	1:10	Peptone Salt-90 mL
SLC00375	10 g	1:10	Butterfields buffer-90 mL
SLC00381	10 g	1:10	Peptone Salt- 90 mL
SLC00385	10 g	1:10	Butterfields buffer-90 mL
SLC00387	10 g	1:10	Butterfields buffer-90 mL
SLC00389	10 g	1:10	Butterfields buffer-90 mL
SLC00396	10 g	1:10	Peptone Salt-90 mL
SLC00408	10 g	1:10	Butterfields buffer-90 mL
SLC00415	10 g	1:10	Butterfields buffer-90 mL
SLC00425	10 g	1:10	Peptone Salt-90 mL
SLC00508	10 g	1:10	Butterfields buffer-90 mL
SLC00575	10 g	1:10	Butterfields buffer-90 mL
SLS00226	10 g	1:10	Butterfields buffer-90 mL
SLS00305	10 g	1:10	Butterfields buffer-90 mL
SLS00306	10 g	1:10	Peptone Salt-90 mL
SLS00307	10 g	1:10	Peptone Salt-90 mL
SLS00348	10 g	1:10	Butterfields buffer-90 mL
SLS00355	10 g	1:10	Butterfields buffer-90 mL
SLS00370	10 g	1:10	Butterfields buffer-90 mL
SLS00394	10 g	1:10	Butterfields buffer-90 mL
SLS00395	10 g	1:10	Butterfields buffer-90 mL
SLS00405	10 g	1:10	Butterfields buffer-90 mL
SLS00427	10 g	1:10	Peptone Salt-90 mL
SPW00003	10 g	1:10	Butterfields buffer-90 mL
SPW00008	10 g	1:10	Butterfields buffer-90 mL
SPW00080	10 g	1:10	Butterfields buffer-90 mL
SPW00149	10 g	1:10	Butterfields buffer-90 mL
SPW00157	10 g	1:10	Butterfields buffer-90 mL

Attachment 7 – Finished Product Summary for Petrifilm Validated Dilution and Diluent (continued)

Product Profile Number	Product (g)	Dilution	Diluent
SPW00193	10 g	1:10	Peptone Salt-90 mL
SPW00278 CA	10 g	1:10	Butterfields buffer-90 mL
SPW00278 US	10 g	1:10	Butterfields buffer-90 mL
SPW00339	10 g	1:10	Peptone Salt-90 mL
SPW00342	10 g	1:10	Peptone Salt-90 mL
SPW00352	10 g	1:10	Butterfields buffer-90 mL
SPW00353	10 g	1:10	Butterfields buffer-90 mL
SPW00354	10 g	1:10	Butterfields buffer-90 mL
SPW00365	10 g	1:10	Butterfields buffer-90 mL
SPW00366	10 g	1:10	Butterfields buffer-90 mL
SPW00367	10 g	1:10	Butterfields buffer-90 mL
SPW00376	10 g	1:10	Peptone Salt-90 mL
SPW00383	10 g	1:10	Butterfields buffer-90 mL
SPW00400	10 g	1:10	Butterfields buffer-90 mL
SPW00413	10 g	1:10	Butterfields buffer-90 mL
SPW00417	10 g	1:10	Peptone Salt-90 mL
SPW00429	10 g	1:10	Peptone Salt-90 mL + 25 mL of 1N NaOH
SPW00430	10 g	1:10	Peptone Salt-90 mL + 25 mL of 1N NaOH
SPW00431	10 g	1:10	Peptone Salt-90 mL + 25 mL of 1N NaOH
SPW00432	10 g	1:10	Peptone Salt-90 mL + 25 mL of 1N NaOH
SPW00434	10 g	1:10	Peptone Salt-90 mL + 25 mL of 1N NaOH
SPW00435	10 g	1:10	Peptone Salt-90 mL + 25 mL of 1N NaOH
SPW00436	10 g	1:10	Peptone Salt-90 mL
SPW00437	10 g	1:10	Peptone Salt-90 mL
SPW00445	10 g	1:10	Butterfields buffer-90 mL
SPW00458	10 g	1:10	Butterfields buffer-90 mL
SPW00460	10 g	1:10	Butterfields buffer-90 mL
SPW00468	10 g	1:50	Butterfields buffer + 8 mL of 1N NaOH
SPW00469	10 g	1:50	Butterfields buffer + 7 mL of 1N NaOH
SPW00488	10 g	1:100	Butterfields buffer-900 mL
SPW00489	10 g	1:50	Butterfields buffer + 4 mL of 1N NaOH
SPW00490	10 g	1:100	Butterfields buffer-900 mL
SPW00523	10 g	1:50	Peptone Salt + 3 mL of 1N NaOH
SPW00530	10 g	1:50	Butterfields buffer + 6 mL of 1N NaOH
SPW00565	10 g	1:50	Butterfields buffer + 4 mL of 1N NaOH
SSG00087	10 g	1:10	Peptone Salt-90 mL
STB00134	10 g	1:10	Butterfields buffer-90 mL
STB00177	10 g	1:10	Peptone Salt-90 mL
NFIG00001	10 g	1:10	Butterfields buffer-90 mL

Attachment 7 – Finished Product Summary for Petrifilm Validated Dilution and Diluent (continued)

Product Profile Number	Product (g)	Dilution	Diluent
NFIG00007	10 g	1:10	Butterfields buffer-90 mL
NFYO00003	10 g	1:10	Butterfields buffer-90 mL