



Laboratory Guidebook Notice of Change

Chapter new, **revised**, or archived: MLG 3.02

Title: Quantitative Analysis of Bacteria in Foods as Sanitary Indicators

Effective Date: 01/05/15

Description and purpose of change(s):

FSIS validated an automated MPN system for addition to this chapter for the enumeration of sanitary indicators in meat carcass sponges, poultry rinses, and raw beef, raw pork, and raw poultry products.

Section 3.5 was revised to provide clarification on the dilution factors and preparation of serial dilutions.

The methods described in this guidebook are for use by the FSIS laboratories. FSIS does not specifically endorse any of the mentioned test products and acknowledges that equivalent products may be available for laboratory use. Method validation is necessary to demonstrate the equivalence of alternative tests as detailed in the document titled “FSIS Guidance for Evaluating Test Kit Performance” available on the FSIS website.

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3.1 Introduction

The laboratory methods contained in this section of the Guidebook are used to detect and, when desired, quantify elected microorganisms in meat, poultry, catfish and pasteurized egg products. They generally follow the Compendium of Methods for the Microbiological Examination of Foods and AOAC International's Official Methods of Analysis. The methods presented in this section may be used to analyze the following types of samples:

- a. fresh, frozen, smoked, cured or dehydrated meat and poultry products;
- b. prepared/ready-to-eat products such as pot pies, luncheon meats, dinners, battered or breaded meat and poultry products;
- c. refrigerated meat or poultry salads;
- d. dehydrated soups and sauces containing meat or poultry;
- e. meat snacks, hors d'oeuvres, pizza and specialty items;
- f. various ingredients incorporated with meat and poultry products such as spices, vegetables, breading material, milk powder, dried egg, vegetable proteins;
- g. pasteurized egg products;
- h. environmental samples from areas in which any of the above are processed or manufactured.
- i. fresh and frozen catfish

The quantity and types of mesophilic microorganisms present in or on any of these products may offer a means of evaluating the degree of sanitation.

Unless otherwise stated all measurements cited in this method have a tolerance range of $\pm 2\%$.

3.2 Safety

The organisms referenced in this procedure are generally categorized as Biosafety Level 2 (BSL-2) pathogens. CDC guidelines for manipulating Biosafety Level 2 pathogens

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should be followed whenever live cultures are used. A Class II laminar flow biosafety cabinet is recommended for procedures in which infectious aerosols or splashes may be created. All available Safety Data Sheets (SDS) must be obtained from the manufacturer for the media, chemicals, reagents and microorganisms used in the analysis.

3.3 Quality Control Practices

Positive and sterility controls and an air plate for environmental monitoring will be used with each set of sanitary indicator tests. Use 1 ml of Butterfield's Phosphate diluent to inoculate an APC Petrifilm™ or APC pour plate. Leave open on bench for 15 minutes. Incubate with samples. APC plate count should be ≤ 15 CFU/plate in 15 ± 2 minute exposure. Refer to the methods below for additional quality control information. Air plates for environmental monitoring are not required for closed systems such as TEMPO®.

3.4 Equipment, Media, Reagents and Test Kits

3.4.1 Equipment

- a. Balance, capacity ≥ 2 kg, sensitivity ± 0.1 g
- b. Blender and sterile blender jars
- c. Stomacher® and sterile stomacher bags, Whirl-Paks®, or equivalent
- d. Incubators at $35 \pm 1.0^\circ\text{C}$, and $20 \pm 1.0^\circ\text{C}$
- e. Water bath at $45.5 \pm 0.5^\circ\text{C}$ and $37 \pm 1.0^\circ\text{C}$
- f. Freezer at $\leq -10^\circ\text{C}$
- g. Manual or Automatic colony counter and tally register
- h. Sterile, disposable/reusable dishes, pans or trays for sample cutting
- i. Sterile forceps, spoon, knife, scissors and other sterile sampling equipment
- j. Sterile 1, 5 and 10 ml pipettes, pipettors, or equivalent
- k. Sterile 100 x 15 mm petri dishes
- l. Transfer loop, 3 mm
- m. Refrigerator at $2-8^\circ\text{C}$
- n. TEMPO® System

3.4.2 Media

- a. Plate count agar (PCA) in containers suitable for making pour plates
- b. Baird-Parker Medium

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- c. Brain heart infusion (BHI) broth

3.4.3 Reagents and Test kits

- a. Butterfield's phosphate diluents (or BPW if required)
- b. Desiccated rabbit plasma (coagulase) EDTA
- c. Aerobic Plate Count Petrifilm™
- d. Coliform/*E. coli* Petrifilm™
- e. *Enterobacteriaceae* Petrifilm™
- f. TEMPO® AC (Aerobic Count)
- g. TEMPO® CC (Coliform Count)
- h. TEMPO® EB (*Enterobacteriaceae*)
- i. TEMPO® EC (*E. coli*)

3.5 Preparation and Dilution of Samples

3.5.1 General Guidelines for Testing Fresh or Prepared Foods

- a. The quantity, condition and suitability of the sample are very important:
 - i. The quantity should be sufficient to perform the analysis and for reserve to repeat testing if needed.
 - ii. The condition of receipt should be in keeping with good microbiological practices for the analysis requested.
 - iii. The sample should be, to the greatest extent possible, representative of the whole of the original product at the time the sample was taken.
 - iv. When appropriate and if possible, samples should be received at the laboratory in their original unopened package(s) (intact sample).
- b. For multi-component RTE products, follow the appropriate sample preparation list below:
 - i. If the meat or poultry component is separate and distinct from other non-meat ingredient, analyze only the representative meat/poultry portion of the RTE product.
 - ii. When meat/poultry is combined with other ingredients to form the product, analyze representative meat/poultry portions in combination with other ingredients.

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3.5.2 Food Homogenates

- a. Using sterile spoons, forceps, scissors, etc., aseptically weigh 50 ± 0.1 g of the sample into a sterile blender jar or stomacher bag.
- b. If the sample is frozen, remove portions, whenever possible, without thawing the larger sample and weigh 50 ± 0.1 g of the sample into a sterile blender jar or stomacher bag. It is well known that freeze/thaw cycles are damaging to bacteria. This is particularly important when a re-examination of the product may be necessary. Otherwise, partially thaw the sample at $2-8^{\circ}\text{C}$ for about 18 h, or by placing the sample in a watertight container and immersing it in cold water for 1-2 h.
- c. Add 450 ml sterile Butterfield's phosphate diluent or Buffered Peptone Water (BPW) and stomach for 2 minutes, or blend at high speed for two minutes. The total volume in the blender jar must completely cover the blades. This becomes the 1:10 dilution.
- d. From the prepared food homogenate make appropriate serial dilutions. For example: Pipette 10 ml of the blended 1:10 dilution into a 90 ml dilution blank to make the 1:100 dilution. Avoid pipetting foam. Repeat this procedure to prepare serial dilutions of 10^{-3} , 10^{-4} , etc. Shake all dilutions about 25 times in an approximately one-foot arc or vortex thoroughly. Use a separate pipette to prepare each dilution. Pipettes must deliver accurately the required volumes. Do not deliver less than 10% of a pipette's volume. For example, to deliver 1 ml, do not use a pipette of more than 10 ml volume.
- e. The analyst should strive to minimize the time from when the sample is stomached or blended until all the dilutions were placed in or on the appropriate medium; this time should not exceed 20 minutes.
- f. If the sample consists of less than 50 g, weigh about half the sample, and add the amount of diluent required to make a 1:10 dilution (nine times the weight of the portion of sample used) and proceed as above.
- g. Hold reserves of each sample at $\leq -10^{\circ}\text{C}$, unless the product is stored normally at ambient temperature or unless a specific protocol specifies otherwise.

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3.5.3 Whole Bird Rinse

- a. Since there are differences between sample types and sizes (e.g. chicken vs. turkey carcasses), be sure to check the specific program protocol before using this procedure. Note: BPW may be substituted for the Buffered Phosphate Diluent (BPD).
- b. Aseptically transfer the carcass to a sterile Stomacher 3500 bag (or equivalent), draining as much excess fluid as possible during the transfer.

Note: Larger (24 x 30-36 in.) bags will have to be used with turkeys.
- c. Add 400 ml (chickens) or 600 ml (turkeys) of BPD to the carcass in the bag. Pour approximately one-half the volume into the interior cavity of the bird and the other half over the skin.
- d. Rinse the bird, inside and out, with a rocking motion for 1 min at a rate of approximately 35 forward and back swings per minute. This is done by grasping the carcass in the bag with one hand and the closed top of the bag with the other. Rock the sample with a reciprocal motion in an approximately one-foot arc, assuring that all surfaces (interior and exterior) are rinsed.
- e. Aseptically remove the carcass from the bag, draining excess rinsed liquid into the bag, dispose of the carcass.
- f. From the prepared rinsate make appropriate serial dilutions. For example: Pipette 10 ± 0.1 ml of the rinse sample into a 90 ml dilution blank to make a 1:10 dilution. Repeat this procedure to prepare serial dilutions of 10^{-2} - 10^{-6} . More dilutions may be required if a sample is suspected of having a high microbial load $> 10^7$ CFU/g.
- g. Shake all dilutions 25 times in an approximate a one-foot arc or vortex thoroughly. Use a separate pipette to prepare each dilution.
- h. The analyst should strive to minimize the time from when the sample is stomached or blended until all the dilutions were placed in or on the appropriate medium; this time should not exceed 20 minutes.

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3.5.4 Pasteurized Egg Products

- a. Mix the sample with a sterile spoon, spatula, or by shaking.
- b. Aseptically weigh 100 g of egg sample into a sterile blender jar or sealable bag containing 900 ml of the appropriate enrichment or buffer. If a specific protocol requires a sample size other than 100 g, the 1:10 ratio must be maintained in the same enrichment or buffer.
- c. Mix the 1:10 sample enrichment/buffer well by shaking, stomaching, or blending.
- d. With dried egg products, gradually add diluent to the sample. Add a small portion of sterile diluent and mix to obtain a homogeneous suspension. Add the remainder of the diluent. Mix until a lump-free suspension is obtained.
- e. From the prepared egg sample make appropriate serial dilutions. For example: Pipette 10 ± 0.1 ml of the mixed 1:10 dilution into a 90 ml dilution blank to make a 1:100 dilution. Repeat this procedure to prepare serial dilutions of 10^{-3} , 10^{-4} , 10^{-5} , and 10^{-6} .
- f. Shake all dilutions 25 times in an approximate a one-foot arc or vortex thoroughly. Use a separate 10 ml pipette to prepare each dilution.
- g. The analyst should strive to minimize the time from when the sample is stomached or blended until all the dilutions were placed in or on the appropriate medium; this time should not exceed 20 minutes.

3.5.5 Catfish Products

- a. Weigh 25 ± 2.5 g of tissue into a sterile blender jar, other sterile jar or a Whirl-Pak™ or Stomacher™ bag.
- b. Add 225 ± 22.5 ml of BPD or BPW for a 1:10 dilution. Stomach or blend, as required, for approximately two minutes or shake thoroughly.
- c. From the prepared catfish sample make appropriate serial dilutions. For example: Pipette 10 ± 0.1 ml of the stomached 1:10 dilution into a 90 ml dilution blank to make a 1:100 dilution. Repeat this procedure to prepare

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serial dilutions of 10^{-3} , 10^{-4} , 10^{-5} , and 10^{-6} . More dilutions may be required if a sample is suspected of having a high microbial load of $> 10^7$ CFU/g.

- d. Shake all dilutions approximately 25 times in an approximate a one-foot arc or vortex thoroughly. Use a separate 10 ml pipette to prepare each dilution.
- e. The analyst should strive to minimize the time from when the sample is stomached or blended until all the dilutions were placed in or on the appropriate medium; this time should not exceed 20 minutes.

3.6 Aerobic Plate Count (APC)

3.6.1 APC Controls

- a. For the positive control, prepare a stock of *S. aureus* ATCC 25923 or equivalent. Dilute the stock to a countable range and dispense 1 ml of the stock dilution onto Petrifilm™ or equivalent.
- b. For the sterility control, dispense 1 ml of the diluent on Petrifilm™ or equivalent.

3.6.2 Dry APC Rehydratable Film (Aerobic Plate Count Petrifilm™) Method

- a. Plate $1 \pm .01$ ml from each dilution onto duplicate APC Petrifilm™ for each sample. Samples should be dispensed onto plates within 20 minutes of preparing the initial dilution.
- b. Incubate the APC Petrifilm™ at $35 \pm 1^{\circ}\text{C}$ for 48 ± 3 hours.
- c. Count colonies on the duplicate Petrifilm™ plates. Count all red colonies on Petrifilm™ regardless of their size or color intensity. The countable range is 25-250 CFU. Refer to Section 3.11 Calculating Results and Reporting.

3.6.3 Plate Count Agar (PCA) Pour Plates

Pour plates can be used as an alternative method to APC Petrifilm™ for aerobic plate counts.

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- a. Using the dilutions prepared in Section 3.5, pipette 1 ml from the 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} etc. dilutions into each of 2 Petri dishes. Plate additional prepared dilutions when expecting higher bacterial levels.
- b. Use separate sterile pipettes for each dilution.
- c. Add molten Plate Count Agar cooled in a water bath to $45 \pm 1^{\circ}\text{C}$. Pour enough of the tempered agar to cover bottom of a Petri dish and uniformly mix the agar and the inoculum by gently swirling or tilting each plate, taking care not to generate bubbles.
- d. Allow the agar to harden and then incubate at $35 \pm 1^{\circ}\text{C}$ for 48 ± 2 h.
- e. Count colonies on the duplicate plates. The countable range is 30-300 colonies per plate. Refer to Section 3.11 Calculating Results and Reporting.

3.7 *Escherichia coli* and Coliform Group

3.7.1 *Escherichia coli* and Coliform Group Controls

- a. For a positive *E. coli* control with blue colonies and gas, prepare a stock of *E. coli* ATCC 25922 or equivalent. Dilute the stock to a countable range and dispense 1 ml of the appropriate dilution onto Petrifilm™.
- b. For a positive coliform control with red colonies and gas, make a stock of *Enterobacter aerogenes* ATCC 13048 or equivalent, dilute to a countable range, and dispense 1 ml of the stock dilution onto Petrifilm™.
- c. For a negative sterility control, dispense 1 ml of the diluent on Petrifilm™.

3.7.2 *Escherichia coli* and Coliform Dry Rehydratable Film Count (*E. coli*/coliform Petrifilm™) Method

- a. Plate $1 \pm .01$ ml from each dilution onto duplicate *E. coli*/coliform Petrifilm™ for each sample. Samples should be dispensed onto plates within 20 minutes of preparing the initial dilution.
- b. Incubate *E. coli*/coliform Petrifilm™ at $35 \pm 1^{\circ}\text{C}$ for 24 ± 2 hours.

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- c. The following day, count colonies on the duplicate Petrifilm™. On *E. coli*/coliform Petrifilm™, count blue to red-blue colonies with entrapped gas for *E. coli* and for coliforms count red coliform colonies with gas. Do not count colonies that appear on the foam barrier. The countable range is 15-150 CFU. Refer to Section 3.11 Calculating Results and Reporting.
- d. Record “Total Coliforms” by adding *E. coli* and coliform counts.

3.8 *Staphylococcus aureus*

3.8.1 *Staphylococcus aureus* Plating/Coagulase Controls

- a. For a positive culture control, prepare a stock of *S. aureus* ATCC 25923 or equivalent. Dilute the stock to a countable range and dispense 0.1 ml of the stock dilution onto Baird Parker. For a negative culture control, prepare a stock of *S. epidermidis* ATCC 12228 or equivalent, dilute down to a countable range and dispense 0.1 ml onto Baird-Parker.
- b. For the sterility control, dispense 1 ml of the diluent on Baird-Parker and/or in BHI broth.

3.8.2 Direct Plating for *S. aureus*

Baird-Parker plates need to be at room temperature and labeled prior to inoculations.

- a. Pipet $1 \pm .01$ ml from the 1:10 sample dilution and split between three Baird-Parker plates. Pipet 0.1 ml from the 1:10 dilution in duplicate on Baird-Parker plates to represent the 10^{-2} dilution in duplicate.
- b. Pipet 0.1 ml from each remaining dilution on the corresponding Baird-Parker agar plates in duplicate. Use separate pipettes for each dilution.
- c. Distribute the inoculum evenly over the surface of the plates using sterile hockey sticks or equivalent for each dilution.
- d. Invert plates and incubate at $35 \pm 1^{\circ}\text{C}$ for 45-48 hours.
- e. Count typical *S. aureus* colonies. The countable range is 20–200 colonies on all plates. Typical colonies are circular, convex, smooth, grey-black to jet-black

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and frequently have an off-white margin surrounded by a zone of precipitation (turbidity) followed by a clear zone. The colonies usually have a buttery to gummy consistency.

- f. Select 10 colonies, if available, from those counted and inoculate each into separate tubes containing 0.2 ml of BHI broth for coagulase testing. Test for coagulase as in Section 3.8.3.
- g. Calculate the total number of colonies represented by coagulase positive cultures and multiply by the appropriate sample dilution factor to record the number of coagulase positive staphylococci per gram.

3.8.3 Coagulase Test for *S. aureus*

- a. Use an inoculating needle to obtain a small amount of growth from each suspect colony and place it into tubes containing 0.2 ml of BHI Broth.
- b. Incubate each tube at $35 \pm 1^\circ\text{C}$ for 18-24 h.
- c. Add 0.5 ml of rabbit plasma with EDTA, reconstituted according to the manufacturer's directions, to the BHI cultures.
- d. Mix thoroughly and incubate at $35 \pm 1^\circ\text{C}$ or in accordance with manufacturer's instructions.
- e. Examine these tubes periodically over 6 h interval for clot formation. Any degree of clotting should be interpreted as a positive reaction. Coagulase-positive cultures are considered *S. aureus*. Tubes that do not display any degree of clotting after the 6 h incubation are negative.

3.9 *Enterobacteriaceae*

3.9.1 *Enterobacteriaceae* Petrifilm™ Controls

- a. For a positive control, prepare a stock of *Klebsiella pneumonia* ATCC 13883 or equivalent. Dilute the stock to a countable range and dispense 1 ml of the stock dilution onto Petrifilm™.
- b. For a negative sterility control, dispense 1 ml of the diluent on Petrifilm™.

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3.9.2 *Enterobacteriaceae* Dry Rehydratable Film (Petrifilm™) Method

- a. Plate 1 ± 0.01 ml from each dilution onto duplicate *Enterobacteriaceae* Petrifilm™ for each sample. Samples should be dispensed onto plates within 20 minutes of preparing the initial dilution.
- b. Incubate *Enterobacteriaceae* Petrifilms™ at $35 \pm 1^{\circ}\text{C}$ for 24 ± 2 hours.
- c. The following day, count colonies on the duplicate Petrifilm™ in the suitable range. Typical colonies produce gas and/or acid. Count red colonies associated with gas bubbles or colonies surrounded by yellow zones with or without gas. The countable range is 15-100 CFU. See Section 3.11 for calculating results and reporting.

3.10 Lactic Acid Organisms

3.10.1 Lactic Acid Controls

- a. For a positive control, prepare a stock of *Lactobacillus plantarum* ATCC 14917 or equivalent. Dilute the stock to a countable range and dispense 1 ml of the stock dilution in an APT agar pour plate.
- b. For a negative sterility control, dispense 1 ml of Butterfield's Phosphate diluents in an APT agar pour plate.

3.10.2 APT Pour Plate Method

- a. Plate 1 ± 0.01 ml from each dilution into duplicate Petri dishes for each sample. Plate additional dilutions when expecting higher bacterial levels.
- b. Use separate sterile pipettes for each dilution.
- c. Add molten APT Agar cooled in a water bath to $42-45^{\circ}\text{C}$. Pour enough of the tempered agar to cover bottom of a petri dish and uniformly mix the agar and the inoculum by gently swirling or tilting each plate, taking care not to generate bubbles.

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- d. Allow agar to harden and then incubate plates at $20 \pm 1^{\circ}\text{C}$ for 4-5 days.
- e. Count colonies on the duplicate plates in a suitable range (30-300 colonies per plate). See Section 3.11 for Calculating Results and Reporting.

3.11 Calculating Results and Reporting

Note: When plating the 1:10 dilution of *Staphylococcus aureus*, 1 ml is split among three plates. To calculate the number of colony forming units of *Staphylococcus aureus* **at the 1:10 dilution only**, add the total number of colonies from the three plates at the 1:10 dilution and multiply by the reciprocal of the dilution factor (10).

Express plate count results as colony forming units (CFU) per gram or per ml reflecting the original sample measurement. Select plates that appear to have the appropriate range of colonies for counting. The countable range may vary by method. See the chart below for a brief listing of the countable ranges for several methods.

Test – Medium	Countable Range (CFU)
APC: Petrifilm™	25 to 250
APC: Pour Plates	30 to 300
<i>Enterobacteriaceae</i> : Petrifilm™	15 to 100
APT (20°C psychrotrophic lactic bacteria)	30 to 300
<i>E.coli</i> /coliforms: <i>E. coli</i> /coliform Petrifilm™	15 to 150
<i>S. aureus</i> : Baird-Parker agar plates	20 to 200

3.11.1 All Plates in the Countable Range Are From the Same Dilution

If the plates with colonies in the countable range are from the same dilution, multiply the average number of colonies per plate by the reciprocal of the dilution used. *Example:* counts at the 1:100 dilution are 45 and 49. The average is 47. (The reciprocal of 1/100 is 100.) Multiply 47 by 100. The result is 4700.

$$\left[\left(\frac{45 + 49}{2} \right) * 100 \right] = 4700$$

Divide the result by the fraction of a micro liter, if any, which was used to inoculate the plate. This will yield the count per ml.

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Example: The calculated result is 4700. If the plates were inoculated with 0.1 ml, divide 4700 by “0.1”. The result is 47,000. The result is the count per ml. If 1 ml was used to inoculate the plates, this step is not necessary.

$$\frac{4700}{0.1} = 47,000 / ml$$

3.11.2 Plates in the Countable Range Are from Different Dilutions

If plates have colonies within the countable range at more than one dilution, calculate the CFU/gram for each dilution and arithmetically average the result. The exception is that if one calculated value is more than twice the other, use the result from the lower dilution.

Example of counts for multiple dilutions (1 ml inoculum on APC Petrifilm™):

Dilution	Colonies	
1:100	239, 249	(239 + 249) / 2 = 244 at 1/100 = 24400
		(26 + 28) / 2 = 27 at 1/1000 = 27000
1:1000	26, 28	(24400 + 27000) / 2 = 25700 = 2.6 x 10 ⁴ CFU/ml

Example for the exception (1 ml inoculum on APC Petrifilm™):

Dilution	Colonies	
1:10	115, 117	(115 + 117) / 2 = 116 at 1/10 = 1160
1:100	25, 27	(25 + 27) / 2 = 26 at 1/100 = 2600
		Record 1160 CFU/mL

3.11.3 All Plates Have Fewer Colonies than the Minimum of the Countable Range

- a. If there are no colonies on any plates, report the result as less than one times the corresponding lowest dilution, taking into account the inoculum quantity. For example, if the lowest dilution tested is 1/10 and the inoculum is 1 ml, report as < 10 CFU/g. If the inoculum is 0.1 ml report as < 100 CFU/g.
- b. If there are colonies on the plates for the lowest dilution, but less than the countable range, record the actual number of average colonies on the lowest dilution. Perform the same mathematical conversions as for colonies in the countable range. Report result as an estimated count by adding the word “estimated,” or equivalent verbiage, before the result.

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Example: Aerobic Plate Count (Petrifilm™) Countable Range of 25–250 CFU

Dilutions	1:100	1:1000	Result (CFU/g)
Count	18, 20	2	estimated 1900

3.11.4 All Plates Have More Colonies than the Maximum of the Countable Range

- a. Some examples include; *E.coli* Petrifilm™: (more than 150 CFU), *Enterobacteriaceae* Petrifilm™: (more than 100 CFU), and APC Petrifilm™: (more than 250 CFU)
- b. Determine the count for one or more representative squares. Calculate the average per square. Multiply the average by 20 to determine the resulting CFU per plate.
- c. Report results from these plates as an estimated count, indicated by adding the word “estimated” before the value.
- d. If the plate is too crowded to distinguish individual colonies and to accurately count, record as too numerous to count (TNTC) and report as > (greater than) the maximum countable range.

Note: Refer to the Compendium of Methods for more explicit instructions, if needed.

3.11.5 One Count in the Countable Range

If two or more plates are inoculated at each dilution and only one plate has colonies in the countable range, count all plates with countable colonies at that dilution and average the counts. This also applies if more than one dilution yields colonies in the countable range as described in Section 3.11.2.

Example: S. aureus spread plates, 0.1 ml inoculum,
One plate with colonies in the countable range of 20–200 CFU

Dilution	Colonies	Calculation
1:100	198, 258	$(198+258) / 2 = 228$ times 100 $(21+12)/2 = 16.5$ times 1000

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1:1000	21, 12	$(22800 + 16500) / 2 = 19650$ Divide result by 0.1 = 2.0×10^5
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3.11.6 No Counts in the Countable Range (Counts above and below the range)

Average the two counts closest to the maximum countable range. For crowded plates, use the procedure described in Section 3.11.4. Adjust for inoculum volume if necessary. Report the result as an estimated count by inserting the word “estimated” before the value or equivalent approach.

Example: E. coli on 3M Petrifilm™, countable range of 15-150 CFU,
No plates with colonies in the countable range

Dilution	Colonies	N = $(182+165) / (2)(10^{-2})$ = $347 / (2)(10^{-2})$ = 17350, Reported value: estimated 1.7×10^4
1:100	182, 165	
1:1000	9, 11	

3.11.7 Records and Reporting Results

Records shall be maintained for all activities described in this procedure. For official sample analyses, report only the first two significant digits, rounding off as described below (unless otherwise stated). For baseline studies and food safety assessments, report the raw data unless otherwise specified by the study or client.

- a. Raise the second digit to the next highest number only when the third digit from the left is 5, 6, 7, 8, or 9.
- b. Report counts obtained from plates outside the countable range as **estimates** as described in the previous section.

Examples:

Calculated Count	Rounded Count	Value To Report
12700	13000	1.3×10^4
12400	12000	1.2×10^4
14510	15000	1.5×10^4
15500	16000	1.6×10^4
14500	15000	1.5×10^4

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3.12 TEMPO®

3.12.1 Control Preparation

Appropriate controls will be prepared by dilution to a reportable range following the TEMPO® instructions for each sanitary indicator and aerobic count. Include a sterility control for each type of TEMPO® card.

3.12.2 Preparation of Samples

Samples may need to be filtered by various means, dependent on product type, to avoid clogging. For ground pork, poultry and beef product samples, remove a sufficient amount of the primary enrichment, e.g., BPW for poultry and mTSB for beef, after stomaching from the filter bag to a suitable container. The enumeration may be started immediately or refrigerated to facilitate work flow. Follow the TEMPO® instructions for each sanitary indicator and aerobic count.

3.12.3 Determination of Results on TEMPO®

Dilution schemes may be modified based on an expected level of contamination for each sample type. Typically for the TEMPO® the dilution scheme is 1:4, 1:40, and 1:400 but this should be adjusted based on expected bacterial load of the samples tested. If the primary dilution is not 1:10 then either multiply the final result by the factor used or input in the TEMPO® the final dilution used. Results are reported out from the TEMPO® as CFU/ml and may need to be changed to reflect CFU/sq cm or CFU/gram. For more detailed information follow the TEMPO® manufacturer instructions.

3.13 Selected References

Official Methods of Analysis of AOAC International, Current Edition. AOAC International Inc., Gaithersburg, MD 20877.

Lancette, G. A., and S. R. Tatini. 1992. *Staphylococcus aureus*, p. 533-550. In C. Vanderzant and D. F. Splittstoesser (ed.), Compendium of Methods for the Microbiological Examination of Foods. Amer. Publ. Hlth. Assoc., Washington, D.C. 20005.
AOAC Official Method 998.08 for *E. coli*

AOAC Official Method 990.12 for Aerobic Plate Counts

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Compendium of Methods for the Microbiological Examination of Foods, 4th edition, 2001, Frances Pouch Downes and Keith Ito, Eds. American Public Health Association, Washington D. C.

TEMPO[®] Preparation Station User's Manual

TEMPO[®] Reading Station User's Manual

TEMPO[®] AC (Aerobic Count) Package insert

TEMPO[®] CC (Coliform Count) Package insert

TEMPO[®] EB (Enterobacteriaceae) Package insert

TEMPO[®] EC (E. coli) Package insert