SECTION XI. MEMBRANE FILTER METHOD FOR *C. perfringens*

1. Scope and Application

1.1 This procedure enumerates *Clostridium perfringens* spores from surface and drinking water. Since *C. perfringens* is present in large numbers in human and animal wastes and its spores are resistant to wastewater treatment practices, extremes in temperature and environmental stress, it is an indicator of present fecal contamination as well as a conservative tracer of past fecal contamination. Some investigators have proposed *C. perfringens* as an indicator of the presence and the density of pathogenic viruses and possibly other microorganisms.

1.2 It is the user's responsibility to insure the validity of this method for untested matrices.

2. Summary of Method - An appropriate volume of water sample is passed through a membrane filter that retains the bacteria present in the sample. The membrane filter is placed on mCP agar and incubated anaerobically for 24 h at 44.5°C using a medium modified by Armon and Payment from Bisson and Cabelli (1,2). Upon exposure to ammonium hydroxide, the yellow straw-colored *C. perfringens* colonies turn dark pink to magenta and are counted as presumptive *C. perfringens*. Because of the selectivity of the mCP medium, a presumptive count is normally reported for routine monitoring purposes. Verification is not required for ICR monitoring, but if desired, colonies are confirmed by anaerobic growth in thioglycollate, a positive gram stain reaction and stormy fermentation of iron milk. The mCP counts are adjusted based on the percent confirmation. This method was originally prepared by Irwin Katz, U.S. EPA Region 2 for ASTM Subcommittee D19.24, Water Microbiology.

3. Definitions

3.1 *C. perfringens* - An obligate anaerobic gram-positive, spore forming, non-motile bacillus that ferments lactose with stormy gas production and ferments sucrose but does not ferment cellobiose. *C. perfringens* produces acid phosphatase and also produces exotoxins which cause gas gangrene and gastroenteritis.

3.2 Spores - *C. perfringens* produces single oval subterminal spores less than 1 µm in diameter during adverse conditions. Sporulation can also occur in the intestinal tract. The endospore that develops is a highly refractile body formed within the cell. Spores are resistant to heat, drying and chemical disinfectants, which would kill the vegetative cells of *C. perfringens*. This resistance to unfavorable conditions preserves the organisms for long periods of time.

4. Interferences

4.1 Waters containing sediment or large quantities of colloidal or suspended materials such as iron, manganese, alum floc or algae can clog the filter pores and prevent filtration, or can cause the development of spreading bacterial colonies that mask other colonies and prevent accurate counting.

4.2 When bacterial densities are high, a smaller sample volume or sample dilution can be filtered to minimize the interference of turbidity or high background (non-target) bacterial densities. Replicates of smaller sample volumes or dilutions of sample may be filtered and the results combined. However, the membrane filter technique may not be applicable to highly turbid waters with low *Clostridium* densities.

4.3 Toxic materials such as metals, phenols, acids, caustics, chloramines, and other disinfection by-products may also adversely affect recovery of *Clostridium* vegetative cells on the membrane filter. Although most probable number (MPN) methods are not usually expected to generate results comparable to membrane filter methods, an MPN method should be considered as an alternative procedure if the membrane filter method is not useable for these samples (3).

4.4 Some lots of membrane filters produce low recoveries or poor differentiation of target and non-target colonies due to toxicity, chemical composition, or structural defects. Quality control checks should be made on new lots of membranes (4).

5. Health and Safety

5.1 This method does not address all safety problems associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and determine regulatory limitations prior to use.

5.2 The analyst/technician must know and observe normal good laboratory practices and safety procedures required in a microbiology laboratory while preparing, using and disposing of cultures, reagents and materials and while operating sterilizers and other equipment and instrumentation.

5.3 Mouth-pipetting is not permitted.

6. Instruments, Equipment and Supplies

6.1 Sample container, sterile, non-toxic glass or rigid plastic with screw cap, or plastic bag, minimum of 125 mL capacity.

6.2 Pipet container, stainless steel, or aluminum, for sterilization and storage of glass pipets.

6.3 Pipets, sterile T.D. bacteriological or Mohr, glass or plastic, of appropriate volumes.

6.4 Graduated cylinders, 100 to 1000 mL, tops are covered with aluminum foil or kraft paper and sterilized.

6.5 Bottles, milk dilution, borosilicate glass or non-toxic heat stable plastic, screw-cap with neoprene liners, marked at 99 mL for 1:100 dilutions. Dilution bottles marked at 90 mL or tubes marked at 9 mL may be used for 1:10 dilutions.

6.6 Membrane filtration units, (filter base and funnel), glass, plastic or stainless steel, wrapped with aluminum foil or kraft paper and sterilized.

6.7 Membrane Filters - sterile, white, grid marked, 47 mm diameter, with 0.45 ± 0.02 µm pore size or other pore sizes for which the manufacturer provides data demonstrating equivalency.

6.8 Ultraviolet unit for disinfecting the filter funnel between filtrations in a series (optional).

6.9 Line vacuum, electric vacuum pump or aspirator as a vacuum source.

6.10 Flask, vacuum, usually 1 L, with appropriate tubing, to hold filter base. Filter manifolds to hold a number of filter bases are optional.

6.11 Flask, safety trap, placed between the filter flask and the vacuum source.

6.12 Forceps, straight or curved, with smooth tips to permit handling of filters without damage.

6.13 Petri plates, plastic or glass, 50 × 9 mm, with tight-fitting lids, or 60 × 12 mm, with loose fitting lids (dimensions are nominal).

6.14 Test Tubes, 20 × 150 mm, borosilicate glass or disposable plastic.

6.15 Caps, aluminum or autoclavable plastic, for 20 × 150 mm test tubes.

6.16 Test Tubes, screw cap, 16 × 125 mm or other appropriate size.

6.17 Inoculation loops, 3 mm diameter, and needles, nichrome or platinum wire, 26 B & S gauge, in suitable holders. Sterile disposable applicator sticks or plastic loops are acceptable alternatives to inoculation loops.

6.18 Thermometers, 0-50°C, graduated to 0.2 degrees, and 0-100°C for heat shock which has been checked against the appropriate National Institute of Standards and Technology (NIST) certified thermometer, or against a thermometer traceable to NIST.

6.19 Waterbath, that maintains 46-48°C for tempering agar.

6.20 Waterbath with gable cover that maintains 60°C ± 0.5°C for heat shocking samples.

6.21 Anaerobic system (anaerobic jar, reaction chamber, hydrogen/carbon dioxide disposable generator and anaerobic indicator), or any other system capable of producing the appropriate anaerobic conditions to support the growth of the organisms[1].

6.22 Filter Paper, circular, 11 cm, Whatman 40 or 110, or equivalent, for separation of mCP agar plates during anaerobic incubation.

6.23 Incubator, that maintains 44.5°C ± 0.2°C and is large enough to hold the anaerobic chamber.

6.24 Incubator, Water Bath, that maintains 44.5°C ± 0.2°C for incubation of Iron Milk Medium.

6.25 Microscope, stereoscopic, wide-field type, with magnification of 10 to 15X.

6.26 Microscope lamp, that produces diffuse light from a cool white fluorescent or tungsten lamp adjusted to give maximum visibility.

6.27 Counting device, hand tally or electronic.

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[1]BBL 60460 or BBL 60466 GASPAK Anaerobic System with BBL 70308 Disposable Hydrogen and Carbon Dioxide Generator Envelopes, BBL Microbiological Systems, Cockeysville, MD 21030, or equivalent.

6.28 Sonication unit, to aid in dissolving reagents.[2]

7. Reagents, Standards and Media

7.1 **Purity of Reagents** - Use reagent grade chemicals in all tests. Unless otherwise indicated, all reagents must conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available (5). Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Use microbiological grade agar in preparation of culture media. Whenever possible, use commercial culture media as a means of improved quality control.

7.2 **Purity of Water** - Unless otherwise indicated, references to water mean reagent water as defined by Type II of Specification D1193 (6).

7.3 **Buffered Dilution and Rinse Water**

7.3.1 **Phosphate Buffer Dilution Water**

7.3.1.1 **Stock Phosphate Buffer Solution** - Dissolve 34.0 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 mL of water. Adjust pH to 7.2 with 1 N NaOH and bring to 1000 mL with water. Dispense aseptically into screw-cap bottles and autoclave for 15 min at 121°C. Alternatively, sterilize by filtration through a 0.2 µm pore membrane filter and dispense aseptically into sterile screw-cap bottles. Store in refrigerator and handle aseptically. If cloudiness, a marked change in pH, or other evidence of contamination appears, discard the stock. Confirm that pH is 7.2 ± 0.5 before use.

7.3.1.2 **Magnesium Chloride Solution** - Dissolve 81.4 g of hexahydrate magnesium chloride ($MgCl_2 \cdot 6H_20$) in 1000 mL of water. Mix well and sterilize by filtration or autoclave for 15 min at 121°C. Store in refrigerator and handle aseptically. If cloudiness, or other evidence of contamination occurs, discard the stock solution.

7.3.1.3 **Phosphate Buffered Dilution Water** - Add 1.25 mL of stock phosphate buffer solution and 5 mL of magnesium chloride solution to 1000 mL of water in a volumetric flask and mix well. Dispense dilution water in amounts which will provide 99 ± 2 mL after sterili-

[2]Bronson Sonifier, 500 W, or Tekmar Sonic Disrupter, 500 W with 3 mm tip set at 18 W, or equivalent.

zation in screw-cap dilution bottles, or in larger volume containers for use as rinse water. Autoclave dilution bottles for 15 min at 121°C. Autoclave larger volumes for longer periods as appropriate. Alternatively, sterilize by filtration through a sterile 0.2 µm pore membrane filter unit and dispense aseptically into sterile screw-cap bottles.

7.3.2 **Peptone Dilution and Rinse Water** - Dissolve 1.0 g of peptone[3] in 100 mL of water, and bring to 1000 mL with water. Dispense in screw-cap bottles in volumes to produce 99 ± 2 mL after autoclaving. Autoclave for 15 min. at 121°C. Final pH should be 6.8 - 7.0. Adjust as necessary.

7.4 **Ethanol** - 95%, pure, for flame-sterilization of forceps and for preparation of acetone alcohol for gram stain.

7.5 **Ammonium Hydroxide Solution** (29.2% NH_4OH) - commercially available.

7.6 **Ferric Chloride Solution** - Weigh out 4.5 g of $FeC1_3 \cdot 6H_20$ and dissolve in 100 mL of water. Filter sterilize and store in refrigerator.

7.7 **Phenolphthalein diphosphate Solution** - Weigh out 0.5 g of phenolphthalein diphosphate and dissolve in 100 mL of water. Filter sterilize and store in refrigerator.

7.8 **Indoxyl β-D Glucoside Solution** - Weigh out 0.06 g of Indoxyl β-D Glucoside and dissolve in 80 mL of water (0.075 solution). Sonicator (item 6.28) can be used to speed dissolution. Filter-sterilize and use in 7.9.2.

7.9 **Modified mCP Agar** (1)

7.9.1 **Composition/L**

Tryptose	30.0	g
Yeast Extract	20.0	g
Sucrose	5.0	g
L-cysteine Hydrochloride	1.0	g
$MgSO_4 \cdot 7H_20$	0.1	g
Bromcresol Purple	0.04	g
Agar	15.0	g

7.9.2 **Preparation of Modified mCP Agar:** Add medium ingredients from 7.9.1 to 900 mL water in a liter Erlenmeyer flask. Stir and heat to dissolve in a boiling water bath. Bring the pH to 7.6 with 1 N NaOH. Autoclave for

[3]**Peptone** (Difco 0118), Difco Laboratories, Detroit, MI, or equivalent.

15 min at 121°C (15 lbs pressure). Cool to 50°C. Add the following reagents aseptically and mix well:

D-cycloserine	0.4	g
Polymyxin B sulfate	0.025	g
4.5% $FeCl_3 \cdot 6H_20$ solution	2.0	mL
0.5% Phenolphthalein diphosphate solution	20.0	mL
0.075% Indoxyl-β-D-Glucoside solution	80.0	mL

7.9.3 Dispense 4-4.5 mL into each petri plate using a sterile Cornwall syringe or Brewer pipette. Store agar plates inverted in a plastic bag in a refrigerator for no more than one month. It is recommended that the plates be stored in an anaerobic chamber in the refrigerator for optimal preservation.

7.10 **Modified Iron Milk Medium** (7)

7.10.1 **Composition/L**

Fresh pasteurized, homogenized milk (3.5% butterfat)	1.0	L
$FeSO_4 \cdot 7H_2O$	1.0	g

7.10.2 **Preparation:** Dissolve ferrous sulfate in 50 mL water. Add slowly to 1 L milk and mix with magnetic stirrer. Dispense 11 mL of medium into culture tubes. Cap and autoclave 12 min at 118°C. CAUTION: Do not exceed the recommended time and temperature limits to avoid coagulation.

7.11 **Fluid Thioglycollate Medium**[4]

7.11.1 **Composition/L**

L-Cystine	0.5	g
Agar (granulated)	0.75	g
NaCl	2.5	g
Dextrose (anhydrous)	5.0	g
Yeast extract	5.0	g
Tryptone	15.0	g
Sodium thioglycollate	0.5	g
Resazurin	0.001	g

[4]Fluid Thioglycollate Medium (BBL 12461), Becton-Dickinson Microbiology Systems, Cockeysville, MD; (Difco 0432-02-6) Difco Laboratories, Detroit, MI; or equivalent.

7.11.2 **Preparation:** Suspend 29.25 g of medium in 1 L of water. Mix thoroughly and heat to boil for 1-2 min or until solution is complete. Final pH is 7.1 ± 0.1. Dispense 15 mL portions into culture tubes. Cap and autoclave for 15 min at 121°C. Store tubes in the dark at room temperature. Do not refrigerate. If medium becomes oxidized (more than 30% of medium is pink), reheat once only in boiling water bath and cool before use.

7.12 **Gram Stain Reagents**

7.12.1 Gram stain reagent kits are commercially available and are recommended.

7.12.2 **Ammonium oxalate-crystal violet (Hucker's)**: Dissolve 2 g crystal violet (90% dye content) in 20 mL 95% ethyl alcohol. Dissolve 0.8 g $(NH_4)_2C_2O_4 \cdot H_2O$ in 80 mL water; mix the two solutions and age for 24 h before use. Filter through a 0.22 µm membrane filter. Store in a glass bottle.

7.12.3 **Lugol's solution, Gram's modification**: Grind 1 g iodine crystals and 2 g KI in a mortar. Add water, a few mL at time, and grind thoroughly after each addition until solution is complete. Filter solution through a 0.22 µm membrane filter, and rinse into an amber glass bottle with the remaining water (using a total of 300 mL).

7.12.4 **Counterstain**: Dissolve 2.5 g safranin dye in 100 mL 95% ethyl alcohol. Add 10 mL to 100 mL water. Filter through a 0.22 µm membrane filter.

7.12.5 **Acetone alcohol**: Mix equal volumes of ethyl alcohol (95%) with acetone.

8. Sample Collection, Preservation and Holding Times

8.1 **Collection** - Water samples are collected in sterile sample containers with leak-proof lids.

8.2 **Sample Preservation and Holding Conditions** - Hold water samples at a temperature below 10°C during transit to the laboratory by placing them on ice, surrounding them with blue ice or by refrigeration. Use insulated containers to maintain storage temperature during transit. Take care that sample bottle closures are not submerged in water during transit or storage.

8.3 **Holding Time** - Refrigerate samples upon arrival in the laboratory and analyze within 8 h after collection. *C. perfringens* spores can survive for extended periods

at 1-4°C. However, since a correlation is planned with other indicators, the holding time for *C. perfringens* must be limited to that of the other indicators.

9. Quality Control

9.1 Adherence to sampling procedures, preservation procedures and holding time limits is critical to the production of valid data. Reject samples if appropriate sampling, preservation and handling procedures have not been followed

9.2 Check and record temperatures in incubators daily to insure operation within stated limits.

9.3 Check thermometers at least annually against a National Institute of Standards and Technology (NIST) certified thermometer or one traceable to NIST and record the results. Examine mercury columns for separation and reunite before use. Adjust or post correction factors on equipment.

9.4 Use a loop to inoculate mCP agar plates with pure cultures of *C. perfringens* and *E. coli*. Carry these plates through the entire analytical procedure, as positive and negative controls.

9.5 For general quality control recommendations, see "Quality Assurance for Microbiological Analyses" in ASTM Special Technical Testing Publication 867 (8).

10. Procedure for Analyses of Water Samples for Spores

10.1 Prepare mCP Agar according to Section 7.9.

10.2 Mark the bottoms of the petri plates and laboratory data sheets with sample identities and volumes.

10.3 Grasp a sterile membrane filter by its edge using a sterile forceps and place on the filter base, grid side up. Attach the funnel to the base of the filter unit; the membrane filter is now held between the funnel and the base.

10.4. **Procedure for Inactivation of Vegetative Cells** - To obtain a count only of *C. perfringens* spores, hold water samples in a waterbath at 60°C for 15 min to kill all vegetative cells.

10.4.1 Equilibrate a waterbath at 60 C.

10.4.2 Determine the time necessary to bring a blank sample to 60°C. Use the same size container and volume as used for water samples.

10.4.3 Immerse the containers containing the water samples in the waterbath for the time necessary to warm sample to 60°C plus 15 min. Do not allow the container cap or container opening to become contaminated by water in the bath.

10.4.4 Cool the sample containers in cold tap water immediately after heat shock and proceed with the analyses in 10.3.

10.5 For greatest accuracy, it is necessary to filter a sample volume that will yield a countable plate. Select sample volumes based on previous knowledge, which will produce membrane filter plates with 20-80 *C. perfringens* colonies. A narrow range of dilution factors of 4 or 5 can usually be used to achieve the desired number of colonies. An example of such factors is shown in **Table XI-1**. However, if past analyses of specific samples have resulted in confluent growth or "too numerous to count" (TNTC) membranes from excessive turbidity, additional samples should be collected and filtration volumes adjusted to provide isolated colonies from one or more smaller volumes. The counts from smaller volumes can be combined for a final count/total volume filtered.

10.6 Shake the sample bottle vigorously about 25 times and measure the desired volume of sample into the funnel with the vacuum off. To measure the sample accurately and obtain good distribution of colonies on the filter surface, use the following procedures:

10.6.1 Sample volumes of 20 mL or more: Measure the sample in a sterile graduated cylinder and pour it into the funnel. Rinse the graduate twice with sterile dilution water, and add the rinse water to the funnel.

10.6.2 Sample volumes of 10-20 mL: Measure the sample with a sterile 10 mL or 20 mL pipet into the funnel.

10.6.3 Sample volumes of 1-10 mL: Pour about 10 mL of sterile dilution water into the funnel without vacuum. Add the sample to the sterile water using appropriate sterile pipet and filter the sample.

10.6.4 Sample volumes of less than 1.0 mL: Prepare appropriate dilutions in sterile dilution water and proceed as applicable in steps 10.6.1-10.6.3 above.

10.6.5 To reduce the chance for carryover, when analyzing a series of samples or dilutions, filter samples in the order of increasing volumes of original sample. The time elapsing between preparation of sample dilutions and filtration should be minimal and never more than 30 min.

Table XI-1. Sample Volumes to Obtain Colony Count on Membrane Filters * (Range of 20 - 80 Colonies)	
Sample Volume in mL	**Added as:**
0.05	5.0 mL of 10^{-2} dilution
0.20	2.0 mL of 10^{-1} dilution
0.80	8.0 mL of 10^{-1} dilution
3.20	3.2 mL of Undiluted Sample
15.00	15.0 mL of Undiluted Sample
60.00	60.0 mL of Undiluted Sample
*The range of volumes and dilutions selected for filtration of completely unknown samples can be broader, to provide a factor of 10 or more. Prepare at least three sample increments.	

10.7 After adding the sample to filter funnel, turn on vacuum and filter the sample. Rinse the sides of the funnel walls at least twice with 20-30 mL of sterile dilution water. Turn off vacuum and remove the funnel from the filter base.

10.8 Flame forceps, cool and aseptically remove the membrane filter from the filter base. Place the filter, grid side up, on the mCP agar using a rolling motion to prevent air bubbles. Reseat the filter if bubbles occur.

10.9 Remove the lids from mCP agar plates. Invert lids and nest them under the corresponding plate bottom for identification. Stack the plates in layers in the anaerobic chamber, separating each plate with sterile filter paper. Incubate the anaerobic chamber at 44.5°C for 24 h, maintaining anaerobic conditions through the use of a commercial anaerobic system. If visible condensation does not occur within 60 min after the BBL GasPak is activated, the reaction should be terminated by opening the jar, and removing the GasPak. Inspect the chamber seal for alignment and lubricant. Insert a new GasPak and seal the chamber. The disposable anaerobic indicator (moistened flat fiber wick impregnated with 0.35% methylene blue solution) is white to pale blue upon opening foil envelope. It turns blue upon exposure to air. Under anaerobic conditions the methylene blue indicator will decolorize (turn white) within 2 - 4 h. It should remain white through the incubation period.

10.10 After 24 h, remove one agar plate at a time from the chamber and reclose the chamber. Examine the mCP plate for straw-yellow colonies. If such colonies are

present, invert and expose the open agar plate 10-30 sec to the fumes from an open container of concentrated ammonium hydroxide.

10.11 If *C. perfringens* colonies are present, the phosphate in the phenolphthalein diphosphate will be cleaved from the substrate by acid phosphatase and typical colonies of *C. perfringens* will turn a dark pink or magenta after exposure to fumes of ammonium hydroxide.

10.12 Count pink or magenta colonies as presumptive *C. perfringens*.

10.13 Repeat steps 10.10 to 10.12 with the other culture plates.

11. Confirmation Tests

11.1 Pick at least 10 typical isolated *C. perfringens* colonies from the mCP plate and transfer each into a separate thioglycollate tube. Incubate at 35°C for 24 h. Examine by gram stain and for purity. *C. perfringens* are short gram-positive bacilli. Retain tubes for further testing.

11.2 Inoculate ten tubes of iron milk medium with 1 mL from the ten fluid thioglycollate tubes and incubate in a 44.5°C waterbath for two h. Examine hourly for stormy fermentation with rapid coagulation and fractured rising curd.

11.3 Those colonies which are gram-positive, non-motile, and produce stormy fermentation of milk in these confirmatory tests are considered confirmed *C. perfringens*.

12. Data Analyses, Calculations and Reporting Results

12.1 Pink or magenta colonies counted on mCP medium are adjusted to a count/100 mL and reported as: Presumptive *C. perfringens* colony forming units (CFU)/100 mL. The presumptive count is normally used for routine monitoring.

12.2 If confirmation tests are performed, original counts on mCP agar are adjusted based on the percent of colonies picked and confirmed. Report as confirmed *C. perfringens* CFU/100 mL of water sample.

13. Method Performance Characteristics

13.1 The detection limit is one *C. perfringens* CFU per sample volume or sample dilution tested.

13.2 The false positive rate is reported to be 7-9% by Bisson and Cabelli (2) and Fujioka and Shizumura (10). The false negative rate is reported to be 3% by Fujioka and Shizumura (10).

13.3 The single laboratory recovery is reported to be 79-90% by Bisson and Cabelli (2).

13.4 In a collaborative study, sixteen analysts from nine laboratories analyzed a sediment, a non-chlorinated wastewater and three spiked waters (marine water, lake water and a finished drinking water), as unknowns. Analysts were provided range values to reduce the number of dilutions necessary for the analyses.

13.4.1 The single operator precision as % Relative Standard Deviation (RSD) ranged from 14-28% while the overall precision (as % RSD) ranged from 24-41%, for S_t/S_o (overall precision/single operation precision) ratios of 1.13-1.80. The larger RSD values were not generated with the more difficult sample matrices of sediment and wastewater. Rather, they occurred with the seeded finished drinking water sample and are believed to have been caused by overestimates of the concentration of *C. perfringens*, which resulted in marginally low plate counts with inherently greater deviations. Overall, the S_t and S_o values were similar across sample types and concentration levels of *C. perfringens*.

13.4.2 Although there were no "standards" available for this RR study, sample 5, a seeded drinking water, had a reference count of 78 *C. perfringens* CFU/100 mL. The laboratories in this study achieved a mean recovery of 67 CFU from Sample 5 for an 86 percent recovery.

13.4.3 **Table XI-2** contains the statistical summary of the collaborative study results.

Table XI-2. Statistical Evaluation of Results (CFU/100 mL) (After Rejection of Outliers)

Sample	Initial n	Final n	X	S_0	S_t	%RSD (S_o)	%RSD (S_t)
1	30	30	2893.63	397.78	715.45	13.75	24.73
2	36	35	108.09	20.34	26.18	18.82	24.22
3	30	30	73.07	20.29	23.23	27.77	31.79
4	36	35	5985.71	1400.70	1585.80	23.40	26.49
5	27	27	67.22	18.64	27.60	27.73	41.06

14. Pollution Prevention

14.1 Pollution prevention is any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. It is the environmental management tool preferred over waste disposal or recycling. When feasible, laboratory staff should use a pollution prevention technique such as preparation of the smallest practical volumes of reagents, standards and media or downsizing of the test units in a method.

14.2 The laboratory staff should also review the procurement and use of equipment and supplies for other ways to reduce waste and prevent pollution. Recycling should be considered whenever practical.

15. Waste Management - The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water and land by minimizing and controlling releases from hoods and bench operations, complying with the letter and spirit of sewer discharge permits and regulations and by complying with solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions.

16. Key Words - *Clostridium*, *Clostridium perfringens*, anaerobic bacteria, spore-forming bacteria, indicator organisms, pollution, water quality.

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