



Albuquerque Bernalillo County

Water Utility Department

WATER RECLAMATION DIVISION
4201 2ND STREET SW, ALBUQUERQUE, NEW MEXICO 87105

**WATER QUALITY LABORATORY
STANDARD OPERATING PROCEDURE APPROVAL FORM**

WQL SOP **SOP 301**

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MODIFICATIONS AND REASONS FOR REVISION

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| ❖ 2006 A2LA Audit Requirements |
| ❖ Deficiency 26; SOP revised to specify that detergents designed for lab use be used. |
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STANDARD OPERATING PROCEDURE

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Microbiology

SCOPE AND APPLICATION: These methods are applicable to drinking, surface and saline waters, domestic and industrial wastewater, solids (after extraction). The practical range of determination is 0 cfu to >500 cfu. The methods are used to enumerate or determine the presence and absence of the following groups of organisms: heterotropic bacteria, total coliform, E.coli, fecal streptococci and fecal coliform.

APPLICABLE METHOD REFERENCES:

18th ed. of Standard Methods, 9060B, 9215A, 9215B, 9221E, 9222A, 9222B, 9222D, 9223B, 9230B

DISPOSAL OF SAMPLES: Dispose of liquid samples at an acid sink. Dispose of solid samples in trash container. All contaminated or used microbiological media and bacterial cultures shall be autoclaved at 121°C prior to disposal in a trash container.

1.0 EQUIPMENT AND SUPPLIES

1.1 pH measurements: All pH measurements conducted for microbiological testing will be made in accordance to the procedure as specified in WQL Standard Operating Procedure AC-022. All measurements will be made and reported to within ± 0.1 pH units. Buffer aliquot shall be used only once per calibration. Electrodes shall be maintained as specified in SOP AC-22. The pH meter shall be standardized each use period with pH 7.0 and either 4.0 or 10.0 buffers, with date and buffers used recorded in log book. Commercial buffer solutions shall be dated when received and opened and discarded before expiration date.

1.2 Balance (top loader): The balance sensitivity must be checked monthly. The balance sensitivity is checked by zeroing the balance. Then placing a 150g load on the balance. Then place a 0.1g weight on the balance pan with the 150g load. The final weight of both weights should be $150.1g \pm 0.1g$, if not take corrective action. The balance shall be calibrated monthly using three ASTM type II weights. The weights used should bracket laboratory weighting needs. The weights selected are 5g, 25g and 50g. Annual service and calibration is to be conducted by a certified Balance Service and the records of service and calibration filed and retained by the laboratory manager.

1.3 Temperature Monitoring Devices: Temperature monitoring devices are to be graduated in 0.5°C increments and 0.2°C increments for tests which are incubated at 44.5°C . Thermometers exhibiting separation in the fluid column will not be used for continued temperature monitoring. All glass thermometers will be calibrated annually with a reference NIST thermometer. All thermometers that are calibrated will be tagged as specified in WQL SOP AC-028 and the results recorded in the Thermometer Calibration Log.

1.4 Incubator Unit: Incubators are to be maintain at $35 \pm 0.5^{\circ}\text{C}$ and $44.5 \pm 0.2^{\circ}\text{C}$. The 35°C incubator shall have two thermometers for recording internal temperature. One thermometer will be placed on the top shelf and the other thermometer placed on the bottom shelf, both thermometers are to have the bulb of the thermometers immersed in liquid. The 44.5°C incubator (water bath) shall have one thermometer with the bulb of the thermometer fully immersed in the water bath. The incubator thermometer temperature will be recorded daily in the temperature log at 10am and 2pm. The temperature recorded for the 35°C incubator will be an average temperature of both thermometers.

1.5 Autoclave: The autoclave shall maintain sterilization temperature (121°C) during a cycle and complete entire cycle within 45 minutes when 12-15 minute sterilization period is used. The autoclave when on slow exhaust shall depressurize slowly enough to ensure media will not boil over and bubbles will not form in inverted tubes. If the autoclave performance does not meet the two preceding conditions, corrective action is to be conducted. The date, contents, sterilization lot number, sterilization time, temperature, total cycle time, and analyst's name are to be recorded for each cycle in the sterilization log. Maintenance and inspection of the autoclave will be done by the service contractor. The records of maintenance will be filed and

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maintained in the autoclave maintenance log located in the microbiology lab. A maximum-temperature-registering thermometer and continuous recording device shall be used each autoclave cycle and the temperature of the maximum-temperature-registering thermometer recorded in the sterilization log and the continuous recording device temperature shall be recorded and dated on the circular chart. To avoid overcrowding in the autoclave chamber never stack items to be sterilized. To check autoclave sterilization performance check autoclave at a minimum of once monthly using spore strips or kill ampules. The autoclave automatic timing mechanism shall be checked quarterly with a stopwatch and the results of the timing test recorded in the sterilization log. Operation procedure for the autoclave refer to appendix I.

1.6 Conductivity meter: All conductivity measurements conducted for microbiological testing will be made in accordance to the procedure as specified in WQL Standard Operating Procedure AC-012. The Cell constant will be determined monthly and the result recorded in the conductance log.

1.7 Refrigerator: The refrigerator used for storing microbiological materials shall maintain a temperature of 1-5°C. The thermometer used to measure the refrigerator temperature shall be graduated in at least 1°C increments with the bulb immersed in liquid. The refrigerator thermometer temperature will be recorded daily in the temperature log at 10am and 2pm.

1.8 Membrane Filtration Equipment: The graduations on filtering funnels will not be used to measure sample volumes. Sterile graduated cylinders will be used for large sample volumes and sterile pipets used for volumes less than 50mL. A 10x to 15x stereo microscope with a fluorescent light source shall be used to count green sheen colonies. Membrane filters approved by manufacturer for use in total coliform analysis of water and are made of cellulose ester, are grid-marked, have a 47mm diameter, and 0.45um pore size will be used for membrane filter coliform analysis. Lot number and date received shall be recorded for membrane filters in the Membrane Sterility Check book located in the microbiology lab. All lots of membrane filters received will be checked for sterility. Membrane sterility check: Aseptically, remove a membrane filter from its wrapper. Place filter into a tube containing 50mL of TSB broth and recap the tube. Incubate the TSB tube containing the filter at 35±0.5°C for 24 hours. If the broth appears turbid after 24 hours, this is a positive result indicating the filter is not sterile. Repeat the sterility check using two more filters from the same lot to confirm the first result. If one or both of the repeat filters are positive then the lot is condemned and discarded. The results of the membrane sterility check are recorded in the Membrane Sterility Check book located in the microbiology lab. Opened packs of disposable culture dishes shall be resealed between use periods.

1.9 Pipets: Pipets used for microbiological analysis shall be sterile individually wrapped disposable serological pipets. All lots of pipets received will be checked for sterility. Pipet sterility check: Aseptically remove a pipet from its wrapper. Place pipet into a tube containing 50mL of sterile TSB broth. Using a pipet bulb withdraw one pipet volume from the tube, then let flow back into the tube of TSB and re-cap the tube. Incubate the TSB tube at 35±0.5°C for 24 hours. If the broth appears turbid after 24 hours, this is a positive result indicating the pipet is not sterile. Repeat the sterility check using two more pipets from the same lot to confirm the first result. If one or both of the repeat pipets are positive then the lot is condemned and discarded. The results of the pipet sterility check are

recorded in the Sterility Check book located in the microbiology lab

1.10 Culture Tubes & Closures: Culture tubes and containers used must be of sufficient size to contain medium plus sample without being more than three quarters full. Tube closures used must be of stainless steel, plastic or aluminum screw caps with non-toxic liners; cotton plugs are not to be used as closures.

1.11 Sample Containers: Sample containers will be wide-mouth 120 mL(4oz) bottles. The bottles used for sampling microbiological sample shall be constructed of polypropylene or suitable plastic material that can be repeatedly autoclaved at 121°C without loss of structural integrity. All bottles prior to sterilization will have added to the bottle 1 mL of 1% sodium thiosulfate solution to prevent air-lock sterilization failure and destroy chlorine if present in the sample.

1.12 Glassware and Plasticware: Glassware must be made of borosilicate glass or other corrosion-resistant glass, free of chips and cracks, with all markings legible. Plastic items must be clear and non-toxic to microorganisms. Sterile graduated cylinders used to measure sample volumes must have a tolerance of 2.5% or less. Graduations on sample containers and filtration funnels will not be used to measure sample volumes.

1.13 Ultraviolet Lamps: Ultraviolet lamps shall have each unit cleaned monthly by wiping with a soft cloth moistened with ethanol. The ultraviolet lamps used of sterilization will be tested quarterly with a UV light meter. Lamps should be replaced when the uW/cm² measured are <70% of the initial reading. The initial reading is the first reading recorded in the UV lamp log or the reading first appearing in the log after lamp replacement.

2.0 GENERAL LABORATORY PRACTICES:

2.1 Sterilization Procedure: Required times for autoclaving material at 121°C (except for membrane filters and pads, if used and carbohydrate-containing media indicated times represent minimum times, dependent upon volumes, containers, and loads):

| | |
|--------------------------------|----------------|
| -CARBOHYDRATE CONTAINING MEDIA | 12-15 MINUTES |
| -CONTAMINATED TEST MATERIALS | 30 MINUTES |
| -MEMBRANE FILTER ASSEMBLIES | 15 MINUTES |
| -SAMPLE COLLECTION CONTAINERS | 15 MINUTES |
| -DILUTION WATER BLANK | 15 MINUTES |
| -INDIVIDUAL GLASSWARE | 15 MINUTES |
| -RINSE WATER(0.5-1 LITER) | 15-30 MINUTES* |

* time depends upon water volume per container and autoclave load

Autoclaved media must be removed immediately after completion of sterilization

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cycle. All sterilized prepared media, glassware, membrane filters, pipets, dilution water, petri dishes, filtering funnels, sample bottles must be assigned a lot number and be tested for sterility. The results of each lot tested for sterility shall be recorded in appropriate logs. Results for prepared media shall be documented in the individual media books. The assignment of lot numbers for all prepared media will be documented in the prepared media log. Glassware, membrane filters, pipets, dilution water, petri dishes, filtering funnels, sample bottles must be assigned a lot number and be tested for sterility and the results of the testing and the assignment of lot numbers documented in the Sterility log. All lots must be labeled with the assigned lot number. The procedure for assignment of lot numbers is as follows:

SAMPLE BOTTLES: Prefixed with a **B** and a four digit number beginning with 0001, numbered consecutively thereafter for subsequent lots.

FILTER FUNNELS: Prefixed with a **F** and a four digit number beginning with 0001, numbered consecutively thereafter for subsequent lots.

PETRI DISHES: Prefixed with a **PD** and manufacture's lot number.

DILUTION WATER: Prefixed with a **W** and a four digit number beginning with 0001, numbered consecutively thereafter for subsequent lots.

PREPARED MEDIA: Prefixed with a **M** and a four digit number beginning with 0001, numbered consecutively thereafter for subsequent lots.

MEMBRANE FILTERS: Prefixed with a **MF** and manufacture's lot number.

GLASSWARE: Prefixed with a **G** and a four digit number beginning with 0001, numbered consecutively thereafter for subsequent lots.

PIPETTS: Prefixed with a **P** and manufacture's lot number.

2.2 Sample Containers: Sterility of each lot of sample containers shall be confirmed by adding 25mL of a sterile non-selective broth (TSB) to at least one container, incubating at 35±0.5°C for 24 hours and checked for growth, turbidity or gas bubbles.

2.3 Reagent-Grade Water: Only satisfactorily tested reagent water from the laboratory deionization/RO unit will be used to prepare media, reagents and dilution/rinse water. Quality of reagent water shall be tested to meet the following criteria and schedule of testing:

| TEST PERFORMED | ACCEPTABILITY LIMIT | TESTING SCHEDULE |
|-------------------------|---|------------------|
| Conductivity** | <2 umho at 25°C | Weekly |
| Pb, Cd, Cr, Cu, Ni, Zn | not greater than 0.05 mg/L per contaminant, and not greater than 0.1 mg/L total | Annually |
| Total chlorine residual | <0.1 mg/L | Monthly |

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| TEST PERFORMED | ACCEPTABILITY LIMIT | TESTING SCHEDULE |
|---|------------------------------|------------------|
| Heterotropic plate count | <500/L | Monthly |
| Bacteriological quality of reagent water* | ratio of growth rate 0.8:3.0 | Annually |

* Procedure, refer to Appendix II
**Cell constant to be determined monthly

2.4 Dilution/Rinse Water: Stock buffer solution or peptone water prepared as specified in standard methods. Stock buffers autoclaved or filter-sterilized and containers labeled, dated and refrigerated. Store stock buffer which is free of turbidity. Each batch of dilution/rinse water checked for sterility by adding 50mL of double strength non-selective broth, incubating at 35±0.5°C for 24 and checked for growth, turbidity or gas bubbles.

Dilution Water Preparation: Dissolve 34g potassium dihydrogen phosphate in 500mL reagent-grade water, adjust to a pH of 7.2±0.5 with one normal sodium hydroxide, and dilute to one liter with reagent-grade water to prepare stock phosphate buffer solution. Add 1.25mL stock phosphate buffer solution and 5.0mL magnesium chloride solution (81.1g MgCl₂·6H₂O qs 1000mL) to a liter of reagent-grade water. Sterilize by autoclaving using slow exhaust for 30 min, if volume prepared is one liter or less and autoclave 45-60 minutes for volumes greater than a liter.

2.5 Glassware Washing: Detergents, specifically designed for laboratory use, such as Contrad 70™ or Alconox™ shall be used for cleaning glassware used for microbiological analysis. Distilled or de-ionized water shall be used for the final rinse for all glassware used for microbiological analysis. Each lot or batch of dry glassware will be spot-checked for pH reaction with bromothymol blue.

3.0 ANALYTICAL METHODOLOGY:

3.1 General: Only analytical methodology specified in the Total Coliform Rule and Surface Water Treatment Rule shall be used for compliance samples. Water samples are to be shaken vigorously, 25 times before analysis. If no total coliform-positive results occur during a quarter, then the laboratory shall perform a coliform procedure using a known coliform-positive, fecal coliform and/or E.coli-positive control to spike the sample. The Sample volume analyzed for total coliform in drinking water shall be 100 ± 2.5mL.

3.2 Media: Dehydrated or prepared media manufactured commercially shall be the sole source of media used for microbiological analysis and prepared in accordance with the manufactures instructions. Dehydrated media shall be stored in a cool dry location in the microbiology lab and caked or discolored dehydrated media discarded. Unopened dehydrated media will be discarded when it exceeds the manufactures expiration date or if opened and stored in a desiccator discarded one year after the date opened. Laboratory media preparation records shall include: Date of preparation, type of medium, lot number, sterilization time and temperature, final pH and analyst's name. Each new lot of dehydrated media shall be checked before use with positive and negative culture controls, specific organisms for specific media are located in the individual media books, and results recorded in the book designated for

that media. Also see Appendix V for control organisms for specific media and final pH and sterilization of media. Prepared plates are to be refrigerated in sealed plastic bags or containers not longer than two weeks, with bag or container dated with preparation or expiration date. Loose-cap tubes of broth stored at <30°C shall be discarded after two weeks, tightly capped tubes shall be discarded after three months at <30°C (A-1 media to be discarded after 1 week). Refrigerated medium incubated at room temperature overnight shall be discarded if growth is observed. All refrigerated media shall be allowed to reach room temperature prior to inoculation or use.

4.0 MEMBRANE FILTER (MF) TECHNIQUE FOR TOTAL COLIFORM

4.1 General Discussion

4.1.0 Principle: As related to the membrane filter technique, the coliform group may be defined as comprising all aerobic and many facultative anaerobic, gram-negative, non-spore forming, rod-shaped bacteria that develop a red colony with a metallic sheen within 24 hours at 35°C on an Endo-type medium containing lactose. Some members of the total coliform group may produce a dark red or nucleated colony without a metallic sheen. When verified these are classified as atypical coliform colonies. When purified cultures of coliform bacteria are tested they produce a negative cytochrome oxidase (CO) and positive B-galactosidase (ONPG) reaction. Generally, all red, pink, blue, white or colorless colonies lacking sheen are considered noncoliforms by this technique. The coliform group includes the genera: Escherichia, Citrobacter, Enterobacter, and Klebsiella. The purpose of the technique is to enumerate or detect the presence of total coliform. An appropriate volume of water sample or its dilution is passed through a membrane filter that retains the bacteria present in the sample. The filter retaining the microorganisms is placed on M-Endo agar or LES M-Endo agar in a petri dish. The filter and dish are then incubated for 24 hours at 35°C, after which visual detection and enumeration is conducted.

4.1.1 Interferences: Turbidity caused by the presence of algae or other interfering material may not permit testing of a sample volume sufficient to yield significant results. Low coliform estimates may be caused by the presence of high numbers of non-coliforms or of toxic biotoxic substances. This technique is applicable to the examination of saline waters, but not wastewater that have received only primary treatment followed by chlorination because of turbidity in high volume samples or wastewater containing toxic metals or toxic organics such as phenols.

4.1.2 Safety Considerations: Samples and used test materials may be biohazardous, avoid ingestion, inhalation and contact with the skin.

4.1.3 Sample Preservation & Storage: Start microbiological examination of a water sample promptly after collection to avoid unpredictable changes. If samples cannot be processed within 1 hour after collection, use an iced cooler for storage during transport to the laboratory. Hold temperature of all stream pollution, drinking, and wastewater samples below 10°C during a maximum transport time of 6 hours. Refrigerate these samples upon receipt in the laboratory and process within 2 hours. In no case the time elapsing between

collection and examination shall exceed 24 hours.

4.1.4 Sample Preparation: Sample must be shaken vigorously at least 25 times prior to filtration.

4.1.5 Method Performance Criteria: The reference method cites that statistical comparisons of results obtained by the multiple-tube method and the membrane filter technique show the membrane filter is more precise. Although data from each test yield approximately the same water quality information, numerical results are not identical. For raw water sources it would be expected that 80% of the membrane filter test results would be within the 95% confidence limits of the multiple-tube completed test results. Results from the multiple-tube test would be expected to be higher than membrane filter results because of a built-in positive statistical bias.

4.2 Apparatus & Equipment & Supplies: Sample bottles 125mL, Dilution bottles, pipets and graduated cylinders 100mL, culture dishes 50 X 12mm, glass filtration units with metal clamp, membrane filter 0.45µm pore size with a 50mm diameter, forceps smooth-tipped, incubator, microscope, fluorescent light source.

4.3 Reagents & media: M-Endo agar or LES M-Endo agar, Lauryl tryptose broth, brilliant-green lactose bile broth, Dilution water and EC with MUG.
Dilution Water Preparation: Dissolve 34g potassium dihydrogen phosphate in 500mL reagent-grade water, adjust to a pH of 7.2±0.5 with one normal sodium hydroxide, and dilute to one liter with reagent-grade water to prepare stock phosphate buffer solution. Add 1.25mL stock phosphate buffer solution and 5.0mL magnesium chloride solution (81.1g MgCl₂·6H₂O qs 1000mL) to a liter of reagent-grade water. Sterilize by autoclaving using slow exhaust for 30 min, if volume prepared is one liter or less and autoclave 45-60 minutes for volumes greater than a liter.

4.4 Procedure: Mark dishes and bench forms with sample identities and volumes and requested information in the total coliform log. Place a sterile membrane filter on the filter base, grid-side up and attach the funnel to the base of the filter unit; the membrane filter is now held between the funnel and the base and secured by the metal clamp. Shake the sample bottle vigorously about 25 times and measure the desired volume, usually 100mL, into a sterile graduated cylinder. Pour contents of cylinder into the funnel and apply vacuum. Filter sample and rinse the sides of the funnel at least twice with 20-30mL of sterile dilution water. Loosen the lid of an M-Endo agar or LES M-Endo agar petri dish. Turn off the vacuum and using flamed forceps, remove the membrane filter from the filter base and place grid-side up on the agar. Place the inoculated filter directly on the agar surface. Reseat the membrane if air bubbles occur between the agar surface and bottom of the filter. Invert the dish and incubate for 24 ±2 hours. **Under no circumstances will samples be run in series using the same filter funnel and support. All samples will be filtered using only one filter funnel per test.** After incubation remove the dishes from the incubator and examine for sheen colonies using a 10X or 15X binocular microscope with fluorescent light source.

4.5 Verification procedure: Verification of total coliform colonies from M-Endo type media validates sheen as evidence of coliforms. Verification of representative numbers of colonies may be required in evidence gathering or

for quality control procedures.

Using a sterile inoculating needle, pick growth from the centers of a least 5 but, no greater than 10 isolated sheen colonies. Inoculate each into a tube of lauryl tryptose broth and simultaneously into brilliant green bile lactose broth tubes and incubate 24-48 hours at 35°C ±0.5°C. At the 24 and 48 hour readings, confirm gas-positive lauryl tryptose broth and brilliant green bile broth tubes. Cultures that are positive in both media are interpreted as verified coliform colonies. To verify sheen colonies are of fecal origin inoculate culture tubes of EC with MUG simultaneously with the BGB and LTB media and incubate 24-48 hours at 35°C ±0.5°C. E.coli is present if the culture tube is fluorescent when exposed to a Wood's lamp (UV).

4.6 Calculation of coliform density: Compute the count, using membrane filters with 20-80 coliform colonies and not more than 200 colonies of all types per membrane. By the following equation:

$$\text{(Total) coliform colonies/100 mL} = \frac{\text{coliform colonies counted} \times 100}{\text{mL sample filtered}}$$

For verified coliform counts, adjust the initial count based upon the positive verification percentage and report as "verified coliform count per 100 mL."

$$\% \text{ verified coliforms} = \frac{\text{Number of verified colonies}}{\text{Total \# of colonies subjected to verification}} \times 100$$

With water of good quality, the occurrence of coliforms generally will be minimal. Therefore, count all coliform colonies and disregard the lower limit of 20 as cited previously and use the formula given above to obtain coliform density.

If confluent growth occurs, that is, growth covering either the entire filtration area of the membrane or a portion thereof, and colonies are not discrete, report results as 'confluent growth with (or without) coliforms' and request a new sample from the same location. If the total number of bacterial colonies, coliforms plus noncoliforms, exceeds 200 per membrane, or if the colonies are not distinct enough for accurate counting, report results as "too numerous to count" (TNTC). The presence of coliforms in such cultures showing not sheen may be indicated by placing the entire membrane filter culture into a sterile tube of brilliant green lactose bile broth. As an alternative, brush the entire filter surface with a sterile loop, applicator stick or cotton swab and inoculate this growth to the tube of BGB. If gas is produced from this culture within 48 hours at 35°C ±0.5°C, conclude that coliforms are present. For compliance samples, report confluent growth or TNTC with at least one detectable coliform colony as a total coliform positive sample. Report confluent growth or TNTC without detectable coliforms as invalid. Request a new sample from the same location and select more appropriate volumes to be filtered per membrane, observing the requirement that the standard drinking water portion is 100 mL.

4.7 Reporting: Record all results on LIMS. Mail out report forms: Pink copies of BT's are counted and given to the Lab supervisor, the yellow copies of BT's, are placed in a green interoffice mailer addressed to Barbara Gastian

and placed in the Water file at the front desk. File all white copies of all microbiological reports sequential order by log number in an accordion file.

5.0 MEMBRANE FILTER (MF) TECHNIQUE FOR FECAL COLIFORM

5.1 General Discussion

5.1.0 Principle: Definition of the Fecal Coliform group- The fecal coliform are part of the total coliform group. They are defined as gram-negative nonspore-forming rods that ferment lactose in 24 \pm 2 hours at 44.5 \pm 0.2°C with the production of gas in a multiple-tube procedure or produce acidity with blue colonies in a membrane filter procedure. The major species in the fecal coliform group is *Escherichia coli*, a species indicative of fecal pollution and the possible presence of enteric pathogens. Summary of the technique: An appropriate volume of a water sample or its dilution is passed through a membrane filter that retains the bacteria present in the sample. The filter containing the microorganisms is placed on a culture dish containing M-FC agar. The dish is incubated at 44.5 \pm 0.2°C for 24 hours. After, incubation the typical blue colonies are counted under low magnification and the number of fecal coliforms is reported per 100 mL of original sample.

5.1.1 Interferences: Turbidity caused by the presence of algae or other interfering material may not permit testing of a sample volume sufficient to yield significant results. Low coliform estimates may be caused by the presence of high numbers of non-coliforms or of toxic biotoxic substances. This technique is applicable to the examination of saline waters, but not wastewater that have received only primary treatment followed by chlorination because of turbidity in high volume samples or wastewater containing toxic metals or toxic organics such as phenols.

5.1.2 Safety Considerations: Samples and used test materials may be biohazardous, avoid ingestion, inhalation and contact with the skin.

5.1.3 Sample Preservation & Storage: Start microbiological examination of a water sample promptly after collection to avoid unpredictable changes. If samples cannot be processed within 1 hour after collection, use an iced cooler for storage during transport to the laboratory. Hold temperature of all stream pollution, drinking, and wastewater samples below 10°C during a maximum transport time of 6 hours. Refrigerate these samples upon receipt in the laboratory and process within 2 hours. In no case the time elapsing between collection and examination shall exceed 24 hours.

5.1.4 Sample Preparation: Sample must be shaken vigorously at least 25 times prior to filtration.

5.1.5 Method Performance Criteria: The reference method cites that the MF procedure uses an enriched lactose medium and incubation temperature of 44.5 \pm 0.2°C for selectivity and gives 93% accuracy in differentiating between coliforms found in the feces of warm-blooded animals and those from other environmental sources.

5.2 Apparatus & Equipment & Supplies: Sample bottles 125mL, Dilution bottles, pipets and graduated cylinders 100mL, culture dishes 50 X 12mm, plastic filtration units, membrane filter 0.45 μ m pore size with a 50mm diameter,

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forceps smooth-tipped, incubator (water bath), microscope, fluorescent light source, water-proof plastic bags, Bunsen burner.

5.3 Reagents & media: M-FC agar, Dilution water, ethanol (undenatured), 1% rosolic acid.

5.4 Procedure: Mark dishes and bench forms with sample identities and volumes and requested information in the fecal coliform book. Using a sterile forceps place a sterile membrane filter 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 base. Shake the sample vigorously about 25 times and measure the sample into the funnel with vacuum off. If sample volume is <10 mL, add 10mL of sterile dilution water to the filter before adding the sample. Sample volumes use should provide counts of 20-60 colonies on a membrane filter. Do not filter less than 1.0 mL of undiluted sample. Filter the sample and rinse the sides of the funnel walls at least twice with 20-30 mL of sterile dilution water. Turn off the vacuum and remove the funnel from the filter base. Aseptically remove the membrane filter from the filter base. Place the filter, grid side up, on the M-FC agar, using a rolling motion to prevent air bubbles. Incubate the petri dishes for 24 ±2 hours at a temperature of 44.5 ±0.2°C in sealed waterproof plastic bags submerged (with petri dishes inverted) in a waterbath. MF cultures should be placed in the incubator within 30 minutes of filtration. After 24 hours remove dishes from the incubator and examine for blue colonies.

5.5 Calculation of coliform density: Select those plates with 20-60 blue (sometimes greenish-blue) colonies. Non-fecal colonies are gray, buff or colorless and are not counted. Pinpoint blue colonies should be also counted as well as the larger colonies. The colonies are counted using a microscope of 10-15X and a fluorescent lamp. The following rules are used in calculating the fecal coliform count per 100 mL of sample:

$$\text{Fecal coliform colonies/100 mL} = \frac{\text{\#fecal coliform colonies counted} \times 100}{\text{mL sample filtered}}$$

Filter colony count range 20-60 CFU (colony forming units).

Conditions for calculation:

- 1) Only one filter in range: Use the one filter to calculate Fecal coliform colonies/100 mL.
- 2) More than one filter in range: Calculate individual numbers and average results, of those filters within range.
- 3) All filters less than 20 colonies: Use single highest filter count to calculate Fecal coliform colonies/100 mL.
- 4) All Filters are zero: Calculate using highest volume filter filtered, assume a count of one. Report result as a less than value.
- 5) All filters greater than 60 colonies: Calculate using filter count with the smallest volume filtered. Report as 'Estimated Count.'

- 6) All filters 'TNTC': Use upper limit count (60) with smallest filtration volume for calculation, report as greater than value.
- 7) No result, due to confluence, Lab accident, etc.: Report as 'No Result', and specify reason for no result.
- 8) All filters less than 20 and greater than 60: Calculate using filter with the highest volume filtered, with count closest to the ideal range, 20-60 CFU.

5.6 Reporting: Record all results on LIMS. Results for fecal coliform are recorded in the fecal coliform book. Results for the final effluent fecal coliform are recorded in the 'Final Effluent pH and Fecal Coliform' book.

6.0 HETEROTROPIC PLATE COUNT, Pour Plate Method

6.1 General Discussion

6.1.0 Principle: The heterotrophic plate count, formerly known as the standard plate count, is a procedure for estimating the number of live heterotrophic bacteria in water and measuring changes during water treatment and distribution or in swimming pools. Colonies may arise from pairs, chains, clusters, or single cells, all of which are included in the term 'colony-forming units' (CFU). The pour plate method can accommodate volumes of sample or diluted sample ranging from 0.1 to 2.0 mL.

6.1.1 Safety Considerations: Samples and used test materials may be biohazardous, avoid ingestion, inhalation and contact with the skin.

6.1.2 Sample Preservation & Storage: Start microbiological examination of a water sample promptly after collection to avoid unpredictable changes. If samples cannot be processed within 2 hour after collection, use an iced cooler for storage during transport to the laboratory. Hold temperature of all stream pollution, drinking, and wastewater samples below 10°C during a maximum transport time of 6 hours. Refrigerate these samples upon receipt in the laboratory and process within 2 hours. In no case the time elapsing between collection and examination shall exceed 24 hours.

6.1.3 Sample Preparation: Sample must be shaken vigorously at least 25 times prior to plating.

6.1.4 Method Performance Criteria: The reference method cites no method performance criteria.

6.2 Apparatus & Equipment & Supplies: Sample bottles 125mL, Dilution bottles, pipets, culture dishes 100 X 15mm, incubator, Quebec counter, culture tubes, 105°C oven, test tube rack, hand tally.

6.3 Reagents & media: R2A agar, dilution water.

6.4.0 Procedure:

6.4.1 Sample Dilution:

a) *Selecting dilutions*- Select the dilution so that the total number of colonies on a plate will be between 30 and 300. For most potable water samples, plates suitable for counting will be obtained by plating 1 mL and 0.1 mL undiluted sample and plating 1 mL of the 10^{-2} dilution. Higher dilutions may be necessary with some potable waters.

b) *Measuring sample portions*- If dilution of a sample is indicated, shake the sample vigorously about 25 times. Then prepare an initial 1:100 dilution by pipetting 1 mL of the sample into a 99 mL dilution water blank using a sterile 1 mL pipet. The 1:100 dilution bottle is vigorously shaken and further dilutions made by pipetting aliquot into additional dilution blanks. Use a sterile pipet for initial and subsequent transfers from each container.

If pipet becomes contaminated before transfers are completed, replace with a sterile pipet. Use a separate sterile pipet for transfers from each different dilution. Do not prepare dilutions and pour plates in direct sunlight. Use caution when removing sterile pipets from the container; to avoid contamination, do not drag pipet tip across exposed ends of pipets in the pipet container or across lips and necks of dilution bottles. When removing sample, do not insert pipets more than 2.5 cm below the surface of sample or dilution.

6.4.2 Preparation of Agar: Melt prepared plate count agar by heating in an oven or boiling water. Do not allow the medium to remain at these high temperatures beyond the time necessary to melt it. Prepared agar shall be melted only once. Place melted agar in a tempering water bath maintained at temperature of 44-46°C. Do not hold agar at this temperature longer than three hours because precipitates may form which confuse the counting of colonies. Maintain a thermometer immersed in a separate tube or melted media in the water bath to monitor the temperature of the media.

6.4.3 Preparation for plating: Prepare at least duplicate plates for each sample. Mark volume of sample used and identify samples with a marker pen on the lid of petri dishes. Aseptically pipet an aliquot from the appropriate dilution or sample container into the bottom of each petri dish. Disperse the aliquot by placing droplets of sample in a symmetrical pattern in the bottom of the petri dish, start deposition at the center and work outward from center of the dish. Use a separate sterile pipet to transfer an aliquot to each set of petri dishes for each sample or sample dilution used. Vigorously shake the undiluted sample and dilution containers before each transfer is made. Pipet sample or sample dilution into marked petri dish. To minimize bacterial density changes in the samples, do not prepare any more samples than can be diluted and plated within 20-25 minutes.

6.4.4 Pouring Agar Plates: Use the thermometer in the control tube in the tempering bath to check the temperature of the plating medium before pouring. Add not less than 12 mL (usually 12-15 mL) of the melted and cooled (44-46°C) agar medium to each petri dish containing an aliquot of the sample or its dilution. Mix the inoculated medium carefully to prevent spilling. Avoid

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splashing the inside of the cover by using the following technique: rotate the plate five times to the left, five times to the right and five times in a back and forth motion. The technique is performed with the plate remaining on the counter for support. Pipet a one mL volume of sterile dilution water into a petri dish, add agar, mix and incubate with test plates. This control plate will check the sterility of pipets, agar dilution water and petri dishes.

6.4.5 Incubation of Plated Samples: After agar plates have hardened on a level surface, usually within 10 minutes, invert the plates and immediately incubate at 35°C for 48 hours. Stacks of plates should be at least 2.5 cm from adjacent stacks, the top or sides of the incubator, Do not stack plates more than four high. These precautions allow proper circulation of air to maintain uniform temperature throughout the incubator and speed equilibration.

6.5 Counting and Recording: After incubation, examine plates and select those with 30-300 colonies using a Quebec counter and hand tally. The following rules shall be used for reporting results:

a) **Plates with 30-300 colonies:** Count all colonies and divide by the volume tested. If replicate plates from one dilution are countable, sum the counts of colonies on all plates and divide by the volumes tested as in the following equation:

$$\text{Count/mL} = \frac{\text{Sum of Colonies}}{\text{Sum of Volumes Tested, mL}}$$

Record the dilutions used, the number of colonies on each plate and report as count per milliliter. If two or more consecutive dilutions are countable (30-300), independently carry each calculation of plate count to final count per mL, then calculate the mean of these counts/mL for the reported value.

b) **All Plates with Fewer than 30 Colonies:** If there are less than 30 colonies on all plates, record the actual number of colonies on the lowest dilution plated and report the count as: Estimated Heterotrophic Plate Count per milliliter. However, if 1 mL of the original sample produce counts of <30, then the actual counts are reported.

c) **Plate with No Colonies:** If all plates from dilutions tested show no colonies, report the count as <1 times the lowest dilution plated.

d) **All Plates Greater than 300 Colonies:** When counts per plate in the highest dilution exceed 300 colonies, compute the count by multiplying the mean count by the dilution used and report as a greater than (>), Heterotrophic Plate count per milliliter.

6.6 Reporting: Record all results on LIMS. Results for heterotrophic plate count are recorded in the standard plate count book.

7.0 MOST PROBABLE NUMBER (MPN) METHOD for Fecal Coliform & Fecal Streptococci

7.1 General Discussion

7.1.0 Principle: The Most Probable Number procedure estimates the number of specific organisms in water and wastewater by the use of probability tables.

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Decimal dilutions of samples are inoculated in series into liquid tube media.

Positive test are indicated by growth and/or fermentative gas production. Bacterial densities are based on combinations of positive and negative tube results read from the MPN table. The MPN procedure may be carried to three stages of completion:

The Presumptive Test- provides a preliminary; estimate of bacterial density based on enrichment in minimally-restrictive tube media. The results of this test are usually never used without further analyses.

The Confirmed Test- the second stage of the MPN, is the usual extent of testing. Growth from each positive Presumptive Test tube is inoculated into a more selective inhibitory medium. The tubes are incubated at the prescribed temperature and time, the positive reactions noted and counts calculated from the MPN table.

The Completed Test- is the third stage of the MPN used for total coliform analyses only. Positive tubes from the Confirmed Test are submitted to additional test to verify the identification of the isolated microorganisms. Although the Completed Test provides the greatest reliability, the amount of time and the workload restrict its use to periodic substantiation of Confirmed Test results, to other QC checks on methodology and analysts, and to research.

The MPN procedure can be used for water samples with high turbidity or large numbers of algae, which have no apparent deleterious effect on the tube reactions. If a toxic substance is present in the sample, the resultant 1:10 or 1:100 dilution of that sample in the liquid broth may reduce the toxicity to the point of no effect. The MPN may be in many cases the only method applicable to problem sample materials such as bottom sludge, muds, soils and sediments.

7.1.1 Interferences: Background organisms or toxic constituents in 10 mL volumes can interfere and be undetected.

7.1.2 Safety Considerations: Samples and used test materials may be biohazardous, avoid ingestion, inhalation and contact with the skin.

7.1.3 Sample Preservation & Storage: Start microbiological examination of a water sample promptly after collection to avoid unpredictable changes. If samples cannot be processed within 1 hour after collection, use an iced cooler for storage during transport to the laboratory. Hold temperature of all stream pollution, drinking, and wastewater samples below 10°C during a maximum transport time of 6 hours. Refrigerate these samples upon receipt in the laboratory and process within 2 hours. In no case the time elapsing between collection and examination shall exceed 24 hours.

7.1.4 Sample Preparation: Sample must be shaken vigorously at least 25 times prior to culture tube inoculation. For solid samples, place 50g sample in sterile blender container, add 450 mL sterile phosphate or 0.1% peptone dilution water, and blend for 1 to 2 minutes at low speed. Prepare the appropriate decimal dilutions of the homogenized slurry as quickly as possible to minimize settling. For determining fecal coliform density for solid samples refer to Appendix IV.

7.1.5 Method Performance Criteria: The reference method cites that the MPN

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tables are probability calculations and inherently have poor precision and contain a 23% bias at the 5 tube, three dilution levels normally used.

7.2 Apparatus & Equipment & Supplies: Dilution bottles, pipets, culture tubes 150 X 20mm with inverted fermentation vials, incubator (water bath), Bunsen burner, inoculation loops 3mm diameter and needle, culture tube racks 10 X 5 openings, dilution bottles.

7.3 Reagents & media: Dilution water, A-1 broth (used for fecal coliform), Azide dextrose broth, Pfizer selective enterococcus* (PSE) agar (used for fecal streptococci). Lauryl Tryptose broth, Brilliant Green broth, EC (used for total coliform and fecal coliform).

*Bile Esculin Azide agar

7.4 Procedure:

Presumptive test- To perform the Presumptive Test, arrange a series of three or more rows of culture tubes containing the test medium in a rack, providing for five replicates in each row. Use five rows for samples of unknown density. Inoculate each successive row with decreasing decimal dilutions of the sample. When removing sample aliquot or dilutions for further inoculations, do not insert the pipet tip more than 2.5 cm (1") below the surface of the sample. For Fecal Streptococci incubate tubes containing Azide dextrose broth for 24±2 hours at 35°C. For fecal coliform incubate tubes containing A-1 for 3 hours at 35°C. Then transfer tubes to a water bath at 44.5 ±0.2 hours for 21 ±2 hours. A positive presumptive test is gas production for fecal coliforms or growth for fecal streptococci. After 24 hours incubation, examine the tubes for gas formation and/or growth. Inoculate positive streptococci tubes into Confirmed Test media. Fecal coliform using A-1 media is a direct method requiring no conformational step, gas production in any A-1 broth culture within 24 hours or less is a positive reaction indicating coliforms of fecal origin. If there is no growth in the streptococci tubes, reincubate the negative tubes for an additional 24 hours.

If the presumptive fecal streptococci tubes are negative after 48 ±3 hours, discard tubes. If the Presumptive streptococci tubes are positive, the cultures are verified in the Confirmed Test. Record the negative and positive results.

Confirmed Test- The Confirmed Test is performed by verifying positive tubes from the Presumptive Test at 24 and 48 hours. If presumptive tubes are positive at 24 hours, confirm them at that time. Subject all azide dextrose broth tubes showing growth (turbidity) after 24 or 48 hours incubation to the confirm test. Streak a portion of the growth from each positive azide dextrose broth tube on PSE (Bile Esculin Azide) agar. Incubate the inverted petri dish of streaked PSE agar at 35 ±0.5°C for 24 ±2 hours. Brownish-black colonies with brown halos confirm the presence of fecal streptococci.

7.5 Calculation for MPN Value: to calculate density, compute in terms of the Most Probable Number (MPN). The MPN values, for a variety of planting series and results, are given in the table (MPN table refer to appendix III), MPN values for combinations of positive and negative results when five 10 mL, five 1.0 mL and five 0.1 mL volumes of sample are tested. When the series of decimal dilution is different from that in the table, select the MPN value for the combination of positive tubes and calculate according to the following

formula:

$$\text{MPN value (from table)} \times \frac{10}{\text{Largest volume tested}} = \text{MPN}/100\text{mL}$$

When more than three dilutions are used in a decimal series of dilutions, use the results from only three of these in computing the MPN. To select the three dilutions to be used in determining the MPN index, choose the highest dilutions that gives positive results in all five portions tested (No lower dilutions giving any negative results) and the two next succeeding higher dilutions. Use the results at these three volumes in computing the MPN index.

The MPN for combinations not appearing in the table, or for other combinations of tubes or dilutions, may be estimated by Thomas's simple formula:

$$\text{MPN}/100 \text{ mL} = \frac{\text{Number of positive tubes} \times 100}{\sqrt{\text{mL sample in negative tubes} \times \text{mL sample in all tubes}}}$$

7.6 Reporting: Record all results on LIMS. Results for fecal coliform and fecal streptococci are recorded in the MPN book.

8.0 CHROMOGENIC/FLUOROGENIC SUBSTRATE TEST (MMO-MUG TEST, COLILERT⁷) FOR TOTAL COLIFORM

8.1 General Discussion

8.1.0 Principle: Colilert[®] reagent is used for the simultaneous detection and confirmation of total coliforms and E. Coli in water. It is based on patented defined substrate technology. The media utilizes nutrient indicators that produce color and/or fluorescence when metabolized by total coliforms and E. Coli. When the reagent is added to the sample and incubated, it can detect these bacteria at 1 CFU/100mL within 24 hours with as many as 2 million heterotrophic bacteria/100mL present.

8.1.1 Interferences: This technique is not applicable to the examination of saline waters.

8.1.2 Safety Considerations: Samples and used test materials may be biohazardous, avoid ingestion, inhalation and contact with the skin.

8.1.3 Sample Preservation & Storage: Start microbiological examination of a water sample promptly after collection to avoid unpredictable changes. If samples cannot be processed within 1 hour after collection, use an iced cooler for storage during transport to the laboratory. Hold temperature of all stream pollution, drinking, and wastewater samples below 10NC during a maximum transport time of 6 hours. Refrigerate these samples upon receipt in the laboratory and process within 2 hours. In no case the time elapsing between collection and examination shall exceed 24 hours.

8.1.4 Sample Preparation: Sample must be shaken vigorously at least 25 times prior to addition of media.

8.1.5 Method Performance Criteria: The reference method cites no performance

8.2 Apparatus & Equipment & Supplies: Sample bottles 125mL, sterile transparent sample container 125mL, sterile graduated cylinders 100mL, UV lamp (6W, 366nm), incubator 35°C.

8.3 Reagents & media: Colilert® ONPG-MUG media (Storage: 4-30°C away from light). Colilert7 Presence/Absence Comparator,

8.4 Procedure: Mark sample test container and bench forms with sample identities and requested information in the total coliform log. Shake the sample bottle vigorously about 25 times and measure the desired volume, 100mL, into a sterile graduated cylinder. Pour contents of cylinder into sample test container. Carefully separate one Snap Pack of Colilert7 ONPG-MUG media from the strip taking care not to accidentally open adjacent pack. Tap the Snap Pack to ensure that all of the Colilert® powder is in the bottom part of the pack. Open one pack by snapping back the top at the scoreline. Add the reagent to the water sample container. Aseptically replace cap of sterile sample container and shake until powder is dissolved. Incubate for 24 hours at 35°C ±0.5°C. After incubation remove the sample container from the incubator and examine for color change. Compare each result against the Comparator dispensed into an identical vessel. The Comparator is a color/fluorescence reference which acts as a threshold positive control. If the sample becomes as yellow or deeper yellow than the comparator after adding Colilert® media and incubation, then the sample is positive for total coliforms. Any color change which has a lower intensity than the Comparator indicates that the sample is negative for total coliforms. Likewise, after incubation, an E.coli positive sample will fluoresce with an intensity equal to or greater than the comparator. The Comparator is made with color and fluorescent dyes and does not contain any coliforms or E.coli. If no yellow color is observed, the test is negative for total coliform. If the sample has a yellow color equal to or greater than the comparator, the presence of total coliforms is confirmed. If color is not uniform, mix by inversion then recheck. If the sample is yellow, but lighter than the comparator, it may be incubated an additional 4 hours (but no more than 28 hours total). If the sample is coliform positive, the color will intensify. If it does not intensify, the sample is negative for total coliform. If the sample is yellow, check vessel for fluorescence by placing a 6 watt 365 nm UV light within five inches of the sample in a dark environment. Be sure the light is facing away from your eyes and toward the vessel. If fluorescence is greater or equal to the fluorescence of the comparator, the presence of E.coli is confirmed.

8.5 Calculation of coliform: Using the criteria in the procedure determine the Presence or Absence of total Coliforms and E.coli. Report the results of each sample as having total coliform, Present or Absent. If the sample was positive for total coliform then report the results of each positive sample as having E.coli, Present or Absent.

8.6 Reporting: Record all results on LIMS. Mail out report forms: Pink

copies of BTs are counted and given to the Lab supervisor, the yellow copies of BTs, are placed in a green interoffice mailer addressed to Barbara Gastian and placed in the Water file at the front desk. File all white copies of all microbiological reports sequential order by log number in an accordion file.

9.0 RECORDS AND DATA REPORTING

Records of microbiological analyses must be kept by the laboratory or must be accessible to the laboratory for at least five (5) years. Actual laboratory reports may be kept, or data may be transferred to ledgers, provided that the following information is included:

1. Exact date and time of collection.
2. Sample site location.
3. Name of collector.
4. Identification of sample as to whether it is a routine distribution sample, check sample, raw or process water sample, or other special purpose sample.
5. Chlorine residual is taken and recorded.
6. Exact date and time of sample receipt and analysis.
7. Laboratory and persons responsible for performing analysis.
8. Analytical procedure used, and quality control data. The laboratory may only use those procedures that they are certified for.
9. Results of analysis. Base results of coliform analyses on data from confirmation procedure or completed test procedure (for MPN Procedure).

10.0 ACTION RESPONSE TO LABORATORY RESULTS

1. Laboratory must submit a copy of all compliance analytical results within five working days after completion of the analyses to:

- a) Water System
- b) local Environmental Department field office nearest to the water system:

New Mexico Environment Department
Environmental Division District 1
4131 Montgomery Blvd. N.E.
Albuquerque, New Mexico 87109

2. Laboratory must test all total coliform-positive cultures for presence of either fecal coliforms or E. coli.

3. Laboratory must notify state Environmental Department within 24 hours of a positive total coliform, fecal coliform, or E. coli result, so that appropriate follow-up action (e.g., collection of repeat samples) can be conducted. Total coliform-positive result is based on verified test for the

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Multiple Tube Fermentation Technique, Presence- Absence (P/A) Coliform Test or Membrane Filter Technique.

4. Laboratory must notify state Environmental Department when results indicates that high levels (TNTC or Confluent Growth) of noncoliforms are present and may interfere with the total coliform analysis. Sample is invalid and a resample from the same location is required.

5. Laboratory must notify the Water Utility Div. immediately of a positive total coliform, fecal coliform, or E. coli result, so that appropriate follow-up action (e.g., collection of repeat samples) can be conducted. Total coliform-positive result is based on verified test for the Multiple Tube

Fermentation Technique, Presence- Absence (P-A) Coliform Test or Membrane Filter Technique. Laboratory must notify the Water Utility Div. when results indicates that high levels (TNTC or Confluent Growth) of noncoliforms are present and may interfere with the total coliform analysis. Sample is invalid and a resample from the same location is required. All 'Compliance Samples' with positive results are to be reported to WUD using the Internal Communication Pathway for the Total Coliform Rule found in **Table I**.

11.0 MICROBIOLOGICAL QUALITY ASSURANCE PLAN

11.1 Unscheduled Activities

- 1) Confirm all compliance total coliform up to 5 colonies.
- 2) Each filtration series will use a 'control blank' at the beginning and end of the series.
- 3) Complete documentation is required for all logs and workbooks.
- 4) Use lower dilutions on final effluent when total chlorine is < 1 mg/L and TSS > 25 mg/L.
- 5) Document the lot# and date on autoclave temperature recording chart when sterilizing. Use a highest recording thermometer during each sterilization cycle. Record total time in autoclave and temperature in sterilization log.
- 6) Sterilize all used supplies, live cultures and used culture media before final disposal.
- 7) Maintain the autoclave maintenance and repair records. File service records in autoclave maintenance log.

11.2 Lot Testing Activities

- 1) Test all sterile supplies, media, glassware, funnels, filters, pipet and petri dishes. Confirm sterility using TSB medium.
- 2) Test all washed sterile glassware with BTB.
- 3) Test all prepared media with positive and negative control organism.

11.3 Daily Activities

- 1) Record incubators and refrigerator temperatures, at 10am and 2pm on apparatus chart and record temperature in Temperature Book

11.4 Weekly Activities

- 1) Check the conductivity of reagent-grade water.
- 2) Check autoclave performance with a kill-it ampule or spore strip.
- 3) Transfer live Control cultures to new tube of TSB.

11.5 Monthly Activities

- 1) Start new control cultures from ATCC sets.
- 2) Perform both total coliform procedures on a known coliform positive sample, TP2.6.
- 3) Perform HPC, Total Chlorine testing on reagent-grade water.
- 4) The autoclave automatic timing mechanism shall be check monthly with a stopwatch and the results of the timing test recorded in the Autoclave and Glassware Check Book.
- 5) The balance sensitivity must be checked monthly. The balance shall be calibrated monthly using three ASTM type II weights. The weights used should bracket laboratory weighting needs.

11.6 Annual Activities

- 1) Bacterial testing of reagent-grade water.
- 2) Trace metals analysis must be performed on reagent-grade water.
- 3) Calibrate all thermometers with a NIST traceable thermometer.

12.0 MICROBIOLOGICAL CORRECTIVE ACTION PLAN

12.1 Autoclave- If the autoclave is not functioning properly in regards to sterilization time and temperature or is non-operational, notify section supervisor for corrective action, If supervisor is not available call Medical Scientific Services at 298-6639 for emergency repair service.

12.2 Sterilized material- If any supply, media or other material fails the sterility testing the lot will be condemned. The lot will be reesterilized and retested where applicable or discarded where reesterilization renders the material unusable.

12.3 Biological media- If any prepared media fails to achieve a specified final pH or produce the specified reaction for a negative and or positive control organism for that media or carbohydrate media that has exceeded a

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total autoclave cycle time of 45 minutes, the lot will be condemned and discarded. Media lots that have been condemned will be documented in the Prepared Media Book.

12.4 Incubators, water baths and refrigerators- If any of these apparatus not within the specified temperature range as indicated in the temperature book make an immediate adjustment of the temperature control, as outlined in the operation manual for that device, operation manuals can be located in the laboratory office. Samples resident in the incubator during an episode when the incubator is out of range will be deemed a 'Laboratory Accident' and will be treated as an 'Invalid Sample.' Any corrective action will be documented in the Corrective Action Log.

12.5 Glassware- If any glassware fails the pH test with BTB, that is indicator not blue-green, then re-rinse with reagent-grade water and retest. Failure is indicated when indicator is yellow (acidic) or blue (basic).

12.6 Laboratory Water- If failure of the laboratory water to meet the criteria in the Water Book for any of the test parameters, then the section supervisor is to be notified immediately or is the supervisor in not available call Culligan/Southwest Water Conditioning @ 299-9581 for emergency repair service.

12.7 Balance- If the top loading balance fails to achieve a sensitivity of $100\text{mg} \pm 5\text{mg}$ @ a 150g load, and fails to calibrate with calibration weights, notify section supervisor for corrective action, If the supervisor is not available call QA Balance Service Inc. At 303-693-6419 for emergency repair service.

12.8 pH- If the pH meter fails to calibrate or hold calibration ± 0.1 pH units, notify section supervisor for corrective action, after consulting the troubleshooting guide in the Orion manual.

12.9 Proficiency Samples- If a failure to perform adequately on a proficiency sample set, then a new set will be ordered and tested until a greater proficiency or satisfactory proficiency is achieved.

12.10 Control Blank- If the control blank has present any growth after incubation, all samples tested concurrently with the control blank will be rejected and new samples requested. All material lots used to comprise the control blank will be re-tested for sterility and discarded if determined to be contaminated.