MICROBIOLOGY CHECKLIST

1. PERSONNEL	S	U	N	Comments
The laboratory supervisor/consultant has a bachelor's degree in biology, chemistry medical technology or a related field. [ ] Supervisor has at least 4 credits in Microbiology; or has a [ ] minimum of 2 weeks training from a federal agency, state agency or academic institution in the microbiological analysis of drinking water.				
Supervisor/consultant has one year experience on the job. [ ] If a consultant is used, the consultant is present on site at least one day per month and is available more frequently if needed by the analyst.				
Analyst has a minimum of high school education. Analyst has had training in the microbiological analysis plus minimum 30 days on the job training.				
Data produced by the analyst while in the process of obtaining the required training and experience were reviewed and validated by fully qualified analyst or the supervisor/consultant.				
2. LABORATORY FACILITIES				
Laboratory facilities are clean, have temperature and humidity control, have adequate lighting at bench tops and 150 - 200 sq/ft/analyst is available.				
Provisions have been made for the disposal of biological wastes. _____ _____				
There is sufficient bench top area for processing samples (5 to 6 linear ft/analyst recommended), storage and bench top equipment.				
Separate office space. (Recommended)				
3. LABORATORY SAFETY (Recommended)	**S**	**U**	**N**	**Comments**
Laboratory facilities and practice satisfy applicable state and OSHA standards.				
Passages and aisles are clear and uncongested.				
Fully operable fire extinguisher to cover Class				

A, B, and C fires located within 50 ft. of the laboratory.				
Fire exits from laboratory clear.				
First aid supplies available.				
Personnel indoctrinated in first aid emergency procedures and fire control.				
No smoking, eating or drinking permitted in the laboratory.				
4. LABORATORY EQUIPMENT AND SUPPLIES				
pH METER, ELECTRONIC Manufacturer _____ Model _____.				
pH meter has accuracy and scale graduations within +/- 0.1 units.				
pH buffer aliquots are used only once				
Calibrated to 0.1 pH units and record maintained.				
pH meter is clean and calibrated each use period with 2 certified buffer standards that bracket the lab's normal working range. A pH 7.0 buffer is then measured. The records include the lot number of the buffer solutions.				
Electrodes are maintained according to manufacturer's recommendations.				
Commercial buffer solution containers are dated upon receipt, and when opened. None exceeds the manufacturer's expiration date.				

4. LABORATORY EQUIPMENT AND SUPPLIES (Cont.)	S	U	N	Comments
BALANCE - TOP LOADER AND PAN For general media preparation TYPE:_____				
Balance is able to detect 100 mg at a 150 g load.				
Balance is clean and operable.				
Appropriate weights of good quality available for each balance.				
Calibration of the balance is checked monthly using a minimum of 3 NIST Class S, NIST Class S-1, ANSI/ASTM Class 1, or ANSI/ASTM Class 3 reference weights which bracket the laboratory's weighing needs.				
Non-reference weights are calibrated at least every 6 months with NIST Class S, NIST Class S -1, ANSI/ASTM Class 1, or ANSI/ASTM Class 3 reference weights.				
At least one set of weights is recertified annually by a commercial balance service. Any other sets of weights are checked annually by a commercial balance service or are checked against a set of weights that has been.				
Weight correction data are used where necessary.				
Balance is serviced on at least an annual basis by a commercial balance service that gives written certification that the balances (and weights where necessary) are calibrated using standards traceable to the NIST.				

4. LABORATORY EQUIPMENT AND SUPPLIES (Cont.)	S	U	N	Comments
INCUBATOR OR INCUBATOR ROOM Manufacturer _____ Model _____ Manufacturer _____ Model _____				
Thermometers used in incubators are graduated in increments of 0.1 °C or less. There is no liquid separation and graduations are legible.				
Calibration of glass/mercury thermometers and digital thermometer is checked annually (dial thermometers quarterly) at the temperature used against a reference NIST or NIST traceable thermometer.				
Only dial thermometers that may be adjusted are used.				
The NIST traceable thermometer is graduated in increments equal to or smaller than the units of the thermometers being checked.				
NIST traceable thermometer has been checked at [the zero point at least once annually(NIST) [at all temperature points used at least every 3 years (EPA manual)].				
Each thermometer in use has a tag which identifies the thermometer, the date the thermometer was checked, and any correction factor. The identification must allow tracing back to the calibration check records. If no correction factor is needed, the tag must have 0.0 correction factor listed on the tag.				
NIST thermometer serial number matches the certificate for the thermometer.				

4. LABORATORY EQUIPMENT AND SUPPLIES (Cont.)	S	U	N	Comments
Continuous recording devices are recalibrated annually.				
Incubator used for Total Coliform analyses is able to maintain a temperature of 35 +/ - 0.5°C on shelves in all areas in use.				
A thermometer is placed on a minimum of the top and bottom shelf of compartment or chamber in use.				
Incubator or water bath used for Fecal Coliform analyses able to maintain a temperature of 44.5 +/- 0.2°C on shelves in all areas in use.				
Thermometer bulbs are immersed in liquid (the probe of digital thermometers that may be read outside the incubator need not be in a liquid).				
Culture dishes & tubes fit snugly if an aluminum block is used.				
Incubator temperature is recorded morning and afternoon for days in use with readings at least 4 hours apart.				
AUTOCLAVE: Manufacturer _____ Model_____.				
Autoclave has a temperature and/or pressure gauge with sensor on the exhaust, and an operational safety valve. (Strongly recommend that both gauges be present)				
Autoclave is able to maintain sterilization temperature during the sterilization cycle and completes the entire cycle within 45 minutes when 12-15 minute sterilization period is used.				

4. LABORATORY EQUIPMENT AND SUPPLIES (Cont.)	S	U	N	Comments
Rapid depressurization does not produce air bubbles in fermentation media.				
Date, contents, sterilization time and temperature, and total time of each cycle of each autoclave cycle is being recorded.				
A maximum-temperature thermometer or continuous recording device is used with all autoclave cycles.				
[ ] Autoclave doors are clean and free of caramelized media. [ ] Autoclave drain screens are clean and have all debris removed.				
Spore strips or ampules are used at least once every month. The records include the results after incubating the test and control strips or ampules.				
Automatic timing mechanism of autoclave has been checked against a stopwatch at least once every quarter.				
The correction factor is applied to the time the automatic timer is set to when incubating media.				
An autoclave [ ]service contract or [ ] internal maintenance protocol has been established. Records are maintained.				
HOT-AIR STERILIZING OVEN Manufacturer:_____Model:_____				
Hot air oven is able to maintain a sterilization temperature of 170 -180°C for at least 2 hours.				
Thermometers in hot air ovens are graduated in increments of 10 °C or less. There is no liquid separation and graduations are legible.				

4. LABORATORY EQUIPMENT AND SUPPLIES (Cont.)	S	U	N	Comments
The bulbs of thermometers in hot air ovens are placed in beakers of sand.				
Date, contents, sterilization time and temperature are recorded for each cycle.				
Spore strips or ampules are used at least once every month. The records include the results after incubating the test and control strips or ampules.				
QUEBEC COLONY COUNTER:				
[ ] Dark field model colony counter [ ] or equivalent is used to count HPC				
CONDUCTIVITY METER Manufacturer:_____ Model:_____				
Conductivity meter is readable in ohms or mhos having a range from 2 ohms to 2 megohms or equivalent micromhos +/ - 2%.				
Conductivity meter is calibrated with 0.01 M KCl solution at least once per month using an appropriate certified and traceable low level standard or the cell constant is determined monthly using a method indicated SM 2510.				
In-line conductivity meter is used only if it can be calibrated.				
REFRIGERATOR:				
Refrigerator maintains a temperature of 1 °C to 5°C.				
Thermometer is graduated in 1 °C increments or less and is immersed in a liquid.				
Refrigerator temperature of the upper and lower shelf is recorded at least once per day for days in use.				

4. LABORATORY EQUIPMENT AND SUPPLIES (Cont.)	S	U	N	Comments
INOCULATION EQUIPMENT				
[ ] Metal or [ ]plastic loops,or [ ] wood applicator sticks sterilized by dry heat, [ ] sterile swabs, or [ ] sterile disposable pipette tips are used as inoculation equipment.				
Bunsen burner or electrical incinerator used for loop & needle sterilization.				
Metal loops are 3 mm dia. loops of nichrome or platinum wire.				
MEMBRANE FILTRATION UNITS Manufacturer:_____ Model:_____				
Membrane filtration units are stainless steel, glass or autoclavable plastic that are not scratched or corroded and do not leak.				
OPTICAL EQUIPMENT				
10 to 15 X stereo microscope with a fluorescent light source is used to count sheen colonies.				
MEMBRANE FILTERS AND PADS Manufacturer:_____ Type:_____				
Membrane filters are made from cellulose ester material, white grid marked, 47 mm diameter, 0.45 µm pore size and certified by the manufacturer for total coliform water analysis. Filters used for Fecal Coliform analyses are certified for that analysis.				
Pads are 47 mm diameter and uniformly absorbent				
Lot number and date received are recorded for membrane filters				
New lots of membrane filters are checked by comparing recovery of coliform organisms against membrane filters from previously acceptable lots. (Recommended)				

4. LABORATORY EQUIPMENT AND SUPPLIES (Cont.)	**S**	**U**	**N**	**Comments**
Sterility of each lot number of membranes is checked before use.				
Forcep tips are clean and without pitting or corrugations.				
If graduation marks on clear glass or plastic funnels are used to measure sample volume, their accuracy is checked with a graduated cylinder before use and a record is maintained. It must be replaced if it has a tolerance greater than 2.5%.				
PETRI DISHES (MF ONLY):				
[ ] Presterilized plastic or [ ] sterilized glass culture dishes are used.				
Loose-lid dishes (with 90% controlled humidity) are incubated in a tight -fitting chamber.				
Dishes are clear, flat bottom, free from bubbles and scratches				
Open packs of disposable culture dishes are resealed between major use periods.				
GLASS, PLASTIC AND METAL UTENSILS FOR MEDIA PREPARATION				
Glass pipets are sterilized in stainless steel or aluminum canisters or individually wrapped in char-resistant paper.				
Pipets have legible markings and are not chipped.				
Pipets are marked TD (to deliver) and made of glass or plastic.				
Open packs of disposable pipets are resealed between major use periods.				

4. LABORATORY EQUIPMENT AND SUPPLIES (Cont.)	S	U	N	Comments
Glass items are made of borosilicate glass or other corrosion-resistant glass, free of chips and cracks.				
Metal utensils are stainless steel or aluminum.				
Utensils are clean and free from foreign residues or dried medium.				
Plastic items are clear with visible graduations.				
Culture tubes used for the Presumptive Test in the MTF technique are large enough to contain the medium plus the sample without being more than 3/4 full.				
Culture tube closures are [ ] stainless steel, [ ] plastic, [ ]aluminum or[ ] screw caps with non-toxic liners.				
Cotton plugs are never used for culture tube closures.				
SAMPLE BOTTLES				
Sample containers are [ ] wide mouth plastic or [ ] non-corrosive glass bottles with ground glass stoppers or screw caps with leak proof non-toxic liners. Sample containers have a capacity of at least 120 mL (4 oz). ~~[ ] Thio whirl pak bags are used~~ .				
Glass stoppered bottle closures are covered with aluminum foil or char-resistant paper for hand delivered samples only.				
Glass bottles are sterilized by [ ] autoclaving or [ ] dry heat. Plastic bottles are sterilized by [ ] autoclaving.				
Empty containers are moistened before autoclaving to prevent an "air lock" sterilization failure.				

4. LABORATORY EQUIPMENT AND SUPPLIES (Cont.)	S	U	N	Comments
Glassware is borosilicate glass or other corrosion resistant glass, free of chips and cracks. Markings on graduated cylinders and pipets are legible.				
Graduated cylinders, and pipets delivering volumes of 10 mL or less, are accurate to a tolerance of 2.5%.				
5. LABORATORY MATERIALS PREPARATION				
AUTOCLAVE STERILIZATION PROCEDURES				
The following minimum times for autoclaving materials at 121 °C are used with necessary adjustments (more time) being made depending on volumes, containers and loads: (Item - Minutes at 121 °C) [ ] Membrane filters and pads (with fast exhaust) - **10 min** not exceeded. [ ] Tube media/reagents (15 min not exceeded) - **12 to 15 min** [ ] Contaminated test materials - **30 min** [ ] Membrane filter assemblies - **15 min** [ ] Sample collection containers - **15 min** [ ] Individual glassware - **15 min** [ ] Dilution water blank - **15 min** [ ] Rinse water - **15 min** (30 min for 1L)				
Autoclaved membrane filters and pads and all media are removed immediately after completion of the cycle.				
[ ] Membrane filter assemblies are autoclaved at the start of the filtration series. [ ] Ultraviolet light sterilization is used only if supplies are presterilized.				

5. LABORATORY MATERIALS PREPARATION (Cont.)	S	U	N	Comments
Membrane filter assemblies are [ ] exposed to UV radiation or [ ] submerged in boiling water for minimum 2 minutes between individual sample filtrations unless a lab is [ ] analyzing chlorinated, municipal samples and can demonstrate that funnel rinsing is adequate to prevent carryover of bacteria from contaminated samples.				
10% sodium thiosulfate solution is added to sample containers prior to sterilization. Records indicate solution is made up properly.				
At least 1 sample container per batch is checked for sterility.				
Tubes are packed loosely in baskets or racks for uniform heating and cooling.				
Timing begins when autoclave reaches 121 °C (250°F) and 15 p.s.i.				
Total exposure of tubed media to heat not over 45 minutes.				
Wire loops, needles, forceps, and spatulas are flame sterilized.				
UV LIGHTS:				
Lamps are cleaned with ethanol monthly.				
[ ] Lamps are tested monthly with UV meter. If less than 70% of initial output, lamps are replaced, -OR- [ ] Spread plate containing 200 to 250 microorganisms exposed for two minutes show at least a 99% count reduction; done at least monthly.				
LABORATORY PURE WATER Source: [ ] Laboratory prepared [ ] Purchased [ ] Other: _____.				
Purchased water has documentation of water quality, as required below, for each lot.				

5. LABORATORY MATERIALS PREPARATION (Cont.)	S	U	N	Comments
Still Manufacturer: _____ Deionizer Manufacturer: _____				
Records of recharge frequency are maintained				
Protected storage tank and construction.				
Distilled water product is not in contact with heavy metals.				
Production rate and quality adequate for laboratory needs.				
Satisfactory record of systems maintenance.				
Inspected, repaired, cleaned by [ ] service contract or [ ] in -house service.				
WATER QUALITY: The quality of the reagent water is tested and meets the following limit and frequency requirements: Parameter - Limits - Frequency				
Conductivity (Monthly) >0.5 megohms < 2 micromhos/cm @ 25 °C				
Pb, Cd, Cr, (Annually) *Cu, Ni, Zn* < 0.05 mg/L/contaminant Collectively < 0.1 mg/L Date Last Tested: _____				
Total Chlorine Residual (Monthly) (If source water is chlorinated) Nondeductible				

5. LABORATORY MATERIALS PREPARATION (Cont.)	S	U	N	Comments
Heterotrophic plate count (Monthly) (by pour plate method) - < 500 cfu/mL				
Biosuitability (Annually) - Ratio 0.8 to 3.0 Date Last Tested: _____ (This test not required if using ASTM Type I water as defined in SM 1080)				
RINSE WATER:				
Stock buffer solution is [ ] autoclaved or [ ] filter sterilized. Stock buffer solution container is [ ] labeled and [ ] dated and [ ] is free of turbidity.				
Stock buffer solution is prepared using 1.25 mL stock buffer and 5 mL $MgCl_2$ per liter) Final pH: 7.2 +/- 0.2				
Stock buffer is stored at 1 - 5°C.				
Rinse water is checked for sterility before use, using double strength [ ] tryptic soy, [ ] trypticase soy, [ ] tryptose broth, [ ] other: _____ to equal volume of rinse water.				
GLASSWARE WASHING				
Distilled or DI water used for the final rinse for glassware washing.				
Laboratory glassware shall be washed with a detergent designed for laboratory use.				
Inhibitory Residue Test has been performed on the initial use of a washing compound,or whenever a different formulation has been used.				
Glassware is spot-checked monthly with bromthymol blue or some other pH indicator. ***The glassware shall be at a neutral pH after washing.***				

5. LABORATORY MATERIALS PREPARATION (Cont.)	S	U	N	Comments
MEDIA STORAGE				
Commercially prepared dehydrated media are stored in a cool, dry location. Media are not caked or discolored.				
Dehydrated media are stored [ ] in a desiccator [ ] on an open shelf [ ] in a refrigerator.				
Dehydrated media dated upon receipt and when initially opened.				
No medium exceeds the manufacturer's expiration date.				
Media are discarded if discolored or caked.				
Each lot of commercial and each batch of laboratory-prepared medium is checked before use for performance with positive and negative culture controls, with the results being recorded.				
Refrigerated prepared plates are stored no more than 2 weeks in sealed plastic bags or containers to minimize evaporation. [ ] The label on the container includes the date prepared or the expiration date clearly indicated.				
6. MEDIA PREPARATION:				
Media preparation records include [ ] date of preparation, [ ] type of medium, [ ] lot number, [ ] sterilization time and temperature, [ ] final pH and [ ] technician's initials.				
Clean, smooth-surfaced glassware or stainless-steel utensils used.				
Laboratory pure water used in media preparation.				

6. MEDIA PREPARATION:(Cont.)	S	U	N	Comments
Media are completely dissolved before dispensing to culture tubes or bottles.				
Tube media with loose -fitting closures are stored less than ~~1~~ ***2*** weeks.				
Tube media in screw -capped tubes held no longer than 3 months at less than 30 °C.				
7. ANALYTICAL METHODOLOGY (GENERAL)				
Only analytical methodology specified in the National Primacy Drinking Water Regulations (See Appendix H, Certification Manual) is used.				
Laboratory is certified for at least one total coliform method plus one fecal coliform or *E. coli* method if laboratory analyzes samples for compliance purposes.				
Absorbent pad, if used, is saturated with liquid medium.				
A 100 +/- 2.5 mL sample is analyzed. (Drinking water)				
Water sample shaken vigorously before analysis.				
Coliform test conducted monthly on known coliform-positive and fecal or *E.coli*-positive sample.				
8. SAMPLE COLLECTION, HANDLING AND PRESERVATION				
Sample collection [ ] is, [ ] is not, [ ] is occasionally a laboratory function.				
The collector is trained in sample collection.				

8. SAMPLE COLLECTION, HANDLING AND PRESERVATION (Cont.)	S	U	N	Comments
The laboratory has sampling instructions available and provides them with sample containers.				
Samples are collected from water taps free of aerators, strainers, hose attachments, mixing type faucets and purification devices.				
Care is exercised in collecting representative samples and avoiding contamination of the sample.				
Water is allowed to run to waste for at least 2 minutes before collection.				
At least 100 mL of sample is collected allowing at least 1/2 inch air space at the top to allow for shaking.				
For known compliance samples, the sample report form includes [ ] location, [ ] sample type, [ ] date & time of collection, chlorine residual, [ ] collector's name and [ ] any special remarks concerning the sample is completed immediately after collection *; or [ ] State supplied form is used.*				
Samples delivered directly to the laboratory are iced immediately after collection.				
Date and time sample arrives at the lab are recorded.				
Date and time sample analysis begins are recorded.				
All samples analyzed within 30 hours of collection.				

8. SAMPLE COLLECTION, HANDLING AND PRESERVATION (Cont.)	S	U	N	Comments
Chain of custody procedures are followed in the laboratory and field, when required .				
Samples not analyzed immediately are refrigerated until analysis.				
Samples are analyzed on the day of submission, except when received after normal operating hours.				
9. QUALITY ASSURANCE				
The laboratory has prepared and is following an approved Quality Assurance Plan that includes the following, where appropriate:				
Sampling procedures.				
Sample Handling procedures used to maintain integrity of all samples. Sample preservation, holding time and container type. Chain of Custody procedures for samples likely to be the basis for an enforcement action.				
Instrument or equipment calibration and frequency of their use.				
Analytical procedures - References such as "Standard Methods" may be cited with any deviations from the procedures noted.				
Data reduction, validation and reporting.				
Internal quality control checks to be used and their frequency.				
Preventive maintenance procedures and schedules.				
Procedures used to determine data precision and accuracy.				
Corrective action taken in response to unacceptable results from analysis of PE samples and internal QC checks.				
Laboratory organization and responsibility.				
Records book containing the results of analytical control tests are available for review.				

10. RECORDS AND DATA REPORTING				
Data entered on the sample report form is checked and initialed.				
Records of Microbiological analyses and Quality Control data are kept at least 5 years.				
Records include [ ] date, [ ] place and [ ] time of sampling, [ ] collector's name, [ ] type of sample, [ ] date and [ ] time of sample receipt, [ ] date and [ ] time of analysis, [ ] laboratory and [ ] person responsible for analysis, [ ] analytical technique/method used and [ ] the results of the analysis.				
When a laboratory uses a method that is not EPA approved for an analysis for which it is certified, the test report and/or sales literature clearly indicates that the method being used is not the EPA method for which they are certified.				
11. ACTION RESPONSE TO LABORATORY RESULTS				
Proper authorities are notified promptly of unsatisfactory results on the basis of MTF confirmed tubes or unverified MF coliform data.				
Data used to determine monthly compliance is adjusted by using MF verified results.				
MEMBRANE FILTRATION PROCEDURE:				
Sample is poured into the funnel with the vacuum turned off.				
After filtering, the walls of the funnels are rinsed 2-3 times with 20-30 mL sterile buffered rinse water.				
Membrane is removed with sterile forceps and placed on agar or pad so that there are no air bubbles trapped.				

MEMBRANE FILTRATION PROCEDURE (cont'd):	S	U	N	Comments
Inoculated medium incubated at 35 +/ - 0.5°C for 22-24 hours.				
All green sheen colonies, all questionable red sheen colonies, and red non-sheen colonies are verified on all samples up to 5 colonies/ 100 mL. When greater than 5 colonies, 5 randomly picked colonies of each of the 3 types are verified.				
Sheen colonies in mixed confluent growth are verified.				
Total coliform colonies are tested for either fecal coliforms or *E. coli* by: _____				
Initial counts are adjusted based only upon verification data.				
All samples with confluent or TNTC growth invalidated, unless total coliform -positive, and a new sample requested.				
Non-coliform bacteria counts are recorded for all samples analyzed by this method. [Recommend if not doing]				
Repeat samples are requested for all samples having Non-coliform counts >200. Repeat samples are analyzed by MTF or chromogenic/fluorogenic substrate test.				
The MF sterility check is run at the beginning and end of each filtration series by filtering 20-30 mL of dilution water.				
If controls indicate contamination, all data on affected samples are rejected and resamples are requested.				
Laboratories having 2 or more analysts count one known coliform-positive sample monthly with the sheen colony counts agreeing within 10%.				
Sample volume filtered for total coliforms in source water yields filters with 20 -200 colonies.				

MEMBRANE FILTRATION PROCEDURE:(Cont.)	**S**	**U**	**N**	**Comments**
MEMBRANE FILTER MEDIA(for total coliforms):				
90% ethanol (not denatured) is used to prepare MF media.				
MF medium is brought to just boiling. (It is not allowed to boil) using [ ] boiling water bath [ ] hot plate with stirring bar, constantly attended. Final pH 7.2 +/ - 0.1 (m-Endo broth) 7.2 +/- 0.2 (m-Endo LES agar)				
MF broth is refrigerated no more than 96 hours; poured plate agar no more than 2 weeks. Ampouled media does not exceed manufacturer's expiration date.				
Uninoculated media discarded if growth or surface sheen is observed.				
FECAL COLIFORM MEMBRANE FILTER (MFC) BROTH/AGAR				
Sterilization by bringing to boiling point; not autoclaved. Final pH 7.4				
If medium is stored, it is refrigerated and used within 96 hours (if broth) or two weeks (if agar)				
Refrigerated medium is incubated overnight at 44.5°C before and plates with growth discarded.				
MULTIPLE-TUBE FERMENTATION				
Concentration of inoculated medium is correct.				
Sample aliquots are accurately pipetted.				
Inoculated medium incubated at 35 +/ - 0.5°C.				

MULTIPLE-TUBE FERMENTATION (cont'd)	S	U	N	Comments
Tubes are observed for gas production after 24 +/- 2 hours.				
All tubes are shaken gently before readings.				
Inoculum from those tubes exhibiting heavy growth with no observable gas is transferred to the confirmed medium, or the sample is invalidated and a resample requested.				
It is recommended that if heavy growth in presumptive tubes occur a second time, the samples are reanalyzed using a chromogenic/fluorogenic substrate procedure				
All positive tubes are submitted to the Confirmed Test immediately. All negative tubes (except those with heavy growth) are reincubated for another 24 hours.				
Confirmed Test:				
Growth from each tube is transferred to Brilliant green broth using a 3 mm loop or sterile applicator stick and incubated for 24 hours at 35 +/- 0.5°C for 24 +/ - 2 hours				
All negative tubes are reincubated for 24 hours.				
Samples from the presumptive test exhibiting heavy growth and no gas production that are total coliform-negative after the confirmed test are invalidated and a resample requested.				
If no positive tubes result from potable water samples, the MTF procedure is performed on all positive confirmed tubes from at least one coliform positive water sample.				
Three sample volumes of source water (10 mL, 1 mL, and 0.1 mL) used.				

MULTIPLE-TUBE FERMENTATION(Cont.)	**S**	**U**	**N**	**Comments**
MULTIPLE TUBE FERMENTATION (MTF OR MPN) MEDIA (for total coliform):				
[ ] Lauryl tryptose (lauryl sulfate) or [ ] lactose broth used presumptive phase dispensed in volumes not less than 10 mL/tube.				
Concentration is adjusted so that medium is single strength after sample addition.				
Autoclaved at 121 °C for 12-15 minutes. Final pH of Presumptive Test media:6.8 +/ - 0.2.				
Inverted vials in sterilized medium are 1/3 to 1/2 covered by medium and free of gas bubbles.				
Brilliant green lactose bile broth used for confirmed phase.				
Autoclaved at 121 C for 12 -15 minutes. Final pH: 7.2 +/- 0.2.				
Sterilized media stored at less than 30 °C in the dark.				
Refrigerated MTF media are incubated overnight at room temperature before use. Tubes showing growth and/or bubbles are discarded.				
Tubes showing growth and/or bubbles or tubes where evaporation exceeded 10% of the original volume are discarded.				

CHROMOGENIC/FLUOROGENIC SUBSTRATE TEST PROCEDURE:	S	U	N	Comments
The laboratory uses the [ ] P -A [ ] MPN format. The laboratory uses[ ]Colilert [ ]Colilert-18 [ ]Quanti-Tray [ ]Quantitray 2000 [ ] Colisure				
Each lot of medium is checked before initial use by inoculating sterile dechlorinated tap water, deionized water, or distilled water with a total coliform positive control, an *Escherischia coli* positive control, and a total coliform negative control. (Use manufacturer's recommended organisms.)				
Colilert samples are incubated at 35 +/ - 0.5°C for at least 24 hours. Samples are incubated an additional 4 hours if no distinct color change has taken place after 24 hours. Colilert-18 samples are incubated for 18-24 hours.				
Colilert samples are incubated no longer than 28 hours. Samples incubated more than 28 hours may be considered negative if they show no color.				
Colisure samples are incubated 28 - 48 hours. (Positive samples remain stable in the incubator up to 48 hours.)				
Colisure samples are incubated no longer than 48 hours. (Samples remain stable in the incubator up to 48 hours.)				
Inoculated test not exposed to prolonged direct sunlight.				
All coliform positive samples are checked for *E. coli* by placing the sample approx. 2 inches from a long wave 366 nm ultraviolet light (6 watt light is recommended).				
UV light identification:				

CHROMOGENIC/FLUOROGENIC SUBSTRATE TEST PROCEDURE (cont'd):	S	U	N	Comments
Samples showing a yellow or fluorescent color after 24 hours so faint that the analyst is uncertain, the color is compared to a color comparator, if available. (Colilert)				
If a color comparator is not available, or the analyst is still uncertain, the sample is considered invalid; another sample from the same site is requested if the sample is a known compliance purpose sample and is tested by an alternate test procedure. (Colilert)				
The chromogenic/fluorogenic substrate test is not used to verify/confirm coliforms on membrane filters or in broth cultures.				
Parallel testing is performed for several months between the chromogenic/fluorogenic substrate test and another EPA approved procedure. (Optional)				
The laboratory has determined the time needed for an air-type incubator to reach 35 °C after samples added to the incubator. If the number of samples varies significantly, the laboratory has determines the time for a variety of samples.				
The laboratory has a contingency plan available for turbid, colored or inconclusive (faint colored results) samples.				
New glassware and media is checked for autofluorescence.				
If this is the only method used, the [ ] autoclave, [ ] incubator, [ ] thermometer, [ ] refrigerator, [ ] balance and [ ] lab pure water records have been checked where applicable.				
[ ] Colilert Medium stored at 4 -30°C, away from light in a cool, dry environment. [ ] Colilert-18 medium is stored at 4-25 °C. [ ] Colisure medium is stored at 2-8 °C				

CHROMOGENIC/FLUOROGENIC SUBSTRATE TEST PROCEDURE (cont'd):	S	U	N	Comments
Media are discarded at or before manufacturer's expiration date.				
Medium not autoclaved.				
Only Colilert, Colilert-18, or Colisure is being used. At this time Coliquick has not been approved				
PRESENCE-ABSENCE (P-A) COLIFORM TEST:				
Inoculated medium incubated at 35 +/ - 0.5°C and observed for yellow color after 24 and (if necessary) 48 hours.				
Yellow cultures are confirmed in BGLB broth.				
All non-yellow turbid cultures are invalidated, and another sample is requested.				
PRESENCE-ABSENCE (P-A) COLIFORM TESTMEDIUM:				
Medium is autoclaved for 12 -15 minutes at 121 °C, with space allowed between bottles. Final pH: 6.8 +/- 0.2. The maximum time in the autoclave does not exceed 30 minutes.				
Medium is stored in culture bottles at less than 30°C in dark, and used within 3 months; discarded if evaporation exceeds 10% of original volume.				
FECAL COLIFORM TEST				
(For distribution system samples) Positive culture from total coliform medium is transferred to EC medium, using an approved transfer technique.				
(For source water samples) Positive culture from total coliform medium is transferred to EC medium, using an approved technique, or A -1 medium is directly inoculated with a water sample (use 3 sample volumes (10 mL, 1 mL 0.1 mL), 5 or 10 tubes/sample volume)				

FECAL COLIFORM TEST (cont'd)	S	U	N	Comments
Water level of water bath is above upper level of medium in culture tubes.				
EC medium is incubated at 44.5 +/ - 0.2°C for 24 +/- 2 hours. A -1 Medium incubated at 35 +/ - 0.5°C for 3 hours, then 44.5 +/ - 0.2°C for 21 +/ - 2 hours.				
Any gas detected in inverted vial of tube that has turbidity is considered fecal coliform -positive.				
EC MEDIUM (for fecal coliforms):				
Autoclaved for 12 -15 minutes at 121 °C Final pH: 6.9 +/- 0.2.				
Inverted tubes following sterilization are ½ to 1/3 covered by medium and of air bubbles.				
Sterile medium is stored at less than 30 °C in tightly closed screw cap tubes and used within 3 months. Medium stored in loose caped tubes are stored at less than 30 °C for up to 2 weeks.				
Refrigerated sterile media incubated overnight at room temperature before use and tubes with growth and/or bubbles discarded.				
A-1 MEDIUM (for fecal coliforms in source water):				
Medium is sterilized at 121 °C for 10 minutes. Final pH: 6.9 +/ - 0.1.				
Inverted tubes are 1/3 to 1/2 covered by the medium and free of air bubbles.				
Sterilized medium is stored in dark at room temperature and used within two weeks. If stored in tightly closed screw cap tubes in the dark, it is stored for no more than three months.				

EC MEDIUM + MUG TEST:	**S**	**U**	**N**	**Comments**
Positive cultures from total coliform presumptive medium is transferred to EC + MUG medium, using an approved transfer technique.				
Water level in water bath is maintained above upper level of medium in culture tubes.				
Inoculated medium is incubated at 44.5 +/ - 0.2°C for 24 +/ - 2 hours.				
Fluorescence examined with ultraviolet lamp (366-nm). MUG-positive and MUG-negative controls used where the medium autofluoresces.				
EC MEDIUM + MUG (for E. coli):				
Tubes and autoclaved medium observed for fluorescence before use with 366 -nm ultraviolet light. If weak fluorescence is observed, either [ ] another lot of medium is used, or [ ] MUG positive and MUG negative controls are used with the analysis.				
Inverted vial in test tube is not used.				
Final MUG concentration: 50 Tg/mL				
Final pH: 6.9 +/ - 0.2				
Refrigerated medium is incubated overnight at 44.5 C before use and tubes with growth discarded.				
NUTRIENT AGAR + MUG TEST:				
Total coliform-positive filters transferred to Nutrient Agar + MUG.				
The position of each total coliform positive colony marked before incubation on Nutrient Agar + MUG.				
Inoculated medium is incubated at 35 +/ - 0.5°C for 4 hours.				
Fluorescence examined with ultraviolet lamp (366-nm). Any amount of fluorescence in a halo around a sheen colony is considered *E. coli*-positive.				

NUTRIENT AGAR MEDIUM + MUG(for E. coli):				
Quality of medium lot/batch evaluated by spot-inoculating with control bacteria.				
Medium sterilization in 100 mL volumes at 121 °C for 15 minutes. Final pH 6.8+/ - 0.2.				
Final MUG concentration: 100 Tg/mL.				
If media are stored in petri dishes, they are refrigerated in plastic bag or tightly closed container, and used within 2 weeks.				
Refrigerated medium incubated overnight at room temperature before use, and plates with growth are discarded.				
HETEROTROPHIC PLATE COUNT (POUR PLATE METHOD):				
Sample volume analyzed is 1.0 mL or 0.1 mL.				
Sample volume aseptically pipeted into bottom of petri dish.				
12 - 15 mL of melted HPC agar tempered at 44 - 46°C in a water bath is added to the 100 mm x 15 or 90 x 15 mm petri dishes.				
Sterile agar medium remelted only once.				
Sample and melted agar is carefully mixed.				
At least two replicate plates prepared per sample.				
After agar plates have solidified, they are inverted and stacked in the incubator in such a manner so as to allow proper air circulation. (Except R2A Medium)				
Plates are not stacked more than 4 high.				
Plates are incubated at 35 +/ - 0.5°C for 48 +/ - 3 hours				
Colonies are counted manually using a counting aid such as a Quebec colony counter.				

HETEROTROPHIC PLATE COUNT (POUR PLATE METHOD):	**S**	**U**	**N**	**Comments**
Only plates having 30 - 300 colonies are considered except when a 1 mL aliquot of undiluted sample is used.				
Counts of less than 30 are acceptable for plates that are inoculated with 1 mL of undiluted sample.				
Each batch of HPC agar is checked for sterility by pouring ending control plates.				
Data are rejected if controls are contaminated.				
HETEROTROPHIC PLATE COUNT(HPC) MEDIUM:				
HPC agar is autoclaved at 121 ° C for 15 minutes. Final pH: Plate Count Agar 7.0 +/ - 0.2; R2A Agar 7.2.				
Melted HPC agar is tempered in a water bath at 44 - 46°C at least 20 minutes before pouring.				
Melted agar is held no longer than 3 hours and is never remelted.				
If media are stored in petri dishes, they are refrigerated in plastic bag or tightly closed container, and used within 2 weeks (one week for R2A medium).				
HETEROTROPHIC PLATE COUNT (SPREAD PLATE METHOD):				
R2A agar medium is used.				
Plates with solidified medium dried before use.				
Medium inoculated in accordance with Standard Methods.				
At least two replicate plates used for each sample.				
Plates inocubated in inverted position at 28 °C for 7 days.				

Plates stacked no more than four high.				
Colonies counted manually using a counting aid such as a Quebec colony counter.				
Counts reported for plates having 30 -300 colonies. (If 1.0 mL of undiluted sample results in fewer than 30 colonies, that count is acceptable.				
Sterility check performed on an uninoculated control plate. Data rejected if control is contaminated.				
HETEROTROPHIC PLATE COUNT (HPC) MEDIUM:				
Agar is autoclaved at 121 °C for 15 minutes. Final pH: —7.2 +/-0.2.				
Media are stored in petri dishes, refrigerated in plastic bag or tightly closed container, and used within one week				
Refrigerated medium incubated overnight at room temperature before use, and plates with growth discarded.				
HETEROTROPHIC PLATE COUNT (MEMBRANE FILTER METHOD):				
Sample volume filtered yields filters with 20 -200 colonies.				
Filter transferred to R2A medium				
Plates incubated at 35 °C or lower for 5 -7 days in close fitting box containing moistened paper towels.				
Sterility check performed on a filter in a control plate. Data rejected if control is contaminated.				
HETEROTROPHIC PLATE COUNT (HPC) MEDIUM				
Agar is autoclaved at 121 °C for 15 minutes. Final pH: —7.2 +/-0.2.				
Melted HPC agar is tempered in a water bath at 44 - 46°C at least 20 minutes before pouring.				

HETEROTROPHIC PLATE COUNT (HPC) MEDIUM (cont'd)				
Melted agar is held no longer than 3 hours and is never remelted.				
~~If~~ Media are stored in petri dishes, refrigerated in plastic bag or tightly closed container, and used within one .				
Refrigerated medium incubated overnight at room temperature before use, and plates with growth discarded.				
FECAL COLIFORM MEMBRANE FILTER PROCEDURE (For fecal coliforms in source water)				
Sample volumes used which yields 60 or fewer fecal coliform colonies/membrane (and preferably at least 20)				
Inoculated medium incubated at 44.5 +/ - 0.2°C for 24 +/- 2 hours.				
Sterility check conducted at beginning and end of each filtration series. If controls indicate contamination, data rejected and a resample requested.				
If two or more analysts, each counts the fecal coliform colonies on the same membrane at least monthly. Colony counts agree within 10%.				
FECAL COLIFORM MEMBRANE FILTER (MFC) BROTH/AGAR				
Sterilization by bringing to boiling point; not autoclaved. Final pH 7.4				
If medium is stored, it is refrigerated in sealed plastic bag or tightly closed container and used within 96 hours (if broth) or two weeks (if agar)				

MICROBIOLOGY SAMPLE TRACKING CHECKLIST

Date & Time - Collected:_____ Received:_____ Analysis Began:_____.

Lab Sample ID	Method	Tests Report Result	Log Book Result

Records Checked: [ ] T. Coliform MF [ ] F. Coliform MF [ ] MMO -MUG

	S	U	N
Incubator Temperature		[ ]	[ ] [ ]
Water Bath Temperature		[ ]	[ ] [ ]
Refrigerator temperature		[ ]	[ ] [ ]
Autoclave Records		[ ]	[ ] [ ]
Media Prep Within Holding Time		[ ]	[ ] [ ]
Media pH		[ ]	[ ] [ ]
pH meter calibrated and checked with pH 7.00 buffer	[ ]	[ ]	[ ]
Balance Checked Within Month of Media Prep		[ ]	[ ] [ ]
Membrane Filter Sterility Check		[ ]	[ ] [ ]
Pre & Post MF Sterility Check		[ ]	[ ] [ ]
Buffered Rinse Water Sterility Check	[ ]	[ ]	[ ]
Sample analyzed within labs holding time.	[ ]	[ ]	[ ]
Report form signed.		[ ]	[ ] [ ]
Clients informed of certification status.	[ ]	[ ]	[ ]

Provisions to insure that samples are not lost and all analyses are performed:

_____

NEW ENGLAND CERTIFICATION OFFICERS
MICROBIOLOGY CHECKLIST

ON-SITE EVALUATION OF

_____

LOCATED IN

_____

LABORATORY TELEPHONE NUMBER

_____

CONDUCTED ON

_____

BY

_____

S = Satisfactory U = Unsatisfactory N = Not Applicable