Laboratory Manual for the Microbiological Analyses of Public Drinking Water

2001

Donald E. LaSota
Todd W. Bidlack
James W. Evans

Christopher Jones
Ohio EPA Director

Bob Taft
Governor of Ohio

Cover Art: Immunofluorescence image of several *Cryptosporidium parvum* oocysts provided by H.D.A Lindquist, USEPA

TABLE OF CONTENTS

Chapter I

Part I Certification Overview

All persons performing analyses of public drinking water in a certified laboratory must be approved by the Ohio EPA. The Ohio EPA/DES Laboratory Certification Section evaluates laboratories. The Ohio EPA then either grants or denies certification to the laboratory.

Analysts are only approved at the laboratory for methods noted on the certificate of approval. If an analyst changes or adds methods from what is noted on their certificates of approval, those methods are not approved. If an analyst moves to another facility that may or may not be certified, that analyst is no longer approved and will need to reapply for certification at the new facility. If the facility is certified and loses all of its approved analysts, the facility is no longer certified and must reapply for certification, when new analysts are hired. It is the laboratory's responsibilities to notify the Ohio EPA/DES of all personnel changes. All certificates of approval remain the property of the Ohio EPA and must be returned to the Ohio EPA/DES when an analyst departs.

Each certified analyst shall participate proportionally in the analyses for which he or she is approved. The minimum acceptable proportion shall be 10%, which is typically three days per month.

Certification covers personnel, equipment, records and facility. A person is certified in his or her laboratory, if that person moves to a new facility, he or she must inform the Ohio EPA Ohio EPA/DES Laboratory Certification Section of the intended change. All approved lab personnel must be present for a survey.

If a certified laboratory moves but retains the same personnel, and if the laboratory plans are approved in writing by the Ohio EPA, certification will remain in effect. If a certified laboratory makes a substantial change in physical facility, such as addition of bench space, without prior plans approval and subsequent on-site survey, the laboratory is subject to loss of certification. If a laboratory has never been previously certified, the initial survey is followed by surveys one year and three years from the initial survey.

Surveys are normally conducted every three years for certification renewal. These "full" surveys will cover all aspects of analyses for which certification is being sought. The surveys will be scheduled upon receipt of an application for certification. The survey fee will only be assessed once every three years. Applications can be obtained by contacting the Ohio EPA/DES Laboratory Certification Section or by accessing the Ohio EPA website at: http://www.epa.state.oh.us/ddagw/labcert.html

In addition to the normally scheduled surveys, surveys may also be conducted on a random unannounced basis. The laboratory facility and records must be available for inspection during normal working hours (8:00 am to 5:00 pm) for unannounced surveys. The approved person(s) need not be present for unannounced surveys as long as the survey officer has access to the laboratory and records.

A responsible person must be designated to provide access to the laboratory (city hall, other operator, police, etc.), and laboratory records must be maintained in the laboratory building. The laboratory records must be clearly labeled and easily accessible, in a conspicuous location. Copies of the records must be made for survey personnel when requested.

Telephone numbers of the responsible personnel must be posted to allow access for emergencies and certification officers.

Part II Requirements for Certification

Rules pertaining to laboratory certification are contained in chapters 3745-89-01 through 3745-89-10 of the Ohio Administrative Code (OAC). Ohio approved test methods are in OAC chapter 3745-81-27. If copies are needed, please contact the Ohio EPA or access the Ohio EPA website: http://www.epa.state.oh.us/ddagw/ddagwmain.html

An application form for a microbiological laboratory survey is to be submitted in writing to the Ohio EPA/DES Laboratory Certification Section. This submittal must be made within 120 days in advance of the expiration of the current certificate of approval.

Applications must be submitted at least 30 days prior to the expiration of the current certificates. If a completed application is not submitted by thirty days prior to the expiration date, the laboratory is not eligible for any extension beyond the expiration date of the certification and a prescheduled date for an on-site survey is subject to cancellation. If the prescheduled date for an on-site survey is canceled, a subsequent survey shall not be **scheduled** until at least fourteen days after the expiration date occurs.

Once an application has been submitted and accepted, the current certificates of approval automatically will have the expiration date extended until an on-site survey has been successfully performed, unless the application was submitted with less than 30 days until the expiration of the current certificates as noted above.

In order for an application to be acceptable, the completed application form must be accompanied by an approval letter for laboratory plans issued by the Ohio EPA. Certification fees will be invoiced **after** the application has been accepted. Fees sent with applications will be returned.

If the above mentioned letter for lab plans approval is not available, it is advisable to contact the Ohio EPA/DES Laboratory Certification Section six months prior to the expiration date of your current certificate.

Part III Laboratory Approval Status

Upon completion of the on-site survey, a conclusion is mailed with the narrative report.

Conclusions are as follows:

Certified - A Certificate of Approval will be issued by the Ohio EPA for the microbiological tests listed in the report. Certificates of approval are valid for a time period not to exceed three years from the date of issue.

Extended Certification - The deviations listed in the report must be corrected before the date listed in the report. A statement detailing the remedial actions taken to correct each of the listed deviations must be forwarded to the Laboratory Certification Section prior to the expiration date of the report to avoid revocation in accordance with the OAC.

Not Certified - The laboratory will not be certified for the microbiological test(s) noted in the report.

Part IV Proficiency Test (PT) Samples

All certified microbiological drinking water laboratories must analyze an annual set of 10 PT tests in the last quarter of the year. As noted a set of 10 PT tests must be analyzed at least once each year, in the fall quarter. One set of 10 PT tests is required for each laboratory. It is not necessary to analyze a set of 10 PT tests for each approved analyst.

It is a requirement of the USEPA that laboratories analyze one set of 10 PT tests via each approved method,

therefore, if your laboratory is approved for both membrane filter (MF) and MMO-MUG, you must obtain two sets of 10 PT tests and analyze one by MF and one by MMO-MUG. All laboratories must analyze PT samples sometime within September-December on PT studies.

PT samples can be obtained from any NIST approved PT supplier. As of August 2000 NIST had approved three suppliers, please check with the Laboratory Certification office or NIST for the current status.
Each laboratory is responsible for all costs associated with PT samples. Please indicate to the PT supplier that **they** must forward PT test results to the attention of:

Ohio EPA/DES
Laboratory Certification Section
1571 Perry Street
Columbus, OH 43201

In order to maintain certification for microbiological methods, you must successfully analyze PT samples for each method your laboratory holds certification. A passing score is 90% (you must successfully analyze 9/10 samples). A "miss" is defined as:

1) Failing to analyze a sample.

2) Failing to detect either total coliforms or *E. coli/*fecal coliforms.

3) Mistakenly identifying the aforementioned bacteria in a negative sample.

Any PT failure will result in a requirement for the laboratory to submit a statement of probable cause and corrective action to the Ohio EPA. The criteria used for invalidation of certification will be based upon USEPA/National Environmental Laboratory Accreditation Conference (NELAC) guidelines. Two consecutive failures will result in invalidation of certification for the parameter. Two failures out of three, but not successive will require a written statement from the laboratory describing the problem that may have caused the failures and corrective action.
There will be one supplemental PT sample allowed after two successive failures. The laboratory must pay for all supplemental samples. If the laboratory passes, they will be recertified for the parameter. If the laboratory misses the parameter, they must wait for the next scheduled PT sample to regain certification.

A sample set may be considered invalid b y the Ohio EPA if >30% of the analysts are out of range, for sets of ten samples. Samples will also be invalidated if there is any other acknowledged problem noted, such as contaminated samples, samples that will not filter, etc.

Laboratories seeking initial approval for a new method must satisfy the following conditions before a certificate of approval will be issued by the Ohio EPA for the new method:

1) Order, analyze a PT sample set and receive a passing score of at least 90% on a scheduled or supplemental PT sample series. The laboratory must request the test results be sent directly to the Ohio EPA prior to analysis. This also applies to new facilities. In this case, PT samples may be obtained at any time of the year they are available. Subsequent samples must be analyzed in the fall quarter of the year.

2) Successfully complete an on-site laboratory survey.

Any laboratory failing their first two PT sample sets must wait until the next regularly scheduled PT study, in the fall, before attempting the certification process again.

PT samples are to be treated as normal drinking water samples for analysis. If you miss the make-up or supplemental samples, you must wait until the next scheduled PT sample (fall quarter) and successfully analyze it

to be considered for certification Failing to participate in a PT study will be treated as an unsuccessful test. Tests submitted in winter, spring or summer quarters, except for “make-up” tests will not be accepted.

The following chart summarizes some possibilities:

PT Results	**Action**
Score of at least 90% on a scheduled study	Pass study-PT requirements are satisfied until the next scheduled study
Did not participate in a scheduled study	Fail the study. Submit statement of probable cause for failure and corrective action to the Ohio EPA. May require a supplemental study.
First score of less than 90% on a scheduled study	Fail the study. Submit statement of probable cause for failure and corrective action to the Ohio EPA. Will not require a supplemental study.
2/3 scheduled study scores of less than 90%	Fail the study. Submit statement of probable cause for failure and corrective action to the Ohio EPA. Will not require a supplemental study.
Two successive studies with a score of less than 90%	Fail the study and invalidation of certification for the methods missed. May be reinstated after: 1) Submit statement of probable cause for failure and corrective action accepted by the Ohio EPA. 2) Order, analyze a PT sample set and receive a passing score of at least 90% on one supplemental PT sample series.
Supplemental PT sample set score of at least 90%	Pass study-The laboratory is recertified for the method.
Supplemental PT sample set score of less than 90%	Fail the study and continued invalidation certification for the methods missed. No recertification will be granted for this method until: 1) Submit statement of probable cause for failure and corrective action accepted by the Ohio EPA. 2) Participate in the next scheduled PT sample set and receive a passing score of at least 90% 3) Successfully complete an on-site survey.
30% or more of the labs do not receive a score of at least 90% from one supplier.	That sample set, for that supplier will be declared invalid by the Ohio EPA. Results will not be used. Supplemental samples must be obtained.

Part V Drinking Water Analysis for Other Entities

A laboratory performing analyses for a water supply other than their own distribution system or distribution system connected by a master meter has additional requirements.

Requirements for such laboratories are:

- Submit microbiological sample submission report (SSR) forms as required by the Ohio EPA Division of Drinking and Ground Waters for each individual sample. Report forms must comply with Ohio EPA DRINKware requirements. Keep the original copy on file.

- Provide a supply of sampling instruction sheets to be given to each new customer. These sheets must contain all necessary sampling information for proper collection and handling of the samples.

- Provide sample containers to the customers. If commercially prepared sample containers are purchased by the customer (pre-sterilized with sodium thiosulfate) the lab may accept them as long as the containers are checked in accordance with Ohio EPA guidelines. The sample bottle QC record from another Ohio EPA approved laboratory, for that lot of containers, must be provided to the testing laboratory.

- Record all samples on the daily log, listing sample number, location, and results. All information needed for the submission of SSR forms must be provided. If any essential information is missing, do not analyze the sample. Essential information includes: PWS number, water supply name, date collected, time collected, name of collector, address of sample tap (with county listed), the tap the sample was collected from, a contact phone number and any other information that the Ohio EPA DDAGW tells you they need. Sample identification slips must not have any of the collection data altered by laboratory personnel. Any corrections to sample data must be done and initialed by the sample collector. A legible, corrected copy of an SSR sent via fax may be accepted by the laboratory if it is received prior to analysis and attached to the original SSR. Because the laboratory is obliged to report all positive microbiological samples to the appropriate district office, by the close of business the day after the test is completed (read), a sample may not be analyzed if collection data is not completed before the sample is too old for analysis.

- The certified drinking water laboratory that accepts samples from the public water system is responsible for reporting the data, including faxing positive results to the Ohio EPA.

- Submission of SSR forms to the Ohio EPA is required to be completed by no later than the tenth day after the end of the month in which analysis is completed (read). If a positive result is indicated or if repeat samples are analyzed, the reports must be submitted by no later than the end of the next business day after analysis is completed (read).

Do not analyze samples for the following reasons:

1. Time elapsed since sample collection >30 hours.
2. Leaking or breakage of sample bottles in transit.
3. Free chlorine residual in dechlorinated sample.
4. Incomplete information for completion of SSRs.

5. Samples frozen.

6. Less than 100 ml of sample available.

7. Any other reason that may affect test results.

Part VI The Microbiological Water Testing Course

The Microbiological Water Testing Course is a hands-on training course presented by the Operator Training Committee of Ohio, Inc. For details on specific dates, fees, schedules, and applications, contact:

Operator Training Committee of Ohio, Inc.
3972 Indianola Avenue
Columbus, Ohio 43214 (614) 268-6826

For laboratories applying for a certificate of approval for the first time: One new analyst, who will be surveyed, from each laboratory must successfully complete this course or equivalent training course.

For laboratories that have lost their certificate of approval: One or more analysts may have to successfully complete this course or equivalent training course prior to resurvey if the loss of certification was due to the analyst's lack of training.

Chapter II

Part I Sampling

Questions about sampling, "sampling plans", reporting data and resample procedures should be directed to the appropriate Ohio EPA district office or the Ohio EPA DDAGW.

A. Transportation and Storage

Sample collection information must be transferred to the daily laboratory worksheet. Potable samples are preferably examined within one hour of collection and kept refrigerated. Potable samples must have analysis completed (incubation initiated) within 30 hours of collection to obtain acceptable results.

B. Record of Laboratory Examination

The laboratory must have available for inspection all laboratory records, worksheets, and monthly operation reports for at least the previous three years. Keep all records on file for at least five years. All records and the laboratory facility must be available for unannounced survey five days per week from 8:00 am to 5:00 pm. If the laboratory is normally closed during any of these hours, provide a key to appropriate personnel.

Part II Instructions for completing the Microbiological Sample Submission Report (SSR) form

This form is to be used by laboratories performing analyses for other entities. The microbiological SSR form must be completed, using either a typewriter or indelible pen. Samples accompanied by illegible, inaccurate, or incomplete forms will result in rejection of those samples for analysis. Electronic submission by DRINKware may also be used.

The following information is to be completed for each sample submission report form:

Laboratory Information:

Reporting Lab Name: ____	Enter the name of the lab reporting the sample submission report.
Reporting Lab Certification #: ____	Enter the certification number of the lab reporting the sample submission report.
Analytical Lab Name: ____	Enter the name of the lab which analyzed the sample.
Analytical Lab Certification #: ____	Enter the certification number of the lab which analyzed the sample.
Sample Number:____	Enter the sample number issued by the reporting lab. Sample numbers should include <u>only</u> numbers (no letters or dashes) and are limited to 10 digits. Sample numbers must start with a non-zero number. The exact same sample number cannot appear from the same lab on more than one report unless a unique numerical prefix is used (e.g. 900 for Bacti and 800 for VOCs).

Public Water System Information:

District Office:_____	Enter the name of the district office to which the form is to be reported.
PWS Name:_____	Enter the name of the public water system (PWS), i.e. a city, restaurant, etc.
PWS ID NUMBER:_____	Enter the six or seven digit public water system identification (PWSID) number assigned by the Ohio EPA.
Address:_____	Enter the street address where the PWS is located.
City/State/Zip:_____	Enter the city, state, and zip code in which the PWS is located.
County:_____	Enter the county in which the PWS is located.
Contact's Name:____	Enter the name of a person who can provide sampling information about the PWS.
Contact's Phone:____	Enter the phone number where the contact person can be reached.

Sample Information:

Sample Collection Date:_____	Enter the date (Month/Day/Year) which the sample was taken.
Time:_____	Enter the time of sample collection in military time, i.e. 1300 for 1 p.m.
Sample Collected by:____	Enter the name of the person who collected the sample.
Collector's Phone:____	Enter the phone number of the person who collected the sample.
Sample Class:____	Mark the box that indicates the reason for analyzing the sample. A Routine sample represents the drinking water being used. A Repeat sample is collected to confirm the results of a positive compliance sample. A Special sample is not representative of the drinking water being used and is not used for compliance. A Raw sample is collected to review a well for approval as a drinking water source, etc.
Sample Monitoring Point:____	Enter the location where the sample was collected. Locations are typically DS000 for a distribution sample or RS00# for a raw sample.
Repeat for Sample # : _____	If applicable, enter the sample number for the previous sample which had the results that the repeat sample was collected to confirm or check.
Tap Address:	Enter the street address where the sample was taken, 1847 Main Street.
Sample Tap ID:	Enter a number or description of the tap where the sample was taken, i.e. Sink tap 2, Gray's Gas Station.

Analytical Information:

Method Used:	Indicate the method used to perform the analysis as membrane filter or MMO-MUG.
Analyst Number:	Enter the number assigned by the Ohio EPA for the approved analyst.
Analysis Date:	Enter the date that incubation was started.
Analysis Time:	Enter the time that incubation was started in military time; 1500 for 3 p.m.
Total Coliform Results:	Mark the appropriate box indicating the results as Positive, Positive-HBC (High Background Count), Positive-CG (Confluent Growth), or Negative. If there were problems with analysis or another type of result was obtained, use the Other Results area instead.
Fecal Coliform Results:	If the Membrane Filtration method is used **,** mark the box indicating the results as positive, negative, or no value. No value indicates that the analysis was not done.
***E. coli* Results:**	If the MMO/MUG method is used, mark the box indicating the results as positive, negative, or no value. No value indicates that the analysis was not done.
LTB 24:	If the Membrane Filtration method is used, mark the box indicating the results as positive, negative, or no value. No value indicates that the analysis was not done.
LTB 48:	If the Membrane Filtration method is used, mark the box indicating the results as positive, negative, or no value. No value indicates that the analysis was not done.
BGB 24:	If the Membrane Filtration method is used, mark the box indicating the results as positive, negative, or no value. No value indicates that the analysis was not done.
BGB 48:	If the Membrane Filtration method is used, mark the box indicating the results as positive, negative, or no value. No value indicates that the analysis was not done.
Other Results:	If "Other Results" are obtained for a total coliform analysis, indicate the appropriate result as one of the following: Broken in Transit, Insufficient Sample, Incomplete Info, Lab Accident, Leaked in Transit, Not Analyzed, Residual Chlorine, Sample Too Old, TC Negative/Confluent Growth-Invalid, or TC Negative/High Background Count-Invalid.
Comments (For ...):	This comment area is only for use when an "Other Result" is given. Include any pertinent information about the analysis.

Information Needed from the Public Water System/sampler

In order to properly complete the public water system information and sample information sections of the required microbiological SSR form, the sampler should provide the laboratory with the following items:

1. The Ohio EPA **District Office** to receive the results.
2. The name of the **PWS** (public water system) that was sampled.
3. The six or seven digit **PWSID** # assigned to public water system by the Ohio EPA.
4. The mailing **Address** of the PWS that was sampled.
5. The **County** in which the PWS is located.
6. The **Contact Name** for the person responsible for the PWS.
7. The number of the **Contact Phone** for the contact person.
8. The **Sample Collection Date**- must be indicated for sample to be acceptable for analysis.
9. The **Time** that the sample was collected- must be indicated for sample to be acceptable for analysis.
10. The full name (not initials) of the person who collected the sample, **Sample Collected By**.
11. The number for the **Collector's Phone.**
12. The **Sample Class** (Routine, Repeat, Special, or Raw).
12. The **Sample Monitoring Point** (usually DS000, or the assigned RS00# code if for a well).
13. If the sample class is Resample, the sample number of the previous sample with positive results that initiated the collection of the resample, **Repeat for Sample #.**
14. The **Tap Address** where the sample was collected for example, 28 Main St.
15. The **Sample Tap ID** where the sample was collected for example, sink tap 2 - Jim's Grocery.

Part III Collection of Microbiological Drinking Water Samples: Total Coliform/Fecal Coliform/E. coli

The prescribed procedures must be followed in detail for a valid laboratory analysis.

1. Select the sampling tap

a. A tap, such as faucet, petcock, or small valve, is preferable. Do not sample from hoses or drinking water fountains.

b. Avoid taps with a leak at the stem or taps with a swivel joint.

c. Aerated or screened nozzles may harbor bacteria. The aerator or screen must be removed before collection of the sample.

d. Place all carbon filters, sediment filters and water softeners on bypass unless operated by a public water system.

e. Sanitize the nozzle of the tap with a chlorine solution.

i. Use a 5.25% sodium hypochlorite solution, such as Clorox™ liquid bleach. Do not use chlorine solutions with special scents. To prepare a sanitizing solution that will contain about 400 mg/L of available chlorine (as hypochlorite) from the 5.25% sodium hypochlorite, add one ounce of bleach to one gallon of water (or 1 tablespoon per half-gallon. Store the mixed solution in a tightly closed screw capped container. The solution should be discarded and remade six months after preparation. Stronger solutions can be used, however, some faucet discoloration may result.

ii. Flush the sample tap to waste for one minute. Close the valve.

iii. Apply the sanitizing solution, prepared in step (i) above to the nozzle. This can be accomplished by either using a spray bottle or a plastic bag.

(1) Using a spray bottle, saturate the tap opening with sanitizing solution then wait at least two minutes before proceeding
or
(2) Place the bag over the nozzle and hold the top of the bag tightly on the tap. Alternately squeeze and release the bag to flush the solution in and out of the tap. Do this for two minutes. A fresh solution and bag must be used to sanitize each tap.

f. Flush the tap. The sample to be collected is intended to be representative of the water in the main. The tap must be opened fully and the water run to waste for at least 3-5 minutes to allow for adequate flushing of the piping between the tap and water main.

g. Reduce the flow from the tap. This will allow the samples bottle to be filled without splashing.

h. Remove the cap from the sample bottle

i. Grasp the bottom of the sample bottle.

ii. Remove the cap and hold the exterior of the cap between fingers while filling the sample bottle. Take care not to touch the mouth of the bottle or the inside of the cap with fingers or the sample could become contaminated.

iii. The bottle must be open only during the collection of the sample.

i. Fill the sample bottles

i. Do not rinse out the bottle before collecting the sample. Do not remove any 'pills' from the bottle. The bottle contains a small amount of sodium thiosulfate to neutralize the chlorine in the water.

ii Do not touch the rim or mouth of the bottle during collection of the sample.

iii. Do not overflow. Fill the bottle to within ½-1" of the top.

j. Immediately recap the sample bottle tightly

k. If there is any question as to whether a sample or bottle has become contaminated during collection of the sample, the sample must be discarded and a new sample collected in a new sample bottle.

l. Deliver the sample to the laboratory as soon as possible. The laboratory must receive the sample so that analysis can be completed (incubation initiated) within 30 hours after collection.
Allow the laboratory adequate time to analyze the sample. Certified laboratories will not test samples >30 hours old because the results will be invalid and the laboratory risks loss of certification.

m. Additional information

i. A sample identification slip (may use microbiological SSR form) is supplied with each sample bottle. The collection portion of the form is to be filled out in a legible manner using either indelible pen, rubber stamp or typewriter. Do not use a fountain pen or other pens having water soluble ink.

ii. Samples must be collected in bottles supplied by the certified laboratory performing the analyses. Bottles may be used from other certified laboratories provided the sample bottle supplying laboratory furnish records of sample bottle sterility tests.

iii. Samples must be accompanied by a properly completed sample identification slips. Sample identification slips that have not been properly completed as to name of water supply, address, date and time of collection, and signature of collector must not be accepted for bacteriological examination.

Chapter III

Part 1 Laboratory Facilities

Laboratory Space

Laboratory space must be adequate to accommodate periods of peak work loads. Adequate bench space for membrane filter is six linear feet of uncluttered bench space in unbroken sections per analyst. Adequate bench space for MMO-MUG testing is defined as 5-6 feet of clear bench space per analyst, up to a maximum of 12 feet for three analysts. Working space requirements must include sufficient benchtop area for analytical equipment, processing samples, storage space for media, glassware and other laboratory items. The space required for both laboratory work and materials preparation in small water plant laboratories may be consolidated into one room with the various functions allocated to different parts of the room.

Facilities must be clean, air conditioned, heated (65-80 ° F), and with adequate lighting at benchtop. Humidity levels must not be excessive. Rugs in laboratories are unacceptable. Bench counter tops must be in good condition. Rusted, unfinished, or badly worn cabinets are unacceptable and must be repaired. Outside windows must be covered with appropriate sun blocks.

Laboratory safety, must be a conscious effort in laboratory operations. The laboratory shall provide safeguards to avoid electric shock, prevent fire and accidental chemical spills, and minimize microbiological dangers, facility deficiencies, and equipment failures.

Bacterial cultures grown from water samples could cause disease. Therefore all inoculated culture tubes, petri dishes, MMO-MUG tests, etc. must be decontaminated in an autoclave (119 - 121 ° C for 30 minutes). All spills must be decontaminated with bactericidal substances. Common household bleach can be used for this (if it does not come into contact with acid).

Work space must be increased proportionally for laboratories engaged in multiple disciplines, i.e. water, milk, and food, so that water and other samples may be processed as necessary throughout the day without the need to program limited work space and time for one or the other type of sample examination.

There is to be no food or drinks stored or consumed in the laboratory. Smoking is not permitted in the laboratory.

Laboratory Construction and Remodeling

It is a requirement that all new laboratory areas, and remodeling, as well as adjacent areas utilized by the laboratory, be approved by the Ohio EPA/DES Laboratory Certification Section.

It will be the responsibility of each laboratory to notify the Ohio EPA/DES Laboratory Certification Section, in advance of intended structural changes or modification of the laboratory area. Examples of remodeling and structural changes are relocation of walls, doors, and addition of analytical benches, cabinets, plumbing, wiring, etc. If in doubt, submit plans. Contact the Ohio EPA/DES Laboratory Certification Section for the guidelines for laboratory construction and remodeling. These guidelines have been prepared by the Ohio EPA/DES Laboratory Certification Section. All of these items may not be applicable to a particular laboratory. If you need clarification or assistance, contact the Ohio EPA/DES Laboratory Certification Section .

Laboratory Construction

In the event that a laboratory is to be relocated within a building or at some other outside location, the Ohio EPA/DES Laboratory Certification Section, must be notified in writing prior to the intended move. Equipment must be operational and all QC tests must be performed and recorded in the new facility for a reasonable time prior to conducting official analysis in the new location. During the interim between moving and the next scheduled survey, the laboratory will remain certified.

Part II Equipment

1. Absorbent Pads

Used only for membrane filter broth medium; forty-eight millimeter diameter, uniform thickness to permit 1.8 to 2.2 mL medium absorption, presterilized or autoclaved at 119 - 121 ° C for 10 minutes, free from bacteriostatic or bactericidal chemicals. Pad dispensers must be wrapped in aluminum foil during storage. The best alternative, when using broth and pads, is to obtain petri dishes from the manufacturer with sterile pads already in each petri dish. NOTE: Agar is preferable to broth and pads.

These items are not required for MMO-MUG testing.

2. Autoclave

Must be of sufficient size to prevent internal crowding (≥3 cubic feet). The autoclave must be equipped with an accurate thermometer with its bulb properly located so as to register maximum temperature. Calibrate the autoclave's outside thermometer following the procedures outlined in this manual if a fast exhaust cycle is used.

The autoclave must be equipped with a pressure gauge and an operational safety valve, as well as a fast/slow exhaust selector. The autoclave must be free of steam leaks.

The autoclave must reach and maintain a temperature of 119 - 121 ° C. Any deviations from this must be rectified.

The sterilization temperature must be checked for all slow exhaust cycles with a maximum registering thermometer in a 20 X 150 mm, or similar sized glass test tube containing 10 mL of water, supported in a wire rack, or in a 25 mL glass graduated cylinder containing 10 mL of water. Do not use plastic vessels to hold the maximum registering thermometer. After the door is opened carefully remove the tube with the thermometer, wait five minutes and read and record the temperature. Record temperature using the outside thermometer for fast exhaust cycles.

When sterilizing carbohydrate broths, the complete sterilization cycle from the time the door is sealed to the time it is opened must not be greater than 45 minutes. Preheating the autoclave may be necessary in order to meet this requirement.

Data for each autoclave run must be recorded on the autoclave sterilization record. Use a copy of the form provided for this in this manual. All entries must be legible, complete, and recorded on a single line of the official form.

The autoclave's automatic timer must be calibrated when new, and every quarter thereafter. Set the timer for 45 minutes and use an accurate watch or stopwatch to time it at appropriate sterilization intervals used within

your lab. Post any correction factor near the timer on the autoclave. Record the timer calibration in the QC log.

3. Balance

Electronic top loading balances, with automatic tare controls, are generally available and reasonably priced. These balances must be used instead of triple beam or other mechanical balances.

When using electronic top-loading balances you may weigh down to 0.5g, if the balance reads to one-tenth of a gram.

Weigh media into clean plastic weighing dish, filter paper, aluminum foil or into the flask. When weighing a mass of less than 0.5g use an analytical balance with 1 milligram sensitivity at 10g load instead of a rough balance.

The analytical balance must be supported by a stone balance table, stone slab, or equivalent. All balances must be on an annual service contract. Proof of such service must be posted on or near the balance. Additionally, balances must be checked each month with certified calibration weights.

To do this you must see if the non-analytical balance responds correctly to a 0.1g mass with a minimum of three weights that bracket the range of weights normally used in the laboratory. Place each mass on the balance add a 0.1g weight. Response must be ±0.1g of the total mass on the balance. For analytical balances the response must be to a 0.01g weight. Multiple weights may be used to arrive at the total load.

Calibration weights must be ASTM type 1, 2 or 3 or NIST Class S or S1 standards. Certified calibration weights typically come in 200, 100, 50, 10, 5 and 1 g mass. Documentation, in the form of a manufacturers certificate or label, must be produced at the time of survey. If appropriate documentation is not available the weights will need to be certified or replaced. If the balance sensitivity is not acceptable, the balance must be serviced or if for some reason the weights have changed mass, the weights must be replaced.

This item may not be required for MMO-MUG testing if culture QC is contracted to another Ohio EPA approved laboratory.

4. Culture Tubes

Culture tubes must be of sufficient size so that the total volume of medium and inoculum does not fill the tube more than 2/3. Tubes more than 2/3 full are subject to contamination and are potentially more hazardous to work with. Tubes must be made of borosilicate glass. The diameter of Durham tubes (small inverted tubes used for trapping gas) must not be less than 40% of the culture tube. For example, a 20 mm test tube requires at least an 8 mm Durham tube. Aluminum, plastic, or stainless steel caps must be used to cover the test tubes. Cotton or foam plugs are not acceptable because they do not prevent contamination as well as caps, and are inconvenient to use.

This item may not be required for MMO-MUG testing if culture QC is contracted to another Ohio EPA approved laboratory.

5. Forceps

Forceps must be round tipped, without corrugations on the inner sides of tips. Sterilize before use by dipping in 95% ethanol or absolute methanol, and then ignite the fluid and let it burn out.

This item is not required for MMO-MUG testing.

6. Hot Air Sterilizing Oven

The oven must be of sufficient size to prevent internal crowding. The oven must be constructed to give a uniform sterilization temperature of 170 ° C or greater. Kitchen type stoves are not acceptable in the laboratory. Equip oven with an accurate thermometer that can be read while the oven is in operation, that includes the range of 160 - 180 ° C. If the oven is not equipped with a thermometer, it will be necessary to install one by drilling a hole through the top or side of the oven.

Calibrate the oven thermometer at the higher ranges of the NIST thermometer using sand, or in a thermostatically controlled water bath set within the higher range of the NIST thermometer.

This item is not required for MMO-MUG testing if disposable glassware is used.

7. Total Coliform Incubator

The incubator must provide sufficient space for the daily work load. The minimum acceptable size of an incubator is 7,200 cubic inches (inner chamber). Commercial laboratories and laboratories analyzing many samples will need even larger units. Laboratories performing MMO-MUG tests on a large scale will need substantially more incubator space as compared to MF.

The incubator must maintain a uniform and constant temperature of 35±0.5 ° C. An accurate thermometer calibrated in at least 0.5 ° C increments with the bulb continuously immersed in a 20mm or 25mm X 150mm stoppered test tube of water or mineral oil must be maintained on each shelf in the incubator (Carefully insert thermometer in rubber stopper; insert stopper with thermometer into a test tube of hot water; cool; place a thermometer on each shelf of the incubator). Optionally, thermometers in square bottomed, liquid-filled vials may be purchased.

Twice daily readings of the temperature on each shelf must be recorded. Record the temperature in the early morning followed by another reading at least 4 hours later. Include any correction factor of the thermometer in the recorded reading. That is, if the temperature of the thermometer registers 35.5 ° C, and the thermometer has a correction factor of minus 0.5 ° C, record "35" as the true temperature. This record must include the date, the analyst's initials and temperature reading on each shelf. Any deviations greater than 0.5 ° C from 35 ° C must be corrected by the proper thermostat adjustment. Maintaining such a record of the incubator temperature will alert laboratory personnel to any gradual changes that may reflect a faulty thermostat or other problem.

The incubator must not be subject to excessive room temperature variations beyond a range of 65 - 80 ° F. This will require the laboratory to be air conditioned and heated.

8. Inoculating Equipment

Wire loops and swabs are used for verification procedure. Wire items must be composed of 22 or 24 gauge chromel, nichrome, or platinum-iridium. Presterilized, single service plastic loops and needles are also acceptable. The loop's diameter must be at least 3 mm or slightly larger. The loop must be a full, unbroken circle. The loop and inoculating needle wire must have a length of 2 to 3.0 inches.

Swabs used for the verification test must be individually, or doubly wrapped and presterilized. Handles may

be made of wood or plastic. The tip must be covered with cotton or calcium alginate.

This item is not required for MMO-MUG testing.

9. Media Preparation Utensils

Use borosilicate glass flasks and graduated cylinders. Flasks and cylinders used for preparation of membrane filter media must be hot-air sterilized prior to use. All graduated cylinders must be calibrated "To Deliver" (TD). Plastic cylinders marked TD/TC or TC/TD are acceptable and are sterilized in the autoclave. Utensils must be clean and free from foreign residues or dried medium.

These items may not be required for MMO-MUG testing if culture QC and sample bottle sterility are contracted to another Ohio EPA approved laboratory.

10. Membrane Filters

Pore size 0.45 micrometer, diameter 47 mm, grid marked with a nontoxic ink, presterilized or autoclaved at 119 - 121° C for 10 minutes. The filters must be certified as meeting USEPA requirements by the manufacturer. Record the date each lot is received. Test each lot for sterility upon receipt, then annually if the supply lasts ≥ 12 months. To do this aseptically remove one filter from the sterile pack with sterile forceps and transfer it to a sterile beaker, or suitable container, containing 50 mL of sterile TSB or BHI (aseptically measure this volume from the Erlenmeyer flask the media was prepared in). Incubate 24 hours at 35± 0.5 ° C and observe for cloudiness. Record the results.

If the results are positive, contact the manufacturer and do not use the filters.

This item is not required for MMO-MUG testing.

11. Membrane Filtration Units

Filtration units may be constructed of stainless steel, glass, or plastic. The unit must not leak during filtration. Causes of leakage may be due to worn or loose lock wheels or a poor seal. Replace or refurbish the funnel if it becomes scratched, worn, corroded, dented, chipped, cracked, or broken because rough surfaces will act as lodging places for bacteria. Stainless steel funnels that are scratched may be polished by a professional metal polishing company. Wire support screens from the funnel receptacle must be replaced if damaged. The stainless steel funnels may be cleaned with a non-abrasive pad. Plastic, magnetically sealed funnels are only acceptable if: they do not leak; if they have been modified to accept a stainless steel receptacle support screen; and if they can be hung inverted without contamination. The funnel and receptacle must be wrapped and sterilized unassembled in the autoclave. Pyrex funnels are sterilized in the hot-air oven. A spare glass unit must be available when only glass funnels are used. Single service disposable funnels are not acceptable.

This item is not required for MMO-MUG testing.

12. Microscope and Lamp

A binocular stereoscopic microscope must be used. The microscope must have a magnification of 10X plus at least one magnification greater than 10X, such as 15X, 20X, 25X, etc.

A fluorescent light source with the light rays projected perpendicular to the surface of the plate must be used

to give maximum sheen discernment of total coliform colonies.

These items are not required for MMO-MUG testing.

13. Petri Dish Containers

Glass petri dishes, which may be used only in emergencies, must be sterilized and stored inside aluminum or stainless steel cans, Kraft paper, or aluminum foil.

These items are not required for MMO-MUG testing.

14. Petri Dishes

Use sterile 50 X 12mm or 48 X 8.5mm tight-fitting plastic dishes for the membrane filter test, having clean, flat bottoms free from bubbles and scratches. Glass petri dishes may only be used in emergencies.

Petri dishes that contain prepacked sterile pads are preferred, when m-Endo broth is used. Store petri dishes in their original box.

These items are not required for MMO-MUG testing

15. pH Meter

The electronic pH meter must be accurate to 0.1 pH units. When agar is used, a flat surface pH electrode must be used. Digital pH meters eliminate parallax errors when reading values, therefore, analog meters are not acceptable. Keep the fill hole covered when the electrode is not in use. Uncover it when it is being used. Potassium chloride crystals must not be allowed to build up on or in the electrode.

Automatic temperature compensators may be used on a pH meter if all test materials are kept at room temperature. Do not use "tri-combination" electrodes, i.e. combination electrodes with a temperature sensor built in.

The pH meter must be standardized (calibrated) once before each day's (or shift) use with at least two buffers. Check the pH meter's linearity, at least once each month, with all three buffers (7, 10, & 4) and record the results. The linearity will either be a "% slope", a pH value (i.e. 4.03) or noted as efficiency. Manufacturers have different ways of identifying the linearity. For more information on checking linearity with a specific meter consult your owner's manual for details.

When performing a two point calibration, bracket the expected test range with two buffers. For example, to check the pH of m-Endo, with an expected pH of 7.2, use pH 7 and pH 10 buffers. If your meter does not have a means of standardizing with at least two buffers (some models use the slope control for the second point) and checking linearity you must obtain a new pH meter with these capabilities. If the meter has a slope control, but no "%" indicator, set up a two point calibration, and check with a third buffer; it must be within 0.1 pH unit of the third buffer. If the meter has a slope control, and a "%" indicator, set up a two point calibration, then check against a third buffer; it must be within 0.1 pH units and be 95-104% slope. If the meter is capable of a three point standardization, use three buffers for calibration. The slope must be 95-104%. Replace electrodes that show variances exceeding >0.1 pH units or outside 95-104% slope.

Always begin pH standardization with a 7.0 pH buffer unless the manufacturer recommends otherwise. This is the "zero" or isopotential point of a meter. For linearity, follow this with the 10.0 buffer, then finally, go to the 4.0 buffer.

Meters from different manufacturers, capable of three point calibration, have different procedures for standardization/calibration. Consult your owner's manual for details.

16. Pipet Containers

Metal cans or boxes must be constructed of aluminum or stainless steel. Copper cans are not acceptable as copper is very toxic to bacteria. To reduce breakage, you may place a pad of glass wool or Teflon in both ends of pipet cans. Carefully remove each sterile pipet from the can or box so that the remaining pipets are not contaminated. Move the pipet container near the work area. Do not carry sterile, individual pipets across the room. Do not sterilize and store unprotected pipets on the shelf of the hot air oven. Unprotected pipets stored in the oven are subject to contamination and cannot be considered sterile.

These items may not be required for MMO-MUG testing if culture QC and sample container sterility are contracted to another Ohio EPA approved laboratory.

17. Pipets

The calibration error must not exceed 2.5%. Serological pipets, Mohr pipets, and water pipets meet this requirement. Tips must be unbroken and graduation marks legible. All pipets must be sterile. The pipet top may be plugged with cotton to help prevent liquid from being pulled into the pipet bulb.

When using serological pipets marked "TD" with two bands; after drainage, blow out any remaining liquid in the tip one time with a pipet bulb to deliver total volume. Mohr pipets are touched off one time to deliver total volume.

Always use a bulb or mechanical pipet aid, **never mouth pipet**.

These items may not be required for MMO-MUG testing if culture QC and sample bottle sterility are contracted to another Ohio EPA approved laboratory.

18. Refrigerator

The refrigerator must be of sufficient size for the work load. It may not be used for volatile chemicals that may be toxic to bacteria. The laboratory refrigerator may not be used for storing food or beverages for employee consumption. It must be equipped with an appropriate thermometer immersed and sealed in liquid in a 20 mm X 150 mm or similar sized test tube on the top shelf. The refrigerator must maintain 0.0 - 5.0 ° C. Record refrigerator temperatures daily.

19. Sample Containers

Laboratories may prepare wide mouth borosilicate glass or autoclavable plastic containers of at least 125 mL (4 ounce) capacity for use as sample containers. Glass stoppered containers must be protected with metal foil or Kraft paper, and a piece of string or paper strip must be inserted between the container and closure before sterilizing to facilitate easy opening during sample collection. All samples of water must be dechlorinated at the time of collection. Failure to do so may result in bacterial elimination before the bacterial analysis is begun, which would result in an underestimation of contamination. For dechlorination, add 0.1 mL of 10%

sodium thiosulfate to each 125 mL (4 ounce) container prior to sterilization.

This will provide an approximate concentration of 100 mg dechlorination agent per liter of water, which will neutralize a sample containing about 15 mg residual chlorine per liter. Prepare an approximate 10% solution of sodium thiosulfate by dissolving 10g of sodium thiosulfate in 100 mL distilled water in an Erlenmeyer flask. Adding the 10g to a class "A" volumetric flask and bringing the water to volume would give an exact 10% solution and is optional. However, the approximate procedure (10g to 100 mL) is acceptable for preparing sample containers for this program. To reduce the possibility of microbial growth, sterilize the dechlorinating reagent at 119 - 121 ° C for 15 minutes and store it in the refrigerator.
Cloudy solutions or solutions with particles must be discarded.

Add 10% sodium thiosulfate to containers using a sterile 1.0 mL pipet. Non-sterile sodium thiosulfate may be used if it is prepared and used on the same day, with the excess discarded.

At least one container per batch of sample containers sterilized must be checked for sterility as follows: Prepare sterile TSB or BHI in an Erlenmeyer flask as outlined in this manual. Using a sterile 25 mL pipet or sterile graduated cylinder measure 25 mL of TSB or BHI and aseptically transfer it to the sample container. Optionally TSB or BHI may be predispensed in individual screw cap Erlenmeyers at the time of preparation and aseptically transferred to a sample container. Then incubate at 35 ±0.5 ° C for 24 hours, and check for growth. Growth will be indicated by even the slightest turbidity in the TSB or BHI. When opaque plastic containers are used, the TSB or BHI must be poured into a glass vessel after incubation, in order to look for turbidity.

Containers used for MMO-MUG testing must also be tested for autofluorescence. In other words, the containers must not fluoresce by themselves. This would cause a false positive E. coli test. Test 5% of plastic containers received and each glass container used for autofluorescence.

To do this take the containers to a dark room and examine them under the UV light for fluorescence. Record the results. If the containers fluoresce, they must not be used for drinking water samples. Replace any glass containers that test positive. For plastic containers, do not use the batch if any fluoresce and call the manufacturer.

Several types of commercially prepared presterilized disposable single use sample containers that contain sodium thiosulfate are available. Please contact the Lab Cert Section at Ohio EPA/DES prior to purchasing this type of container to see if the containers you are planning to obtain are acceptable. Presterilized containers must be checked for sterility in an Ohio EPA approved laboratory and have the results documented in the QC log.

From each box of disposable containers received, check a minimum of one container or 1% of the box for sterility, which ever is greater. Disposable containers with 100 mL ±2.5% volume marks may be substituted for 100 mL graduated cylinders.

20. Thermometers

The accuracy of all mercury-in-glass and electronic thermometers used for any laboratory function must be verified when new and at least once every year. Dial thermometers must be calibrated at least once every three months. The only exception to this is the autoclave's maximum registering thermometer which will be recalibrated for you during the laboratory survey.

Protective coatings are recommended for mercury-in-glass thermometers to help prevent mercury spills in the event the thermometer is broken. Non-mercury, liquid-in-glass thermometers may be used if documentation

that their accuracy meets NIST tolerance specifications is kept in the QC log along with their annual calibration. Alcohol column thermometers are not acceptable. Discard thermometers having correction factors of >1.0°. Thermometers must have a minimum graduations as follows: oven thermometers, refrigerator, autoclave thermometers-1.0° C; 35° C incubator-0.5° C; 44.5 degree incubator-0.2° C. Use only thermometers graduated to read in the Celsius scale. Fahrenheit thermometers may not be used in this program (except when they are permanently installed on an autoclave or oven).

Total and partial immersion thermometers may be used interchangeably for this program. The correction factor, as determined by using the formula to correct for emergent stem of the thermometer, is very low. This will not affect the accuracy of thermometers for bacteriological tests.

Verification of accuracy must be conducted within the minimum and maximum range of intended use by comparison with the readings of a reference thermometer certified by the National Institute of Standards and Technology (NIST), formerly NBS, or one with a manufacturer's certificate of traceability to NIST specifications. The NIST certificate, or equivalent, must be kept on file. The NIST thermometer must not have any mercury separations or unclear graduation lines. Reference thermometers must be checked annually to verify they are still in calibration. Record this in the thermometer calibration log. Replace the reference thermometer if found to be out of calibration. Oven thermometers may be calibrated at the upper range of the available lab NIST thermometer. Keep a QC log for all thermometers calibrated. Record the date, type of thermometer, serial number, location, temperature reading of the NIST thermometer, temperature of in use thermometer, and the corresponding correction factor. Tag all thermometers with a piece of tape indicating any correction factors, even if the correction factor is 0.0 °.

Suggested procedures for calibrating thermometers:

Reference (certified) Thermometers: Submerge the thermometer in an ice water bath (distilled water or deionized ice cubes plus distilled or deionized water) in an insulated container. The thermometer should read 0.0° C or go to the "ice point" calibration mark after a few minutes. The "ice point" calibration mark is found on the stem of thermometers not having a 0.0 ° C mark. It is below the lowest point of the range.

Incubator thermometers: Carefully place all incubator thermometers, plus the NIST thermometer, in a closed beaker or flask of water and incubate overnight at 35 ° C (overnight incubation allows water and thermometers to stabilize). Read and record all thermometer readings the following morning. Optionally, mineral oil may be substituted for the water and eliminates the need for a covered test vessel.

Fecal Coliform Incubator thermometer: (Water Bath) Keep the water bath incubator set at 44.5 ° C immerse both thermometers in a covered vessel, in the water. Allow to stabilize for an hour or so and record readings.

(Aluminum Block) Keep the incubator set at 44.5 ° C immerse each thermometer in a separate test tube containing mineral oil with the level not exceeding the top of the block's well. Use two center wells next to each other. Allow to stabilize for an hour or so and record readings.

Hot air oven thermometer: Place oven thermometer and NIST thermometer inside an oven with the bulbs of both thermometers side by side in a cylinder of sand. Heat oven to approximately 170 ° C (or as high as NBS thermometer will allow). Read and record the thermometer readings through the oven window. If oven does not have a window, preposition the thermometers in sand so they may be quickly read when the door is opened. Optionally, if the NIST thermometer has a maximum range of only 55 - 60 ° C, a water bath may be used. Leave the water bath at 44.5 ° C, immerse both thermometers in a covered vessel, in the water bath, allow to stabilize for an hour or so and record readings. (Optionally, use the Aluminum Block thermometer procedure as outlined above)

Refrigerator thermometer: Follow the same procedure as for incubator thermometers, except replace the incubator with the refrigerator. Optionally, calibrate the refrigerator thermometer at the "Ice Point" with the NIST thermometer in an insulated container with a saturated ice/water solution. This will give you a zero degree point for calibration on the bench top and is also a method of verifying the NIST thermometer has not been damaged.

Maximum Registering Thermometer: This will be calibrated for you on your survey date. The laboratory must have at least one spare maximum registering thermometer. Tag it with the proper correction factor. Please do **not** mail these to The Lab Certification office for calibration.

External autoclave thermometer: Place a calibrated maximum registering thermometer in a test tube, or 25 mL graduated cylinder, containing 10 mL of water. Run the autoclave for 15 minutes in the normal temperature range. When the autoclave reaches timing temperature, observe the external thermometer during the complete sterilization cycle, and note the highest temperature that is attained. After slow exhaust and cooling to below 100 ° C (212 ° F), open the door, wait five minutes for stabilization, and read the temperature. Take into account the correction factor of the maximum registering thermometer, and record the true temperature. Compare this to the highest temperature reached on the external thermometer, and apply the difference as the correction factor. Record the results, and tag the external thermometer with this correction factor.

21. MMO-MUG Test Vessels

Use sterile wide mouth borosilicate glass sample containers or equivalent clarity presterilized test vessels from a commercial vendor. The presterilized test vessels can double as sample containers and eliminate the need for individual 100 mL graduates if they have an accurate mark at 100 mL ±2.5%. Check test vessels for sterility at the same frequency and with the same procedure as sample containers. Refer to the section above "Sample Containers" for details on checking sterility and autofluorescence.

22. Resistivity/Conductivity Meter

Suitable range for checking laboratory pure water; readable in megohms/cm, micromhos/cm, or microSiemens/cm and accurate to ±2%. Use a meter with a glass, plastic or metal probe/cell.

Use only an extremely clean borosilicate glass beaker for this test. Rinse the beaker several times before collecting at least a 500 mL sample of water.

Insert the probe/cell several times and swirl in the sample to eliminate air bubbles that will give an erroneous reading. The resistivity/conductivity test should be performed on-site since the water quality decreases shortly after exposure to both air and the sample container.

The meter must be calibrated at least once each month following the manufacturer's recommendations for calibration in the appropriate range for lab pure water. Calibration solutions of <100us/cm are recommended. If a specific procedure is not available contact the manufacturer to obtain a suitable calibration procedure for low range samples such as lab pure water. Record the calibration data in the QC log. The meter may be calibrated by another Ohio EPA approved laboratory with appropriate documentation.

23. Equipment Timers

All laboratory timers that control equipment must be calibrated once each quarter. Record the results of each

calibration in the QC log. Generally, the only timers that need to be calibrated are the autoclave's automatic timer and the hot-air oven timer, if it is used to control the sterilization cycle. To calibrate the autoclave timer set the timer for 45 minutes and use an accurate watch or stopwatch to time it at appropriate sterilization intervals (i.e. 12, 15, 30, & 45 minutes) used by your laboratory. To calibrate a hot-air oven timer verify the timer does not start until the unit is $\geq 170^{\circ}$ C, then time the 2 hour setting with an accurate watch or stopwatch. Tag the timers with the corresponding correction factor.

24. Fecal Coliform Incubator

This incubator must be either a water bath incubator or aluminum block heat sink incubator. It must be capable of maintaining 44.5 $\pm 0.2^{\circ}$C. Take the morning and afternoon temperature with a thermometer calibrated in 0.1 $^{\circ}$C increments. The thermometer must be calibrated within the range of use with an NIST traceable thermometer when new and once every year thereafter. If a water bath is used it must be capable of holding the maximum number of tube racks that will be used.
The water level must remain above the media level for the entire incubation time. Follow the manufacturer's recommendations as to the type of water used in the unit. The water bath must be equipped with a recirculation system so that uniform temperature is maintained in all parts of the bath. It must be equipped with a cover to prevent evaporation. The water bath must be operational at all times and be a dedicated for fecal coliform testing. Do not turn the bath off when not in use. Do not adjust the temperature for other purposes. The water level must not be allowed to evaporate when not in use for actual tests. The water bath must be cleaned regularly.

A disinfectant made specifically for water bath incubators may be used in the water to keep contamination to a minimum.

If a heat sink incubator is used, the test tubes used must fit tightly in each well. The well must be deep enough so that the level of the medium is within the well. The heat sink must be equipped with a thermometer that is graduated in 0.1 $^{\circ}$C increments or be marked from the manufacturer of the heat sink with lines at 44.3 $^{\circ}$C and at 44.7 $^{\circ}$C. Due to the elevated incubation temperature, place the thermometer in a tube of mineral oil to prevent evaporation. The level of mineral oil in the tube with the thermometer must not extend above the top of the aluminum block. The heat sink must be operational at all times and be dedicated for fecal coliform testing. It will be necessary to locate the heat sink incubator in a non-drafty area or provide a draft shield for the unit.

25. Ultraviolet (UV) Light

Must be a long wave (365 - 366 nm) UV light equipped with a 6 watt bulb.

Chapter IV

Part I Materials and Media Preparation

1. Cleaning Glassware

Wash glassware by hand or in an automatic dishwasher with an appropriate laboratory detergent. Use a non-bactericidal detergent manufactured for use in laboratories. Any detergent that is not designated as a laboratory detergent or has questionable suitability for use in a bacteriology laboratory cannot be used.

If washing glassware by hand, follow these steps:

(1)Wash in hot soapy water using a brush to remove residues.

(2)Rinse in hot tap water several times.

(3)Rinse two to three times in laboratory pure water.

(4)Air dry.

Glassware with stains or residue after washing can be rinsed in chromic acid or an equivalent cleaning solution and then re-washed and checked for acid residual. Since chromic acid solution needs to be prepared in a safety hood, commercially available acid cleaning preparations such as "Chromerge" or "Nochromix", are more practical. M-Endo stains may often be removed by brushing with 95% ethanol eliminating the need for an acid rinse.

Use bromthymol blue indicator to determine if pipets and glassware have a toxic acid residual. Put a small volume of bromthymol blue indicator into the top of pipet or on the glassware, invert or swirl, allowing the indicator to run down inside. If the indicator changes color from blue to yellow, acid residual is present, or if the color changes to deep blue, alkaline residual from the detergent is present. In either instance the glassware must be re-washed.

Dishwashers must be of laboratory design to have the appropriate wash and rinse cycles. Dishwashers must be used with laboratory type detergents specifically made for dishwashers. Using hand wash detergents in a dishwasher results in unacceptable foaming. The dishwasher must have a final lab pure water rinse cycle, that is connected and functional, if it is to be used in this program. Pipets can be washed by an automatic pipet washer which attaches to the faucet.

Use a tablet type detergent designed for pipet washers, such as "Alcotabs", for this. Wash the pipets in hot soapy tap water, followed by many hot tap water rinses, and several laboratory pure water rinses.

2. Sterilization of Materials

Steam sterilization is generally considered a more effective type of sterilization than dry heat. Steam under pressure provides a more uniform temperature and penetrates most materials more rapidly and thoroughly than hot air. The presence of moisture also hastens the coagulation of bacterial protoplasm, which is an effective means of killing microorganisms.

Dry heat sterilization is, however, more suitable for the sterilization of glassware, since contact with steam during autoclaving may introduce water condensate and/or chemical contamination from boiler water. Dry-heat sterilize all glassware, including glass membrane filter funnels and pipets, for at least two hours at $\geq 170^\circ$ C.
Liquids, stainless steel membrane filtration units, plastics, and other materials which might be distorted or destroyed by hot air must be sterilized by steam under pressure.

The following times and temperatures must be used for autoclaving materials:

Material	Temperature	Time	Cycle
Carbohydrate Media (LTB, BGB, EC)	119-121° C	12-15 minutes	Slow
Stainless Steel MF Funnels, Stir Bars	119-121° C	30 minutes	Fast
Rinse/Negative Control Water (500mL)	119-121° C	30 minutes	Slow
Rinse/Negative Control (500-1000mL)	119-121° C	45 minutes	Slow
Rinse/Negative Control (>1000mL)	119-121° C	90-180 minutes	Slow
Contaminated Material	119-121° C	30 minutes	Slow
Stock buffer, sodium thiosulfate, BHI, TSB	119-121° C	15 minutes	Slow
Plastic bottles	119-121° C	30 minutes	Fast

Note: membrane filter assemblies must be sterilized at the end of each sample filtration series. A filtration series ends when 30 minutes or longer elapses between sample filtrations.

3. Microbiologically Suitable (MS) Water

Each laboratory must be equipped with water conditioning apparatus which produces water that is free from traces of dissolved metals, contaminating nutrients, and bacteriostatic or bactericidal compounds. Many laboratories purchase or rent a deionizing system to meet this requirement.

The deionizing unit offers an unlimited supply without the usual care a distilling unit requires and eliminates an additional source of heat in the laboratory. The supply must be adequate for all laboratory needs.

Requirements for MS Water	
Conductivity (or resistivity)	<2.0 micromhos/cm (or us/cm)
Resistivity (or conductivity)	>0.5 megohm/cm
Total Chlorine	Not Found
pH	5.5 - 7.5
Pb, Cd, Cr, Cu, Ni, Zn	Not >0.05mg/L each or not >0.1 mg/L collectively

It will be necessary to add one to two drops of saturated KCl solution (without silver chloride) to 10-20 mL of the lab pure water, then wait twenty minutes, prior to checking pH. Do not stir or swirl when the probe is in the lab pure water, as this will lower the pH significantly. Keep a written monthly record of pH, resistivity or conductivity and total chlorine residual in the QC log.

Use only natural (amber) latex tubing or "tygon" plastic tubing on laboratory pure water and rinse water dispensing systems. Do not use black rubber or copper tubing.

Use any DPD method, with the appropriate fresh reagent, for checking the total chlorine residual (colorimetric, spectrophotometric, "Hach Pocket Colorimeter" or other acceptable method). Report the presence or absence of total chlorine in the water. Since you are only required to report the presence or absence of total chlorine there is no need to calibrate the equipment, as long as you are not reporting quantified levels of chlorine. This only applies to non-quantified QC tests of distilled/deionized water and is not applicable for drinking water chlorine level analysis when microbiological samples are collected.

The annual trace metals analysis must be done in a laboratory certified by the Ohio EPA for these parameters.

4. Buffered Rinse Water

Add 1.25 mL of stock buffer solution and 5.0 mL magnesium chloride stock solution (preparations to follow) per liter of laboratory pure water, to be used as rinse water for the membrane filtration procedure. Allow the stock buffer and $MgCl_2$ solutions to come to room temperature in order to accurately measure the volume. Use a 2.00mL pipet (calibrated in 1/100's) to deliver 1.25 mL, and use a 5.0 mL pipet to deliver 5 mL. Use a one liter graduated cylinder, or a one liter volumetric flask to measure one liter of laboratory pure water. If larger volumes are prepared, it is permissible to mark the rinse water container at the calibrated volume level with a permanent mark. Sterilize the rinse water as soon as the buffer has been added. Do not store the stock buffer, $MgCl_2$, distilled water solution in non-sterile condition for >4 hours. Store sterile rinse water for up to six months at room temperature. Do not refrigerate the rinse water.

Stock phosphate buffer with a pH of 7.2 ±0.2 is prepared as follows: Dissolve 34 G of monobasic potassium phosphate in 500 mL of laboratory pure water in a 1 liter volumetric flask. Do not use phosphate buffer used for BOD analysis because it has a different composition. After dissolving the reagent; adjust the pH to 7.2 ±0.2 with 1.0 or 0.1N NaOH. Let the solution stand for a few hours. Recheck the pH; note initial pH. Dilute the volume to 1000 mL. Optionally, BBL APHA Phosphate Buffer 7.2 may also be used. Follow the manufacturer's instructions for preparation. The pH of this solution is self adjusting and cannot be adjusted before or after autoclaving.

Dispense stock buffer solution in 10 to 20 mL volumes in screw cap tubes or vessels. Autoclave for 15 minutes at 119 - 121 ° C. The pH will generally drop during sterilization. Check and record final pH when cool. If the final pH is not 7.2 ±0.2, discard it and prepare a second batch. If you are not sacrificing a complete container for the final pH reading, use a sterile pipet to remove the pH portion, do not put the electrode into the sterile solution. Date each batch of stock buffer with the date of preparation. Store in the refrigerator at 0.0 - 5.0 ° C for not more than six months. Prepare fresh stock buffer at least every six months, or if the pH varies from 7.2 ±0.2, or if turbidity indicates microbial growth.

Magnesium Chloride Stock Solution: Dissolve 81.1 g $MgCl_2 * 6H_2O$ with laboratory pure water in a 1 liter volumetric flask. Dilute to a final volume of 1 liter in the volumetric flask with laboratory pure water.

If you experience this difficulty you may use the following procedure. Magnesium Chloride Stock Solution

Optional Procedure: Dissolve 81.1 g $MgCl_2 * 6H_2O$ with laboratory pure water by swirling in a wide mouth Erlenmeyer flask or mixing in a beaker with a magnetic stir bar.
The magnesium chloride hexahydrate will readily dissolve with less than the full volume of water. Once dissolved, transfer the solution to a 1 liter volumetric flask (This may require a clean laboratory funnel). Rinse the flask or beaker with three separate portions of laboratory pure water; adding each rinse to the volumetric flask. Dilute to a final volume of 1 liter in the volumetric flask with laboratory pure water. Note: You may prepare other size batches by adjusting proportions and glassware accordingly. Date each batch of stock $MgCl_2$ solution with a date of preparation and store in the refrigerator at 0.0 - 5.0 ° C for not more than 6 months. Prepare fresh stock solution at least every 6 months or if turbidity indicates microbial growth.

5. pH Measurements

Calibrate pH meter at approximately 25 ° C using the procedures previously outlined under "pH Meter". Use each buffer aliquot only once.

The temperature of the medium must be approximately 25 ° C (room temperature) when pH is checked. At this temperature agar medium will be solidified.

Use a surface electrode for agar. Touch the electrode to the surface of the solid agar. The surface electrode may be used for liquids in the normal manner. Keep the pH probe's fill hole covered when not in use. Prepare a sufficient quantity of medium so that the portion of medium used to check pH can be discarded.

Check and record the final pH of each medium or reagent batch prepared (including LTB, EC, BGB, stock buffer, m-Endo, BHI, TSB and others) in the QC log.

Monitoring final medium pH will help identify errors in proportions weighed, excessive sterilization resulting in lactose hydrolysis, chemical contamination or deterioration of ingredients. Media that have a final pH value after sterilization greater than or less than 0.2 of the correct pH must be discarded and the source of the difficulty located.

6. Sterilization of Media

Carbohydrate media: (LTB, BGB, EC) must be sterilized at 119 - 121 ° C for 12 to 15 minutes. Tubes must be packed loosely in baskets or racks for uniform heating and cooling. Timing starts when autoclave temperature reaches sterilization temperature. The maximum elapsed time for exposure of carbohydrate broths (or other sugar broths, such as TSB) to any heat (from the time autoclave door is closed until the medium is removed from autoclave) is 45 minutes. Excessive exposure of lactose contained in LTB, BGB and EC to heat may result in hydrolysis with formation of the sugars glucose and galactose. False positive reactions could result if non-coliforms were present which can ferment and form gas from glucose and/or galactose, but not ferment lactose. Further heating will form aldehydes and ketones, which would result in false negatives. Media must therefore be removed and cooled to room temperature as soon as possible after sterilization.

7. Storage of Media, Reagents, and pH Buffers

All bottles of media, reagents, and pH buffers must be dated on receipt and when opened. Date bottles in this manner: "Received→(month- day-year); Opened→(month-day-year)". Except for large volume uses, media should be purchased in 1/4 pound bottles. Bottles of media must be used within six months after opening unless they are stored in a desiccator, in which case they may be stored for one year. Shelf life of unopened bottles of media is two years. Bacto-agar may be kept for five years after receipt, and two years after opening , as long as it remains in its original free-flowing state.

Chemical reagents may be kept for six years after receipt. pH buffers may be stored for one years after receipt

or six months after opening. If a manufacturer's expiration date occurs prior to any in house labeled expiration date, discard the material based on the earliest expiration date.

You may not extend storage time based on optimistic manufacturer's expiration dates, or by opening an otherwise expired bottle to "extend" the shelf life based on the date of opening. Discard bottles of media, reagents, and pH buffers when they expire. If expired bottles are in the laboratory, they will be assumed to be in use. Do not use 'white out' on bottles. Bottles with 'white out' or dates scratched off or erased, must be discarded. Bottles of dehydrated media and reagents must be kept tightly closed and stored at less than 30 ° C (86° F). Media and reagents must be kept in their original containers. Dehydrated medium and reagents must be discarded if they become discolored, caked, or are no longer in their original free flowing form.

Store Colisure reagent in the refrigerator , even though the manufacturer may state otherwise, following the manufacturer's listed expiration date. Colilert may be stored at room temperature out of direct sunlight.

Sterile culture media must be stored in a clean area free from contamination and excessive evaporation. Store broth media at room temperature and out of direct sunlight.

Sterile batches of LTB, BGB and EC stored at room temperature must be used within one week or be discarded. Optionally, LTB, BGB and EC, in screw capped tubes, may be stored for up to 3 months in the refrigerator. Preincubate refrigerated tubes overnight at 35 ° C and discard any tubes with air bubbles prior to use. Preincubated tubes may then be held in reserve for one week, stored at room temperature.

Store BHI or TSB in screw capped tubes, flasks, or vessels for up to six months in the refrigerator. Optionally, store TSB or BHI in slip cap tubes or aluminum foil covered flasks, at room temperature, for up to one week.

Store m-Endo agar plates inverted, in a suitable sealed container, in the laboratory refrigerator for up to two weeks. m-Endo agar plates not stored in a sealed container, but stored on a shelf in the refrigerator, must be discarded after 96 hours (four days). Store m-Endo broth in a covered Erlenmeyer flask for 96 hours in the refrigerator.

All racks/baskets of media tubes and plates, as well as reagents, must be clearly labeled as to the type of medium or reagents and the preparation/expiration dates.

8. Media Quality Control

Each new lot of media prepared/received in the laboratory must be checked for sterility and performance prior to use. This includes m-Endo, LTB, BGB, EC, BHI and TSB. To check performance of m-Endo, LTB, BGB, EC, BHI and TSB take two plates or tubes from each new lot and inoculate one plate or tube with a known coliform (fecal coliform for EC broth) culture. Leave the other plate or tube blank. Incubate m-Endo, LTB, BGB, BHI and TSB for 24 hours at 35 ±0.5 ° C (for LTB and BGB incubate an additional 24 hours to complete the test). Incubate EC broth at 44.5 ±0.2 °C for 24 hours. If the uninoculated items show growth or the inoculated items do not show growth, contact the Laboratory Certification Section at Ohio EPA/DES and do not use the medium.

Each lot of MMO-MUG reagent must be checked before use with three known stock or commercial cultures of the following: for the *E.coli* control use *Escherichia coli*; for a total coliform that is not a fecal coliform, *Klebsiella pneumoniae* or *Enterobacter aerogenes*; and for a non-coliform use Pseudomonas aeruginosa or Proteus mirabilis. You may use other organisms if the fit the appropriate general classification.

Record the results of this QC procedure. Aseptically fill three test vessels with 100 mL sterile deionized or distilled water.

Add a tube of MMO-MUG reagent to each test vessel and mix to dissolve. Touch a sterile inoculating needle to an 18-24 hour pure culture slant, or equivalent, for each of the three specified bacteria and inoculate the

three corresponding test vessels. See "Culture Methods" for alternative procedures.

For Colilert: after 24 hours incubation at 35 ±0.5 ° C the test results should be as follows:

1. *Escherichia coli* → Yellow, total coliform positive; Fluorescence, E. coli positive.
2. *Klebsiella pneumoniae* (or other total coliform) → Yellow, total coliform positive; No Fluorescence, E. coli negative.
3. *Pseudomonas aeruginosa* (or other non-coliform) → No color, total coliform negative; No Fluorescence, E. coli negative.

For Colisure: after 24-48 hours incubation at 35 ±0.5 ° C the test results should be as follows:

1. *Escherichia coli* → Red, total coliform positive; Fluorescence, E. coli positive.
2. *Klebsiella pneumoniae* (or other total coliform) → Red, total coliform positive; No Fluorescence, E. coli negative.
3. *Pseudomonas aeruginosa* (or other non-coliform) → Yellow, total coliform negative; No Fluorescence, E. coli negative.

The test results listed above, with the corresponding three organisms, verify that the test system is viable across the range of potential results. If these results are not observed initially repeat the procedure. If the correct results are not observed on retest then contact the Lab Certification Section at Ohio EPA/DES for further assistance.

9. Culture Methods

Viable pure cultures of the above listed organisms are required by the USEPA as part of the MMO-MUG quality control protocol. These bacteria are opportunistic pathogens. This necessitates strict adherence to good microbiological practices to prevent contamination of laboratory personnel.

If laboratory personnel do not have the time or bacteriological expertise to keep and maintain pure stock cultures, the best option is to purchase commercially prepared cultures. The cultures are stored in the refrigerator and reactivated for use in the laboratory as necessary.

If you choose to purchase commercial culture preparations you may choose any manufacturer producing the three required organisms. Follow the manufacturer's directions for use. Label and date all cultures.

Another choice is to obtain three pure stock cultures. Keep them viable by transferring them monthly. Label and date all stock culture tubes. The use of TSB and TSA slants in screw cap tubes is the preferred method of keeping stock cultures.

Place an inoculum in TSB and incubate for 18 - 24 hours at 35 ±0.5 ° C. Streak the surface of a TSA slant with a two loop inoculum from the freshly incubated TSB. Incubate the TSA slant for 18 - 24 hours and use this tube as the stock culture to test a new lot of MMO-MUG reagent or as the start of the next month's culture transfer. Store the stock culture tubes in the refrigerator at 0 - 5 ° C until next months transfer. An alternative procedure is to place a two loop inoculum in a screw cap tube of TSB and incubate for 18 - 24 hours.

A loop of fresh culture grown in TSB would then serve as the stock culture for testing a lot of MMO-MUG reagent or as the start of the next months transfer. Store the stock culture tubes in the refrigerator at 0 - 5 ° C until next months transfer.

Laboratories not using a commercial culture preparation with specific manufacturer's instructions must grow pure test cultures for 18 - 24 hours at 35 ±0.5 ° C and use them fresh. To standardize the inoculation of MMO-MUG test vessels with stock cultures grown in the laboratory adopt one of the following procedures:

For growth on agar; touch a sterile inoculating needle to the culture and inoculate 100 mL of sterile water in a test vessel.

For growth in broth; swirl the tube to suspend the culture growth and inoculate 100 mL of sterile water in a test vessel with one 3 mm loop of broth. Note: Any contamination of a stock culture by another organism will ruin the stock culture.

If laboratory personnel do not wish to do the required reagent QC testing they may contract with another Ohio EPA approved water laboratory for this service. Send several individual MMO-MUG reagent packs, with the corresponding lot number and expiration date, to the contract lab for analysis. The laboratory must receive and retain a copy of the contracted reagent QC test results, with the contract lab's name and Ohio EPA approval number, before using each new lot of reagent. NOTICE: Any mention of a commercial product does not signify an endorsement. References are made as examples only.

Chapter V

Part I Culture Media Specifications and Preparation

Lauryl Tryptose Broth

LTB is used for verification of total coliform colonies from MF procedure. The ingredient sodium lauryl sulfate inhibits growth of aerobic spore forming, gas forming lactose fermenters other than coliform, thus eliminating some of the false positives that lactose broth would yield.

Composition

Tryptose	20g
Lactose	5g
Dipotassium hydrogen phosphate	2.75g
Potassium dihydrogen phosphate	2.75g
Sodium chloride	5g
Sodium lauryl sulfate	0.1g
Laboratory pure water	1000 mL

Final pH after sterilizing (119 - 121 ° C for 12 - 15 minutes): 6.8 ±0.2.

Weigh out 35.6g of medium on appropriate balance. Add 500 mL of water to an Erlenmeyer flask. Transfer the medium to the flask. Rinse down the sides of the flask with the remaining water. Allow the medium to rehydrate. Swirl. Pipet 11-12 mL per culture tube (containing a Durham tube); cap tubes. Sterilize 12 - 15 minutes at 119 - 121 ° C. Promptly remove tubes from the autoclave. Rapidly cool a small portion; check and record final pH. Final pH must be 6.8 ±0.2. There must be at least 10 mL of liquid in each tube. Store a maximum of one week at room temperature.

Optionally, carbohydrate media may be stored for up to 3 months in the refrigerator in screw cap tubes. Before using refrigerated tubes, you must incubate them overnight and discard any tubes with air bubbles in the Durham tube. The laboratory may date and preincubate a week's supply of tubes and then hold them in reserve at room temperature. However, after one week these tubes must be discarded if they are not used.

Brilliant Green Lactose Bile Broth

Brilliant Green Lactose Bile Broth (BGB) is used for verification of total coliform colonies from MF Procedure. The ingredients brilliant green dye and bile inhibit the growth of bacteria other than coliform.

Composition

Peptone	10g
Lactose	10g
Dehydrated Oxgall	20g
Brilliant Green	0.0133g
Laboratory pure water	1000 mL

Final pH after sterilizing (119 - 121 ° C for 12 - 15 minutes): 7.2 ±0.2.

Follow the same general procedures as in preparing and storing LTB, except the final pH must be 7.2 ± 0.2; and when weighing, add 40.0g per liter of water.

m-Endo Medium

Tryptose of polypeptone	10g
Thiopeptone or thiotone	5g
Casitone or trypticase	5g
Yeast extract	1.5g
Lactose	12.5g
Sodium chloride	5g
Dipotassium hydrogen phosphate	4.375g
Potassium dihydrogen phosphate	1.375g
Sodium lauryl sulfate	0.05g
Sodium desoxycholate	0.1g
Sodium sulfite	2.1g
Basic fuchsin	1.05g
Laboratory pure water	1000 mL
95% ethanol	20 mL
Final pH - 7.2 ± 0.2	

Preparation

4.8g of dehydrated medium plus 100 mL laboratory pure water, plus 2 mL 95% pure ethanol, plus 1.5g of bacto-agar. Use pure 95% ethanol (not denatured). Do not use vodka or other alcohols because they contain sugars and other contaminants. You may be able to obtain 95% pure ethanol from a local drug store, hospital, state liquor store, or chemical supply company. Do NOT use absolute (100%) ethanol for m-Endo preparation. In some cases, you will need a written explanation from your board of public affairs or director of health.

1. Measure the distilled water into a sterile graduated cylinder. All liquids must be at room temperature. All measuring instruments are calibrated to deliver accurate volumes at room temperature. (Sterilize cylinder by covering top with aluminum foil and heating to at least 170 ° C for a minimum of two hours. Foil must remain in place until cylinder is ready to be used.)
2. Add 95% pure ethanol to the water in the cylinder. The alcohol must be at room temperature to accurately measure the volume.
3. Transfer approximately one-half of the water-ethanol mixture to a sterile Erlenmeyer flask (the flask must be sterilized and covered as the graduated cylinder).
4. Weigh out the dehydrated medium on an appropriate laboratory balance. When weighing any medium, once you have removed a portion of the dehydrated medium from its original bottle, it must be used or discarded. Do not return media or reagents to their original containers. Use a clean weigh boat, or filter paper, or weigh directly into the flask. If you choose to weigh directly into the flask, and accidentally add too much to the flask, you must discard the flask and medium, and begin again. Do not prepare <100 mL batches of medium.
5. Transfer the medium to the flask; rinse down the sides of flask with the remainder of the water-ethanol mixture. Do not rinse out the weigh boat unless it contains a significant amount of media. If you must rinse out the weigh boat and spill any water-ethanol you must discard the batch and start over. Replace the sterile foil cover on the flask.
6. Allow the medium to rehydrate (absorb water). The mixture may be swirled to hasten this process. The use of a presterilized magnetic stir bar is an efficient method of mixing the media with out getting agar on the flask above the level of the solution. Magnetic stir bars may be wrapped in aluminum foil, sterilized in the autoclave (same cycle as MF funnels), and aseptically transferred to the flask of media. Magnetic stir bars with the proper coating may be sterilized in the glass flask in the hot-air oven.
7. Checking initial pH is not required.

8. Heat medium to the first bubble of boiling over a gas burner using a tripod and screen, a hot plate set on "high", or a hot plate with a presterilized magnetic stirring bar. Do not use double boilers. Swirl flask while heating to dissolve medium. Never allow the liquid in the flask to be on the heat source while the liquid is not moving. The medium will burn to the bottom of the flask. Prolong the heating for the time it takes to dissolve all of the agar. All agar must dissolve without additional heating. After one bubble of boiling is observed, immediately remove from heat. If any granular material is still on the sides of the flask and will not go into solution after the medium has boiled, a new batch must be prepared. One bubble of boiling is defined as the first boiling bubble that comes from the bottom of the flask, and breaks the surface of the liquid. When this is seen, immediately remove the flask from the heat. Additional bubbles may be seen after removal from heat. This is normal. However, it is not acceptable to have the flask of medium boil over. If this happens, discard the medium, and begin again.
9. Remove the pH portion with a sterile pipet. To prevent contamination do not pour this media from the flask. Rapidly cool the pH portion of medium, in the refrigerator or freezer to room temperature (25 ° C). At room temperature the medium will solidify, then check and record the final pH.
10. Touch a surface probe to the surface of the agar, do not press a bulb probe into the agar to get the pH reading. If pH is not 7.2 ±0.2, the medium must be discarded and a new batch must be prepared.
11. Disinfect a suitable bench area, and set out the required number of petri dishes. Uncover the dishes, but leave the covers right side up, with one portion of the "lip" touching the bench, and another section supported by the bottom half of the dish. If too much of the bottom is covered, excess condensation will result. The petri dishes can be uncovered for up to 30 minutes.
12. Cool the remaining agar slightly.
13. Using a sterile pipet, aseptically transfer 6-10 mL of the warm, liquid m-Endo agar into each sterile petri dish. Flame any air bubbles that develop with an inverted gas burner, at once. Optionally, plates with air bubbles may be discarded.
14. Allow the agar to solidify, then cover the petri dishes.
15. Refrigerate m-Endo agar plates, in a covered container, inverted.
16. m-Endo agar may be used up to two weeks after preparation when stored in a sealed container. Store m-Endo broth in the refrigerator, in the Erlenmeyer flask for up to 96 hours.

EC Broth

EC Broth is used for fecal coliform confirmation of total coliform cultures taken from m-Endo in drinking water samples.

Composition

Tryptose or trypticase	20g
Lactose	5.0g
Bile salts mixture or bile salts #3	1.5g
Dipotassium hydrogen phosphate	4.0g
Potassium dihydrogen phosphate	1.5g
Sodium chloride	5.0 g
Laboratory Pure water	1000 mL

Final pH after sterilizing (119 - 121 ° C for 12 - 15 minutes): 6.9 ±0.2.

Weigh out 37.0g of medium on appropriate balance. Add 500 mL of water to an Erlenmeyer flask. Transfer the medium to the flask. Rinse down the sides of the flask with the remaining water. Allow the medium to rehydrate. Swirl. Warm slightly to aid in dissolving the medium if necessary. Pipet 11-12 mL per culture tube (containing a Durham tube); cap tubes. Sterilize 12-15 minutes at 119 - 121 ° C. Promptly remove tubes from the autoclave. Rapidly cool a small portion; check and record final pH. Final pH must be 6.9 ±0.2. There must be at least 10 mL of liquid in each tube. Store a maximum of one week at room temperature.

Optionally, carbohydrate media may be stored for up to 3 months in the refrigerator in screw cap tubes. Before using refrigerated tubes, you must incubate them overnight and discard any tubes with air bubbles in the Durham tube. The laboratory may date and preincubate a week's supply of tubes and then hold them in reserve at room temperature. However, after one week these tubes must be discarded if they are not used.

BHI (Brain Heart Infusion or Bacto Brain Heart Infusion)

Infusion of calf brains 200g
Infusion of beef heart . . . 250g
Proteose peptone 10g
Glucose 2g
Sodium chloride 5g
Disodium hydrogen phosphate . . . 2.5g
Laboratory Pure Water 1000mL
Final pH 7.4 ±0.2

1. Weigh out 37g of dehydrated medium into a weigh boat or into a large flask (or 3.7g/100 mL).
2. Transfer medium to large flask if a weigh boat was used.
3. Add 1000 mL of laboratory pure water (distilled or deionized).
4. Allow medium to completely dissolve, swirling if necessary
5. Dispense into screw capped Erlenmeyer flasks, or vessels (25 mL final volume for sample bottle sterility or 50 mL final volume for membrane filter sterility). Dispense smaller volumes into test tubes for pH and quality control tests, 10 mL final volume.
6. Autoclave at 119-121 ° C for 15 minutes on slow exhaust.
7. Allow to cool, check and record final pH using one of the 10 mL test tubes. If it is not 7.4 ±0.2 then discard the batch and re-prepare.
8. Store screw capped flasks, tubes, or vessels for up to six months in the refrigerator. This media contains no Durham tubes so it does not need to be preincubated before use. Optionally, prepare the media in slip cap tubes or aluminum foil capped flasks or vessels and store at room temperature for up to one week. Label each batch with the preparation and expiration date and type of medium.

TSB (Tryptic Soy Broth or Bacto Tryptic Soy Broth)

Tryptone	17g
Papaic digest of soybean meal	3g
Dextrose	2.5g
Sodium chloride	5g
Disodium hydrogen phosphate	2.5g
Laboratory Pure Water	1000mL

Final pH 7.3 ±0.2

1. Weigh out 30g of dehydrated medium into a weigh boat or into a large flask (or 3.0g/100 mL).
2. Transfer medium to large flask if a weigh boat was used.
3. Add 1000 mL of laboratory pure water (distilled or deionized).
4. Allow medium to completely dissolve, swirling if necessary.
5. Dispense into screw capped Erlenmeyer flasks, or vessels (25 mL final volume for sample bottle sterility or 50 mL final volume for membrane filter sterility). Dispense smaller volumes into test tubes for pH and quality control tests, 10 mL final volume.
6. Autoclave at 119 - 121 ° for 15 minutes on slow exhaust.
7. Allow to cool, check and record final pH using one of the 10 mL test tubes. If it is not 7.3 ±0.2 then discard the batch and re-prepare.
8. Store screw capped flasks, tubes, or vessels for up to six months in the refrigerator. This media contains no Durham tubes so it does not need to be preincubated before use. Optionally, prepare the media in slip cap tubes or aluminum foil capped flasks or vessels and store at room temperature for up to one week. Label each batch with the preparation and expiration date and type of medium.

Chapter VI

Part I Membrane Filter (MF) Procedures

Filtering Procedures

1. Using vacuum tubing, set up the water collection apparatus.
2. Unwrap the filter receptacle and place onto the primary filtering flask.
3. Unwrap the funnel and hang it upside down by its neck from a ring support and ring stand. If more than 30 minutes will elapse between filtering one sample and another, the funnel and receptacle must be resterilized or replaced with another sterile unit before continuing. The use of a UV light box may not extend this 30 minute time period.
4. Label the petri dish with its sample number. If using broth medium, insure the absorbent pad has one excess drop of medium; pour out the excess if there is more than one drop.
5. Sterilize the forceps by dipping them in 95% ethanol, or absolute methanol, then passing them through a flame to ignite the alcohol, and allowing the flame to extinguish. Then place the membrane filter, grid side up, onto the center of the filter receptacle.
6. Place the funnel over the filter receptacle and gently tighten the locking apparatus.
7. Vigorously shake the sample bottle 25 times within seven seconds, each shake a complete vertical or horizontal movement of about one foot. The interval between shaking and removing the test portion must not exceed three minutes.
8. Using a graduated cylinder or calibrated bottle, accurately marked at 100 mL ±2.5 %, measure the sample volume and transfer the sample to the center of the funnel; avoid splashing the sides. Do not rinse out the cylinder or bottle.
9. If samples are being analyzed, where the chlorine residual is suspected to be high, or known to be very high ($\geq$10 mg/l) a portion of the sample must be tested for the presence of free chlorine in excess of what the sodium thiosulfate will tie up. Examples of samples that should be checked are: super chlorinated or disinfected wells; chlorine sterilized new lines, not fully flushed disinfected supplies, etc. For these tests, a qualitative test may be used. Add free chlorine DPD reagent to the water remaining in each bottle, and look for a color change. If the color does change and indicates chlorine present, the sample must not be filtered. Report the results as "residual chlorine present."
10. Turn on the pump and pull the sample through the filter.
11. Rinse all sides of the funnel three times with 20-30 mL of sterile, room temperature, rinse water. Allow the first volume of rinse water to be pulled through the filter before following with the second rinse. After the second rinse, rinse again for a total of three separate rinses.
12. Carefully remove the funnel and hang it, inverted.
13. Open the petri dish, flame the forceps, and then gently pick up the filter.
14. Roll the filter (grid side up) over the agar so that air bubbles will not form. Do not touch the area of the filter where the sample was filtered. Air bubbles between the filter and the agar may prevent bacteria on the filter from absorbing needed growth nutrients.
15. Invert the plate immediately and incubate the petri dish within 30 minutes.

Part II Membrane Filter (MF) Quality Control

A start and finish MF negative control test (rinse water, medium, and supplies) must be conducted for each filtration series. A known polluted water sample must be run at the end of each filtration series as a positive control. If sterility controls indicate contamination, all samples that are positive, and precede, or follow the negative control with growth, are to be recorded as: "laboratory accident." Positive in this case means: coliforms are present. If non-coliforms are seen occasionally (1-3 clear colonies): investigate the cause. If negative controls are routinely positive, or show non-coliforms routinely, all tests must be suspended until the problem can be discovered. Call the Laboratory Certification Section for assistance. Perform a negative control by filtering a portion of sterile phosphate buffered rinse water, rinsing the funnel 3 times, and then incubating as normal. There is no need to accurately measure this portion. Many laboratories fill the funnel to the "neck", apply vacuum, then rinse three times in the customary manner. This is acceptable. When testing untreated water, it is advisable to add intermediate negative controls after every six samples, along with the required start and end negative controls. List these controls and results on the sample log. Record the results of the positive control as + or -. If the results are negative investigate the cause and correct it.

Part III Incubation

Incubate plates in an electric incubator at 35 ±0.5 ° C, in saturated humidity (tight-fitting box with moist towels on bottom), 22 - 24 hours.

Incubate all plates within 30 minutes of filtering

Stack plates no more than three high.

Part IV Filtration Volumes

Potable water (finished drinking water): Filter 100 mL of sample (one m-Endo plate per sample is needed).

When a sample is too turbid to filter a full 100 mL volume you may split 100 mL over two or four plates.

If less than 100 mL of sample is available, mark the sample result as "not analyzed - insufficient sample".

Part V Bacterial Colony Identification Procedures

Equipment

Binocular stereoscopic microscope with a magnification of 10X plus at least one magnification greater than 10X, such as 15X, 20X, 25X, etc.

Fluorescent light with the light rays perpendicular to the plate being read (highlights the golden-green sheen of total coliform on m-Endo medium).

Data sheet for recording results.

Colony Morphology

Total coliforms on m-Endo medium: Look for all colonies that have any amount of golden-green sheen. The sheen may cover the complete surface of colony or only one section. Tipping plate occasionally helps to reveal sheen. Do not confuse the golden-green sheen of total coliform with the "light shine" non-coliform colonies which just reflect the white fluorescent light. Colonies which are growing outside the area where the sample was deposited on the filter could have developed due to contamination from the funnel, forceps, or some other source.

Part VI Determination of MF Results

Definitions :

Total count - all of the total coliform colonies plus all of the other colonies on a plate.

Total coliform - all of the typical coliform colonies on a plate also called typical count.

Background count - all of the colonies on a plate that are not typical coliform colonies also called atypical colonies.

Examination of Plates

Examine plates with the microscope set on 10X. If the plate has any bacterial colonies, the growth must be verified. If the plate is covered by a film of continuous bacterial growth, the plate must be verified. See following sections for the verification procedures. All typical gold green sheen colonies must be verified to confirm that total coliforms are present and to see if fecal coliforms are present. If only clear colonies are present and the total bacterial count is less than or equal to 200, there is no need for further testing, report: "Total Coliform Negative".

Part VII Verification Test Procedures

Verification Test

This test is designed to confirm whether growth on m-Endo medium is total coliforms and/or fecal coliforms.

Verification as a Monthly Quality Control

At least one positive total coliform colony plate and fecal coliform positive plate must be verified from m-Endo each month. A positive verification must be performed monthly by each analyst on non-potable samples if none are produced in routine work.

Verification of Colonies Seen on Routine Samples

Verify all plates that appear to positive or are suspected to be positive for total and fecal coliforms. It is not a requirement that clear colonies be subject to verification, however all other growth must be verified.

When colony counts exceed 200 or when there is a continuous film of bacterial growth, the plates must be verified for total and fecal coliforms.

Verification Test Procedures

Equipment/Materials

1. m-Endo plate with growth.
2. 1-2 sterile swabs, they must be individually, or doubly wrapped. They can be cotton or calcium alginate tipped. You may use plastic or wooden swabs.
3. LTB, BGB and EC tubes
4. Total coliform incubator (35 ±0.5 ° C)
5. Fecal coliform incubator (44.5 ±0.2 ° C)
6. Verification test record.

Procedure

1. BGB is the test medium used to confirm total coliform growth. EC medium is the test medium used to confirm fecal coliform growth. LTB is an enrichment medium to facilitate coliform growth. Gas in LTB is only a "presumptive positive" not a confirmed positive. Gas in BGB is a confirmed positive for total coliforms. Gas in EC is a confirmed positive for fecal coliforms.

2. Make entry in verification test record. Include date, sample number, description of plate.
3. Aseptically remove a sterile swab. Handle the swab only on the end of the handle.
4. Remove all growth from the plate using 1-2 swabs.
5. Transfer the swab in a tube of LTB by swirling the swab, trying not to remove all of the growth, then using the same swab swirl it into a tube of EC broth again trying not to remove all the growth, finally make the last transfer to BGB in the same manner. Do not leave the swab in the tube. Discard the swab in disinfectant or an autoclave bag and decontaminate in an autoclave.
6. Incubate the LTB and BGB tubes at 35 ±0.5 ° C for 24 ±2 hours, swirl tube, and look for any amount of gas production in the Durham tube. Incubate the EC tube in a water bath or heat sink at 44.5 ±0.2 ° C for 24 ±2 hours. After 24 ±2 hours the test for fecal coliforms is completed.
7. If no gas has formed in the LTB and BGB tube, reincubate these tubes for an additional 24 hours, and again swirl the tube and look for any amount of gas formation in the Durham tube. If no gas has formed in the tube within 48 ±3 hours, both tests are negative. Incubate EC tubes for 24 ±2 hours only. Never incubate EC tubes for 48 hours.
8. If gas has formed in BGB within 48 ±3 hours after inoculation, the tube is considered positive for total coliforms. Gas in BGB is a confirmed positive for total coliforms.
9. If gas has formed in EC within 24 ±2 hours after inoculation, the tube is considered positive for fecal coliforms. Gas in EC is a confirmed positive for fecal coliforms.
10. If there is gas in the LTB but no gas in both the BGB and EC tubes, you must reinoculate fresh tubes of BGB and EC. If BGB is negative but EC is positive, reinoculate a fresh tube of BGB only. If EC is negative but BGB is positive, reinoculate a fresh tube EC only. Swirl the LTB tube and transfer 1-3 loopfuls of LTB to a fresh tube of BGB and 1-3 loopfuls of the same LTB to a fresh tube of EC broth.
11. Incubate the fresh BGB tube at 35 ±0.5 ° C for 24 ±2 hours, swirl tube, and look for any amount of gas production in the Durham tube. If no gas has formed in the tube, reincubate the tube for an additional 24 hours, and again swirl the tube and look for any amount of gas formation in the Durham tube. If no gas has formed in the tube within 48 ±3 hours, the test is negative for total coliforms. If gas has formed, the test is positive for total coliforms.
12. Incubate the fresh EC broth tube in a water bath or heat sink at 44.5 ±0.2 ° C for 24 ±2 hours. If no gas has formed in the tube within 24 ±2 hours, the test is negative for fecal coliforms. If gas has formed, the test is positive for fecal coliforms. A test cannot be positive for fecal coliforms and negative for total coliforms. If there is gas in the EC tube but none in the BGB tube, the total coliform test is reported as "Laboratory Accident". In this case, however, it is permissible to report the fecal coliform results as "Fecal Coliform Positive".
13. Be sure to record all information and results on the verification log.

Part VIII Reporting of MF Results

Use the following table to determine what must be verified:

m-Endo Results	Verification Necessary?
No Growth	No
≤200 Clear Colonies	No
≤200 Typical Coliforms	Yes
≤200 Atypical Coliforms	Yes
≤200 Non-Coliforms (Red, pink)	Yes
Confluent Growth With Coliforms	Yes
Confluent Growth Without Coliforms	Yes
>200 of Any Colonies	Yes

Use the following tables to determine proper reporting procedures:

m-Endo Plate	Reported Result
No Growth	Total Coliform Negative
≤200 Clear Colonies	Total Coliform Negative
Typical Gold-Green Sheen Colonies With no Gas in BGB	Laboratory Accident

Plates that are verified with:

≤200 colonies

LTB Results	BGB Results	EC Results	Reported Results
Negative	Negative	Negative	Total Coliform Negative
Positive	Negative	Negative	Total Coliform Negative
Positive	Positive	Negative	Total Coliform Positive; Fecal Coliform Negative
Positive	Positive	Positive	Total Coliform Positive; Fecal Coliform Positive
Positive	Negative	Positive	Total Coliform Lab Accident; Fecal Coliform Positive

Plates that are verified with:

>200 colonies or Confluent Growth

LTB Results	BGB Results	EC Results	Reported Results
Negative	Negative	Negative	"HBC" or "Confluent" Invalid
Positive	Negative	Negative	"HBC" or "Confluent" Invalid
Positive	Positive	Negative	Total Coliform Positive, HBC or Confluent; Fecal Coliform Negative
Positive	Positive	Positive	Total Coliform Positive HBC or Confluent; Fecal Coliform Positive
Positive	Negative	Positive	Total Coliform Lab Accident; Fecal Coliform Positive

When analyzing samples, the testing laboratory is not to designate "safe" or "unsafe", on the sample report form. The regulatory agency will interpret the laboratory results, reducing the potential liability for the laboratory.

Other Reported Results:

Conditions	Reported Result
Chlorine Detected	Residual Chlorine Present: Not Analyzed
Sample >30 Hours Old	Sample Too Old: Not Analyzed
Sample Leaked	Leaked in Transit: Not Analyzed
Sample Broken	Broken in Transit: Not Analyzed
Sample Frozen	Sample Frozen: Not Analyzed
Incomplete Information	Incomplete Information: Not Analyzed
Negative Control is Positive	Laboratory Accident
Positive Control is Negative	Laboratory Accident
Growth outside or under the filter area	Laboratory Accident
Incubator Broken, or other lab error	Laboratory Accident

Chapter VII

Part I MMO-MUG Test Procedures

Initial Procedure

Vigorously shake the water sample bottle 25 times within seven seconds. Each shake is a complete vertical or horizontal movement of about one foot. This will ensure a homogenous sample before the 10ml test portion is measured. The interval between shaking and measuring the test portion can not exceed three minutes.

Procedure

1. Measure 100 mL of sample into a sterile, transparent, borosilicate glass or equivalent clear plastic test vessel. If using bottles calibrated at 100 mL ±2.5 mL, aseptically adjust the volume to the 100 mL mark. Note: The laboratory may combine the collection and test vessel and eliminate the need for 100 mL graduates by purchasing presterilized calibrated clear plastic bottles containing sodium thiosulfate. This type of bottle can not practically be prepared in the laboratory since the clear plastic is not heat stable and therefore must be sterilized with radiation. However, purchasing this type of prepared bottle will condense laboratory supplies and preparation time.
2. Number the test vessel with the corresponding sample number.
3. Observe the sample for any innate yellow or amber color (for Colilert) that may interfere with reading the test results. Most samples generally contain no interfering color. If an innate interfering color is present, reserve the remaining sample portion (min. of 10 mL) to serve as an color control blank for reading the final water sample test results. Do not add test reagent or incubate the color control blank. Assign this portion the corresponding sample number and hold at room temperature until the test is read. A clean test tube facilitates storage and color comparisons with low volume innate color control blanks.
4. Aseptically open and add a tube of MMO-MUG reagent to the 100 mL water sample in the test vessel. Recap the test vessel and shake vigorously to dissolve the reagent. Some particles may remain undissolved initially, however dissolution will continue during incubation.

Part II Controls

A positive and a negative control are required at the end of each set of samples tested.

1. Aseptically fill a sample collection bottle with sterile deionized or distilled water. Do not use phosphate buffered rinse water with the MMO-MUG test. This will serve as the negative control. Fill another bottle with water that contains coliforms. This can be deionized water inoculated with a culture or a low turbidity polluted water sample. You may collect a polluted water sample in a large volume and keep it in the refrigerator. You may take smaller samples from this large volume as long as the water still produces positive results.
2. Mix and measure 100 mL of sterile control water and polluted water into separate test vessels following the same procedure used for water samples.
3. Aseptically add a tube of MMO-MUG reagent to both control samples. Label both bottles.

Part III Incubation of MMO-MUG Tests

Incubate all test vessels at 35 ±0.5 C for the required time. For Colilert incubate for 24 hours. For Colisure, incubate 24-48 hours. Do not pack the incubator full. The test vessels must be a minimum of one inch apart and one inch from adjacent incubator surfaces. Additional shelves may be added to the incubator, providing they contain calibrated thermometers and maintain 35 ±0.5 C.

Part IV Interpretation of MMO-MUG Tests - Total Coliforms

A positive color comparator is required with Colilert to determine the minimum amount of color development necessary to equal 1 CFU/100 mL. The color comparator is available from the test reagent manufacturer and has an expiration listed on the bottle. The laboratory must dispense the positive color comparator into the same type of test vessel they are using for sample analysis. Label the comparator and include the manufacturer's expiration date. The positive color comparator is to be stored at room temperature in the dark between sample readings.

After 24 hour incubation of Colilert compare each test vessel to the positive color comparator and negative control. After 24-48 hour incubation of Colisure compare each test vessel to the negative control. A white background card may facilitate color comparisons. If the negative control displays any color change (yellow for Colilert, red for Colisure) the set of test samples are invalid and must be reported as laboratory accidents (LA). The positive control should be positive (yellow for Colilert, red for Colisure) . If not, choose another positive sample source. If that does not solve the problem, contact the certification office.

1. If no color change (yellow for Colilert, red for Colisure) is observed in a test vessel the sample is considered negative for total coliforms.
2. If a Colilert sample displays yellow color greater than or equal to the positive color comparator the presence of total coliforms is confirmed. If a Colisure sample develops a color change from yellow to red, the presence of total coliforms is confirmed.
3. If a Colilert sample is slightly yellow colored after 24 hour incubation, but the color is less than the positive comparator for Colilert, reincubate the test vessel for 4 additional hours (28 hours total incubation). A total coliform positive sample will display further color development. If the color does not intensify, consider the sample total coliform negative and report as such.
4. Colilert samples that displayed an initial innate color must have the color development of the test vessel, after incubation, compared to the unincubated innate color control blank from that specific sample. Assign a value of "Zero" to the level of color observed in the color control blank and follow Steps 1 and 2 to interpret the test results.
5. If the color control (original sample) has the same color as the incubated test portion the sample is considered negative for total coliforms.
6. If the color control (original sample) has less color than the incubated test portion then follow the procedure for interpreting a Colilert sample with the positive color comparator.

Part V Interpretation of MMO-MUG Tests - Escherichia coli (E. coli)

1. After incubation (24 hours for Colilert, 24-48 hours for Colisure) examine the test vessels for fluorescence.
2. Using the 6 watt, 365-366 nm UV light in a darkened room examine all total coliform positive samples to see if they fluoresce.
3. If no fluorescence is observed in a test vessel the sample is considered negative for E. coli.
4. If a sample displays fluorescence the presence of E. coli is confirmed.

Part VI Reporting MMO-MUG Test Results

Use the following tables to determine proper reporting procedures:

Yellow (Colilert) or Red (Colisure) Color	Fluorescence	Reported Results
No Color Change	None	Total Coliform Negative
Color < Comparator or No Color Change	None	Total Coliform Negative
Color ≥ Comparator or Color Change	None	Total Coliform Positive; E. coli Negative
Color ≥ Comparator or Color Change	Positive	Total Coliform Positive; E. coli Positive

Other Reported Results:

Conditions	Reported Result
Chlorine Detected	Residual Chlorine Present: Not Analyzed
Sample >30 Hours Old	Sample Too Old: Not Analyzed
Sample Leaked	Leaked in Transit: Not Analyzed
Sample Broken	Broken in Transit: Not Analyzed
Sample Frozen	Sample Frozen: Not Analyzed
Incomplete Information	Incomplete Information: Not Analyzed
Negative Control is Positive	Laboratory Accident
Positive Control is Negative	Laboratory Accident
Incubator Broken, or other lab error	Laboratory Accident

When analyzing samples, the testing laboratory is not to designate "safe" or "unsafe", on the sample report form. The regulatory agency will interpret the laboratory results, reducing the potential liability for the laboratory.

Part VII Quality Control Procedures for MMO-MUG Tests

Before a certified membrane filter laboratory may receive an interim authorization for the MMO-MUG procedure, a minimum of 20 parallel tests must be completed with comparable results recorded in the corresponding log. Parallel testing involves analyzing duplicate samples by both the approved membrane filter procedure for total coliform and the MMO-MUG procedure concurrently. The set of 20 parallel tests should include 15 potable and 5 non-potable (total coliform positive) water samples.

The initial set of parallel tests must be sent to the Lab Certification Section of Ohio EPA/DES for review, before interim authorization will be granted. A certificate of approval for the new procedure will be issued after successful completion of an on-site survey.

Before a new lot of MMO-MUG reagent may be used it must be checked with three specific organisms as outlined under Media QC.

Each approved analyst must test a minimum of one total coliform and one E. coli positive sample every month, along with the corresponding positive and negative controls, and record the results in the QC log.

MMO-MUG Procedural Comments

Do not autoclave MMO-MUG reagent prior to use. The reagent system is heat labile and will be destroyed.

Avoid prolonged exposure of inoculated test vessels to direct sunlight. Sunlight may hydrolyze the indicator compounds resulting in a false positive test.

Do not incubate Colilert test vessels beyond 28 hours and Colisure beyond 48 hours. After these times heterotrophic bacteria could overcome the suppressant system yielding a false positive result.

Do not dilute a sample in phosphate buffered water (MF rinse water or dilution blank water) for the MMO-MUG test. The test reagent contains a buffer and additional buffers may adversely affect the test performance.

Do not use the MMO-MUG test to verify organisms grown from other procedures. The pre-enrichment could cause the inoculum to overload the MMO-MUG suppressant reagent system and yield erroneous test results.

MMO-MUG is primarily a potable water test for coliforms. Some non-coliform bacteria can overload the suppressant reagent system and give false positive test results when they occur in high concentrations (>1,000,000 CFU/100 mL). Bacterial counts of this magnitude should not occur in treated potable water.

Colilert samples containing a gross excess of free chlorine will display a transient blue color when the MMO-MUG reagent is added. These samples should be discarded and reported as Not Analyzed - Residual Chlorine.

High concentrations of calcium salt in some water supplies may cause a slight precipitate. This should not affect test results.

The Colilert must be stored at 4 - 30 ° C, in the dark, in a dry environment. Colisure must be stored in a refrigerator at 0 - 5 °C. Discard after the manufacturer's expiration date.

If any of the individual reagent containers appears discolored, do not use that pack. Contact the manufacturer for instructions.

Chapter VIII

Part I. Quality Control Forms

Included on the following pages are master copies of quality control forms. Keep these forms in three ring binders with indexed separators between each form. Keep all records from survey to survey in the binders, after which they are to be transferred to a safe, accessible storage place, and kept for five years. Current equipment records may be posted on, or near the equipment to be monitored. When a sheet is full, it is to be transferred to the three ring binder. Do not separate complete sets of QC records and file by months. Do not store individual sets of QC record sheets in difficult to remove, individual, clear pocket binders. Keep running records for three year blocks of time readily available. Records for different tests may be kept in a combination of separate notebooks if this is physically more convenient. Over all, the three ring binder method has proven to be the best way to keep records. Therefore, it is required unless the laboratory has received approval for an alternative method. Some of the larger commercial and municipal laboratories have other methods, such as computerized systems, for test results. All computer generated logs must be reviewed, signed and dated by an approved analyst. This may be acceptable, because of the large amount of paperwork involved. Alternate record systems will be reviewed on a case-by-case basis and must be approved before they are implemented.

Please copy and fill in all data on the forms in the most recent revision of the manual. Some of the forms may have small changes, that are required. Using exact copies of these forms is not mandatory. However, if you make your own forms, they must be in exactly the same format and contain all of the information, as the forms in this manual. You may "stretch" certain columns or rows to fit your needs.

Fill out the forms completely and legibly; do not use ditto marks or arrows unless they represent a common block of data that was generated together. Use a complete space for a complete entry, i.e. do not record entries on half lines or above or below the limits of the form. This practice indicates that the records may have been modified in an attempt to avoid deviations on a survey. Accidentally forgetting to record an occasional temperature, etc., is not a criminal offense, even though it may be listed as a deviation on the survey report. Please keep in mind that knowingly, falsifying official state documents such as these is a criminal offense. Falsification of data, when discovered, will be dealt with through the criminal court system. Additionally, falsification of results will always result in loss of certification for the facility, and most likely permanent decertification for the individual involved. The individual involved also risks the loss of all plant operational certification.

Do not recopy from a rough data sheet onto the official log. The official log must be the original entry. Data should be recorded at the time the test is read to reduce the number of transcription errors. Deviations will not be given if the data is not perfectly neat. It is better to have an original record that is a little sloppy (but legible) than a record that has been recopied, possibly with errors.

Keep one set of handwritten records only. If you feel better about xeroxing a copy of the original record for safety's sake each month, it is permissible, but not required.

Laboratories are required to have a quality assurance plan (QA plan) covering the test parameters for which they are certified. The microbiological test methods and quality control procedures for this program are documented in this manual. In lieu of each laboratory drafting an individual QA plan, laboratories may use the sample QA plan outline from this manual and adopt if for their use. The QA plan must be updated as necessary.

Quality Assurance Plan of the Granville Water Plant Laboratory

The quality assurance plan of the Granville WTP laboratory is based wholly on the microbiological certification manual supplied by The Ohio EPA. All QC procedures and test procedures are contained in that document.

_____

Supervisor's Signature Date

Organizational Chart

1. Superintendent -(Name, test(s) approved)

Lab Supervisor -(Name, test(s) approved)

Analyst -(Name, test(s) approved)
Analyst -(Name, test(s) approved)
Analyst -(Name, test(s) approved)
Analyst -(Name, test(s) approved)
Analyst -(Name, test(s) approved)
Analyst -(Name, test(s) approved)
Analyst -(Name, test(s) approved)
Analyst -(Name, test(s) approved)
Analyst -(Name, test(s) approved)

2. Clerical/Lab Support

Office Staff -(Name)
Office Staff -(Name)

Microbiological Test Data Sheet

Laboratory		Year		Page #	
Certificate Number		Test Method	Membrane Filter (MF)	MMO-MUG/Colilert (CL)	MMO-MUG/Colisure (CS)

	Collected		Incubated				Total Coliform		Fecal Coliform		E. coli		Controls			Chlorine	
Lab #	Date	Time	Date	Time	Analyst	Sample Location	+	-	+	-	+	-	+	-	Test Method	Free	Total

Calibration Record To Be Recorded for Each Thermometer

Laboratory		Year		Page #	

Name	Date	Themometer Location & Number	Observed Temperature	Temp of NIST Thermometer	Correction Factor

Monthly Total/Fecal Coliform Verification for MF

Laboratory		Year		Page #	

Date	Colony Description/Sample #/Source	LTB		BGB		EC	Name
		24	48	24	48	24	

Sample Bottle Sterility/Fluorescence Record To be recorded for each lot received or batch sterilized

Laboratory		Year		Page #	

Name	Date Rec'd/ Sterilized	Lot Number	Number of Bottles Rec'd/Sterilized	Date Tested	Growth		UV	
					#(+)	#(-)	#(+)	#(-)
*Note action taken if results are not acceptable								

Microbiological Laboratory Schedule

Month→		1	2	3	4	5	6	7	8	9	10	11	12
Monthly													
Lab Pure Water QC	C												
Verification/TC & FC	B												
+ Total Coliform/E. coli	A												
*Balance Check	C												
pH Linearity	C												
Quarterly													
Dial Thermometers	B												
Timers	C												
Semiannual													
Stock Buffer Prep	B												
Rinse Water Prep	B												
+ or- Control Water	A												
*TSB/BHI Prep	C												
Open pH Buffers	C												
*Open Media (Room Temp)	C												
Annual													
Thermometer Calibration	C												
*Balance Service	C												
Trace Metals: LP Water	C												
*Opened Media (Dessicator)	C												
As needed													
New Lot media QC	C												
*New Lot MMO-MUG Reagent	A												
*New Lot Sample Bottle QC	C												
New Lot MF QC	B												
Update QA Plan	C												

A=MMO-MUG only B=MF Only C=Both *Can be elimintaed by subcontracting QC

Fecal Coliform Incubator Temperature Record

To be recorded twice each day, am/pm 44.5±0.2°C

Laboratory		Year		Page #	

Date	Temperature	Name	Date	Temperature	Name	Date	Temperature	Name
am			am			am		
pm			pm			pm		
am			am			am		
pm			pm			pm		
am			am			am		
pm			pm			pm		
am			am			am		
pm			pm			pm		
am			am			am		
pm			pm			pm		
am			am			am		
pm			pm			pm		
am			am			am		
pm			pm			pm		
am			am			am		
pm			pm			pm		
am			am			am		
pm			pm			pm		

*Note action taken if temperature is out of range

Total Coliform Incubator Temperature Record To be recorded twice each day, am/pm for each shelf, 35 ±0.5°C

Laboratory		Year		Page #	

Date	Name	Temperature °C Location_____	Location_____	Location_____	Date	Name	Temperature °C Location_____	Location_____	Location_____
am					am				
pm					pm				
am					am				
pm					pm				
am					am				
pm					pm				
am					am				
pm					pm				
am					am				
pm					pm				
am					am				
pm					pm				
am					am				
pm					pm				
am					am				
pm					pm				
am					am				
pm					pm				

*Note action taken if temperature is out of range

Refrigerator Temperature Record To be recorded once each day

Laboratory		Year		Page #	

Date	Temperature	Name	Date	Temperature	Name	Date	Temperature	Name

*Note action taken if temperature is out of range

Autoclave Temperature Record To be recorded for each run

Laboratory		Year		Page #	

Date	Time In	Cycle Time @ Temp	Time Out	Temp (°C)	Material	Name

Monthly Total Coliform/E.coli Positive for MMO-MUG

Laboratory		Year		Page #	

Date	Sample #/ Sample ID/Sample Source	Total Coliform		E. coli		Name
		+	-	+	-	

Monthly Balance Calibration Record

Check each balance monthly with certified calibration weights. A minimum of three weights that bracket normal weighing needs are required. Record balance response to reference weight and sensitivity to reference weight plus test load. Non-analytical must be sensitivite to a 0.1g test load. Analytical balances must be sensitive to a 0.01g test load.

Laboratory			Year		Page #	
Balance Model		Date of annual Servicing				

Date	Analyst	Test Load Readings with Appropriate Mass in grams											
		200	200+L	100	100+L	50	50+L	10	10+L	5	5+L	1	1+L

*Note action taken if calibration is not acceptable

Note: “L” refers to “Test Load”

MF Receipt/Sterility Record To be recorded for each new lot or annually

Laboratory		Year		Page #	

Dates		Sterility				Dates		Sterility			
Rec'd	Test	Lot#	+	-	Name	Rec'd	Test	Lot#	+	-	Name

*Action taken if filters are not sterile

Media Quality Control Record To be recorded for each new lot

Laboratory		Year		Page #	

Date Tested	Medium	Quality Control			Name	Date Tested	Medium	Lot #	Quality Control		Name
		Lot #	+	-					+	-	

*Note action taken if results are unacceptable

Oven Temperature To be recorded for each run

Laboratory		Year		Page #	

Date	Time In	Time to 170°C	Time Out	Temperature °C	Material	Name

pH Record to be checked and recorded for each batch of medium/reagent prepared

Laboratory		Year		Page #	

Date	Name	Medium	Lot #	Final pH	Date	Name	Medium	Lot #	Final pH

*Note action taken when pH is out of range

Monthly Laboratory Pure Water Record

Laboratory		Year		Page #	

Date	Total Chlorine Residual	pH	Resistivity/Conductivity	Meter Calibration Date	Annual Trace Metals Date	Name

MMO-MUG Quality Control Record To be recorded for each new lot

Laboratory		Year		Page #	

Date	Analyst/Testing Lab	Lot # of MMO-MUG Reagent	Reagent: Colilert/Colisure	Test Results		
				E. coli	*Klebsiella*	*Pseudomonas*

pH Linearity Record to be checked and recorded at least once each month

Laboratory		Year		Page #	

Date	Name	Slope%	pH 4	pH 7	pH 10	Date	Name	Slope %	pH 4	pH 7	pH 10

*Note action taken when linearity is not acceptable

Calibration Record To Be Recorded for Each Equipment Timer

Laboratory		Year		Page #	

Name	Date	Timer Location	Timer Setting	Actual Time (Watch)	Correction Factor