



Common Findings in Microbiology

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Presentation Overview

TCEQ Accreditation Update

Presentation Format for Findings

References and Acronyms

Used throughout the presentation

Common Findings

Proper Microbiological Nomenclature

Since I've got your attention!

Questions?!

Accreditation Update

Revision of the
TCEQ Microbiology
Drinking Water
Fields of Accreditation

TCEQ Accreditation Update

- ▶ Effective October 15, 2016.
- ▶ FoA now includes *E. coli* as an analyte in addition to total coliforms for presence/absence analyses.
- ▶ Reflects the Revised Total Coliform Rule (rTCR) which went into effect on April 1, 2016.
 - ▶ References.
- ▶ Provides clarity to accredited laboratories.
Now includes:
 - ▶ Applicable sections of reference method.
 - ▶ Media used for the target analytes.

Presentation Format

Discussion for each finding will include:

- ▶ The Commonly Seen Deficiencies
- ▶ The Requirements & References
 - ▶ EPA
 - ▶ SM
 - ▶ TNI
- ▶ The Important Points
 - ▶ Things to think about.
 - ▶ Reasons why.

References

▶ EPA

- ▶ Chapter V, *Manual for the Certification of Laboratories Analyzing Drinking Water*, 5th Edition (2005)
- ▶ Title 40, Code of Federal Regulations, Part 141 (40 CFR 141)

▶ SM

- ▶ 22nd Edition of *Standard Methods for the Examination of Water and Wastewater*

▶ TNI

- ▶ Volume 1 (2009) of the standards adopted by TNI
- ▶ *Some requirements *may* only be applicable to TNI accredited laboratories.

* Required in TNI accreditation.

Common Finding #1

Using Improper
References.

Commonly Seen Deficiency - Improper References

Package inserts cited as the approved DW methods.

The most commonly seen package inserts referenced as methods include:

- ▶ Colilert[®], Colilert-18[®], and *Colisure[®]
 - ▶ SM 9223 is the proper reference
 - ▶ *After the rTCR went into effect in April 2016.
- ▶ SimPlate[™]
 - ▶ Method was approved by EPA in November 2000.
 - ▶ *IDEXX SimPlate[™] HPC Test Method for Heterotrophs in Water*

Requirements and References

- ▶ EPA Section 5, *Analytical Methodology* and 40 CFR 141
 - ▶ Systems must use methods listed in applicable sections of 40 CFR 141. For example:
 - ▶ 40 CFR 141.74 - Enhanced SWTR
 - ▶ 40 CFR 141.402 - Ground Water Rule
 - ▶ 40 CFR 141.852 - rTCR
 - ▶ Appendix A to Subpart C of Part 141
- ▶ SM approved editions/years differ depending on the rule.
 - ▶ Must check the CFR for requirements.
- ▶ TNI V1M2-5.4.2
 - ▶ The laboratory shall use test methods which are appropriate and meet the needs of the customer.

Important Points

40 CFR 141 – National Primary Drinking Water Regulations

- ▶ Review method tables.
 - ▶ Read the footnotes.
- ▶ Method procedures and associated QC requirements change.
 - ▶ Colisure® is now a SM 9223 media.
- ▶ Some editions of SM are **not** approved.
 - ▶ For example: 22nd Edition of SM 9222.
- ▶ Package inserts are not the approved method.
 - ▶ Contain important supplemental information.
- ▶ Be proactive, stay informed, and ask questions.
 - ▶ Use your resources!

Common Finding #2

Using the Incorrect
Volume for
Presence/Absence
Testing.

Commonly Seen Deficiency - Incorrect Volume for P/A Testing

- ▶ Analyzing 100 ± 2.5 mL sample volumes.
- ▶ Analyzing *any* volume varying from 100 mL.
 - ▶ Such as volumes greater than verified 100 mL line to below the neck of the bottle.
- ▶ Using volume comparators for sample analysis.

Requirements and References

- ▶ 40 CFR 141
 - ▶ Regardless of method used, sample volume is 100 mL.
- ▶ EPA 5.1.5
 - ▶ Volume analyzed for total coliforms must be 100 mL.
- ▶ SM 9221.A.1 (2006)
 - ▶ Replicate tubes total 100 mL sample volume.
- ▶ SM 9222.A.2 (2006)
 - ▶ Standard volume to be filtered for drinking water samples is 100 ± 2.5 mL.
- ▶ SM 9223.B.2.b (2004)
 - ▶ Add enzyme medium to 100 mL sample.

Important Points

- ▶ ***Most stringent** requirement applies.
 - ▶ 40 CFR 141
 - ▶ All analyses on 100 mL sample volume.
- ▶ There is an allowable *tolerance* of 2.5% for the accuracy of the 100 mL line on sample analysis vessels.
 - ▶ This is related to the uncertainty of the line on the vessel.
 - ▶ It is *not* related to the sample volume analyzed.

* Required in TNI accreditation.

Common Finding #3

Using Improper
Procedures for
Testing the
Sterility of Water.

Commonly Seen Deficiency - Water Sterility Testing

- ▶ Using single strength broth.
 - ▶ Significantly diluting the media in the water.
- ▶ Using a selective media.
 - ▶ Such as Colilert[®], Colilert-18[®], Colisure[®].
- ▶ Using a Certificate of Analysis (CoA) which does not attest to having been actually analyzed with a non-selective media.
 - ▶ ISO 11137 Sterility Assurance Level (SAL).
- ▶ Not testing for sterility prior to use.

Requirements and References

- ▶ EPA QC 4.4.3
 - ▶ 50 mL to 50 mL double-strength, non-selective broth.
- ▶ SM 9020 B.9.d (2005)
 - ▶ Check each new batch (or lot) **before first use**.
 - ▶ 50 mL of water to 50 mL of a double-strength broth.
 - ▶ Filter ≥ 100 mL and place on non-selective medium.
- ▶ TNI V1M5-1.7.3.1.b.iv
 - ▶ A sterility check shall be performed on each batch/lot with non-selective growth media.

Important Points

- ▶ Procedures should create a single-strength medium after the addition of non-selective broth to water.
- ▶ Media is very specifically formulated with nutrient concentrations to encourage the growth of microorganisms present.
 - ▶ Too few nutrients make it difficult for any microorganisms to reproduce to a concentration that would result in turbidity, the visible growth in a broth.
 - ▶ Too many nutrients can overwhelm microorganisms and cause an inhibitory effect.
- ▶ Perform QC prior to use for sample analysis.

Common Finding #4

Not Recording
Temperatures
Every Day of Use.

Commonly Seen Deficiency – Temperature Recording

- ▶ *Temperatures not recorded over weekends and holidays, when the equipment is “in use”.
- ▶ If *anything* pertaining to the analysis is kept in the piece of equipment, then that equipment is considered to be “in use”.
 - ▶ For example, storage of:
 - ▶ Media.
 - ▶ Samples.
 - ▶ PT Samples.
 - ▶ QC organisms.
 - ▶ Reagents.

* Required in TNI accreditation.

Requirements and References

- ▶ EPA QC 3.9.2
 - ▶ Days the refrigerator is in use, and the laboratory is staffed, the temperature should be recorded daily.
- ▶ SM 9020 B.4.i and B.4.j (2005)
 - ▶ Check and record temperature daily when in use.
- ▶ TNI V1M2-5.5.13.1.d
 - ▶ On each day the equipment is used it shall be checked and documented.

Important Points

- ▶ Differing requirements between EPA, SM, and TNI.
 - ▶ *Most stringent requirement applies.
- ▶ Options for recording temperatures when laboratory is not staffed include:
 - ▶ Minimum/Maximum (min/max) recording thermometers.
 - ▶ Have procedures for proper use.
 - ▶ Continuous recording data loggers.
 - ▶ *Ensure the original observations are not overwritten by the software.

* Required in TNI accreditation.

Common Finding #5

Using Improper
Incubation
Procedures for
Colilert-18[®].

Commonly Seen Deficiency – Colilert-18[®] Incubation Issues

- ▶ Incubating longer than 10 minutes when using the 44.5 °C warm-up option.
- ▶ *Not recording the time in and out of the 44.5 °C waterbath warm-up step.
 - ▶ Must ensure sample is not exposed to a high temperature for an extended period of time.
 - ▶ Could significantly impact results.
 - ▶ Total incubation time starts with the warm-up step.

* Required in TNI accreditation.

Requirements and References

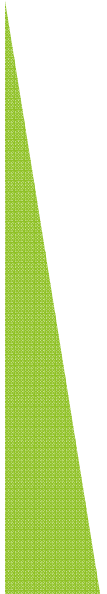
- ▶ EPA QC 5.3.1.5
 - ▶ Table includes manufacturer's instructions to minimize false negatives due to improper incubation procedures.
 - ▶ Incubators may not bring a cold 100 mL water sample to incubation temperature.
- ▶ SM 9223
 - ▶ Incubate as specified in manufacturer's instructions.
 - ▶ Colilert-18 Package Insert.
- ▶ TNI V1M2-4.13.3.f
 - ▶ Lab shall keep records necessary for historical reconstruction of the data.

Important Points

- ▶ Warm-up step is initiated *after* the addition of media.
 - ▶ The warm-up step is the beginning of the incubation period.
- ▶ 44.5 °C for 7-10 minutes.
- ▶ *Records of incubation are necessary to reconstruct the history of the sample pre-warming steps.
- ▶ Total coliforms are temperature sensitive.
 - ▶ Do not expose to high temperature for long periods.
- ▶ Waterbath water levels should reach the level of the top of the sample.

* Required in TNI accreditation.

Proper Microbiological Nomenclature



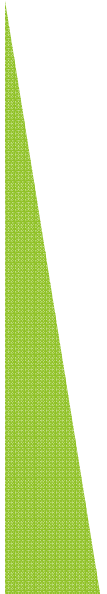
Microbiological Nomenclature

- ▶ Laboratory management should ensure the use of proper microbiological nomenclature in quality documentation.
 - ▶ Genus names are always capitalized.
 - ▶ Species names are always lower case.
 - ▶ **Both** genus and species should always be:
 - ▶ Italicized (*Genus species*)
 - OR
 - ▶ Underlined (Genus species)

Microbiological Nomenclature

- ▶ When referring to a microorganism in a document for the first time:
 - ▶ Both genus and species should be included in full.
 - ▶ *Escherichia coli*
 - ▶ *Klebsiella pneumoniae*
 - ▶ *Pseudomonas aeruginosa*.
- ▶ Any following references to a microorganism in a document may be made in the form of:
 - ▶ An abbreviated genus with full species name.
 - ▶ *E. coli*
 - ▶ *K. pneumoniae*
 - ▶ *P. aeruginosa*.

Questions?!



TCEQ Laboratory Accreditation Program

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