QA/QC of Fecal Coliforms
by Membrane Filtration
SM 9222D

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Introductory Concepts

How is Microbiology QC Different than Wet Chem QC?

Living Organisms in a Living System

Extreme Variability

Difficulty in Preparing Known Standards

Control of Variables Becomes Extremely Important

False Positives and False Negatives

Different Types of Calculations to Account for Variability

The Parts of SM that nobody reads…

SM Section 9020B – Intra-Laboratory QC

SM Section 9030 – Laboratory Apparatus

SM Section 9040 – Washing & Sterilization

SM Section 9050 – Prep of Culture Media

SM Section 9060 – Samples

SM Method 9222B – Total Coliform by MF

Review of Methodology

Choice of Methodology

Collection and Aliquoting of Sample

Sample Filtration/Filtration Sequence

Contamination Blanks

Incubation

Reading the Plates

Calculation of Coliform Density

Review of Calculations

Target Range – 20 to 60 cfu per plate

Three Usual Scenarios:

One or More Plates in Range

All Plates Below Range

All Plates Above Range

One or More Plates in Range

Example:

100ml = 95 ⟶ *Do not use*

50ml = 42 ⟶ *Use Result*

25ml = 20 ⟶ *Use Result*

10ml = 6 ⟶ *Do not use*

(Count/Volume) x 100

(42/50)x100 = 84 *(20/25)x100 = 80*

Average 84 + 80 = (164/2) = 82 Final Answer.

All Plates Below Range

Example:

100ml = 18 ⟶ *Do not use*

50ml = 7 ⟶ *Do not use*

25ml = 2 ⟶ *Do not use*

10ml = 0 ⟶ *Do not use*

Divide Total Counts by Total Volume (x 100)

(18+7+2+0)/(100+50+25+10) x100

(27/185) x 100 = 14.59 = 15 Final Answer

Note: If no colonies on any plates, report as "<1 cfu/100ml"

All Plates Above Range

Example:

100ml = TNTC ⟶ *Do not use*

50ml = TNTC ⟶ *Do not use*

25ml = 187 ⟶ *Do not use*

10ml = 80 ⟶ *Do not use*

Calculate plate with highest count, report as “>”

(187/25)x100 = 748 = >748 Final Answer

Note: Report “TNTC” if colony count exceeds 200

Geometric Mean

Data Set for Five Days:

$D1 = 15,\ D2 = 8,\ D3 = 28,\ D4 = 182,\ D5 = 22$

Arithmetic Mean:

$(15+8+28+182+22)/5 = (255/5) = 51$

Geometric Mean:

$(15 \times 8 \times 28 \times 182 \times 22)^{1/5} = (13{,}453{,}440)^{1/5} = 26.7 = 27$

Duplicate Precision

Standard Methods Section 9020B8a4:

"Perform duplicate analyses on 10% of samples and on at least one sample per test run…"

Standard Methods Section 9020B8b:

"Calculate precision of duplicate analyses for each different type of sample examined…"

Standard Methods Section 9020B8a2:

"For routine performance evaluation, repeat counts on one or more positive samples at least monthly…should agree within 5% for same analyst and 10% for different analysts…"

Duplicate Precision

Calculation of Duplicate Precision

1. Perform Duplicate Analyses on 15 Positive Samples
2. Record Results as D1 and D2
3. Calculate the Logarithm of Each Result. For Any Results of "<1", Add 1 to Each Duplicate Result.
4. Calculate the Range of the Log Values.
5. Calculate the Average Range.
6. Calculate the Control Limit, Which is Defined as:

   3.27 x Average Range.

Duplicate Precision

Example:

Sample Set	D1	D2	L1	L2	Range (L1-L2)
1	89	71	1.9494	1.8513	0.0981
2	38	34	1.5798	1.5315	0.0483
3	58	67	1.7634	1.8261	0.0627
15	110	121	2.0414	2.0828	0.0414

Avg = 0.0626

Duplicate Precision Control Limit = 3.27 x 0.0626

Duplicate Precision Control Limit = **0.2047**

Analyze Daily Duplicate, Transform, Compare!

9020B Personnel

Trained Microbiologist or Access to One for Guidance

Supervisory Review

Training and Skills

9020B Facilities

Ventilation

Space Utilization

Bench Area

Walls and Floors

Laboratory Cleanliness

9020B Equipment

Thermometers

Balances

pH Meters

Autoclaves

Refrigerators

Freezers

Membrane Filtration Equipment

Water Bath Incubators

9020B Laboratory Supplies

Glassware

Detergent

Reagents

Volumetric Sample Containers

Membrane Filters

Media

Lab-Prepared vs Ready-to-use

pH Verification

Receipt & Storage

Positive & Negative Controls

9020B SOPs

Document Analytical Procedures

Document Quality Assurance Procedures

Document Sample Handling

Document Data Handling

9020B Sampling

Sampling Plan

Proper Technique to Avoid Contamination

Container, Preservation, Holding Times

9020B Analytical Methods

40CFR Part 136

Multiple Tube Fermentation

Membrane Filtration

Defined Substrate Methodology

9020B Verification

Standard Methods Section 9020B9b2:

"Verify positives monthly by picking at least 10 blue colonies from one positive sample. Verify in Lauryl tryptose broth and EC broth as in 9221B3 and 9221E…"

9020B Documentation

Quality Assurance Manual

SOPs

Data Quality Management Plan

Benchsheets

Ask This Question:

"Can I trace all equipment, reagents, media, and samples from the final result back through any preparations to the origins of the test?"

Questions?

Thank you for attending!

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