Common Findings and How to Fix Them, or Better, Avoid Them

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Top Findings

- Sterility Checks - everything
- Controls for Media
- Demonstration of Capability
- Traceability
- Thermometers
- Duplicates – plates and counts

Sterility Check ≠ Blanks

- Blanks Check:
  - Method
  - Analyst
- Sterility Checks:
  - Materials
  - Media
  - Anything that will “touch” the sample

Sterility Checks – What?

- Funnels
- Sample Container
- Pipets and Pipet tips
- Graduated cylinder
- Membrane filters
- Petri dishes
- Multi-well plates or trays
- Test tubes
- Media
- Diluent – peptone, buffer, DI, reagent water

General Tips

- Once Per Lot
- Typically Non-selective Media
  - Enzyme Substrate Media – Use Sterile DI Water
  - Some manufacturer materials *
- Keep the Records
- Intermediates might not need to be sterile

Media Checks

- Tested for performance to include
  - Selectivity
  - Sensitivity
  - Sterility
  - Growth promotion or inhibition
- Document

Media Checks

- Blank = No organisms
- Negative = No target response
- Positive = Appropriate response for each (total coliform vs. *E coli*)

Media Records

- For all media records must have
  - Performance check
  - Manufacturer, lot #, amount purchased, etc.
  - Sterility
  - pH
  - Expiration date
- For lab prepared media same as above plus:
  - Recipe
  - Preparer with date of prep
  - Autoclave where appropriate.

Microbiology Media Prep Log

Media: Tryptic Soy Broth Media Tracking # : **MP19 -**

Strength: (circle one) Single (1x) Double Strength (2x)

Prep Date	Containers and Expiration Time	Storage Location	Expiration Date	Prepared By
	Screw top tubes, Milk dilution bottles (circle one) Expire 3 Months From Date Made	Refrigerator #		

Instructions for Single Stength (1x):	Weigh out 15g dehydrated media and add to clean flask with 500mL deionized water on stirring hot plate with stir bar. Stir solution with low heat until media is dissolved and let cool. Dispense ~20mL of media into 20X125mm screw capped test tubes. Within 2 hours of prep time, cap loosely, place a piece of autoclave tape on rack and autoclave on "slow" for 12-13 minutes at 121°C and 15 psi. Allow to cool to room temperature then tighten all caps. Sacrifice three, two for quality control checks and one for final pH measurement. Store in the dark at 6°C or less.			
Vol DI Used (mL)	Stock Media Trace #	Weight of Tryptic Soy Broth Base Used (g)	Autoclave (Time-Temp-Pres) min / °C / psi	Final pH† (Acceptable Range 7.1 - 7.5)

Instructions for Double Stength (2x):	Weigh out 18g dehydrated media and add to clean flask with 300mL deionized water on stirring hot plate with stir bar. Stir solution with low heat until media is dissolved and let cool. Dispense 25mL of media into milk dilution bottles. Within 2 hours of prep time, cap loosely, place a piece of autoclave tape on rack and autoclave on "slow" for 12-13 minutes at 121°C and 15 psi. Allow to cool to room temperature then tighten all caps. Sacrifice three, two for quality control checks and one for final pH measurement. Store in the dark at 6°C or less.			
Vol DI Used (mL)	Stock Media Trace #	Weight of Tryptic Soy Broth Base Used (g)	Autoclave (Time-Temp-Pres) min / °C / psi	Final pH† (Acceptable Range 7.1 - 7.5)

Controls : Inoculate one tube or bottle with the specified culture (positive control) and leave the other un-inoculated. Incubate at 35°C for 24 hours. Remove from incubator and look for turbidity as a sign of growth.

Set Up Date / Time	Read Date / Time	Incubator 35° C	Analyst
		# 6	

Sterility Control Check		Positive Control Check	
No Culture Used	Results (NO Growth = PASS. Any growth = FAIL)	P.aeruginosa Trace #	Results (Growth = PASS. No Growth = FAIL)
		PA	

D.I. WATER USED:	LAB A DI WATER CHECK LOG date	pH	CONDUCTIVITY umhos/cm

Comments: **TSB (1x) is used to check the sterility of petri dishes, membrane filters, and sample containers.**

TSB (2x) is used to check the sterility of prepared dilution buffer water.

†See pH meter calibration log on _____

Microbiology Prepared Media Confirmation Log

Media: **mFC Broth with rosolic acid** - 2mL amps Mfg:_____ Media Tracking # : **MP20 -** _____

Part#:_____

Date Received	MFR Lot Number*/ # Units Received	Expiration Date	Date Consumed/Disposed	Open Date	pH† 7.2-7.6

Controls : Inoculate plates with the specified cultures and incubate at 44.5°C for 24 +/- 2 hours. Remove from incubator and look at colony morphology. Blue (various shades) colonies constitute a positive test for mFC broth.

Incubator 44.5° C	Set Up Date / Time	Read Date / Time	Dilution Buffer Water	Analyst
#			MP	

Positive Control Check	Results
Escherichia coli Trace # EC_____	(Blue colonies = PASS No blue colonies = FAIL)

Negative Control Check	Results
Pseudomonas aeruginosa Trace # PA_____	(No blue colonies = PASS Blue colonies = FAIL)

Sterility Control Check (No culture)	Results
No Culture Used	(No growth = PASS Any growth = FAIL)

Comments: *COA ____

†See pH meter calibration log on _____

Track Your Supplies

- Micro Supplies - One way of tracking
  - Sterility check
  - Volume check
  - Container chlorine check
  - Media Checks

City of Daytona Beach EML
Micro Supplies

Tracking number : M25-00 11 Quality Certificate____ SDS___

DESCRIPTION:_____ # of pieces:_____

Date Received:_____ Date Open:_____ Date Consumed/Disposed:_____

Vendor:_____ Cat#:_____ Lot#:_____ Expiration date:_____

Sterility Check:

1X TSB lot #:_____ 35°C Incubator #:_____ Date/Time in:_____ by:_____

Date/Time out:_____ by:_____

Results Observed:_____ Acceptable for use: YES_____ NO_____

pH Check:

pH meter:_____ Date/Time:_____ Analyst:_____

Buffer 4 lot #:_____ Observed value:_____

Buffer 7 lot #_____ Observed value:_____ Slope:_____

Buffer 10 lot#:_____ Observed value:_____

pH result:_____ (Acceptable range) Acceptable for use: YES_____ NO_____

Postive/Negative Check: Acceptable for use: YES_____ NO_____

Positive Control: _____ Negative Control:_____ Sterility Control: No culture used

_____ °C Incubator #:_____ Date/Time in:_____ by:_____

Date/Time out:_____ by:_____

Appropriate Biochemical Results observed:

YES_____ NO_____ COMMENTS:_____

Performance Check:

A. 100mL graduation line verified using a clean, dry 100mL class A volumetric pipet on _____ by _____

B. No fluorescence observed in vessel filled with 100mL DI H2O and dissolved Colilert-18 on _____by_____

C. _____mg/L chlorine neutralized using _____mL HACH free chlorine standard TN:_____ @ conc of _____mg/L brought to 100mL in volumetric flask and transferred to a vessel; then checked with DPD TN:_____ on _____ by_____.

see back of calculator for formula

ATCC Culture Performance check:

On_____:1 KwikStik hydrated per manufacturer's instructions/transferred to TSA slant (M).

Simultaneously spiked_____ media (MP)dissolved in sterile DI water ( ) to verify viability.

_____°C Incubator #:___ Date/Time in:_____ by:_____

Date/Time out:_____ by:_____

Results observed: Acceptable for use: YES_____ NO_____

TSA Slant =

Media =

NA = NOT APPLICABLE

Demonstration of Capability

- Prove the analyst is capable
- Prove the right method is being used
- Public health and Safety decision are made based on this data

Demonstration of Capability

- Prior to use
- Change in analyst
- Change in instrument
- Change in method
- If more than 12 months expires

Demonstration of Capability

- 4 aliquots approach
- Blind study to analyst
- PT samples
- Quantitative vs qualitative – fitness for use

Thermometers

- Appropriate graduations
- Appropriate for use – range
- Verified annually - N.I.S.T.
- Single point calibration – if it represents the method conditions
- Keep the records
- Use Correction Factors

Thermometers – Tips

- Can use data loggers or other devices
- Should read at normal intervals during normal operations
- Keep the records
- Make sure the data can tie to the temperature reading.

Duplicates

- Method Requirements - plates
  - Repeated Tests
  - Used for Precision
- Analyst Counts – all quantitative
  - Repeated counts on the sample plate/tray
  - Used for identification

Precision Criterion for Fecal Coliform by MF 3rd Quarter 2008

Date/ Replicate number	replicate 1	replicate 2	log replicate 1	log replicate 2	diff inlog
1	1	2	0	0.301029996	-0.301029996
2	1	1	0	0	0
3	12	19	1.079181246	1.278753601	-0.199572355
4	1	1	0	0	0
5	25	18	1.397940009	1.255272505	0.142667504
6	1	1	0	0	0
7	1	2	0	0.301029996	-0.301029996
8	1	1	0	0	0
9	1	1	0	0	0
10	1	1	0	0	0
11	1	1	0	0	0
12	10	1	1	0	1
13	1	1	0	0	0
14	1	1	0	0	0
15	1	1	0	0	0
				ave of logs =	0.022735677
				precision criterion=	0.074345664

2025 EML MICRO QUALITY CONTROL CHECKLIST

Completion Date

		JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Dup Count Fecal	monthly	23											
Dup Count Entero	monthly	23											
Fecal Confirmation	monthly												
Air Environment Monitoring	monthly	14											
DI Water Analysis	monthly	14											
Autoclave Sterility Check	monthly	14											
Quanti-Tray Leak Check	monthly	22											

Timer Check/ Autoclave	quarterly				
IR Gun & #13 Temp	quarterly				
Pipettes	quarterly				
Update Precision Criterion	quarterly				

Calibrate Cl2 meter	bi-annually		
Micro Thermometers	annual		
Metals for DI Water**	annual		w/o:
Autoclave P.M.	annual		

NA = none available for counting

(**1L rectangular from Tom's top shelf; 1mL conc HNO3)

Final Thoughts

- Examples provided are not the only way
- Be creative
- Use the KISS method
- Talk to the Assessors
- Be your own advocate
- Phone-a-friend
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Questions?