Standard Method Microbiology QC Sections required by 2024 MUR

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1

Housekeeping Items

- ◊ Please keep your microphone muted except when called on
- ◊ Please “raise your hand” electronically or post any questions into chat
- ◊ Remember to put your hand down once your question/comment is addressed
- ◊ Questions that need more extensive answers will be addressed in writing later

2

Here’s the plan for today

- ◊ Discuss Requirement Changes in the Microbiology Quality Control Sections
  - ◊ Some comparisons to old version
  - ◊ Some comparisons to TNI
- ◊ Answer as many questions as we can
- ◊ Reminder-This session is about the Quality Control Sections, not the methods

3

QC Sections Overview

- The QC Sections allow for broad guidance and requirements
  - – Allow additions of QC requirements without re-writing every method
  - – Allow application of general requirements without increasing book size dramatically

4

Current Situation

- This most recent version of the Standard Methods compendium(24th Edition) includes updated versions of 9020, 9030, 9040 and 9050.

5

This Presentation

- This presentation reviews the most recent two versions
  - – 9020: 2015, 2022
  - – 9030: 2015, 2022
  - – 9040: 2013, 2022
  - – 9050: 2015, 2022

6

General

- Overall, the changes to these sections are minor
- Many noted have no practical impact
- A few could have significant impact
  - We'll discuss those
  - I can't always tell you how they'll be interpreted
  - There are a few conflicts

7

Note on this presentation

- Many subsections have no significant changes to the text
  - These will not be mentioned
  - If you have concerns about a section not mentioned, feel free to ask

8

Sections for Today

- 9020-Quality Assurance/Quality Control [for microbiological analyses]
- 9030-Equipment
  - Much overlap with the equipment section of 9020

9

Sections for Today

- 9040-Washing Labware
- 9050-Culture Media and Buffered Dilution Water
  - Much overlap with the requirements in 9020
- Let's get started!

10

9020A-Introduction

- The introduction section has four subsections (plus references and a bibliography)
  - General Considerations
  - Guidelines for a Quality System
  - Quality System Objectives
  - Elements of a Quality System Manual

11

9020A-Introduction

- While there is some rewording, there are no significant requirement differences between the versions
- The wording changes give greater emphasis to understanding uncertainty
- Practically, the TNI (-2) Standard covers these points in greater detail

12

9020B-Intralaboratory Quality Control Guidelines

- Section 1-Personnel
  - No significant changes
- Table 9020:I
  - This Table summarizes quality control requirements and gives a reference to find them in this 28-page (yikes!) section

13

Table 9020:I Balances

2015	2022
Check zero-Daily	Check zero-Daily
Check accuracy with at least 2 weights-Daily	Check accuracy with at least 2 weights-Monthly
Service and Calibrate-Monthly	Service and Calibrate-Monthly (preferably)
Working weights – Check with reference weights annually	Working weights – Check with reference weights monthly
Reference weights – Recertify annually	Reference weights – Recertify every 5 years

14

Section 2-Biosafety Criteria

- This section experienced significant reorganization
  - Requirements changed to one list from repetitious lists with each Biosafety level
- No practical changes in requirements

15

Section 3-Facilities

- b. Space Utilization
- The 2022 version adds to clauses
- Ensure only compatible functions are performed in the same laboratory workspace.
  - New, but probably has little impact

16

Section 3-Facilities, cont.

- If the laboratory plans to conduct PCR or other nucleic acid amplification procedures, the laboratory should consider implementing unidirectional workflow, where analysts move through areas dedicated to each step of the procedure to minimize the risk of cross contamination by amplified nucleic acid.

17

3.c Laboratory Bench Areas

2015	2022
Install even, glare-free lighting with about 1000 lux (100 ft-c) intensity at the working surface; test using a photometer	Ensure that there are no shadows over the work area and that analysts have sufficient light to accurately view test reactions and discern test results.

18

3.d Walls, Floors and Ceilings

- The 2022 version adds
- Air vents must be kept free of rust, dust, and mold
  - New requirement but probably not a new practice

19

3.e Work area (error fix)

2015	2022
In general, airborne bacterial populations should not exceed colonies/plate/15 min exposure, or 1 colony-forming unit (CFU) per minute	In general, airborne bacterial populations should not exceed **15** colonies/plate/15 min exposure, or 1 colony-forming unit (CFU) per minute

20

3.f Laboratory Cleanliness

2015	2022
Regularly clean laboratory rooms and wash benches, shelves, floors, windows, overhead lights, and exposed pipe surfaces	Regularly clean laboratory rooms and wash benches, shelves, floors, windows, overhead lights, exposed pipe surfaces, and **air vents**

21

4. Laboratory Equipment and Instrumentation

- The 2022 version added one clause in the introduction
- All files [data] must be available for review by auditors, despite the format used
  - This requirement is not new

22

4.a: Temperature-sensing and -recording devices

- Many laboratories use infrared (IR) thermometers
  - Water samples
  - Actually measure container temperature
  - Critical to be used consistently
  - Follow Mfr's instructions for distance to sample
    - Attach a ruler?

23

IR Thermometers, cont.

- Verify every 6 months over full range of use
  - Ambient (20-30 ^{o}C)
  - Iced (4 ^{o}C)
  - Frozen (0 - -5 ^{o}C)

24

IR Thermometers, cont.

- Single check daily
  - Bottle of water containing verified thermometer
  - If difference > 0.5 °C, recalibrate IR thermometer

25

4.a: Temperature-sensing and -recording devices

- New version adds record-keeping requirements for thermometer verifications
- The laboratory must record in a QC record book the following information:
  - serial number of laboratory thermometer
  - serial number of SI-traceable thermometer (or other reference thermometer)

26

Verification records, cont.

- temperature of laboratory thermometer
- temperature of SI-traceable thermometer (or other reference thermometer)
- correction factor and adjusted (corrected) temperature
- date of verification
- analyst's initials

27

4.a: Temperature-sensing and -recording devices

- New version requires ice-point verification of Reference Thermometer if using liquid in glass
  - Annually
  - Ensures no changes to column
  - Maintain records of the check
  - Procedure is detailed-get from SM
  - Derive correction factor

28

Conflict with TNI

- SM says to record only corrected temperatures
- TNI says to record *all* raw data, which includes the uncorrected temperature data
  - Personal opinion: having both corrected and uncorrected data is more defensible

29

4.b Balances and Weights

- New version makes several changes, including changing section name from "Balances" to "Balances and Weights.
- Adds this clause:
  - Use balances that provide a sensitivity of at least 0.1 g for a load of 150 g, and 1 mg for a load of 10 g or less

30

4.b Balances and Weights

2015	2022
Check balance routinely (preferably daily before use) using at least two working weights that bracket the normal usage range.	Each day the balance is used, perform a verification using reference masses at approximately the same nominal mass to be determined. Perform verifications each weighing session unless it can be shown that fluctuations in the environment do not affect the calibration

31

4.b Balances and Weights

2015	2022
Use only plastic-tip forceps to handle weights	Use only plastic-tip forceps or cotton gloves to handle weights

32

4.b Balances and Weights

2015	2022
Check working weights monthly for accuracy, precision, and linearity against a set of reference weights of known tolerance accompanied by appropriate calibration certificate. Record results along with date and technician's initials	Check working weights monthly for accuracy, precision, and linearity against a set of reference weights of known tolerance (e.g., ASTM Class 1, 2, or 3, accompanied by a verification certificate). Record results, along with correction factors, the date and analyst's initials in a log book. The correction factors must be on file and used

33

4.b Balances and Weights

2015	2022
Recertify reference weights as often as specified in the calibration certificate, or at least once every 5 years. Some regulators or accreditors may require that reference weights be recertified more frequently	If a laboratory does not wish to have two sets of weights, they can use a single set of weights and have them certified annually Recertify reference weights as often as specified in the calibration certificate, or at least once every 5 years

34

4.c pH Meter

- The new version adds a clause
- Record the date the electrode is put into service
  - Hopefully, you've been tracking this already, but, if not, please start
  - pH meters require maintenance records (logs)

35

4.c pH Meter

2015	2022
Replace pH buffer supply containers by the expiration date, preferably 6 months after opening because the solution may absorb carbon dioxide	Replace the pH buffers by the expiration date marked on the container because the solutions may absorb carbon dioxide. A pH 10 buffer is especially susceptible to degradation; minimize its exposure to air. If practical, purchase this buffer in small quantities

36

4.d Water Purification System

2015	2022
Perform the use test [see 9020B.5f2)] whenever there is a new source of water or new water system employed in the laboratory	For systems meeting the requirements for medium quality water, perform the use test (see 9020 B.5f2) whenever there is a new source of water, the water purification system is repaired (including cartridge changes) or for a new water system in the laboratory

37

4.d Water Purification System

2015	2022
Replace cartridges at manufacturer-recommended intervals based on the estimated usage and source water quality. Do not wait for column failure	Replace cartridges at manufacturer-recommended intervals based on the estimated usage and source water quality. Do not wait for column failure. Record any maintenance (such as the replacement of consumables) or service performed in a logbook

38

4.g Hot air sterilizing oven

- The new version adds this clause
- Ovens must maintain a uniform temperature of 170 °C or greater for 2 hours, with a temperature tolerance of 10 °C (160 to 180 °C). Record the date, contents, sterilization time, temperature, and analyst's initials for each cycle

39

4.h Autoclave

- The new version opens this section with a description of an autoclave that is a great familiarization but contains no new requirements.

40

4.h Autoclave

2015	2022
After each run cycle, record the items sterilized, sterilization temperature, total run time (heat exposure), programmed/ preset sterilization period, actual pressure readings, and analyst initials	For each run cycle, record: the date the contents of the load sterilization time and temperature (might be maximum temperature reached if using maximum temperature registering device)

41

4.h Autoclave

2015	2022
	total run time programmed or preset sterilization time actual pressure readings, and analyst initials

42

4.j Freezers

2015	2022
A recording thermometer and alarm system are highly desirable	A recording thermometer and alarm system are highly desirable, especially in ultra-low temperature freezers

43

4.k Membrane Filtration Equipment

2015	2022
Before initial use, assemble filtration units and check for leaks. Discard units if chipped or inside surfaces are scratched. Units that leak should be repaired accordingly or discarded	Membrane filtration units must be stainless steel, glass, porcelain, autoclavable or disposable plastic, not scratched or corroded, and must not leak. Discard units if they are chipped or the inside surfaces are scratched

44

4.k Membrane Filtration Equipment

- Old version:
  - When measuring sample volumes using funnels with volumetric graduation marks, initially check the marks' accuracy using a Class A graduated cylinder or volumetric pipet. Record results. For presterilized single-use funnels, check one per lot or a set percentage (e.g., 1 to 4%) to confirm the accuracy of volumetric graduation mark.

45

4.k Membrane Filtration Equipment

- New Version
  - If graduation marks on clear glass or plastic funnels are used to measure sample volume, check their accuracy with a Class B graduated cylinder or better (or other Class B glassware), and retain a record of this calibration check.

46

4.k Membrane Filtration Equipment

- New version, cont.
  - When measuring sample volumes using funnels with volumetric graduation marks, initially verify the accuracy of the marks using a Class A graduated cylinder or volumetric pipet and record the results. For presterilized single-use funnels, check one per lot or a set percentage (e.g., 1%-4%) to confirm the accuracy of volumetric graduation mark and sterility.

47

4.k Membrane Filtration Equipment

- New Version, cont.
  - A 2.5% error tolerance is acceptable in the graduated marks on the vessel.
  - Confirm the sterility of disposable funnels at least once per lot.

48

4.n Water Bath Incubator

2015	2022
Maintain water level so it is above the upper level of the medium in either tubes or flasks.	Maintain the water level so it is above the upper level of the medium in either tubes or flasks, but below the lids to avoid potential contamination

49

4.o Incubator

- New version adds this clause
  - Incubator units must have an internal temperature monitoring device and maintain the temperature specified by the method used.

50

4.o Incubator

2015	2022
It may take longer for media to reach the set incubation temperature in gravity convection hot-air incubators.	Gravity convection hot-air incubators must not be used.

51

4.o Incubator

- New version adds
  - Load studies may also need to be done to determine how effectively incubators can maintain temperature and how quickly they rebound after the door is opened or samples are added.
  - As a general practice, while in use, open incubator doors as infrequently as possible to prevent temperature fluctuations.

52

4.q Conductivity Meter

- New version adds a scale requirement
  - Meters must be suitable for checking laboratory reagent-grade water and readable in units of either microsiemens per centimeter (µS/cm) or micromhos per centimeter (µmho/cm)

53

4.q Conductivity Meter

- New version also adds
  - Use an extremely clean borosilicate glass beaker for calibration that is rinsed several times with the water to be tested before collecting the test sample. Swirl the sample gently to eliminate air bubbles. Perform conductivity measurements on-site because water quality decreases shortly after exposure to both air and the sample container

54

4.r Microwave Ovens

2015	2022
Vary in power	Vary in power
Have been used to melt presterilized agar media	May be used to melt presterilized agar media
	Antibiotics or other heat-labile inhibitors may not be microwaved
	Use minimum settings necessary to melt

55

5.a Glassware

- New version adds this clause
  - Plastic items must be clear and nontoxic to microorganisms

56

5.a.2 Inhibitory Residues

2015	2022
If each batch of glassware is pH tested, then this test is only needed when changing washing compounds or procedures	If each batch of glassware is pH tested, then this test is only needed when changing washing compounds (including new lots) or procedures

57

5.a.2 Inhibitory Residues

- Text from SM 9040, paragraph 6
  - Because the presence of detergent residual could have a deleterious effect… Perform the inhibitory residue test annually, before initial use of a washing compound or whenever a new detergent formulation or washing procedure is used.

58

5.a.2 Inhibitory Residues

- Lab Cert Manual
  - V. QC 4.5.3 A glassware inhibitory residue test (Standard Methods, Section 9020B, under Laboratory Supplies) should be performed before the initial use of a washing compound and whenever a different formulation of washing compound, or washing procedure, is used. Record results. This test will ensure that glassware is free of toxic residue.

59

5.a.2 Inhibitory Residues

- TNI 2016 7.3.7.b.vi.c
  - Labware that is washed and reused shall be tested for possible presence of residues that may inhibit or promote growth of microorganisms by performing the Inhibitory Residue Test initially and each time the laboratory changes the detergent formulation or washing procedures.

60

5.c Dilution Water Bottles

2015	2022
Discard by expiration date	Discard by the expiration date (3 months if tightly capped and stored at <30 °C)

61

5.d Sample Bottles

2015	2022
Test for sterility at least one or a set percentage (e.g., 1 to 4%) of each batch sterilized in the laboratory or of each presterilized lot purchased from a vendor	Test for sterility at least one or a set percentage (e.g., 1% to 4%) of each batch sterilized in the laboratory or of each presterilized lot purchased from a vendor by adding 25 mL of a sterile nonselective broth (e.g., tryptic soy, trypticase soy, or tryptone broth) and incubating at 35 ± 0.5 °C for 24 to 48 hours

62

5.e Multi-well trays and sealers

2015	2022
When using multi-well trays for growth studies, check one per lot for sterility beforehand by aseptically adding 100 mL of sterile tryptic soy broth or other non-selective medium, sealing, and incubating at 35 ± 0.5°C for 24 and up to 48 h. No growth indicates sterility. If the wells become very turbid (indicating nonsterile condition), there could be gas production and concomitant blowout between wells.	Check one tray per lot for sterility by aseptically adding 100 mL of sterile nonselective medium (e.g., tryptic soy, trypticase soy, or tryptone broth), sealing, and incubating at 35 ± 0.5 °C for 24 to 48 h. Document the results. If growth occurs, discard the entire batch or lot

63

5.e Multi-well trays and sealers

2015	2022
Every month, evaluate the heat sealer's performance by adding one to two drops of a food-color dye to 100 mL deionized water sample, run the multi-well tray through the sealer, and visually check each well for leakage.	Every month, evaluate the heat sealer's performance by adding 1 to 2 drops of a food-color dye to 100 mL reagent-grade water, run the multiwell tray through the sealer, and visually check each well for leakage. If dye is observed outside the wells, either perform maintenance or use another sealer

64

5.f Reagent Grade Water

2015	2022
Use reagent-grade water to prepare solutions and media, and for final glassware rinses. The water must be proven to be free from inhibitory and bactericidal substances	Only satisfactorily tested reagent-grade water from stills or water purification systems may be used to prepare media, reagents, dilution and rinse water, and for final glassware rinses for performing microbial analyses. The water must be proven to be free from inhibitory and bactericidal substances

65

5.f Reagent Grade Water

- Water Suitability Test shows water to be free of inhibitory and bactericidal substances
  - Not required for medium quality water and above
  - Best to subcontract it if needed

66

5.j.4 Culture media

- The older version had this clause
  - If prepared ready-to-use commercial medium has an expiration date later than that noted in Table 9020:V, have the manufacturer supply evidence of medium quality for that entire period. Verify usability weekly by testing recoveries with known densities of culture controls that will also meet QC check requirements.
  - Removed from new version

67

5.j.4 Culture media

2015	2022
Discard all Petri dishes with solid media that have been stored for >2 weeks; discard earlier if they are dried out (e.g., wrinkled, cracked, or pitted)	Discard all petri dishes with solid media that have been stored for >2 weeks; discard earlier if they are dried out (e.g., wrinkled, cracked, or pitted), unless ongoing QC procedures show that there is no loss in selectivity or growth promotion (5.j.5)

68

5.j.5 Media Use Test

2015	2022
Subject laboratory-prepared media to the use test	Do not prepare media in the laboratory from basic ingredients when commercially prepared bulk dehydrated media is available (5.j.6)

69

5.j.7 Media

- The new version offers this clause:
  - Often, manufacturers of commercially prepared ampouled broth specify much longer expiration dates than those recommended here. In these cases, the laboratory may hold those media as recommended by the manufacturer if quantitative QC testing demonstrates no loss in selectivity or growth promotion.

70

9.a Colony-Counting Variability

2015	2022
If counts do not agree within the acceptable margin, determine why and correct as needed. Chart these results in a QC chart.	If counts do not agree within the acceptable margin, determine the reasons and correct with additional training as needed. Calculate the precision of duplicate counts using the best dilution for reading each type of sample examined (e.g., drinking water, ambient water, or wastewater) according to the procedure in paragraph c, and record results.

71

Section 6-SOPs

- No substantive changes from 2015 to 2022 versions.
- It should be noted that most applicable requirements for standard operating procedures for the CA ELAP are found in the TNI standard, which are more stringent than those found in Standard Methods.

72

Section 7-Sampling

- The 2015 and 2022 versions are mostly the same and contain only a few requirements that are applicable to the testing laboratory.
- The few differences noted are listed in the next few slides.

73

7.c Sample Acceptance

2015	2022
Sample-receipt information should include names or identifiers of both sampling site and sampler, turbidity, and date and time of sample collection.	Sample-receipt information **must** include names or identifiers of both sampling site and sampler, **temperature, disinfectant residual**, turbidity, and date and time of sample collection, as applicable.

74

7.d Sample analysis using analytical methods

2015	2022
The laboratory is responsible for ensuring that analyses are initiated within an acceptable holding time.	Only analyze samples that are within the acceptable holding time and temperature. Samples outside of the acceptable holding time and temperature must be rejected.

75

7.d Sample analysis using analytical methods

- The new version contains these clauses
  - Shake samples approximately 25 times in a 7 second timeframe using a 1-foot arc.
  - If a bottle lacks sufficient headspace for adequate mixing, pour the sample into a larger sterile vessel so it can be mixed properly.
  - Do not remove a sample portion until proper sample mixing has occurred.

76

Section 9-Analytical Quality Control for Established Methods

- The section has some re-ordering and some changes in section titles, but only a few changed requirements
- Paragraph 9.d.2 from the previous version, sterility checks for MTF and P/A procedures, was dropped as it just referred to previous procedures

77

9.d Sterility checks (MF Funnels)

2015	2022
If a processing interruption lasts >30 min, use new sterilized funnels and repeat sterility test.	Ideally, filter a blank for each funnel set used during a filtration series. Membrane filter equipment must be autoclaved before the beginning of a filtration series. A filtration series ends when 30 minutes or longer elapses after a sample is filtered.

78

Section 10,Verification

- The old version had confirmation procedures for Fecal streptococci. These have been dropped from the new version.

79

11. Validation of New or Nonstandard Methods

2015	2022
Use commercial laboratory-prepared cell suspensions of the target microorganism from a reputable source. The supplier **should** provide third-party evidence of competence and compliance with global standards	Use commercial laboratory- prepared cell suspensions of the target microorganism from a reputable source. The supplier **must** provide third-party evidence of competence and compliance with global standards

80

SM 9030

Equipment

81

1. Incubators

- The new version has additional wording
  - If a moist chamber environment must be maintained within a specified range, water can be placed in a flat pan or tray (approximately 2 in. high) with maximized surface area of exposure (preferably with heated air flowing directly across water surface).

82

1. Incubators, cont.

- However, do not allow condensate to pool on the floor of walk-in incubators. Monitor the chamber temperature with temperature measuring devices (glass or electronic digital thermometers) placed on a single shelf or upper and lower shelves of use. When using only a walk-in incubator, record the daily temperature range within or adjacent to areas where the samples are incubated.

83

6. pH Equipment

- The new version adds this clause
  - There are two types of pH meters. One type is capable of performing a 2-point standardization (generating one slope) and the newer type is capable of performing a 3-point standardization (generating either one or two slopes).

84

6. pH Equipment

2015	2022
The pH meter must be calibrated with at least 2 buffers that bracket the measurement range.	The pH meter must be calibrated with at least 2 buffers **that includes the pH 7 buffer standard** and brackets the measurement range.

85

6. pH Equipment

- The new version adds this clause
  - Discard used aliquots daily and discard buffers after the manufacturer's expiration date. Contact the pH meter manufacturer or review its operator's manual for the slope determination procedure.

86

7. Balances

- The new version adds this clause
  - Only use balances that have been leveled according to manufacturer's instructions.

87

Media-Preparation Utensils

2015	2022
Refers to "Glassware"	Refers to "Labware"

88

11. Refrigerator and Freezers

2015	2022
Do not store volatile solvents, food, or beverages in the same refrigerator with reagents, media, or cultures.	Do not store volatile solvents, food, or beverages in the same refrigerator with reagents, media, cultures, **or samples.**

89

11. Refrigerator and Freezers

2015	2022
Monitor freezer temperature.	Monitor refrigerator and freezer temperature using a traceable, calibrated temperature sensor placed in liquid.

90

11. Refrigerator and Freezers

- The new version adds this clause:
  - Frost-free freezers incur temperature spikes that may damage biological materials such as cultures, enzymes, DNA or samples. Ensure that the temperature remains within the acceptable limits by monitoring it during the defrost cycle. Temperature excursions are reduced by storing materials in insulated containers.

91

12 Temperature-monitoring Devices

2015	2022
Uses the term “NIST-certified”	Uses the phrase “traceable to the SI through a national metrology institute.”

92

14. Petri Dishes

- The new version includes the minor clarification that Petri dishes must be “pre-sterilized”

93

15. Multiwell Trays and Sealer Units

- The new version includes the minor clarification that multiwell trays must be “pre-sterilized”

94

15. Multiwell Trays and Sealer Units

2015	2022
Sealer unit should be cleaned monthly	Clean the sealer units monthly (or at a frequency established by laboratory).

95

16. Membrane Filtration Equipment

2015	2022
If metal grids break, replace them	If metal grids have **surface defects** or breaks, replace them. Replace gaskets if leaking.

96

20. Microscopes

- The new version add this clause:
  - Change the bulb or external fluorescence source if a loss in fluorescence is noted

97

21. Centrifuges

- The new version adds this clause:
  - De-rate the rotors as required
  - Full disclosure: while this appears to be a process to lower the maximum speed as the centrifuge ages, there is no further requirements on how one does this

98

24. Spectrophotometer

- The new version adds this clause:
  - If used for absorbance, calibrate at least annually using certified reference standards following the manufacturer's instructions

99

27. PCR Instruments

- The new standard adds these clauses:
  - Follow the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines or the US EPA quality assurance/quality control guidance for laboratories performing PCR analyses on environmental samples.
  - Ensure that the temperatures in individual wells match those on the digital display.

100

27. PCR Instruments

- The publication referenced on the previous slide can be linked here. There is a cost to get the entire article, so we haven't read it.
- https://academic.oup.com/clinchem/article-abstract/55/4/611/5631762?redirectedFrom=fulltext&login=false

101

SM 9040

Washing and Sterilization

102

SM 9040

- Wording is extensively rephased, but has few significant changes

103

SM 9040-Glassware/Labware

2013	2022
Limits glassware to glass and heat-resistant plastic	Refers to “labware” and includes glass, plastic or metal

104

SM 9040-Bromothymol Blue

2013	2022
Paragraph 3: Perform bromothymol blue pH check on each batch	Paragraph 5: Perform the test by adding a few drops of 0.04% bromothymol blue solution to the inside of a piece of cleaned labware while it is still wet. Swirl to maximize contact on the inside surface and observe for a color change. Bromothymol blue is blue-green at a circumneutral pH.

105

SM 9040-Inhibitory Residue Test

2013	2022
…if the bromothymol blue test is not done consistently, also run the toxicity test annually	Perform the inhibitory residue test annually, before initial use of a washing compound or whenever a new detergent formulation or washing procedure is used.

106

SM 9040-Glassware/Labware

2013	2022
Glassware is defined in a footnote as being borosilicate glass and heat-resistant plastic materials, but autoclaving requirements only say glass	Glassware that is sterilized in the autoclave must be composed of hard borosilicate glass Plasticware that is sterilized in the autoclave must be composed of heat-resistant plastics

107

SM 9050

Culture Media and Buffered Dilution Water

108

9050 Introduction

- The introduction section has greatly expanded editorial information, but no requirements.
- In the new version (2022), some of the section names have changed, but the content in the sections is similar to the 2015 version

109

1. Purchase of Media

- The new version contains this new section which is editorial/definitional and does not contain requirements

110

Storage of Media

2015	2022
A.1 Storage of Culture Media	A.2 Storage of Dehydrated Media
Store media out of direct sunlight	Protect culture media from strong light

111

Media Preparation

2015	2022
A.1 Storage of Culture Media	A.3 Media Preparation
No specific instructions about preparation bottles or reconstitution.	Many good technique suggestions that were not previously included. These are typically well known to experienced microbiologists, but are worth reading, e.g. – Size of prep vessels – Reconstitute slowly

112

pH Adjustment

2015	2022
A.2 pH Adjustment	A.4 pH Adjustment
This section appears to assume pH will be measured with tempered agar in liquid form with a temperature corrected probe	This section recommends allowing a small aliquot of medium to solidify at room temperature and measuring the pH using a flat electrode, but alternatively allows for measurement in tempered agar.

113

pH Adjustment

2015	2022
A.2 pH Adjustment	A.4 pH Adjustment
This section provides no specific limit for adjusting pH of media	A slight pH adjustment is typically acceptable; however, do not adjust the pH excessively. For example, if the measured pH is >0.5 units from the target pH, discard the batch of medium rather than adjusting.

114

Sterilization

2015	2022
A.3 Sterilization	A.5 Sterilization
Adjust Autoclave times as volumes/ loads increase or decrease.	Adjust Autoclave times as volumes or loads increase or decrease, but do not increase the autoclave temperature.

115

Sterilization, con.

2015	2022
A.3 Sterilization	A.5 Sterilization
After sterilization, examine media to determine whether any unanticipated color or clarity variations occurred, or media components precipitated	Gently stir media after autoclaving to ensure distribution of agar throughout medium. Then, examine media to determine whether any unanticipated color or clarity variations occurred, or media components precipitated

116

Sterilization

2015	2022
A.3 Sterilization	A.5 Sterilization
Mark media containers with preparation date and records all pertinent information in appropriate logbooks.	Label media containers with preparation date and the final pH, and as a good laboratory practice, include the expiration date based on day of preparation.

117

Sterilization, cont.

- The new version adds this clause:
- After pouring agar plates, place 1 or 2 plates in a 35 °C incubator overnight as a sterility check.

118

A.6 Storing Prepared Media

- The new version adds these clauses:
  - Do not store media-containing petri dishes in cardboard boxes, which may serve as a growth substrate for molds in moist environments.
  - Refrigerators containing media must not contain volatile solvents whose absorption into media may be toxic to bacteria.

119

B.1 Water specifications

- The new version adds this clause:
  - If a commercial water purification system is used to generate reagent water, the laboratory must change the consumables (e.g., purification cartridges, UV lights, point of use filters) as recommended by the manufacturer. Record the replacement of these components and any other service in a logbook. Perform a use test to ensure that water of comparable quality is produced after changes to the system.

120

B.2 Buffered Dilution Water Solutions

- The new version adds these clauses:
  - Be sure to use high quality glassware for the preparation and storage of buffered water.
  - Added a maximum 3-month storage time to
    - phosphate buffer stock solution
    - magnesium chloride stock solution
    - peptone water recipes

121

Questions?

If we don't have time, submitted questions will be answered in writing in the next few weeks

122

Thank you!

123

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124