

Housekeeping Items

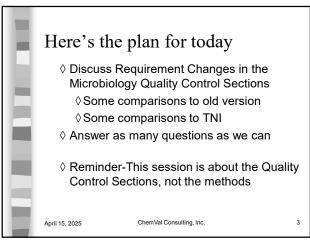
Please keep your microphone muted except when called on
Please "raise your hand" electronically or post any questions into chat
Remember to put your hand down once your question/comment is addressed
Questions that need more extensive answers will be addressed in writing later

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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

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Current Situation

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

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This Presentation

This presentation reviews the most recent two versions

- 9020: 2015, 2022

- 9030: 2015, 2022

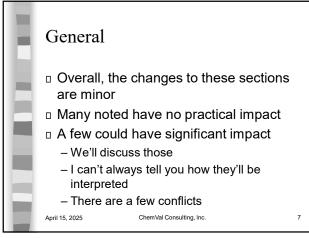
- 9040: 2013, 2022

- 9050: 2015, 2022

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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

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Sections for Today

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

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Sections for Today

- 9040-Washing Labware
- 9050-Culture Media and Buffered
Dilution Water
- Much overlap with the requirements in
9020

- Let's get started!

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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

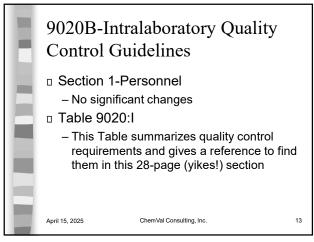
9020A-Introduction

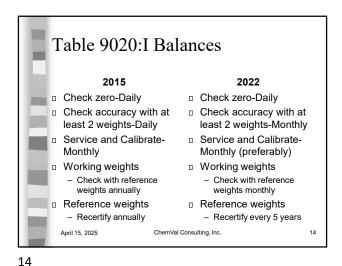
While there is some rewording, there are no significant requirement differences between the versions
The wording changes give greater emphasis to understanding uncertainty
Practically, the TNI (-2) Standard covers these points in greater detail

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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

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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

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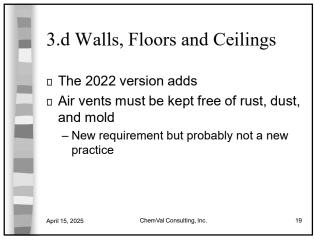
Section 3-Facilities, cont.

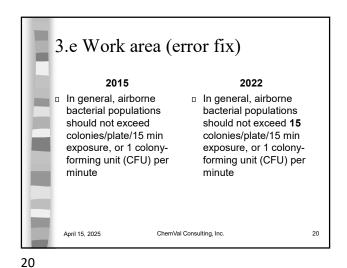
If the laboratory plans to conduct PCR or other nucleic acid amplification procedures, the laboratory should consider implementing unidirectional workflow, where analysts move through areas dedicated to each step of the procedure to minimize the risk of cross contamination by amplified nucleic acid.

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3.c Laboratory Bench Areas 2015 2022 Install even, glare-free Ensure that there are lighting with about 1000 no shadows over the lux (100 ft-c) intensity at work area and that the working surface; test analysts have sufficient using a photometer light to accurately view test reactions and discern test results. April 15, 2025 ChemVal Consulting, Inc. 18

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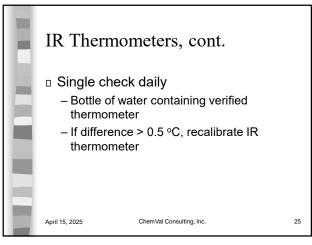
3.f Laboratory Cleanliness 2015 2022 Regularly clean Regularly clean laboratory rooms and laboratory rooms and wash benches, shelves, wash benches. floors, windows, shelves, floors, overhead lights, and windows, overhead exposed pipe surfaces lights, exposed pipe surfaces, and air vents April 15, 2025 ChemVal Consulting, Inc. 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 April 15, 2025 ChemVal Consulting Inc. 22

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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? April 15, 2025 ChemVal Consulting, Inc. 23 23

IR Thermometers, cont. Verify every 6 months over full range of - Ambient (20-30 °C) - Iced (4 °C) - Frozen (0 - ⁻5 °C) April 15, 2025 ChemVal Consulting, Inc.

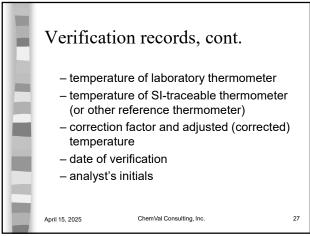


4.a: Temperature-sensing and recording devices New version adds record-keeping requirements for thermometer verifications The laboratory must record in a QC record book the following information: - serial number of laboratory thermometer - serial number of SI-traceable thermometer (or other reference thermometer) April 15, 2025 ChemVal Consulting, Inc. 26

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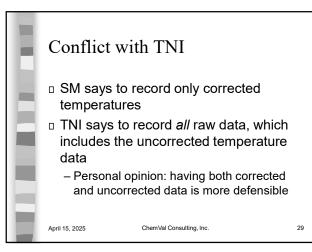
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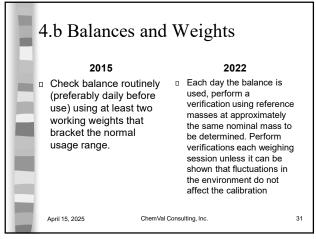
4.a: Temperature-sensing and recording devices New version requires ice-point verification of Reference Thermometer if using liquid in glass - Annually - Ensures no changes to column - Maintain records of the check - Procedure is detailed-get from SM - Derive correction factor April 15, 2025 ChemVal Consulting Inc 28

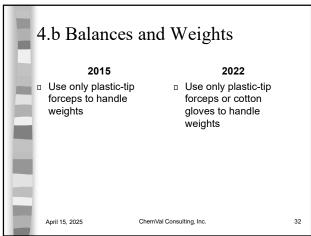
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4.b Balances and Weights New version makes several changes, including changing section name from "Balances" to "Balances and Weights. Adds this clause: - Use balances that provide a sensitivity of at least 0.1 g for a load of 150 g, and 1 mg for a load of 10 g or less April 15, 2025 ChemVal Consulting, Inc. 30

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4.b Balances and Weights 2015 2022 Check working weights Check working weights monthly for accuracy, monthly for accuracy, precision, and linearity precision, and linearity against a set of reference against a set of reference weights of known tolerance weights of known (e.g., ASTM Class 1, 2, or 3, tolerance accompanied accompanied by a by appropriate calibration verification certificate). Record results, along with certificate. Record results correction factors, the date along with date and and analyst's initials in a log technician's initials book. The correction factors must be on file and used April 15, 2025 ChemVal Consulting, Inc.

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

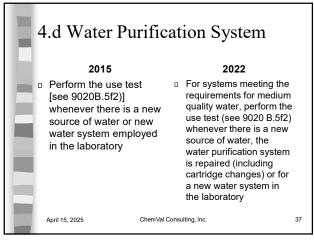
The new version adds a clause
Record the date the electrode is put into service
Hopefully, you've been tracking this already, but, if not, please start
pH meters require maintenance records (logs)

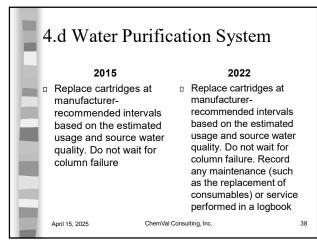
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4.c pH Meter 2015 2022 Replace pH buffer Replace the pH buffers supply containers by the by the expiration date expiration date, marked on the container because the solutions preferably 6 months may absorb carbon after opening because dioxide. A pH 10 buffer the solution may absorb is especially susceptible carbon dioxide to degradation; minimize its exposure to air. If practical, purchase this buffer in small quantities April 15, 2025 ChemVal Consulting, Inc.

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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

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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.

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4.h Autoclave 2015 2022 After each run cycle, For each run cycle, record the items record: sterilized, sterilization the date temperature, total run the contents of the load time (heat exposure), sterilization time and programmed/ preset temperature (might be sterilization period, maximum temperature actual pressure reached if using readings, and analyst maximum temperature initials registering device) April 15, 2025 ChemVal Consulting, Inc.

4.h Autoclave

2015

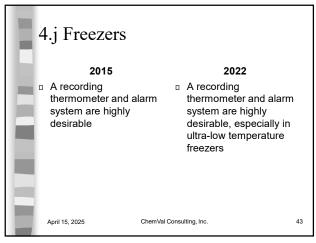
2022

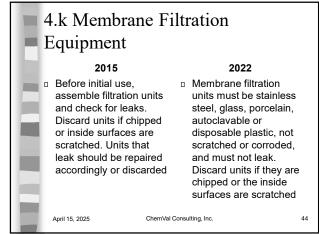
total run time
programmed or preset sterilization time
actual pressure readings, and
analyst initials

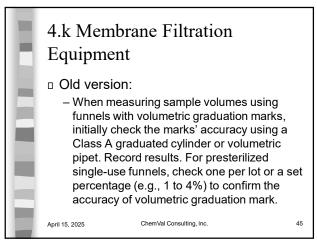
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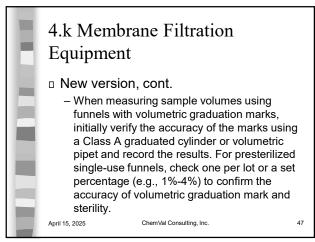
4.k Membrane Filtration
Equipment

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.

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4.k Membrane Filtration
Equipment

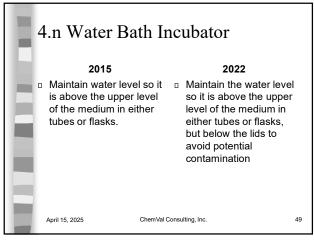
New Version, cont.

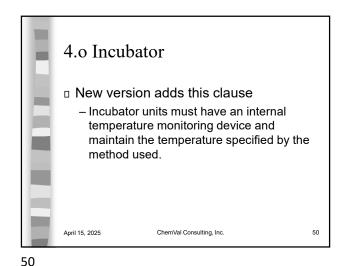
- A 2.5% error tolerance is acceptable in the graduated marks on the vessel.

- Confirm the sterility of disposable funnels at least once per lot.

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4.0 Incubator

2015

It may take longer for media to reach the set incubation temperature in gravity convection hot-air incubators.

Gravity convection hot-air incubators.

Gravity convection hot-air incubators must not be used.

4.0 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.

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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)

4.q Conductivity Meter

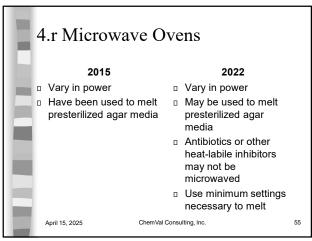
New version also adds

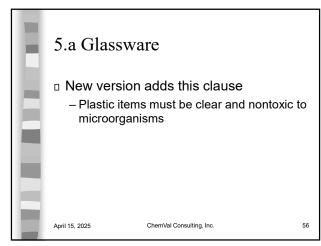
- Use an extremely clean borosilicate glass beaker for calibration that is rinsed several times with the water to be tested before collecting the test sample. Swirl the sample gently to eliminate air bubbles. Perform conductivity measurements on-site because water quality decreases shortly after exposure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

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5.a.2 Inhibitory Residues 2015 2022 If each batch of If each batch of glassware is pH tested, glassware is pH tested, then this test is only then this test is only needed when changing needed when changing washing compounds or washing compounds procedures (including new lots) or procedures April 15, 2025 ChemVal Consulting, Inc. 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.

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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.

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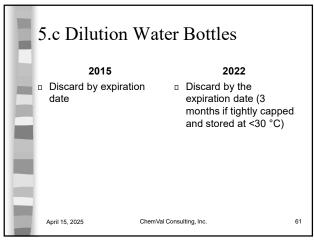
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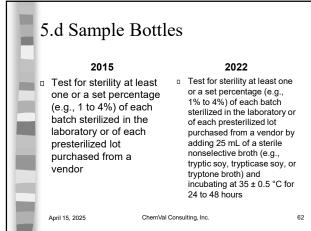
5.a.2 Inhibitory Residues

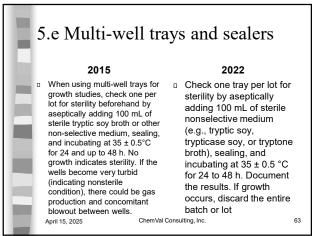
TNI 2016 7.3.7.b.vi.c

- 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.

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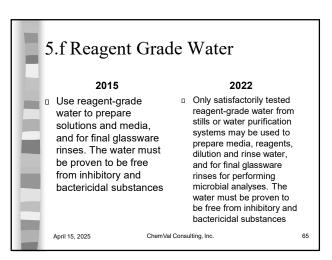




5.e Multi-well trays and sealers 2015 2022 Every month, evaluate the Every month, evaluate heat sealer's performance the heat sealer's by adding 1 to 2 drops of a performance by adding food-color dye to 100 mL one to two drops of a reagent-grade water, run food-color dye to 100 mL the multiwell tray through deionized water sample, the sealer, and visually run the multi-well tray check each well for through the sealer, and leakage. If dve is observed visually check each well outside the wells, either for leakage. perform maintenance or use another sealer April 15, 2025 ChemVal Consulting Inc

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5.f Reagent Grade Water

Water Suitability Test shows water to be free of inhibitory and bactericidal substances

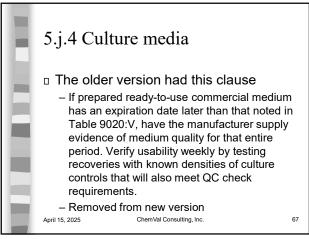
Not required for medium quality water and above

Best to subcontract it if needed

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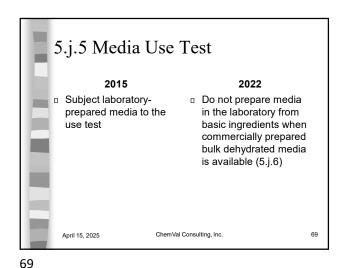


5.j.4 Culture media 2015 2022 Discard all petri dishes Discard all Petri dishes with solid media that with solid media that have been stored for >2 have been stored for >2 weeks; discard earlier if weeks; discard earlier if they are dried out (e.g., they are dried out (e.g., wrinkled, cracked, or wrinkled, cracked, or pitted), unless ongoing pitted) QC procedures show that there is no loss in selectivity or growth promotion (5.j.5) April 15, 2025 ChemVal Consulting, Inc.

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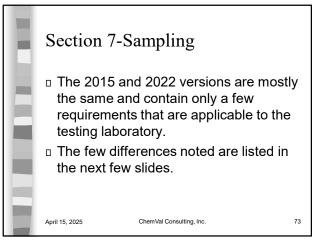
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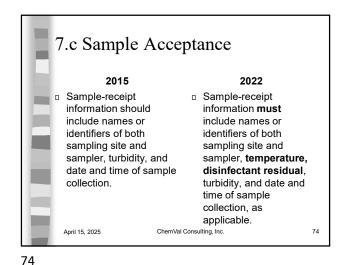


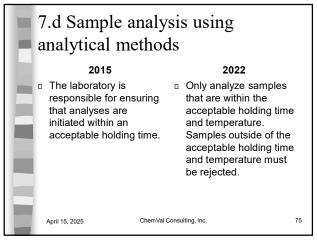
5.j.7 Media The new version offers this clause: - Often, manufacturers of commercially prepared ampouled broth specify much longer expiration dates than those recommended here. In these cases, the laboratory may hold those media as recommended by the manufacturer if quantitative QC testing demonstrates no loss in selectivity or growth promotion. April 15, 2025 ChemVal Consulting Inc

9.a Colony-Counting Variability 2015 2022 If counts do not agree within If counts do not agree the acceptable margin, within the acceptable determine the reasons and margin, determine why correct with additional training and correct as needed. as needed. Calculate the Chart these results in a precision of duplicate counts using the best dilution for QC chart. reading each type of sample examined (e.g., drinking water, ambient water, or wastewater) according to the procedure in paragraph c, and record results April 15, 2025 ChemVal Consulting, Inc. 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. April 15, 2025 ChemVal Consulting, Inc. 72

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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.

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Section 9-Analytical Quality
Control for Established Methods

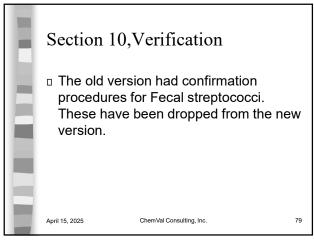
The section has some re-ordering and some changes in section titles, but only a few changed requirements
Paragraph 9.d.2 from the previous version, sterility checks for MTF and P/A procedures, was dropped as it just referred to previous procedures

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9.d Sterility checks (MF Funnels) 2015 2022 If a processing Ideally, filter a blank for each funnel set used interruption lasts >30 min, use new sterilized during a filtration series. Membrane filter funnels and repeat equipment must be sterility test. autoclaved before the beginning of a filtration series. A filtration series ends when 30 minutes or longer elapses after a sample is filtered. April 15, 2025 ChemVal Consulting, Inc.

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11. Validation of New or Nonstandard Methods 2015 2022 Use commercial Use commercial laboratory-prepared cell laboratory- prepared suspensions of the cell suspensions of the target microorganism target microorganism from a reputable source. from a reputable The supplier should source. The supplier provide third-party must provide thirdevidence of competence party evidence of and compliance with competence and global standards compliance with global standards April 15, 2025 ChemVal Consulting, Inc.

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SM 9030
Equipment

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).

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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.

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6. pH Equipment

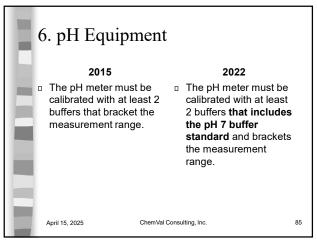
The new version adds this clause

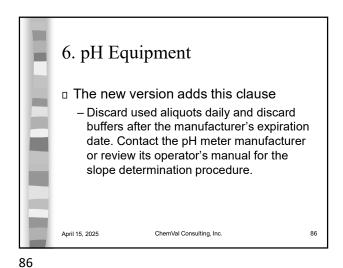
There are two types of pH meters. One type is capable of performing a 2-point standardization (generating one slope) and the newer type is capable of performing a 3-point standardization (generating either one or two slopes).

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7. Balances

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

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Media-Preparation Utensils

2015
2022

Refers to "Glassware"
Refers to "Labware"

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11. Refrigerator and Freezers 2015 2022 Do not store volatile Do not store volatile solvents, food, or solvents, food, or beverages in the same beverages in the same refrigerator with refrigerator with reagents, media, or reagents, media, cultures. cultures, or samples. April 15, 2025 ChemVal Consulting, Inc. 89 11. Refrigerator and Freezers

2015

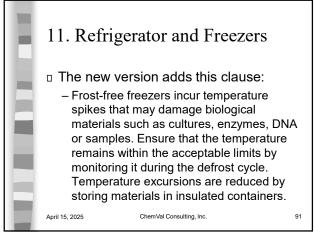
Monitor freezer temperature.

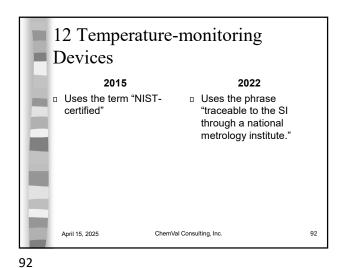
Monitor refrigerator and freezer temperature using a traceable, calibrated temperature sensor placed in liquid.

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14. Petri Dishes

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

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15. Multiwell Trays and Sealer Units

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

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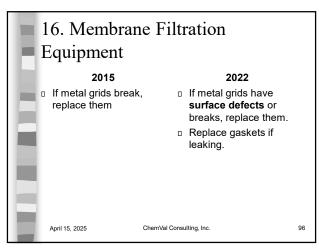
15. Multiwell Trays and Sealer
Units

2015
2022

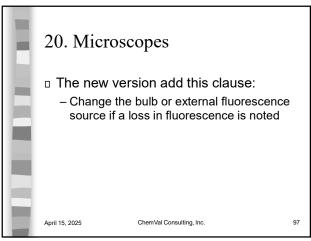
Sealer unit should be cleaned monthly
Clean the sealer units monthly (or at a frequency established by laboratory).

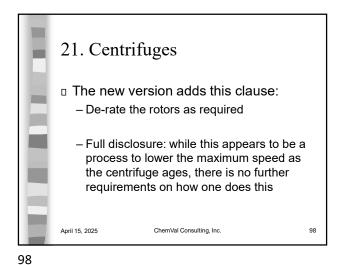
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24. Spectrophotometer

The new version adds this clause:

If used for absorbance, calibrate at least annually using certified reference standards following the manufacturer's instructions

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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.

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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.

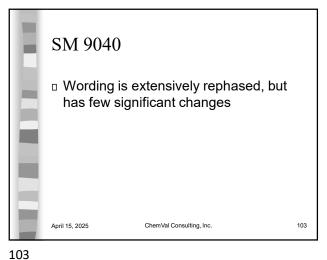
□ https://academic.oup.com/clinchem/artic le-abstract/55/4/611/5631762?redirectedFr om=fulltext&login=false

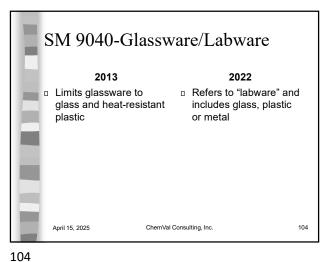
□ the publication referenced on the previous slide can be linked here. There is a cost to get the entire article, so we haven't read it.

□ https://academic.oup.com/clinchem/artic le-abstract/55/4/611/5631762?redirectedFr om=fulltext&login=false

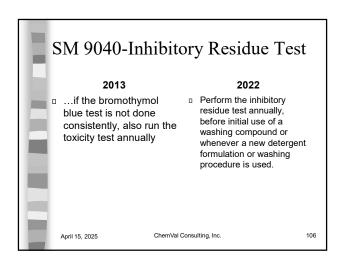
SM 9040
Washing and Sterilization

101 102





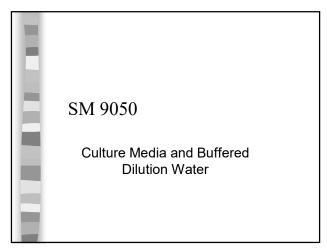
SM 9040-Bromothymol Blue 2013 2022 Paragraph 3: Perform Paragraph 5: Perform the test by adding a few drops bromothymol blue pH of 0.04% bromothymol check on each batch blue solution to the inside of a piece of cleaned labware while it is still wet. Swirl to maximize contact on the inside surface and observe for a color change. Bromothymol blue is blue-green at a circumneutral pH. April 15, 2025 ChemVal Consulting, Inc. 105



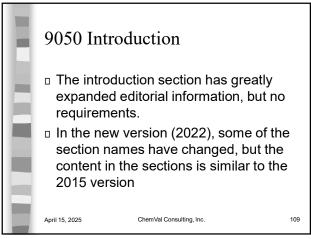
106

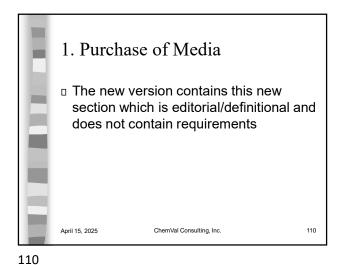
105

SM 9040-Glassware/Labware 2013 2022 Glassware is defined in Glassware that is sterilized in the a footnote as being borosilicate glass and autoclave must be heat-resistant plastic composed of hard materials, but borosilicate glass autoclaving Plasticware that is requirements only say sterilized in the glass autoclave must be composed of heatresistant plastics April 15, 2025 ChemVal Consulting, Inc. 107



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Storage of Media

2015
2022

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

April 15, 2025

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Media Preparation 2015 2022 A.1 Storage of Culture A.3 Media Preparation Media Many good technique No specific instructions suggestions that were about preparation not previously included. bottles or reconstitution. These are typically well known to experienced microbiologists, but are worth reading, e.g. - Size of prep vessels - Reconstitute slowly April 15, 2025 ChemVal Consulting Inc. 112

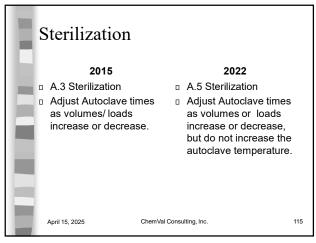
112

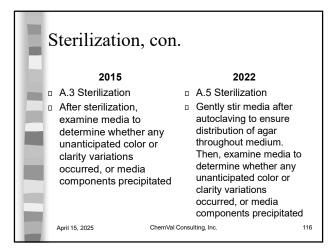
111

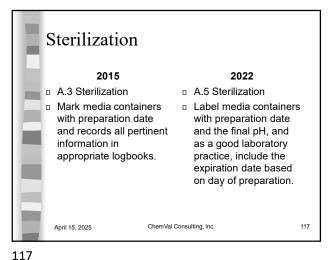
pH Adjustment 2015 2022 A.2 pH Adjustment A.4 pH Adjustment This section This section appears to assume pH will be recommends allowing a small aliquot of medium measured with tempered to solidify at room agar in liquid form with a temperature and temperature corrected measuring the pH using probe a flat electrode, but alternatively allows for measurement in tempered agar. April 15, 2025 ChemVal Consulting, Inc. 113

pH Adjustment 2015 2022 A.2 pH Adjustment A.4 pH Adjustment A slight pH adjustment This section provides no specific limit for is typically acceptable; however, do not adjust adjusting pH of media 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. April 15, 2025 ChemVal Consulting, Inc 114

113 114

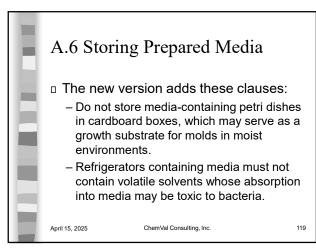






Sterilization, cont. The new version adds this clause: After pouring agar plates, place 1 or 2 plates in a 35 °C incubator overnight as a sterility check. April 15, 2025 ChemVal Consulting Inc. 118

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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. ChemVal Consulting, Inc. April 15, 2025 120

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