NYSDOH ELAP MICROBIOLOGY CHECKLIST

This checklist incorporates references to both 'The NELAC Institute' 2016 Standards, where applicable, and specific method and state and / or federal regulatory requirements.

Directions: Place a mark (e.g., /, √ or X) in the appropriate column (Yes (Y), No (N), or Not Applicable (NA)). If it is an observation on areas for possible improvement, place a mark under the Suggestion (S) column. In database, use code "SGST."

Lab ID: _____ Assessment ID: _____

Lab Name: _____

Personnel Interviewed: Reports Reviewed:

_____ _____ _____

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At the time of the assessment, a question marked 'yes' indicates that no evidence of a deficiency was observed.

Assessment Date(s):_____ Assessor (Signature): _____

If this was a team assessment, indicate the Lead Assessor's name. _____

Microbiological Testing Detailed Method Review	Data Records observed	Comments
Method Number: **SOP Number:** **Rev.:** **SOP date:** **Personnel records observed:**		
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Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
The laboratory is in adherence to the Quality Control procedures specified in the NELAC standard, method, regulation and project.	M5,1.1-1.2						
a. SM9215A, 5 & 7-8: **Heterotrophic Plate Count** SM9215B: **Pour Plate Method**						**000D30**	
_1__ All dilution plates analyzed in duplicate.							
_2__ Incubated at 35.0 ± 0.5 ºC for 48 ± 3 hours.							
_3__ Colonies counted with a dark-field colony counter, or one with equivalent magnification & illumination. (SM9215A, 8.a. & b.& ANSI/AAMI RD52:2004, 7.2.3)						**0d31a1** **0d31a2** **0d31a3**	
_4__ Incubated at 35.0 +/- 0.5 degrees Celsius for 72 ± 4 hours for finished bottled water. (EPA 600/8-78-017, Part III, Sec. 5.5.2)						**0d31a4**	
_5__ Incubated at 35-37 ºC for 48 hours (for dialysis product water - ANSI/AAMI RD52:2004, 7.2.3 and RD62:2006, 5.1)							
_6__Sample volume chosen yields between 30 and 300 colonies. (SM9215A, 8.a. & b. & ANSI/AAMI RD52:2004, 7.2.3)						**0d31a10**	
SimPlate						**0d31a11**	
_7__ Inverted and incubated at 35.0 ± 0.5 ºC for 48 hours. (Results can be read from 48 to 72 hours after start of incubation.)							
_8__ When doing unit dose, 10 ± 0.2 mL sample is added to media tube.						**0d31a5**	
_9__ When doing multi dose, 1 mL of sample and 9 mL of rehydrated media is pipetted onto center of the plate.						**0d31a6**	
SM9215C & ANSI/AAMI RD52:2004 & RD62:2006: **Spread Plate**						**0d31a7**	
_10__							
_11__ An inoculum of at least 0.5 mL of sample spread equally over the surface of the agar. (ANSI/AAMI RD52:2004, 7.2.3)						**0d31a1**	
_12_ Inoculated agar plate with glass rod or pipette. Calibrated loop is not allowed. (ANSI/AAMI RD52:2004, 7.2. & RD62:2006, 5.1.1)						**0d31a8**	
_13_ Incubated at 35-37 ºC for 48 hours. (ANSI/AAMI RD52:2004, 7.2.3 and RD62:2006, 5.1)						**0d31a9**	
_14_ Colonies counted with a dark-field colony counter, or one with equivalent magnification & illumination. (SM9215A, 8.a. & b.&						**0d31a10**	

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ANSI/AAMI RD52:2004, 7.2.3) _15_ Sample volume chosen yields between 30 and 300 colonies. (SM9215A, 8.a. & b. & ANSI/AAMI RD52:2004, 7.2.3) Note: If colony yield is not met, lab can use smaller or larger volumes. Smaller volumes can be reached by doing 1:10 serial dilutions using sterile phosphate buffer. If larger volumes are required, the MF method should be generally be used SM9215D & ANSI/AAMI RD52:2004 & RD62:2006: **Membrane Filter Method** _16_ Dispensed 5-mL portion of sterile agar into 50- x 9- mm petri dishes Note: m-HPC agar may not be sterile. _17_ Incubated at 35-37 ºC for 48 hours. (ANSI/AAMI RD52:2004, 7.2.3 and RD62:2006, 5.1) _18_Colonies counted with a stereoscopic microscope at 10 to 15 x magnification. (SM9215A, 8.. b.& ANSI/AAMI RD52:2004, 7.2.3) _19_ Sample volume chosen yields between 20 and 200 cfu. (SM9215A, 8. & b. & ANSI/AAMI RD52:2004, 7.2.3) Note: If colony yield is not met, lab can use smaller or larger volumes. Smaller volumes can be reached by doing 1:10 serial dilutions using sterile phosphate buffer. If larger volumes are required, the MF method should be generally be used. **b**. SM9221A&B, 1.b.: **Total Coliform Multiple Tube Fermentation with Lauryl Tryptose Medium** _1__ SDWA: 100 mL sample analyzed. (five-20 mL tubes, ten 10 mL tubes, or one 100 mL bottle) _2__ CWA: 5-tube per dilution for each sample. _3__ Incubated at 35.0 ± 0.5 ºC for 24 +/- 2 hours. _4__ SDWA: If no gas detected after 24 hours, incubate for another 24 hours. Note: For other waters (NW), pull positives after 24 +/- 2 hours, transfer them, and still check the ones that are negative after 24 hours at 48 +/- 3 hours. _5__ All samples producing turbid cultures with no gas production invalidated, with another sample requested.						**0d31a3** **0d31a11** **0d31a12** **0d31a10** **0d31a13** **0d31a11** **0d31b1** **0d31b2** **0d31b3** **0d31b4**	

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c. SM9221D, 1.a. & b.: **Total Coliform with Presence/Absence Medium** _1__ 100 mL sample analyzed _2__ Incubated at 35.0 ± 0.5 ºC for 24 hours _3__ If purple color indicator does not turn yellow, incubate for another 24 hours _4__ All samples producing turbid cultures with no color change invalidated, with another sample requested						**0d31b5** **0d31c1** **0d31c2** **0d31c3** **0d31c4**	
d. SM9221E, 1.a. & b:Thermotolerant (**Fecal) Coliform Most Probable Number with EC Medium** _1__ 3-dilution (sample volumes), 5-tube (per sample volume) technique for each sample _2__ Each tube inoculated from positive culture grown on m-Endo or LTB medium _3__ Incubated at 44.5 ± 0.2 ºC for 24 ± 2 hours _4__ Gas formation indicates Fecal Coliform; no further verification needed **e**. SM9221E, 2.a. & b: Thermotolerant (**Fecal) Coliform Most Probable Number with A-1 Medium** _1__ 3-dilution (sample volumes), 5-tube (per sample volume) technique for each sample _2__ Direct inoculation with sample possible _3__ Incubated at 35.0 ± 0.5 ºC for 3 hours, then at 44.5 ± 0.2 ºC for 21 ± 2 hours _4__ Gas formation indicates Thermotolerant (Fecal) Coliform; no further verification needed **f**. SM9221F **PW/NW E. coli enumeration & NW Thermotolerant coliform with EC-MUG** _1__Tube contains inverted Durham tube _2__Culture transferred to EC-MUG using sterile 3- or 3.5 mm diameter sterile loop or sterile wooden applicator inserted at least 2.5 cm to transfer growth from fermentation tube to culture tube. Note: Wooden applicator must be plunged to bottom of EC-MUG tube.						**0d31d1** **0d31d2** **0d31d3** **0d31d4** **0d31e1** **0d31e2** **0d31e3** **0d31e4** **0d32a** **0d32b** **0d32c**	

Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
_3__Incubate 44.5 ± 0.2° C for 24 ± 2 hours. _4__Growth and gas indicates thermotolerant coliform _5__Blue fluorescence under 6 W 365-366 nm UV light indicates E. coli **g**. SM9222B, 5.a.-d.: **Total Coliform by Membrane Filtration** _1__ SDWA: 100 mL sample filtered _2__ CWA: Filter 3 different sample volumes so that at least one dilution will give 20-80 colonies, but not more than 200 colonies. _3__ Enhancement recovery required for stressed organisms in chlorinated samples (e.g., spas and swimming pools). _4__ Incubated at 35.0 ± 0.5 ºC for 22-24 hours **h**. SM9222D, 2.a.-d.: Thermotolerant (Fecal) Coliform by Membrane Filtration _1__ Filter volumes or dilutions that will give 20-60 fecal coliform colonies per membrane filter _2__ Incubated at 44.5 ± 0.2 ºC for 24 ± 2 hours						**0d32d** **0d32e** **0d31f1** **0d31f2** **0d31f3** **0d31f4** **0d31g1** **0d31g2**	
i. SM9223B, 2: **Total Coliform by MMO-MUG** _1__ 100 mL sample analyzed (for drinking waters) _2__ Colilert: Incubated at 35.0 ± 0.5 ºC for 24 hours. _3__ Colilert: When indeterminate after 24 hours, incubate for another 4 hours. _4__ Colisure: Incubated at 35.0 ± 0.5 ºC for >= 24 hours, but <= 48 hours. _5__ Colilert-18: Incubated at 35.0 ± 0.5ºC for 18 hours (up to 22 hours if indeterminate after 18 hours); first 20 minutes MUST be in 35 ºC water bath or 7-10 minutes in 44.5 ºC water bath. _6__ Readycult: Incubated at 35.0 ± 0.5 ºC for 24 hours ±1 hour. _7__ Fluorocult LMX: Incubated at 35.0 ± 0.5 ºC for 24 hours ± 1 hour. _8__ Colitag: Incubated at 35.0 ± 0.5 ºC for 24 hours ± 2 hours. _9__ E*Colite: Incubated at 35.0 ± 0.5 ºC for 28 hours. _10__ Color change indicates Total Coliform; 366-nm blue-light fluorescence indicates E. coli; and no further verification needed. _11__ When enumerating coliforms using Colilert, the lab uses a Quanti-Tray for each sample dilution tested. _12__ The lab checks the Quanti-Tray sealer monthly by adding a dye to						**0d31h1** **0d31h2** **0d31h3** **0d31h4** **0d31h5** **0d31h6** **0d31h7** **0d31h8** **0d31h9** **0d31h10** **0d31h11** **0d31h12**	

Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
the water. The lab reports quantitative (aka estimate of bacterial Density or enumeration) data for E. coli for source water under the SDWA Surface Treatment Rule.							
j. **Enterococci by Enterolert** _1__ 100 mL sample analyzed (for drinking waters) _2__ Incubated at 41.0 ± 0.5 ºC for 24 hours (up to 28 hours if indeterminate after 24 hours) **k**. EPA 1600, 9.5.2, 11.5 & 11.8: **Enterococci by Membrane Filtration with mEI Medium** _1__ Filter volumes or dilutions that will give 20-60 enterococci colonies per membrane filter _2__ Incubated at 41.0 ± 0.5 ºC for 24 hours +/- 2 hours SM 9230C: **Enterococci by Membrane Filtration with mE → EIA Medium** _3__ If mE agar is used, incubated inverted plate for 48 hours at 41°±0.5°C, ± 3 hours and then transfer filter to EIA medium. Incubated at 41°± 0.5°C for 20 minutes.						**0d31i1** **0d31i2** **0d31j1** **0d31j2** **0d31j3**	
l. ISO 11731:2017(E), 8.2 – 8.5: Legionella *Concentration of Water Samples* _1__ Filtered sample using vacuum filtration or positive pressure filtration, or centrifuged sample where concentration by filtration is not possible. _2__ Filtered an appropriate volume of sample based on particulate content or desired detection level. _3__ MF and direct plating: Filtered water sample (without treatment, after acid treatment, and if required, after heat treatment) through cellulose nitrate or mixed cellulose esters membrane filter, and placed filter (right-side up) directly onto culture media, ensuring no air bubble is trapped. _4__ MF followed by washing: Filtered water through polycarbonate or polyethersulfone membrane filter. Note: Placed filter right side down in a screw cap, sterile container with or without sterile beads. _5__ Washed filter using 5 to 10 ml of sterile diluent, or sample, and vortexed for at least 2 minutes, or alternatively, placed the container in an ultrasonic bath, ensuring the level of diluent is below the level of the water in the bath, for an optimum time interval for maximum recovery.						**0d31k1** **0d31k2** **0d31k3** **0d31k4** **0d31k5**	

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Note: Filters may be cut into pieces using sterile scissors to aid elution. Also, refer to NOTES 1-3 in method.							
_6__ Divided concentrate into one portion untreated, one portion with heat, and one portion for treatment with acid solution.						**0d31k6**	
Sample Pre-Treatment							
_7__ Heat: Added sample (concentrated or unconcentrated) into a sterile container and placed in a water bath at 50 ± 1 ºC for 30 ± 2 min. Note: Small volumes (<= 5 ml) should be used.						**0d31k7**	
_8__Acid: Diluted one volume of the sample (concentrated or unconcentrated) with nine volumes of an acid solution, mixed well and left to stand for 5.0 ± 0.5 min.						**0d31k8**	
or							
_9__Acid: Transferred around 30 ml acid solution onto membrane filter, left for 5.0 ± 0.5 min, and rinsed the filter with at least 20 ml of the diluent.						**0d31k9**	
Plating and Inoculation							
_10_ For samples expected with high concentration of Legionella (>10^4 cfu/l) and low concentration of interfering microorganisms: plated sample directly and inoculated and spread 0.1 ml to 0.5 ml sample onto one plate of BCYE and one plate of BCYE+AB agar.						**0d31k10**	
_11_ For samples expected with low concentration of Legionella and low concentration of interfering microorganism: placed untreated, filtered sample onto one plate of BCYE agar. For acid treated filters, the samples are placed on one or more plates of BCYE+AB agar or GVPC or MWY agar.						**0d31k11**	
_12_ Samples expected with low concentration of Legionella and low concentration of interfering microorganism: inoculated and spread 0.1 ml to 0.5 ml of each concentrated portion of untreated, heat treated and acid treated sample from membrane filtration with washing onto one plate of BCYE agar and on one or more plates of BCYE+AB agar or GVPC or MWY agar.						**0d31k12**	
_13_ Samples expected with a high concentration of interfering microorganisms: spread 0.1 ml to 0.5 ml of each portion of untreated, heat treated, and acid treated subsample onto one plate of GVPC or MWY agar.						**0d31k13**	
_14_ Samples expected with an extremely high concentration of interfering microorganisms: spread 0.1 ml to 0.5 ml of each portion of subsample, that has been heat treated then acid treated and mixed well by a vortex mixer or in an ultrasonic water bath, onto one plate of GVPC or MWY agar.						**0d31k14**	

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Incubation							
_15_ Plates inverted and incubated subcultured plates at 36 ± 2 ºC for 10 d in a humid atmosphere to prevent desiccation of plates.						**0d31k15**	
Note: Inoculated media left to stand until inocula is absorbed. It is acceptable to stop the incubation at day 7 for those samples that do not contain wild strains of Legionella. ***Natural samples*** *containing wild strains of Legionella can, however, require the full incubation period of 10 d to present growth.*						**0d31k16**	
Examination of Plates						**0d31k17**	
_16_ Plates inspected for the first time on day 2, 3, 4, or 5 followed by a final inspection at the end of the incubation period (i.e. day 7 or day 10, dependent on the nature of the sample), and the # of each colony type recorded. Check the plates on day 2 to determine if dilutions are needed.						**0d31k18**	
Note: With outbreak investigations, it is advisable for samples with expected high concentration of interfering microorganisms to check the plates on day 2.						**0d31k19**	
Subculturing/Confirmation						**0d31k20**	
_17_ Subcultured 3 presumptive colonies when there is only one colony type. First inoculate BCYE-cys (or alternate media note 6.1.2) and then BCYE.						**0d31k21**	
_18_ Subcultured at least 1 colony type if more than 1 presumptive type of colony is growing. . First inoculate BCYE-cys (or alternate media note 6.1.2) and then BCYE.						**0d31k22** **0d31k23** **0d31k24**	
_19_ Incubated subcultured plates at 36 ± 2 ºC for 2 d to 5 d in a humid atmosphere to prevent desiccation of plates.							
Note: It is acceptable to stop the incubation at day 2 for those samples that are easily confirmed.						**00d335b**	
_20_ With outbreak investigations, subcultured and incubated at least 5 presumptive colonies if only one morphology is present, or 2 presumptive colonies for each type of morphology.							
Recording Results							
_21_ Recorded the results of all plates. Regard as Legionella those colonies that grow on BCYE agar but fail to grow on BCYE-cys agar.							
_22_ Recorded volume filtered.							
_23_ Recorded volume concentrated and final volume.						**00d335t**	

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_24_ Recorded the inoculated volume. Note: Record issues can also be cited using a code in the Quality System checklist, Section 13 – Control of Records. **Reagents and media** **a.) Phosphate Buffered Saline** (ISO 11731, 6.2 Annex C): ___ Stock buffer autoclaved at 121 ºC for 15 minutes. ___ Stock buffer final pH pH 7.5 . ___ Dilution rinse water prepared from stock buffer & $MgCl_2$. ___ Sterility check on dilution rinse water with double-strength non-selective medium, 35 ºC, 24 hour. ___ A commercially available preparation can also be used. **._b.)__BCYE** (ISO 11731:2017(E), Annex B.1): ___ L-cysteine and iron solutions prepared fresh, decontaminated through filtering, and stored at -20 ± 3 °C for not more than 3 months. ___ ACES buffer is prepared by mixing 2 solutions – 1) ACES granules dissolved in 500 ml distilled water using a water bath (45-50 °C) and 2) KOH pellets dissolved in 480 ml distilled water using gentle shaking. ___ Charcoal, yeast extract and α-ketoglutarate added sequentially to ACES buffer. ___ H2SO4or KOH used to adjust pH to 6.8 ± 0.2. ___ Agar added and mixed to ACES solution, autoclaved at 121 ± 3 °C for 15 ± 1 min, and cooled in a water bath to 48 ± 3 °C. ___ L-cysteine and iron solutions added aseptically, mixing well between additions. ___ Final pH is 6.8 ± 0.2 at 25 °C. ___ Stored at 5 ± 3 °C in airtight containers and protected from light for 3 months. **c.) ___BCYE-Cys** (ISO 11731:2017(E), Annex B.2): ___ Prepared as noted above for BYCE, except that L-cysteine is omitted. ___ Stored at 5 ± 3 °C in airtight containers in the dark for 3 months. **d.)___BCYE+AB** (ISO 11731:2017(E), Annex B.3): ___ Prepared as noted above for BCYE, except that 3 antibiotics supplements are added (Polymyxin B sulfate, Sodium cefazolin, and Pimaricin syn Natamycin). ___ Added Polymyxin B sulfate to 100 ml of water to achieve a						**00d335u** **00d335v** **00d335w**	

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concentration of 14,545 IU/ml. Sterilized the solution by filtration through 0.2 um or lower pore size filter. ___ Added 180 mg of Sodium cefazolin to 20 ml of water. Sterilized the solution by filtration through 0.2 um or lower pore size filter. ___ Added 1.75 g of Pimaricin to 100 ml of water. Sterilized the solution by filtration through 0.2 um or lower pore size filter ___ Prepared antibiotic supplements are stored in sterile containers at -20 ± 3 °C for not more than 3 months.							
e)___GVPC (ISO 11731:2017(E), Annex B.4): ___ Prepared as noted above for BYCE except that ammonia-free glycine and 3 antibiotic supplements are added. ___ Ammonia-free glycine added after α-ketoglutarate. ___ H2SO4or KOH used to adjust pH to 6.8 ± 0.2 at 25 °C. ___ Stored at 5 ± 3 °C in airtight containers in the dark for up to 4 weeks. ___ 3 antibiotics - Polymyxin B sulfate, Vancomycin HCl and Cycloheximide - prepared fresh, decontaminated through filtering, and stored at -20 ± 3 °C for up to 3 months when frozen, and thawed at room temperature for use. ___ 3 antibiotics are added and mixed well to the final medium after the aseptic addition of L-cysteine and iron solutions.						**00d335x** **00d335y**	
f**.) ___Acid Buffer** (ISO 11731:2017(E), Annex D): ___ Prepared using HCl and KCl. ___ pH is adjusted to 2.2 ± 0.2 using KOH. ___ Stored in the dark at room temperature for no longer than 1 month.						**00d335aa**	
g)___Diluents – Page's Saline, Diluted Ringer's Solution, and Phosphate-buffered Saline (ISO 117311:2017(E), Annex C): ___ Page's Saline – 5 chemicals (NaCl, $MgSO_4 \cdot 7H_20$, $CaCO_2 \cdot 2H_2O$, Na_2HPO_4, and KH_2PO_4) added to distilled water, dissolved, mixed well and autoclaved at 121 ± 3 °C for 15 ± 1 min. ___ Diluted Ringer – Use a commercially available preparation (1:10 dilution of ¼ strength Ringer's solution). ___ Phosphate-buffered saline – Use a commercially available preparation at pH 7.5 ___ Sterile tap water						**00d335bb**	
h.) ___ Modified Wadowsky Yee (ISO 11731:2017(E). Annex B.5):							

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___ Prepared as noted above for BCYE, except the 3 antibiotics supplements are added (Polymyxin B sulfate, Vancomycin hydrochloride, Anisomycin), two indicators (Bromothymol blue, Bromocresol purple), and ammonium-free glycine. ___ Polymyxin B sulfate, Vancomycin hydrochloride - sterilized through filtration with a 0.2 um or lower pore size, and stored at -20 ± 3 °C for not more than 3 months. ___ Anisomycin – prepared fresh solution ___ Indicators - sterilized through filtration with a 0.2 um or lower pore size, and stored at 5 ± 3 °C for a maximum of 1 year. **i).** ___ **Agars –** Blood, Nutrient and Tryptic soy agar (ISO 11731:2017(E), Annex B.6, B.7 and B.8): ___ Blood Agar – pH adjusted to 6.8 ± 0.2 at 25 °C, autoclaved at 3 °C for 15 ± 1 min, cooled in a water bath (48 ± 3 °C), poured to a depth of 4 mm, and stored at in the dark at 5 ± 3 °C for up to 4 weeks. ___ Nutrient Agar – pH adjusted to 6.8 ± 0.2 at 25 °C, autoclaved at 121 ± 3 °C for 15 ± 1 min, cooled at 48 ± 3 °C, poured to a depth of 4 mm, and stored in the dark 5 ± 3 °C for up to 8 weeks. ___ TSA - pH adjusted to 6.8 ± 0.2 at 25 °C, autoclaved at 121 ± 3 °C for 15 ± 1 min, cooled at 48 ± 3 °C, poured to a depth of 4 mm, and stored in the dark 5 ± 3 °C for up to 8 weeks.							
Quality Control							
The quality control protocols specified by the laboratory's method manual are followed by all analysts.	M2,5.9.3 c)					**000d12**	
All essential quality control measures are incorporated in the lab's method manual.	M2,5.9.3(c)					**000d13**	
All quality control measures are assessed and evaluated on an on-going basis and quality control acceptance criteria are used to determine the validity of the data.	M2,5.9.3(b)					**000d14**	
The laboratory has procedures for developing acceptance/rejection criteria for each test where no method or regulatory criteria exist.	M2,5.9.3(c)					**000d15**	
Bacteriology samples from **known chlorinated water sources, unknown sources where disinfectant usage is suspected, and all potable water supplies** are checked in the laboratory for residual chlorine, unless the	M5,1.7.5.2(a-d)						

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following conditions are met: a.)__ sufficient sodium thiosulfate is added to each container to neutralize at minimum 5 mg/L of chlorine for drinking water and 15 mg/L chlorine for wastewater, b.)__ one container from each batch of laboratory prepared containers or lot of purchased ready-to-use containers is checked to ensure efficacy of the sodium thiosulfate to 5 mg/L chlorine or 15 mg/L chlorine as appropriate and the check is documented, and c.)__ chlorine residual is checked in the field and actual concentration is documented with sample submission. d)-the laboratory can show that the received sample containers are from its laboratory or have been appropriately tested and documented. Note: Lab must meet all these conditions.						**55818ar** **55818br** **55818cr** **55818dr**	
The laboratory has checked samples for **proper preservation** (e.g. pH, absence or free chlorine) prior to or during sample preparation or analysis. Note: Refer to deficiency 51117 in Section 23 of the general Quality System checklist, too.	M4,1.7.4(b)					**000d35r**	
The **maximum hold time** has not been exceeded for the bacteriological samples analyzed by the laboratory. Note: Refer to ELAP Certification Manual Item 245.	SWTR, BWR, TCR, GWR, NPDES, AAMI/ANSI					**000d335z**	
Temperatures of **incubators and water baths** are recorded twice daily separated by **at least 4 hours.** Note: There is no intent to take the temperature of incubation units during periods where there are no samples under test.	M5,1.7.3.7(b)(v)(b)					**000d32r**	
The following support equipment associated with microbiological testing is checked with NIST traceable materials (where possible): a.)__ pH meter b.)__ Balance(s) c.)__ Conductivity meter d.)__ Refrigerator(s) for sample storage and/or media storage e.)__ Incubators f.)__ Water baths g.)__Freezers	M2, 5.5.13.1M5,1.7.3.7(b)					**5916r** or **00d34ar** **00d34br** **00d34cr** **00d34dr** **00d34er** **00de4fr** **00d34gr**	
Does the lab demonstrate and document the quality of reagents and media	D.3.1.a					**000d37r**	

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used is appropriate for the test?	[M5,1.7.3.1]						
Materials and supplies that are required to be sterile prior to use that are needed to process samples (whether sterilized in the laboratory or purchased as sterilized) are checked by the laboratory once per purchased or prepared lot using non-selective growth media.	M5,1.7.3.1(a)					**000d37ar**	
Certificates of Analysis (COA) provided by vendors documenting sterility are verified by the laboratory and documentation available for review.	M5,1.7.3.1(a)					**000d37br**	
Excess sample over 100ml is not removed by pouring off.	MCLADW 5.1.5					**000d37cr**	

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1.__ For **sterility checks and blanks**, the laboratory analyzes a sterility check for each lot of pre-prepared, ready-to-use media(including chromofluorogenic reagent) and for each batch of media prepared in the laboratory, at minimum with first use.	M5,1.7.3.1(a)(i),(b)(i)					**00d381r**	
a.)_For chromo/fluorogenic media, the media is added to sterile deionized water and incubated at the appropriate temperature and time for the method used and documented.	M5,1.7.3.1(a)(i)(a)					**00d382r**	
b.)_ For all other media, the media is incubated uninoculated at working strength (single strength).	M5,1.7.3.1(a)(i)(b)					**0d382a1r**	
2.__ For each lab **sterilized filtration unit** used in a filtration series, the laboratory prepares at least one beginning and one ending blank. The filtration series may include single or multiple filtration units, which have been sterilized prior to beginning the series.	M5,1.7.3.2(a)					**0d382ar**	
a)__ For pre-sterilized single use funnels, a sterility check shall be performed on one funnel per lot.						**0d382br**	
b)__ When an interruption of more than 30 minutes occurs, the filtration funnels are re-sterilized.	M5,1.7.3.1(a)(ii)					**0d382cr**	
c)__ Filtration units are rinsed with three 20-30 mL portions of sterile rinse water after each sample filtration.	M5,1.7.3.1(b)(iii)]						
d)__ The laboratory [ ] inserts a method blank after every 10 samples or [ ] sanitizes filtration units by UV light after sample filtration.	M5,1.7.3.2(b)					**00d383r**	
Note: Lab needs to use filters with diameter of 47 mm and pore size of 0.45 um or better.							
3.__ For the **pour plate technique**, method blanks of the medium are made by pouring, at a minimum, one uninoculated plate for each lot of pre-prepared, ready-to-use media and for each batch of medium prepared in the laboratory.	M5,1.7.3.2.(c)					**00d384r**	
Note: The above is applicable to all plated media used for pour plate, spread plate, and MF techniques.							
4.__ **Sterility checks on sample containers** are performed on at least one container for each lot of purchased, pre-sterilized containers with non-selective growth media. For containers prepared and sterilized in the	M5,1.7.3.1(a)(iii)						

Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
laboratory, a sterility check is performed on one container per sterilized batch with non-selective growth media. Note: Using a non-selective broth, incubate at 35 +/- 0.5 degrees Celsius for 24 and 48 hours and check for growth. 5.__ A sterility **blank** is performed on each batch of dilution water prepared in the laboratory and on each lot of pre-prepared, ready-to-use dilution water with non-selective growth media. 6.__ At least one filter from each new lot of **membrane filters is checked** for sterility with non-selective growth media? 7.__ A sterility check on one (1) funnel per lot of pre-sterilized single use funnels using non-selective growth media. The laboratory shall perform a sterility check on one (1) funnel per batch of laboratory-sterilized funnels, using non-selective growth media.	M5,1.7.3.1(a)(iv) M5,1.7.3.1(a)(v) M.,1.7.3.1 (a)(ii)					**00d385r** **00D387r** **00d386r** **00d388r**	
A **known negative culture** is analyzed(cultured) using an appropriate non-target organism for each lot of pre-prepared, ready-to-use medium (including chromofluorogenic reagent) and for each batch of medium prepared in the laboratory, prior to first use on samples.	M5,1.7.3.6(d)(i)(b)					**00d311r**	
Each lot of pre-prepared, ready-to-use medium (including chromofluorogenic reagent) and each batch of medium prepared in the laboratory is tested with at least one pure culture of a **known positive reaction**, prior to first use on samples.	M5,1.7.3.6(d)(ii)(b)					**00d312r**	
For test methods that specify colony counts (i.e. cfu/100ml or MPN/100 ml): 1.__ **Duplicate counts** are performed **monthly** on one positive sample for each month that the test is performed. 2.__If the lab has two or more analysts, each analyst counts typical colonies on the same sample for each month the test is performed. a) __ Counts within **10% difference** are considered acceptable. 3.__In a lab with one microbiology analyst, the same sample is counted twice by the analyst for **each month** the test is performed. a) __ Counts with no more than **5% difference** are considered acceptable.	M5,1.7.3.3					**0d3161r** **0d3162r** **d3162ar** **0d3163r** **d1363ar**	
The laboratory demonstrates validation with the test method prior to first use							

Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
by: 1.__ Determine accuracy by comparison of at least one positive reference culture result to that of a reference method; 2.__ Determine precision by analyzing a minimum of ten replicate analyses spiked with the target microorganism with both the proposed and reference method and determine that the proposed method is statistically equivalent or better that the reference method.; 3.__ Determine selectivity by analyzing a minimum of ten spiked samples using mixed cultures that include the target microorganisms and at various concentrations. Calculate the number of false positive and false negative results.	M5,1.5.1,-1.5.3					**0d364ar** **0d364br** **0d364cr** **0d364d** **0d364er**	
The laboratory maintains the documentation of the validation as long as the method is in use and for **at least five years** past the date of last use.	M5,1.5(c)					**00d319r**	
To evaluate the ability of the laboratory to produce acceptable data, the laboratory participates in a proficiency test program (interlaboratory).	M5,1.5(b)					**00d320r**	
All growth and recovery media are checked to assure that the target organisms respond in an acceptable and predictable manner once per lot or batch.	M5,1.7.3.6(a)					**00d325r**	
To ensure that analysis results are accurate, a target organisms identity is verified as specified in the method, e.g. by use of the completed test, or by use of secondary verification tests such as a catalase test or by the use of a selective medium such as Brilliant Green Bile Broth(BGLG) or EC or EC +MUG broth. **a.** SM9221B, 2b; SM9221D, 2b: **Total Coliform by Fermentation Broth method** _1__ Each positive culture from LTB (gas formation or color change) is inoculated onto BGLB (Note: If all 5 tubes produced gas in 2 or more sample dilutions, only the 5 tubes with gas from the highest dilution need be confirmed) _2__ Incubated at 35.0 ± 0.5 ºC for 24 ± 2 hours _3__ If no gas formation, re-incubate for additional 24 hours (total of 48 ± 3 hours) _4__ Gas formation in BGLB confirms Total Coliform for purposes of MPN calculation or Presence-Absence reporting _5__ SDWA samples also tested according to SM9221E or EPA 1104	M5,1.7.3.6(b)					**0d325ar** **d340a1r** **d340a2r** **d340a3r** **d340a4r** **0d340a5r**	

Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
						d340b1	
b. SM9222B, 5f: **Total Coliform by Membrane Filter method**						**d340b2**	
_1__ Inoculate at least 10 colonies from filter into LTB & BGLB							
_2__ SDWA: Inoculate all colonies (can swab entire filter) into one LTB tube & one BGLB tube						**d340b3**	
						d340b4	
_3__ Incubate at 35.0 ± 0.5 ºC for 48 hours						**d340b5**	
_4__ Gas production in LTB & BGLB confirms Total Coliform							
_5__ SM9222B: May use rapid-test or commercial multi-test verification systems that utilize test reactions for cytochrome oxidase & b-galactosidase; negative reaction for cytochrome oxidase & positive reaction for b-galactosidase confirms Total Coliform						**d340b6**	
_6__ SDWA: Positive cultures from LTB or membrane filter colonies also tested according to SM9221E, EPA 1104, or EPA 1105. (Note: May inoculate m-Endo colonies directly into BGLB medium. However, if gas is observed in LTB, but not in the corresponding BGLB tube, another BGLB tube must be inoculated & tested with the positive culture from the LTB tube							
_7__ SM 9020B, 9.b.1 Membrane Filter Method Confirmation :							
a)__ For drinking water, all colonies from positive samples on m-Endo medium are verified.						**d340b7a**	
b)__ If there are no positives, at least one known positive source water is tested quarterly.						**d340b7b**	
						d340b7c	
c)__ For other types of water, positives are verified monthly (by picking at least 10 sheen colonies) and counts are adjusted based on percent verification.						**d340b7d**	
d)_ False negatives are picked, by and verified.							
c SM9221E, 1b: **Fecal Coliform with EC Medium (A-1 is not allowed for SDWA)**						**d340c1**	
_1__ Incubated at 44.5 ± 0.2 ºC for 24 ± 2 hours						**d340c2**	
_2__ Gas formation confirms that the Total Coliform is a Fecal Coliform							
d. SM 9222G/EPA 1104, 11: **E. coli by EC + MUG Tube Procedure**							
_1__ Incubated at 44.5 ± 0.2 ºC for 24 ± 2 hours						**d340d1**	
_2__ 366-nm blue-light fluorescence confirms that the Total Coliform is E. coli						**d340d2**	
						d340d3	

Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
_3__ Membrane filter is transferred in its entirety to EC + MUG medium. **e.** SM 9222G/EPA 1105, 11: **E. coli by Nutrient Agar + MUG Membrane Filter Procedure** _1__ Membrane filter transferred in its entirety to NA + MUG medium (Note: Some colonies removed for LTB & BGLB tests.) _2__ Incubated at 35.0 ± 0.5 ºC for 4 hours _3__ 366-nm blue-light fluorescent halos around MF colonies confirm that Total Coliform is E. coli **f.** SM9020B, 9b: **Fecal Coliform by Membrane Filter method** _1__ Inoculate at least 10 colonies from filter into LTB _2__ Incubated at 35.0 ± 0.5 ºC for 24 hours (48 hours if no gas production after 24 hr.) _3__ Positive cultures from LTB (gas formation) inoculated into EC medium _4__ EC tubes incubated at 44.5 ± 0.2 ºC for 24 hours _5__ Positives verified monthly (by picking at least 10 blue colonies from one positive sample); and false negatives picked and verified (SM 9020B, 9.b.2) (Note: May inoculate m-FC colonies directly into EC medium. However, if gas is observed in LTB, but not in the corresponding EC tube, another EC tube must be inoculated & tested with the positive culture from the LTB tube.)						**d340e1** **d340e2** **d340e3** **d340f1** **d340f2** **d340f3** **d340f4** **d340f5**	
The calculations, data reduction and statistical interpretations specified by each method are followed. **a. 9221D** - Reported result as presence-absence test positive or negative for total coliforms in 100 mL of sample. **b. 9222B** - Compute the count, using membrane filters with 20 to 80 coliform colonies and not more than 200 colonies of all types per membrane, by the following equation: (Total) coliforms/100 mL = (coliform colonies counted x 100)/mL sample filtered **c. 9215B** - To compute the heterotrophic plate count, CFU/mL, divide total number of colonies or average number (if duplicate plates of the same dilution) per plate by the sample volume. **d. 9223B** - If performing an MPN procedure, calculate the MPN value for	M5,1.7.3.5					**00d326r**	

Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
total coliforms and *E. coli* from the number of positive tubes as described in Section 9221C. If using the presence-absence procedure, report results as total coliform and *E. coli* present or absent in 100-mL sample. **e. EPA 1600** – Compute the count per 100 mL of sample by dividing the # of enterococci colonies by the volume of sample filtered and then multiplying y 100. Refer to rules in Appendix B of method, too. For example, if there is > 1 dilution, calculate the arithmetic mean for those results in the acceptable counting range. **f. ISO 11731:2017 (E)** – For enumeration, select the plate or set of plates from the same culture showing the maximum number of confirmed colonies per water volume and taking any dilutions into account. Do not average the counts from different methods, treatments or culture media as these are not replicates. Calculate the # of colonies in original water per liter using the equations in section 9 for direct plating, MF, indirect filtration, and plating after dilution.								
The laboratory ensures that the quality of the reagents and media used is appropriate for the test concerned including, but not limited to, test conditions and incubation times.	M5,1.7.3.1					**00d328r**	
Culture media are prepared in the laboratory [ ] from different chemical ingredients if not commercially available or specified by the method, [ ] from commercial dehydrated powders, [ ] or purchased ready to use.	M5,1.7.3.1(b)					**00d329r**	
Reagents and commercial dehydrated powders are used within expiration date or the shelf life of the product provided by the manufacturer and documented according to this Standard.	M5,1.7.3.1(b)(ii)					**00d330r**	
Original containers of reagents and media are labeled with an expiration date.	M2,5.6.4.2(b)					**51026r**	
Distilled water, deionized water or reverse osmosis produced water **free from bactericidal and inhibitory substances** are used in the preparation of media solutions and buffers.	M5,1.7.3.1(d)(i)					**00d332r**	
The **quality of the water** (such as chlorine residual, specific conductance, total organic carbon, and heterotrophic bacteria plate count) is monitored (when in use): a__ on a monthly frequency, b__ when maintenance is performed on the water treatment system, or c__ at startup after a period of disuse longer than one month.	M5,1.7.3.1(d)(ii)					**00d333r**	

Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
Analysis for metals (Cd, Cr, Cu, Ni, Pb and Zn) and the bacteriological water quality test (to determine presence of toxic agents or growth promoting substances) are performed **annually**. Note: An exception to performing the bacteriological water quality test shall be given to laboratories that can supply documentation to show that their water source met the criteria, as specified by the method, for High Quality (Type I) or Medium Quality (Type II) reagent water.	M5,1.7.3.1 (d) (iii)					**00d333ar**	
Records are maintained on all laboratory **reagent water monitoring activities** as below. a_ Residual Chlorine < 0.1 mg/L (monthly) Conductivity < 2.0 umho/cm at 25 ºC (monthly) Heterotrophic Plate Count <1000 colony forming units per mL (monthly) Bacteriological ratio 0.8 – 3.0 (annual) e_Cd, Cr, Cu, Ni, Pb, Zn each < 0.05 mg/L, collectively < 0.1 mg/L (annual) f_ Records maintained for the past five years Total Organic Carbon < 1.0 mg/L (monthly) *(SM only)* h. Ammonia /organic nitrogen <0.1mg/L (monthly) i. Use test (new source) Student's t < or equal to 2.78 Note: Reagent water purchased from an outside source and used for the preparations of media, solutions and buffers shall meet the criteria specified above, too. Note: Refer to SM 18th Table 9020:I and 20th-23nd Table 9020:II 'QUALITY OF REAGENT WATER USED IN MICROBIOLOGY TESTING.'	M5,1.7.3.1(d)(iv) M5,1.7.3.1(d)(iii)					**0d334ar** **0d334br** **0d334cr** **0d334dr** **0d334er** **0d334fr** **0d334gr** **0d334hr** **0d334ir**	
The quality of dilution water, including buffer water and/or peptone water, is monitored for sterility, pH, and volume once per batch whether lab prepared or purchased.	M5,1.7.3.1 (E)					**0d334jr**	
Media, solutions and reagents are prepared, used and stored according to a documented procedure following the manufacturer's instructions or the test method as indicated below: **a.** ____ **Heterotrophic Plate Count Medium** (SM9215A, 6, SM9215B, 3a, SM9215C, 2-3, and SM9215D, 2-3): ___ Plate count agar autoclaved at 121 ºC for 15 minutes. R2A agar						**00d335r** **00d335a**	

Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
heated and sterilized at 121 ºC for 15 minutes. ___ Final pH 6.8-7.2 for Plate Count Agar, 7.2 for R2A Agar. ___ Sterile agar medium melted not more than once. ___ Melted agar used within 3 hours; agar tempered at 44-46 ºC before pouring. ___ A separate "temperature" container exposed to same heating and cooling as medium. (Do not depend on the sense of touch.) ___ Blood agar and chocolate agar are not used with dialysis related product water (ANSI/AAMI RD52:2004, 7.2.3 and RD62:2006, 5.1.1). **b. ____ Phosphate buffer** (SM9050C): ___ Stock buffer autoclaved at 121 C for 15 minutes. ___ Stock buffer final pH 7.2 ± 0.5 (SM9050C) ___ Dilution rinse water prepared from stock buffer & $MgCl_2$. ___ Sterility check on dilution rinse water with double-strength non-selective medium, 35 ºC, 24 hours. ___ A commercially available preparation can also be used.						**00d335b**	
c. ____ Peptone water (SM9050B, 1b): ___ 10% peptone stock solution autoclaved or filter sterilized. ___ 0.1% peptone water prepared as dilution rinse water. ___ Final pH 6.6-7.0. ___ Sterility check on dilution rinse water with double-strength non-selective medium, 35 ºC, 24 hours. **d. ____ m-Endo Medium** (SM9222B, 2): ___ Medium brought to a boil, but not boiled, removed immediately; and not autoclaved. ___ Ethanol used is not denatured. ___ Prepared in sterilized flask. ___ Final pH 7.0-7.4 for m-Endo Agar LES; 7.0-7.4 for m-Endo medium. ___ Uninoculated media discarded if growth or surface sheen observed. **e. ____ Lauryl Tryptose (Lauryl Sulfate) or Lactose Broth** (SM9221B, SM9222B, 1a): ___ Formulated so that concentration is single strength after sample addition. ___ Autoclaved at 121 ºC for 12-15 minutes. ___ Final pH 6.6-7.0. ___ Inverted vials in sterilized media, one-third to one-half covered by						**00d335c** **00d335d** **00d335e**	

Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
media, & free of air bubbles. **f.** ____ **Brilliant Green Lactose Bile Broth** (SM9221B, SM9222B, 2a): ___ Autoclaved at 121 ºC for 12-15 minutes. ___ Final pH 7.0-7.4.						**00d335f**	
Prepared **media is prepared, properly stored and used with in the holding time** so that: **a.** ____ Membrane filter broth in screw-cap flasks used within 96 hours and kept at 4 °C, **b.** ____ Membrane filter agar plates with tight-fitting covers used within 2 weeks and kept at 4 °C, ***Note: The expiration on pre-purchased plates for Legionella extend beyond 2 weeks from some manufacturers. Lab needs to maintain C of A for each lot.*** **c.** ____ Media in tubes or containers with loose-fitting closures used within 2 weeks and kept at 4 °C, **d.** ____ Broth media or agar in tightly closed screw-cap tubes or other sealed containers used within 3 months, **e.** ____ Poured HPC agar plates with loose-fitting covers sealed in plastic bags used within 2 weeks and kept at 4 °C, **f.** ____ HPC agar in tightly closed screw-cap flask or container used within 3 months and kept at 4 °C, **g.** ____ Tubes or plates with growth and/or bubbles discarded, and **h.** ____ Liquid medium with evaporation exceeding 10% of original volume discarded? **i.** ____ Refrigerated medium is warmed to room temperature before use.	[M5, 1.7.3.1 (b) (iii) (SM9020B, 4.i.4, Table 9020:IV):					**0d336ar** **0d336br** **0d336cr** **0d336dr** **0d336er** **0d336fr** **0d336gr** **0d336hr** **0d336i**	
Documentation for **media and reagents prepared in the laboratory** includes the following: a.___ Date of preparation, b.___ Preparer's initials, c.___ Type and amount of media prepared, d.___ Manufacturer and Lot #, e.___ Final pH of the media, and f.___ Expiration date	M5,1.7.3.1(f)					**0d337ar** **0d337br** **0d337cr** **0d337dr** **0d337er** **0d337fr**	
Documentation for **media purchased pre-prepared, ready-to-use** includes the following.	M5,1.7.3.1(f)						

Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
a.___ Manufacturer, b.___ Lot #, c.___ Type and amount of media received, d.___ Date of receipt e.___ Expiration date of the media, and f.___ pH of the media						**0d338ar** **0d338br** **0d338cr** **0d338dr** **0d338er** **0d338fr**	
All growth							
In order to demonstrate traceability and identity, the laboratory uses reference cultures of microorganisms obtained from a recognized national collection or an organization recognized by the NELAP Accrediting Authority.	M5,1.7.3.					**00d341r**	
Reference cultures are [ ] revived (if freeze dried) or [ ] transferred from slants and sub-cultured once to provide reference stocks.	M5,1.7.3.6(c)(i)					**00d342r**	
Microorganisms are [ ] single use preparations or [ ] cultures maintained by documented procedures that demonstrate the continued purity and viability of the organism.	M5,1.7.3.6(c)					**00d343r**	
The reference stocks are preserved by a technique that maintains the desired characteristics of the strains. (Examples of such methods are freeze-drying, liquid nitrogen storage and deep-freezing methods.)	M5,1.7.3.6(c)(i)					**00d344r**	
Reference stocks are used to prepare working stocks for routine work.	M5,1.7.3.6(c)(i)					**00d345r**	
When reference stocks are thawed, they are not re-frozen and re-used.	M5,1.7.3.6(c)(i)					**00d346r**	
Working stocks are not sequentially cultured more than 5 times.	M5,1.7.3.6(c)(ii)					**00d348r**	
Working stocks are not sub-cultured to replace reference stocks.	M5,1.7.3.6(c)(ii)					**00d349r**	
Work surfaces of fixtures and fittings are adequately sealed.	[M5,1.7.3.7(a)]					**00d353r**	
Work floors and work surfaces are non-absorbent and easy to clean and disinfect.	M5,1.7.3.7(a)					**00d354r**	
Work surfaces are adequately sealed.	M5,1.7.3.7(a)					**00d355**	
Measures are taken to avoid accumulation of dust by: a___ Providing sufficient storage space and	M5,1.7.3.7(a)					**0d355ar**	

Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
						0d355cr	
The temperature measurement devices have the appropriate quality needed to achieve the specification in the test method.	M5,1.7.3.7(b)(i)					**00d356r**	
The devices temperature calibration are verified to national or international standards at least annually.	M5,1.7.3.7(b)(i)					**00d357r**	
Thermometer verification is accomplished by a single point provided that it represents the method mandated temperature and use conditions.	M5,1.7.3.7(b)(i)					**0d357a**	
The graduation and range of the temperature measuring devices are appropriate for the required accuracy of measurement.	M5,1.7.3.7(b)(i)					**00d358**	
The stability of temperature, uniformity of temperature distribution and time required to achieve equilibrium conditions in **incubators, water baths** prior to first use after installation and service are established. The equilibrium check includes the time required after test sample addition to re-establish equilibrium conditions under full capacity load for intended use. Note: Position, space between and height of stacks of Petri dishes established. Dishes are not to be stacked more than 4 high. Simplate plates can be stacked higher than 4.	M5,1.7.3.7(b)(v)(a)					**00d359r** **00d359b**	
The performance of each **autoclave** is initially evaluated by establishing its functional properties. Note: Heat distribution characteristics established with respect to typical uses.	M5,1.7.3.7(b)(ii)(a)(1)					**00d360r**	
Autoclave(s) meet specified temperature tolerances. Note: Pressure cookers fitted only with a pressure gauge are not allowed for sterilization of media ..	M5,1.7.3.7(b)(ii)(a)(1)					**00d361r**	
Sterilization is demonstrated by continuous temperature recording devices or through the use of a maximum registering thermometer **with every cycle**.	M5,1.7.3.7(b)(ii)(a)(2)					**00d361a**	
Appropriate biological indicators are used at least **once each month** of use to determine effectiveness of sterilization.	M5,1.7.3.7(b)(ii)(a)(2)					**00d361b**	
The biological indicator used is effective at the sterilization temperature and time needed to sterilize lactose-based media.	M5,1.7.3.7(b)(ii)(2)					**00d361dr**	
Temperature sensitive tape is used with the contents of **each autoclave run**						**00d361c**	

Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
to indicate that the autoclave contents have been processed.	M5,1.7.3.7(b)(ii)(a)(2)						
The laboratory maintains records of autoclave operations for every cycle.	M5,1.7.3.7 (b)((ii)(a)(3)					**00d361er**	
Records of **autoclave** operations include the following: a.___ Date, b.___ Contents, c.___ Maximum temperature reached, d.___ Time in sterilization mode, e.___ Total run time (may be recorded as time in and time out), f.___ Analyst's initials, and g.___Pressure Note: At 121 °C, 10 min for membrane filters & pads; 12-15 min for carbohydrate-containing media; 30 min for contaminated materials and discarded cultures; 15 min for MF assemblies and empty sample collection bottles; 15 min for buffered dilution water	M5,1.7.3.7(b)(ii)(a)(3) Note: table 9020:III					**0d362ar** **0d362br** **0d362cr** **0d362dr** **0d362er** **0d362fr** **0d362gr**	
Autoclave maintenance is performed either internally or by service contract, **annually and records maintained**.	M5,1.7.3.7(b)(ii)(a)(4)					**00d363zr**	
The **annual** maintenance of the autoclave includes a pressure check and verification of the temperature device. Note: When it has been determined that the autoclave has no leaks, pressure checks can be calibrated using the formula PV=nRT	M5,1.7.3.7(b)(ii)(a)(4)					**0d363ar**	
The autoclave mechanical timing device is checked **quarterly** against a stopwatch and the actual time elapsed is recorded.	M5,1.7.3.7(b)(ii)(a)(5)					**0d363br**	
Volumetric equipment with movable parts such as automatic dispensers, dispensers/diluters, and mechanical hand pipettes are verified for accuracy **quarterly** and documented.	M5,1.7.3.7(b)(iii)(a)					**00d364r**	
Volumetric equipment such as filter funnels, bottles, non-Class A glassware, and other marked containers are verified **once per lot** prior to first use in the laboratory.	M5,1.7.3.7(b)(iii)(b					**00d365r**	
The volume of disposable volumetric equipment such as sample bottles, disposable pipettes, and micropipette tips are checked **once per lot**.	M5,1.7.3.7(b)(iii)(c)					**0d365ar**	
Verification of volume is within 2.5% of expected volume.	M5, 1.7.3.7 (b)(iii)(d)					**0d365br**	

Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
Note: This verification can be volumetric as compared to Class A or gravimetric.							
UV instruments, used for sanitization, are tested **quarterly** for effectiveness with an appropriate UV light meter or by plate count, or agar spread plates.	M5,1.7.3.7(b)(iv)					**00d366**	
Bulbs are replaced if output is less than 70% of original for light tests or if count reduction is less than 99% for a plate containing 200 to 300 organisms.	M5,1.7.3.7(b)(iv)					**0d366ar**	
The laboratory has a procedure for the calibration, verification, and Q.C. of support equipment according to the method specified requirements. (Note this includes conductivity meters, oxygen meters, pH meters, hygrometers, and other similar measurement instruments)	M5,1.7.1.1					**00d367r**	
Ovens used for sterilization are checked for sterilization effectiveness **monthly** with appropriate biological indicators.	M5,1.7.3.7(b)(ii)(b)					**0d367ar**	
Records are maintained for **each oven cycle** that include: a.___ Date, b.___ Cycle time, c.___ Temperature, d.___ Contents, and e.___ Analyst's initials	[M5,1.7.3.7(b)(ii)(b)					**d367bar** **d367bbr** **d367bcr** **d367bdr** **d367ber**	
Temperature sensitive tape with the contents of each oven run is used to indicate that the contents have been processed.	M5,1.7.3.7(b)					**d367bfr**	
The laboratory has a documented procedure for **washing labware**, if applicable. Note: Labware is glassware and plasticware.	M5,1.7.3.7(b)(vi)(a)					**0d367cr**	
Only **detergents** designed for laboratory use are used.	M5,1.7.3.7(b)(vi)(a)					**0d367dr**	
Glassware used is made of borosilicate or other non-corrosive material, free of chips and cracks, and it has readable measurement graduation marks.	M5,1.7.3.7(b)(vi)(b)					**0d367er**	
The laboratory tests **labware** that is washed and reused for the possible presence of residues which may inhibit or promote growth of microorganisms by performing the Inhibitory Residue Test **initially**, and **each time the lab changes** detergent formulation or washing procedures.	M5,1.7.3.7(b)(vi)(c)					**00d368**	
Each batch of washed **labware is tested at least once daily, each day of washing**, for possible acid or alkaline residue by testing one piece of glassware with a suitable pH indicator such as bromothymol blue, with a	M5,1.7.3.7(b)(vi)(d)					**00d369r**	

Relevant Aspect of Standards	NELAC2016 /ELAP/Regulation/Method Reference	Y	N	N/A	S	Codes	Comments
record of the test maintained.							