DoD/DOE QSM 6.0 Module 5 Microbiological Checklist

Checklists used for this assessment activity:

- ☐ **M1/M2 PT/QMS**
- ☐ **M3 Asbestos Testing**
- ☐ **M4 Chemical Testing**
- ☐ **M5 Microbiological Testing**
- ☐ **M6 Radiochemical Testing**
- ☐ **M7 Toxicity Testing**
- ☐ **M8 Industrial Hygiene Testing**

This checklist is only a tool, and not considered as the requirements of the standard(s)!

If there is a disagreement between this checklist and the standard(s), the standard(s) shall prevail.

Identify conformity for each requirement along with comments/objective evidence for each clause assessed.

A *clarifying statement* provides additional information to help understand a requirement.

A *permission* is an approach that a conformity assessment body can use to achieve compliance.

Assessment Number:

CAB Name:

Physical Address:

Assessment Date(s):

Assessors(s):

DoD/DOE QSM 6.0 Clause	Requirement	Conformity C / NC / NA	Comments/Objective Evidence
M5	Quality Systems for Microbiological Testing		
M5: 5.0	Method Validation Before acceptance and institution of any method for which data will be reported, are all methods validated?		
M5: 5.1	Accuracy Is at least one known pure positive reference culture used at the anticipated environmental conditions and compared to the method results to that of a reference method?		
M5: 5.2	Precision		

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	Are there at least 10 replicate analyses with both the proposed and reference method performed, using a sample containing the target microorganisms of choice? Do the results show that the precision of the proposed method is statistically equivalent or better than that of the reference method?		
M5: 5.3	Selectivity (sensitivity) Are all responses in at least 10 samples using mixed cultures that include the target organism(s) and at varying concentrations verified? Are the number of false positive and false negative results calculated?		
M5: 6.0	Demonstration of Capability (DOC)		
M5: 6.1	General		
M5: 6.1.1	Does an individual who performs any activity involved with preparation and/or analysis of samples have constant, close supervision (as defined in the laboratory's training procedure) until a satisfactory initial DOC is completed?		
M5: 6.1.2	Thereafter, are ongoing DOCs, performed and recorded at least every 12 months?		
M5: 6.1.3	In cases where an individual has prepared and/or analyzed samples using a method that has been in use by the laboratory for at least one year prior to applying for accreditation and where there have been no significant changes in instrument type or method, the ongoing DOC shall be acceptable as an initial DOC.		Clarifying Statement
M5: 6.1.3	Does the laboratory have records on file to demonstrate that an initial DOC is not required?		
M5: 6.2	Initial DOC		
M5: 6.2	Does everyone successfully perform an initial DOC prior to using any method, any time there is a change in instrument type or method that could potentially affect the precision and bias, sensitivity, or selectivity of the output, or any time that a method has not been performed by the individual in a 12-month period? Does the laboratory have a procedure for performing an initial DOC?		
M5: 6.2.1	Does the laboratory maintain records of each initial DOC in a manner such that the following information is readily available for each individual:		
M5: 6.2.1.a	individual(s) involved in preparation and/or analysis;		

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M5: 6.2.1.b	matrix;		
M5: 6.2.1.c	organism(s);		
M5: 6.2.1.d	identification of method(s) performed;		
M5: 6.2.1.e	identification of laboratory-specific procedures used for analysis, including revision number;		
M5: 6.2.1.f	date(s) of analysis; and		
M5: 6.2.1.g	summary of analyses, including information outlined in Section 6.2.2.c?		
M5: 6.2.2	If the method or regulation does not specify an initial DOC, the following procedure is acceptable.		Clarifying Statement
M5: 6.2.2	Does the laboratory document that other approaches to initial DOC are adequate?		
M5: 6.2.2.a	Is the target organism(s) diluted in a volume of sterile, quality system matrix (a sample in which no target organisms or interferences are present at concentrations that will impact the results of a specific method)? When required by method, is the diluent sterile buffered water and/or sterile peptone water unless specified by the manufacturer? Are at least four aliquots prepared at the concentration specified, or if unspecified, to the countable range for plate methods or working range for most probable number (MPN) type methods?		
M5: 6.2.2.b	Are at least four aliquots prepared and analyzed concurrently according to the method?		
M5: 6.2.2.c	Using all the results, are these results converted to logarithmic values, then the mean recovery and standard deviation of the log converted results in the appropriate reporting units calculated for each organism of interest? When it is not possible to determine mean and standard deviations, such as for presence/absence, does the laboratory assess performance against established and documented criteria?		
M5: 6.2.2.d	For qualitative tests, acceptable performance in a blind study, either internally or externally generated, may be used to meet this standard, provided that the study consists of a minimum of a blank, a negative culture, and a positive culture for each target organism.		Permission
M5: 6.2.2.e	Is the information from c) above compared to the corresponding acceptance criteria for precision and accuracy in the method (if applicable) or in laboratory-generated acceptance criteria such as relative standard		

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	deviation (if there are not established mandatory criteria)?		
M5: 6.2.2.e	If all parameters meet the acceptance criteria, the analysis of actual samples may begin. If any one of the parameters does not meet the acceptance criteria, the performance is unacceptable for that parameter.		Permission
M5: 6.2.2.f	When one or more of the tested parameters fail at least one of the acceptance criteria, does the individual proceed according to i) or ii) below.		
M5: 6.2.2.f.i	Is the source of the problem located and corrected and the initial DOC for all parameters of interest beginning with b) above repeated?		
M5: 6.2.2.f.ii	Is the initial DOC for all parameters that failed to meet criteria repeated?		
M5: 6.2.2.g	A repeat failure confirms a general problem with the measurement system. If this occurs, is the source of the problem located and corrected and the test repeated for all organisms of interest beginning with b) above?		
M5: 6.3	Ongoing DOC		
M5: 6.3.1	Does the laboratory have a documented procedure describing ongoing DOC that includes how the laboratory will identify data associated with ongoing DOCs? Does the individual demonstrate ongoing capability by routinely meeting the QC requirements of the method, laboratory procedure, customer specifications, and/or this standard? If the method has not been performed by the individual in a 12-month period, is an initial DOC performed prior to performing analysis? Does the laboratory document other approaches to ongoing DOC?		
M5: 6.3.2	Does this ongoing demonstration include one of the following:		
M5: 6.3.2.a	another initial DOC;		
M5: 6.3.2.b	analysis of one sample of clean matrix that is fortified with a known quantity of the target organism, with results meeting the laboratory acceptance criteria for accuracy and, where applicable to the testing technique, also meeting the observational details expected for the presumptive, confirmed and completed phases defined in the method;		

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M5: 6.3.2.c	analysis of one positive sample in duplicate for each target organism and test, with results meeting the laboratory acceptance criterion for precision;		
M5: 6.3.2.d	acceptable results for a blind proficiency test sample or sample set, as required by the program, for target organisms in each field of accreditation;		
M5: 6.3.2.e	performance of an alternate adequate procedure for the field of accreditation, the procedure and acceptance criteria being documented in the laboratory's quality system;		
M5: 6.3.2.f	following a procedure for reviewing records of QC samples meeting the QC requirements of the method, laboratory procedure, customer requirements, and/or this standard; or.		
M5: 6.3.2.f	A review of these records may be used to identify patterns and determine if implementation of the nonconforming work process and/or retraining is necessary.		Clarifying Statement
M5: 6.3.2.g	if a) through e) are not technically feasible, then does the laboratory perform analysis of real-world samples with results within predefined acceptance criteria (as defined by the laboratory or method)?		
M5: 7.0	Technical Requirements		
M5: 7.1.1	Calibration Does the laboratory have documented procedures for calibration, verification, and QC of auxiliary equipment including conductivity meters, oxygen meters, pH meters, hygrometers, and other similar measurement instruments? Do these procedures refer to applicable reference methods?		
M5: 7.1.2	For instruments that are continuous monitors, such as in-line specific conductance meters:		
M5: 7.1.2.a	Does the laboratory verify calibration at least once a month and retain records? and		
M5: 7.1.2.b	If a calibration verification is unacceptable, or when the instrument is being returned to service after having been taken off-line, is an initial calibration performed?		
M5: 7.2	Continuous Calibration Reserved for specific procedures.		Clarifying Statement
M5: 7.3	Quality Control		
M5: 7.3.1	Quality and Sterility of Standards, Reagents, Materials, and Media		

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	Does the laboratory demonstrate 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? Are records maintained?		
M5: 7.3.1.a	Sterility checks – Are all materials and supplies that are needed to process the sample and required to be sterile prior to use (whether sterilized in the laboratory or purchased as sterilized) checked by the laboratory once per purchased or prepared lot using non-selective growth media as appropriate? Are certificates of analysis provided by vendors verified by the laboratory and retained? Do these checks include, but are not limited to:		
M5: 7.3.1.a.i	Does the laboratory perform a sterility check for each lot of prepared, ready-to-use, media and on each batch of media prepared in the laboratory?		
M5: 7.3.1.a.i.a	For chromo/fluorogenic media: is media added to sterile deionized water and incubated at the appropriate temperature and time?		
M5: 7.3.1.a.i.b	For all other media, is uninoculated media incubated at the appropriate temperature and time? Where media are made as concentrates (e.g., double strength), is the medium then diluted to working strength with sterile deionized water before testing?		
M5: 7.3.1.a.ii	Does the laboratory perform a sterility check on one funnel per lot of pre-sterilized single use funnels using non-selective growth media? Does the laboratory perform a sterility check on one funnel per batch of laboratory-sterilized funnels, using non-selective growth media perform a sterility check for each lot of prepared, ready-to-use, media and on each batch of media prepared in the laboratory?		
M5: 7.3.1.a.iii	Does the laboratory perform a sterility check on at least one container for each lot of purchased, pre-sterilized sample containers with non-selective growth media? Does the laboratory perform a sterility check on one container/object per sterilization batch sterilized in the laboratory with nonselective growth media?		
M5: 7.3.1.a.iv	Does the laboratory perform a sterility check on each batch of dilution water prepared in the laboratory and		

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	on each lot of pre-prepared, ready-to-use dilution water with non-selective growth media? Is the concentration of the non-selective growth media single strength after the addition of dilution water?		
M5: 7.3.1.a.v	Does the laboratory perform a sterility check on at least one filter from each new lot of membrane filters with nonselective growth media?		
M5: 7.3.1.b	Media – Culture media may be prepared from commercial dehydrated powders or may be purchased ready-to-use.		Permission
M5: 7.3.1.b.i	Are all media tested for performance (e.g., for selectivity, sensitivity, sterility, growth promotion, and growth inhibition)? Are these tests performed at a minimum with first use?		
M5: 7.3.1.b.ii	Does the laboratory use all media within the expiration date or shelf-life provided by the manufacturer?		
M5: 7.3.1.b.iii	Does the laboratory use all laboratory-prepared media within the holding time limits specified in the accredited method?		
M5: 7.3.1.b.iv	Does the laboratory have detailed testing criteria information defined in the laboratory's methods, procedures, or similar documentation?		
M5: 7.3.1.c	Does the laboratory use reagents, media, and commercial dehydrated powders within the shelf-life of the product and maintain records?		
M5: 7.3.1.d	Reagent Water		
M5: 7.3.1.d.i	Does the laboratory monitor the quality of the reagent water used in the laboratory, which will encounter test organisms and is used in preparation of media, solutions, and buffers, for bactericidal and inhibitory substances? Is water distilled water, deionized water, or reverse-osmosis-produced water?		
M5: 7.3.1.d.ii	Does the laboratory monitor the quality of the water for disinfectant residual, specific conductance, total organic carbon, and heterotrophic bacteria plate count monthly (when in use), when maintenance is performed on the water treatment system, or at startup after a period of disuse longer than one month? Is analysis performed by another certified laboratory?		
M5: 7.3.1.d.iii	Does the laboratory monitor the quality of the water 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) annually?		

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M5: 7.3.1.d.iii	An exception to performing the Bacteriological Water Quality Test shall be given to laboratories that can supply records to show that its water source meets the criteria, as specified by the method, for High Quality (Type I) or Medium Quality (Type II) reagent water.		Clarifying Statement
M5: 7.3.1.d.iii	Analysis may be performed by another certified laboratory.		Permission
M5: 7.3.1.d.iv	Do results of the above analyses meet the specifications of the required method?		
M5: 7.3.1.d.v	Does reagent water purchased from an outside source and used for the preparations of media, solutions and buffers meet the criteria specified in items ii) and iii) above? Does the laboratory maintain records of this information?		
M5: 7.3.1.d.vi	Is reagent water that has been opened for longer than the testing intervals specified in items i) through iv), or in the accredited method, either re-tested or discarded?		
M5: 7.3.1.e	Does the laboratory monitor the quality of the dilution water for sterility, pH and volume once per lot or batch whether purchased or prepared by the laboratory?		
M5: 7.3.1.f	Do records for media and reagents prepared in the laboratory include date of preparation, preparer's initials, type, manufacturer, lot number, final pH, expiration date, and the amount of reagents used? Do records for media purchased pre-prepared, ready-to-use (including reagent water purchased from outside sources) include manufacturer, lot number, type of media received, date of receipt, expiration date of the media, and pH of the media? Are records maintained?		
M5: 7.3.2	Method Blanks Does the laboratory demonstrate that the filtration equipment and filters, sample containers, media, and reagents have not been contaminated through improper handling or preparation, or environmental exposure?		
M5: 7.3.2.a	For filtration technique, does the laboratory conduct method blanks per the analytical method? At a minimum, do the filtration series include a beginning and ending blank?		

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M5: 7.3.2.a	The filtration series may include single or multiple filtration units, which have been sterilized prior to beginning the series.		Permission
M5: 7.3.2.b	Is the filtration series considered ended when more than 30 minutes elapses between successive filtrations? During a filtration series, are filter funnels rinsed with three 20-30 ml portions of sterile rinse water after each sample filtration? In addition, does the laboratory insert a method blank after every 10 samples or sanitize filtration units by UV light (254 nm) after sample filtration?		
M5: 7.3.2.c	For pour plate technique, are method blanks of the medium 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: 7.3.3	Test Variability/Reproducibility For methods that specify counts, such as membrane filter, plated media or other methods which specify a quantitative result, are duplicate counts performed monthly on one positive sample for each month that the test is performed? If the laboratory has two or more analysts, does each analyst count typical results on the same sample? Are counts within ten percent (10%) difference to be acceptable? In a laboratory with only one microbiology analyst, is the same sample counted twice by the analyst, with no more than a five percent (5%) difference between the counts?		
M5: 7.3.4	Sample-Specific Controls (where applicable)		
M5: 7.3.4.a	Does the laboratory perform matrix spikes per method requirements?		
M5: 7.3.4.b	Does the laboratory perform sample matrix duplicates per method requirements?		
M5: 7.3.5	Data Reduction Are calculations, data reduction and statistical interpretations specified by each method identified and followed?		
M5: 7.3.6	Selectivity		

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M5: 7.3.6.a	Do all growth and recovery media checked to assure that the target organism(s) respond in an acceptable and predictable manner once per lot or batch?		
M5: 7.3.6.b	To ensure that analysis results are accurate, is target organism identity verified as specified in the method?		
M5: 7.3.6.c	To ensure identity and traceability, are reference cultures used for positive and negative controls obtained from a recognized national collection, organization, or manufacturer recognized by the accreditation body? Are microorganisms, either single-use preparations or cultures maintained for their intended use, maintained by documented procedures that demonstrate the continued purity and viability of the organism?		
M5: 7.3.6.c.i	Reference cultures may be revived (if freeze-dried) or transferred from slants and sub-cultured once to provide reference stocks.		Clarifying Statement
M5: 7.3.6.c.i	Are the reference stocks preserved by a technique that maintains the characteristics of the strains? Are reference stocks used to prepare working stocks for routine work? If reference stocks have been thawed, are they not refrozen and re-used?		
M5: 7.3.6.c.ii	Are working stocks not sequentially cultured more than five times and are not sub-cultured to replace reference stocks?		
M5: 7.3.6.d	Culture Controls (i.e., working cultures)		
M5: 7.3.6.d.i	Negative Culture Controls		
M5: 7.3.6.d.i.a	Do negative culture controls demonstrate that the medium does not support the growth of non-target organisms or not exhibit the typical positive reaction of the target organism(s)?		
M5: 7.3.6.d.i.b	Is each pre-prepared, ready-to-use lot of selective medium (including chromo/fluorogenic reagent), and each batch of selective medium prepared in the laboratory, analyzed with one or more known negative culture controls, appropriate to the method? Is this done prior to first use of the medium?		
M5: 7.3.6.d.ii	Positive Culture Controls		
M5: 7.3.6.d.ii.a	Do positive culture controls demonstrate that the medium can support the growth of the target organism(s), and that the medium produces the specified or expected reaction to the target organism(s)?		

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M5: 7.3.6.d.ii.b	Is each pre-prepared, ready-to-use lot of medium (including chromo/fluorogenic reagent) and each batch of medium prepared in the laboratory tested with at least one or more known pure positive culture controls (i.e., target organism) as appropriate to the method and that produce typical results based on the method? Is this done prior to first use of the medium?		
M5: 7.3.7	Selectivity		
M5: 7.3.7.a	Laboratory Facilities Are floors and work surfaces non-absorbent and easy to clean and disinfect? Are work surfaces adequately sealed? Does the laboratory provide sufficient storage space, clean and free from dust accumulation?		
M5: 7.3.7.b	Laboratory Equipment		
M5: 7.3.7.b.i	Temperature Measuring Devices Does the laboratory use temperature measuring devices such as liquid-in-glass thermometers, thermocouples, or platinum-resistance thermometers to assess equipment temperatures? Are records maintained? Are the temperature measuring devices appropriate quality to meet specification(s) in the method? Are the graduation and range of the temperature measuring devices appropriate for the required accuracy of the measurement? Are temperature measuring devices verified to national or international standards for temperature? Is verification performed at least annually? Is this verification accomplished by a single point if it represents the method mandated temperature and use conditions?		
M5: 7.3.7.b.ii	Sterilization Equipment		
M5: 7.3.7.b.ii.a	Autoclaves		
M5: 7.3.7.b.ii.a.1	Does the laboratory evaluate the performance of each autoclave initially by establishing its functional		

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	properties and performance, for example, heat distribution characteristics with respect to typical uses? Do autoclaves meet specified temperature tolerances? Are pressure cookers not used for sterilization of growth media?		
M5: 7.3.7.b.ii.a.2	Does the laboratory demonstrate proper sterilization temperature by use of a continuous temperature recording device or by use of a maximum registering thermometer with every cycle? Does the laboratory, at least once during each month that the autoclave is used, demonstrate the effective sterilization using appropriate biological indicators? Is the selected biological indicator effective at the sterilization temperature and time needed to sterilize lactose-based media? Does the laboratory use temperature-sensitive tape with the contents of each autoclave run to indicate that the autoclave contents have been processed?		
M5: 7.3.7.b.ii.a.3	Does the laboratory maintain records of autoclave operations for every cycle? Do records include: date, contents, maximum temperature reached, pressure, time in sterilization mode, total run time (may be recorded as time in and time out), and analyst's initials?		
M5: 7.3.7.b.ii.a.4	Is autoclave maintenance, internally or by service contract, performed annually, and include a pressure check and verification of temperature device? Are records of the maintenance maintained?		
M5: 7.3.7.b.ii.a.4	When it has been determined that the autoclave has no leaks, pressure checks can be made using the formula PV = nRT.		Clarifying Statement
M5: 7.3.7.b.ii.a.5	Does the laboratory check the autoclave mechanical timing device quarterly against a stopwatch and record the actual time elapsed?		
M5: 7.3.7.b.ii.b	Ovens Does the laboratory check ovens used for sterilization for sterilization effectiveness monthly with appropriate biological indicators?		

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	Does the laboratory maintain records for each cycle that include date, cycle time, temperature, contents, and analyst's initials? Does the laboratory use temperature sensitive tape with the contents of each run to indicate that the contents have been processed?		
M5: 7.3.7.b.iii	Volumetric Equipment Does the laboratory verify equipment used for measuring volume as follows:		
M5: 7.3.7.b.iii.a	Is equipment with movable parts, such as automatic dispensers, dispensers/diluters, and mechanical hand pipettes, verified for accuracy quarterly?		
M5: 7.3.7.b.iii.b	Is equipment, such as filter funnels, bottles, non-Class A glassware, and other containers with volumetric markings (including sample analysis vessels), verified once per lot prior to first use?		
M5: 7.3.7.b.iii.c	Is the volume of the disposable volumetric equipment, such as sample bottles and disposable pipettes, checked once per lot?		
M5: 7.3.7.b.iii.d	Is verification of volume considered acceptable if the accuracy is within 2.5% of expected volume?		
M5: 7.3.7.b.iii.d	This verification may be volumetric as compared to Class A or gravimetric.		Clarifying Statement
M5: 7.3.7.b.iv	UV Instruments Does the laboratory evaluate UV instruments used for sanitization quarterly for effectiveness with an appropriate UV light meter, by plate count, agar spread plates, or other methods providing equivalent results, such as UV-cide strips? Does the laboratory replace bulbs 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: 7.3.7.b.v	Incubators, Water Baths		
M5: 7.3.7.b.v.a	Does the laboratory establish the uniformity of temperature distribution and equilibrium conditions in incubators and water baths prior to first use after installation or service? Do the equilibrium check include time required after test sample addition to re-establish equilibrium conditions under full capacity load appropriate for the intended use?		
M5: 7.3.7.b.v.b	During periods when samples are under test, does the laboratory have a system in place to monitor and		

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	record the temperature of incubators and water baths twice daily, at least four hours apart? Are records maintained?		
M5: 7.3.7.b.v.b	"Under test" is defined as the time period that the sample is in the incubation phase of the method. Data loggers, continuous temperature monitoring devices, or other temperature monitoring equipment may be used as long as they can be calibrated.		Permission
M5: 7.3.7.b.vi	Labware (Glass and Plasticware)		
M5: 7.3.7.b.vi.a	Does the laboratory have a documented procedure for washing labware, if applicable? Are detergents designed for laboratory use, used?		
M5: 7.3.7.b.vi.b	Is glassware made of borosilicate or other non-corrosive material, free of chips and cracks, and have readable measurement marks?		
M5: 7.3.7.b.vi.c	Is labware that is washed and reused 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?		
M5: 7.3.7.b.vi.d	Is washed labware tested at least once daily, each day of washing, for possible acid or alkaline residue by testing at least one piece of labware with a suitable pH indicator such as bromothymol blue? Are records of tests maintained?		
M5: 7.4	Data Acceptance/Rejection Criteria		
M5: 7.4	Are methods criteria and evaluation methods used?		
M5: 7.5	Sample Handling		
M5:7.5	Does the receipt of samples comply with Module 2, as well as:		
M5: 7.5.1	Are microbiological samples from known chlorinated sources (such as wastewater effluent), unknown sources where disinfectant (e.g., chlorine) usage is suspected (such as a new customer or a new source), and all potable water supplies (including source water) checked for absence of disinfectant residual in the laboratory unless all of the following conditions are met:		
M5: 7.5.1.a	The laboratory can show that the received sample containers are from its laboratory or have been appropriately tested and recorded;		
M5: 7.5.1.b	Sufficient sodium thiosulfate was in each container before sample collection to neutralize at minimum 5 mg/L of chlorine for drinking water and 15 mg/L of chlorine for wastewater samples;		

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M5: 7.5.1.c	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 recorded; and		
M5: 7.5.1.d	Disinfectant residual is checked in the field and actual concentration is provided with sample submission?		