

## C205b – APPENDIX CHECKLIST: ENVIRONMENTAL MICROBIOLOGY TESTING LABORATORIES

May 2006

The following pages present the criteria from the NELAC Appendix D requirements in a checklist format as they relate to microbiological testing. The laboratory’s policies and procedures must meet these requirements. Quality system documentation and supporting records must be available for the assessor’s review.

Requirement	Reference	{RESERVED FOR ASSESSORS ONLY}			Comments
		Compliance			
		Y	N	NA	
<b>D.3 MICROBIOLOGY TESTING</b>					
These standards apply to laboratories undertaking microbiological analysis of environmental samples.					
Microbiological testing refers to and includes the detection, isolation, enumeration and identification of microorganisms and their metabolites or confirmation of the absence of growth in materials and media.					
<b>D.3.1 Sterility Checks and Blanks, Positive and Negative Controls</b>					
a) Sterility Checks and Blanks					
The laboratory shall demonstrate that the filtration equipment and filters, sample containers, media and reagents have not been contaminated through improper handling or preparation, inadequate sterilization, or environmental exposure.					

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1) A sterility blank shall be analyzed for each lot of pre-prepared, ready-to-use medium (including chromofluorogenic reagent) and for each batch of medium prepared in the laboratory. This shall be done prior to first use of the medium.					
2) For filtration technique, the laboratory shall conduct one beginning and one ending sterility check for each laboratory sterilized filtration unit used in a filtration series. The filtration series may include single or multiple filtration units, which have been sterilized prior to beginning the series. For pre-sterilized single use funnels a sterility check shall be performed on one funnel per lot. The filtration series is considered ended when more than 30 minutes elapses between successive filtrations. During a filtration series, filter funnels must be rinsed with three 20-30 ml portions of sterile rinse water after each sample filtration. In addition, laboratories must insert a sterility blank after every 10 samples or sanitize filtration units by UV light after each sample filtration.					
3) For pour plate technique, sterility blanks of the medium shall be 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.					
4) Sterility checks on sample containers shall be performed on at least one container for each lot of purchased, pre-sterilized containers. For containers prepared and sterilized in the laboratory, a sterility check shall be performed on one container per sterilized batch with nonselective growth media.					

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5) A sterility blank shall be performed on each batch of dilution water prepared in the laboratory and on each batch 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 shall be checked for sterility with nonselective growth media.					
b) Positive Controls					
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).					
1) Each pre-prepared, ready-to-use lot of medium (including chromofluorogenic reagent) and each batch of medium prepared in the laboratory shall be tested with at least one pure culture of a known positive reaction. This shall be done prior to first use of the medium.					
c) Negative Controls					
Negative culture controls demonstrate that the medium does not support the growth of non-target organisms or does not demonstrate the typical positive reaction of the target organism(s). Each pre-prepared, ready-to-use lot of selective medium (including chromofluorogenic reagent) and each batch of selective medium prepared in the laboratory shall be analyzed with one or more known negative culture					

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controls, i.e. non-target organisms, as appropriate to the method. This shall be done prior to first use of the medium.					
<b>D.3.2 Test Variability/Reproducibility</b>					
For test methods that specify colony counts such as membrane filter or plated media, duplicate counts shall be performed monthly on one positive sample, for each month that the test is performed. If the lab has two or more analysts, each analyst shall count typical colonies on the same plate. Counts must be within 10% difference to be acceptable. In a laboratory with only one microbiology analyst, the same plate shall be counted twice by the analyst, with no more than 5% difference between the counts.					
<b>D.3.3 Method Evaluation</b>					
a) Laboratories are required to demonstrate proficiency with the test method prior to first use. This shall be achieved by comparison to a method already approved for use in the laboratory, or by analyzing a minimum of ten spiked samples whose matrix is representative of those normally submitted to the laboratory, or by analyzing and passing one proficiency test series provided by an approved proficiency sample provider. The laboratory shall maintain this documentation as long as the method is in use and for at least 5 years past the date of last use.					
b) Laboratories shall participate in the Proficiency Test programs identified by NELAP (5.4.1.5k or 5.5.9.1), The results of these analyses shall be used to evaluate the ability of the laboratory to produce acceptable data					

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and the Proficiency Testing Requirements documents (See Appendix A).					
<b>D.3.4 Test Performance</b>					
a) All growth and recovery media must be checked to assure that the target organisms respond in an acceptable and predictable manner (see D.3.1.b).					
b) To ensure that analysis results are accurate, target organism identify shall be 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.					
<b>D.3.5 Data Reduction</b>					
The calculations, data reduction and statistical interpretations specified by each test method shall be followed.					
<b>D.3.6 Quality of Standards, Reagents and Media</b>					
The laboratory shall ensure that the quality of the reagents and media used is appropriate for the test concerned.					
a) Culture media may be prepared from commercial dehydrated powders or may be purchased ready-to-use. Media may be prepared by the laboratory from basic ingredients when commercial media are not available or when it can be demonstrated commercial media do not provide adequate results. Media prepared by the laboratory from basic ingredients must be tested for performance (e.g., for selectivity, sensitivity, information must be defined in either the laboratory's					

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test methods, SOP's, Quality Manual, or similar documentation.					
b) Reagents, commercial dehydrated powders and media shall be used within the shelf-life of the product and shall be documented according to 5.5.6.4.					
c) Distilled water, deionized water or reverse-osmosis produced water free from bactericidal and inhibitory substances shall be used in the preparation of media, solutions and buffers. The quality of the water shall be monitored for chlorine residual, specific conductance, and heterotrophic bacteria plate count on a monthly frequency (when in use), when maintenance is performed on the water treatment system, or at startup after a period of disuse longer than one month.					
Analysis for metals and the Bacteriological Water Quality Test (to determine presence of toxic agents or growth promoting substances) shall be performed annually. Results of these analyses shall be maintained for five years. (An exception to performing the Bacteriological Water Quality Test shall be given to laboratories that can supply documentation to show that their water source meets the criteria, as specified by the method, for Type I or Type II reagent water).					
d) Media, solutions and reagents shall be prepared, used and stored according to a documented procedure following the manufacturer's instructions or the test method. Documentation for media prepared in the laboratory shall include date of preparation, preparer's initials, type and amount of media prepared, manufacturer and lot number, final pH of the media, and expiration date. Documentation for media purchased pre-prepared, ready-to-use shall include manufacturer,					

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lot number, type and amount of media received, date of receipt, expiration date of the media, and pH of the media.					
<b>D.3.7 Selectivity</b>					
In order to ensure identity and traceability, laboratories shall use reference cultures for positive and negative controls shall be obtained from a recognized national collection or an organization recognized by a NELAP Accrediting Authority, if available. Microorganisms may be single use preparations or cultured maintained by documented procedures that demonstrate the continued purity and viability of the organism.					
1) Reference cultures may be revived (if freeze-dried) or transferred from slants and subcultured once to provide reference stocks. Appropriate purity and biochemical checks shall be made with the reference stocks and documented. The reference stocks shall be preserved by a technique which maintains the characteristics of the strains. Examples of such methods are freeze-drying, liquid nitrogen storage and deep-freezing. Reference stocks shall be used to prepare working stocks for routine work. If reference stocks have been thawed, they must not be re-frozen and re-used.					
2) Working stocks shall not be sequentially cultured more than five times.					
3) Working stocks shall not be subcultured to replace reference stocks.					

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<b>D.3.8 Constant and Consistent Test Conditions</b>					
a) Laboratory facilities					
Floors and work surfaces shall be non-absorbent and easy to clean and disinfect. Work surfaces shall be adequately sealed. Laboratories shall provide sufficient storage space, and shall be clean and free from dust accumulation. Plants, food, and drink shall be prohibited from the laboratory work area.					
b) Laboratory Equipment					
1) Temperature measuring devices					
i) Temperature measuring devices such as liquid-in-glass thermometers, thermocouple, platinum resistance thermometers used in incubators, autoclaves and other equipment shall be the appropriate quality to meet specification(s) in the test method. The graduation of the temperature measuring devices must be appropriate for the required accuracy of measurement and they shall be calibrated to national or international standards for temperature. Calibration shall be done at least annually.					
2) Autoclaves					
i) The performance of each autoclave shall be initially evaluated by establishing its functional properties and performance, for example heat distribution					

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characteristics with respect to typical uses. Autoclaves shall meet specified temperature tolerances. Pressure cookers fitted only with a pressure gauge are not recommended for sterilization of media.					
ii) Demonstration of sterilization temperature shall be provided by use of continuous temperature recording device or by use of a maximum registering thermometer with every cycle. Appropriate biological indicators shall be used once per month to determine effective sterilization. Temperature sensitive tape shall be used with the contents of each autoclave run to indicate that the autoclave contents have been processed.					
iii) Records of autoclave operations shall be maintained. This shall be done for every cycle. Records shall 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.					
iv) Autoclave maintenance, either internally or by service contract, shall be performed annually and shall include a pressure check and calibration of temperature device. Records of the maintenance shall be maintained in equipment logs.					
v) The autoclave mechanical timing device shall be checked quarterly against a stopwatch and the actual time elapsed documented.					
3) Volumetric equipment					
Volumetric equipment shall be calibrated as follows:					

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i) Equipment with movable parts such as automatic dispensers, dispensers/diluters, and mechanical hand pipettes shall be verified quarterly for accuracy. ii) Equipment such as filter funnels, bottles, non-class A glassware, and other marked containers shall be calibrated once per lot prior to first use. iii) The volume of the disposable volumetric equipment such as sample bottles, disposable pipettes, and micropipette tips shall be checked once per lot.					
4) UV Instruments					
i) UV Instruments, used for sanitization, shall be tested quarterly for effectiveness with an appropriate UV light meter or by plate count agar spread plates. Replace bulbs if output is less than 70% of the original for light tests or if count reduction is less than 99% for a plate containing 200 to 300 organisms.					
5) Conductivity meters, oxygen meters, pH meters, hygrometers, and other similar measurement instruments					
i) Conductivity meters, oxygen meters, pH meters, hygrometers, and other similar measurement instruments shall be calibrated according to the method specified requirements (see Section 5.5.5.2.1d).					

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6) Incubators, Water Baths, Ovens					
i) The stability and uniformity of temperature distribution and time required after test sample addition to re-establish equilibrium conditions in incubators and water baths shall be established. Temperature of incubators and water baths shall be documented twice daily, at least four hours apart, on each day of use.					
ii) Ovens used for sterilization shall be checked for sterilization effectiveness monthly with appropriate biological indicators. Records shall be maintained for each cycle that include date, cycle time, temperature, contents and analyst's initials.					
7) Labware (Glassware and Plasticware)					
i) The laboratory shall have a documented procedure for washing labware, if applicable. Detergents designed for laboratory use must be used.					
ii) Glassware shall be made of borosilicate or other non-corrosive material, free of chips and cracks, and shall have readable measurement marks.					
iii) Labware that is washed and reused shall be tested for possible presence of residues which may inhibit or promote growth of microorganisms by performing the Inhibitory Residue Test annually, and each time the lab changes the lot of detergent or washing procedures.					
iv) Washed labware shall be tested at least once daily, each day of washing, for possible acid or alkaline					

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residue by testing at least one piece of labware with a suitable pH indicator such as bromothymol blue. Records of tests shall be maintained.					