MODULE 2D
ENVIRONMENTAL MICROBIOLOGICAL LABORATORY ACCREDITATION PROGRAM (EMLAP) ADDITIONAL REQUIREMENTS

2D.1 SCOPE

The AIHA Laboratory Accreditation Programs (AIHA LAP), LLC's Environmental Microbiological Laboratory Accreditation Program (EMLAP) is intended for accreditation of microbiological laboratories specializing in the analysis of microorganisms commonly detected in air (e.g., spore trapping), surface (e.g., tape lifts, swabs, wipes), and bulk (e.g., dust, liquids, building materials) samples collected from schools, hospitals, offices, industrial, agricultural and other work environments. Laboratory accreditation in this program is based upon a review of the laboratory management systems as defined in Module 2A, this program specific module, and successful participation in AIHA PAT, LLC Programs EMPAT program (www.aihapat.org) or an equivalent proficiency testing program approved by AIHA LAP, LLC, as defined in Module 6.

Available FoTs and corresponding PT for the EMLAP shall meet the requirements detailed in the EMLAP section of the *Scope/PT Table* maintained on the AIHA LAP, LLC web site (www.aihaaccreditedlabs.org).

2D.2 FACILITIES

2D.2.1 The laboratory shall have a documented routine monitoring program to verify adequate contamination control. The laboratory shall have proper facilities for biological and chemical storage and disposal of waste.

NOTE: The most current biosafety level guidelines are defined by the Centers for Disease Control (CDC), the World Health Organization (WHO), and the AIHA LAP, LLC.

2D.3 EQUIPMENT

2D.3.1 General

2D.3.1.1 The laboratory shall utilize a microscope/magnification system suitable for performing the methods in use at the laboratory (e.g., capable of the magnifications required).

2D.3.1.1.1 The microscope/magnification system for non-fluorescence microscopy shall consist of one of the following:

a) A compound optical microscope having a high magnification (e.g., 100x) liquid immersion objective having a numerical aperture (n.a.) of at least 1.25; or,
b) An optical microscope having a theoretical or calculated point to point resolution at 0.34 µm or better. The resolution is calculated as follows: 1.22 x 0.55 µm/ [condenser n.a. + objective n.a.]; or,
c) A magnification system having a measured optical resolution of 0.34µm or better. For example, the optical resolution may be measured with resolution target testing slides.

2D.3.1.1.2 Each non-fluorescence microscope shall have an ocular micrometer which is

checked annually with a stage micrometer.

2D.3.1.1.3 A microscope used for fluorescence microscopy shall have a non- immersion objective of at least 40X magnification, and shall be used in conjunction with oculars of at least 10X magnification.

2D.3.1.1.4 The alignment of each microscope/magnification system shall be documented for each day of use.

2D.3.1.2 The laboratory shall have a reference library appropriate to the FoT(s) to be accredited.

2D.3.1.3 The laboratory shall utilize a molecular detection system suitable for performing the methods in use at the laboratory (e.g. qPCR machine for performing real-time qPCR tests, plate reader for ELISA, etc.)

2D.3.2 Additional Requirements for All Culturable FoTs

2D.3.2.1 The laboratory shall have a Class II biological safety cabinet (BSC) whose performance has been certified by a NSF accredited field certifier according to NSF Standard 49 field requirements (or national equivalent outside the U.S.) Annual certification is required.

2D.3.2.2 The laboratory shall have a steam sterilizer (autoclave) with functioning temperature and pressure gauges or a contract with a biohazard waste disposal company for the disposal of potentially viable waste.

2D.3.2.2.1 Laboratories with steam sterilizers shall use indicators to document successful sterilization with each use.

2D.3.2.2.2 Laboratories with steam sterilizers shall use biological indicators (e.g. spore strips or ampoules) with each use or at least once a week, whichever is less to document the sterilization process.

2D.3.2.3 The laboratory shall have incubators, refrigerators and freezers with temperature settings appropriate for the scope of work performed at the laboratory.

2D.4 ANALYTICAL METHODS

2D.4.1 General

The laboratory shall have written Standard Operating Procedures (SOPs), in accordance with the requirements of Module 2A, for the following: processing and analysis of samples; determining analytical sensitivities for each quantitative or semi-quantitative method; appropriate retention, waste treatment and disposal of environmental microbial samples; the identification of fungi and/or bacteria; identification of fungal spores and structures; and biosafety and decontamination for the applicable FoT(s).

2D.4.2 Additional Requirements for Air Fungal Direct Examination FoT

Analytical methods shall include a description of sample trace analysis, scope magnification, counting rules, percentage of trace analyzed and calculations.

2D.4.3 Additional Requirements for Molecular FoT

2D.4.3.1 Analytical methods shall include a description of the primer/probe combinations, the master mix formulation, the thermal cycling program including temperatures and number of cycles, and/or antibody antigen combinations.

2D.4.3.2 To each run of samples the following QC shall be included:

2D.4.3.2.1 One Laboratory Control Sample (LCS) or one per every 20 samples, whichever is greater.

2D.4.3.2.2 One duplicate analysis per every 20 samples, whichever is greater.

2D.4.3.2.3 One reagent blank sample analysis or one reagent blank sample analysis per every 20 samples, whichever is greater.

2D.5 QUALITY ASSURANCE/QUALITY CONTROL

Routine QA/QC procedures shall be an integral part of laboratory procedures and functions. The laboratory Quality Assurance program shall address the elements in Module 2A, Section 2A.8.2.1 and shall also include the following additional elements.

2D.5.1 General

2D.5.1.1 Compliance with acceptable quality assurance and quality control guidelines for microbiology laboratories, such as APHA-AWWA-WPCF guidelines in *Standard Methods for the Examination of Water and Wastewater*, *The Manual of Environmental Microbiology*, or equivalent national guidelines for foreign laboratories.

2D.5.1.2 To assess precision, intra-analyst analyses shall be completed at a minimum of five (5) percent, or at least one (1) each month samples are received, whichever is greater, for each Field of Testing for which the laboratory is accredited, except for Molecular FoTs (see 2D.4.3.2 for requirements specific to Molecular FoTs).

2D.5.1.3 To assess accuracy**,** inter-analyst analyses shall be completed at a minimum frequency of five (5) percent or at least one (1) each month samples are received, whichever is greater, for each Field of Testing for which the laboratory is accredited except for Molecular FoTs (see 2D.4.3. for requirements specific to Molecular FoTs).

2D.5.1.4 The laboratory shall use control charts or quality control databases to compare intra- and inter-analyst analysis performance to established control limits.

2D.5.1.5 The laboratory shall ensure quality control of culture media and analytical reagents per lot number for appropriate sterility, microbial growth and/or analytical reactions. Records shall be maintained. Acceptance criteria shall be documented.

2D.5.1.6 Acceptance criteria on 5%intra-analyst and inter-analyst analyses, daily reference slide analysis (spore traps) and monthly reference culture analysis (all culturable FoTs) shall be documented. Acceptance criteria shall include:

a) Taxon identification acceptability
b) Taxon abundance ranking acceptability
c) Count or concentration acceptability determined statistically (quantitative QC analysis only)

2D.5.2 Additional Laboratory Requirements for All Culturable FoTs

2D.5.2.1 The laboratory shall keep routine temperature documentation of refrigerators, freezers and incubators. Acceptance criteria shall be documented.

2D.5.2.2 The laboratory shall maintain a microbial culture collection of common organisms relevant to the applicable FoT(s). Cultures shall be from recognized sources when possible. Source and date of acquisition for each culture shall be documented. Procedures for maintaining the cultures and using them for training and QC purposes shall be available.

2D.5.2.3 The culture collection shall be used at least monthly to prepare blind cultures to be used as part of the routine QC program to monitor accuracy in culture identification.

2D.5.3 Additional Requirements for Fungal Direct Examination FoTs

2D.5.3.1 A slide collection shall consist of field samples with various count levels and genera/groups of spores shall be maintained and used as part of total spore analysis quality control. Each day of analysis, at least one slide from this collection shall be reviewed by each analyst. Analysis shall be consistent with the method for field samples. Slides shall be reviewed on a rotational schedule such that a different slide is reviewed each day until the entire slide collection has been examined. The analysis of these slides shall be incorporated into the daily QC plan. Acceptance criteria for spore concentration(s) for each reference slide shall be stated. The upper and lower control limits shall be statistically calculated based on three (3) standard deviations from the reference slide means.

2D.5.3.2 For the Fungal Direct Examination Air FoT, the laboratory shall participate in and have documentation of a round robin slide exchange consistent with the requirements of AIHA LAP, LLC Policy Module 6. The following are additional requirements:

2D.5.3.2.1 Analytical data shall include raw counts and final concentrations for each fungal structure observed.

2D.5.3.2.2 Acceptance criteria shall be determined and take into account organism identification, ranking and quantification.

2D.5.3.3 The traverse width or field of view to be used in calculations for each microscope shall be documented at least annually, if applicable.

2D.5.4 Additional Requirements for Molecular FoT's

2D.5.4.1 The laboratory shall maintain a collection of positive controls (either cultures or DNA extracts), antigen/antibody combinations for the molecular tests it provides. Source and date of acquisition for each shall be documented. Procedures for maintaining the cultures

and/or reagents and using them for training and QC purposes shall be available.

2D.6 REPORTING THE RESULTS

The laboratory's results shall address the elements in Module 2A, Section 2A.7.8 and shall also include the following additional elements:

2D.6.1 Reports shall include raw counts. See definition of "Raw Count" in Module 9 – Terms and Acronyms.

2D.6.2 For quantitative results, the analytical sensitivity shall be stated in the final reporting units. See definition of "Analytical Sensitivity" in Module 9 – Terms and Acronyms.

2D.6.2.1 For analyses utilizing multiple dilutions and/or varying percentages of sample and/or trace analyzed, the applicable analytical sensitivities shall be reported.

2D.7 SAFETY, HEALTH, ENVIRONMENTAL AND TRANSPORTATION REGULATIONS

Laboratories accredited under EMLAP are expected to follow jurisdictional regulations regarding safety, health, environment or transportation. Potentially viable microbial waste shall be collected in properly designated biohazard containers and disposed of properly, either by autoclaving, sterilizing, or incinerating, or by contracting with a biohazard waste disposal company. Failure to comply with applicable jurisdictional regulations regarding safety, health, environment or transportation may result in suspension, denial, or withdrawal of EMLAP accreditation.