EPA Consequence Management Advisory Division's Bioanalytical Laboratory: Capabilities and Collaborations

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Introduction

The CMAD Bioanalytical Laboratory, located at the EPA National Enforcement Investigations Center (NEIC) in Lakewood, Colorado, is a certified Biosafety Level 2 Enhanced (BSL-2E) facility. Unknown environmental samples can be accepted in this laboratory for the analysis of biological select agents or toxins (BSATs). The laboratory is self-contained with isolated air supply, exhaust, and liquid waste biotank systems. The laboratory is equipped to provide molecular detection of pathogens as well as traditional microbiological culture methods. Analytical equipment includes two real-time quantitative PCR (qPCR) thermal cyclers used for detection of pathogen-specific gene expression and an electrochemiluminescence (ECL) instrument which uses immunoassay technology to detect specific biological toxins/proteins. The laboratory also possesses a large capacity microbiological incubator capable of holding 300 bacterial media plates for high throughput analysis.

The CMAD Bioanalytical Laboratory provided support to two EPA/Department of Homeland Security (DHS) collaborations. For the Underground Transportation Restoration-Operational Technology Demonstration (UTR-OTD), the Bioanalytical Laboratory analyzed over 300 samples of surface material and gravel ballast wash for the presence of a *B. anthracis* surrogate organism. The laboratory also provided support for the NYC Subway Tracer Particle and Gas Releases for the Underground Transport Restoration (UTR) Project. The laboratory assisted in improving detection methods and optimizing the qPCR analytical protocol prior to particle deployment in the NYC subway system, and following deployment, processed air filters, swabs, gauze wipes, and cloth wipes by DNA extraction and qPCR analysis, resulting in over 250 total samples analyzed for the entire project.

Currently, the laboratory is working on implementing two additional analytical capabilities. Due to the increase in ricin toxin incidents, the laboratory is in the process of developing methods to detect ricin toxin in environmental samples using ECL technology. Additionally, the laboratory is presently collaborating with EPA National Homeland Security Research Center and Office of Water to verify the *B. anthracis* Rapid Viability-PCR method. With the unique facility design and the multiple analytical capabilities, the CMAD Bioanalytical Laboratory can support EPA's mission to protect human health during an emergency response as well as provide a high level of support during studies which seek to improve pathogen method detection.

BSL-2+ LABORATORY CERTIFICATION 2017 TO 2018
U.S. Environmental Protection Agency
National Enforcement Investigations Center (NEIC)
Denver Federal Center, Building 25

*This is to certify that the **U.S. Environmental Protection Agency, NEIC BSL-2+ Laboratory,** located at the Denver Federal Center, Building 25, has met the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories Guidelines (5th Ed.) as surveyed by Council Rock Consulting, Inc. The Principal Investigator is responsible for operation and maintenance of the facility in accordance with the guidelines. The laboratory requires annual certification. The date for re-certification is on or before October 3, 2018.*

COUNCIL ROCK CONSULTING, INC

Certified by:
Theodore J. Traum, P.E.
Date of Survey: October 3, 2017

Biosafety Level 2 Enhanced Certification Requirements:

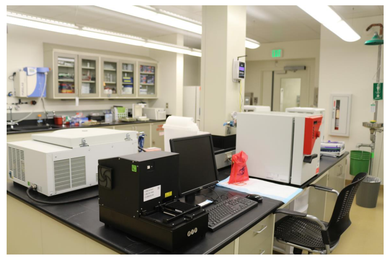
- Biosafety designations and requirements are outlined in the *CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition*
- Typically, this designation means BSL-3 Practices in a BSL-2 facility.
  - No manipulation of culture/sample outside of the biosafety cabinet, in-facility decontamination/sterilization.
- No airflow reversals during HVAC and power failure scenarios.
- Designated laboratory-specific Biosafety Manual and Decontamination SOP.
- Restricted laboratory access.
- Annual certification of containment/sterilization equipment.
- Review of laboratory and facility design plans.
- Visual inspection of laboratory facility.
- Review of laboratory SOPs and operating manual

CMAD BSL-2E Bioanalytical Laboratory Equipment and Capabilities

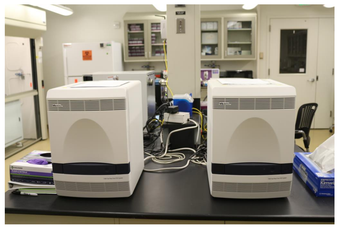
Designed to be self-contained and separate from the rest of the facility as it has its own air supply, exhaust, and liquid waste bio-tank.

Equipped with molecular and microbiological equipment including:

- Two ABI 7500 Fast Real-Time PCR thermal cyclers for specific gene expression analysis
- Janus automated workstation allows for high throughput screening and automated plating/pipetting
- AirClean PCR workstation provides a separate, clean chamber to minimize DNA contamination
- IKA microbial culture shaker is self-contained and environmentally controlled for optimal bacterial growth conditions
- Bead-beater and sonicator are small equipment used for sample extraction of environmentally resistant pathogens
- Qubit fluorometer instantly quantifies DNA, RNA, and protein samples
- Class II biosafety cabinet has a self-contained 100% exhaust with HEPA filtration
- Sensaphone monitoring system notifies laboratory users of power outages and freezer/incubator temperature fluctuations
- Pass-through two door autoclave provides in-laboratory waste sterilization to prevent facility contamination and secondary waste decontamination ensures "cradle to grave" disposal
- Large capacity microbiological incubator allows for high throughput analysis of bacterial media plates
- Cell culture incubator is water-jacketed and can accommodate CO2 tank addition for cell culture needs
- MSD PR2 1800 Electrochemiluminescence (ECL) machine identifies specific biological toxin and proteins, compatible with Department of Defense detection methods for ricin toxin



Upcoming Projects



RV-PCR *B. anthracis* Method

The CMAD Bioanalytical Laboratory is currently collaborating with members from EPA National Homeland Security Research Center (NHSRC) and Office of Water (OW) to verify the *B. anthracis* Rapid Viability-PCR method. Using an anthrax surrogate and qPCR technology, the laboratory will evaluate multiple sampling devices, determine limits of detection, and ensure that this laboratory will be ready to accept environmental samples for analysis in the event of an anthrax release.

Ricin Toxin Detection Using ECL

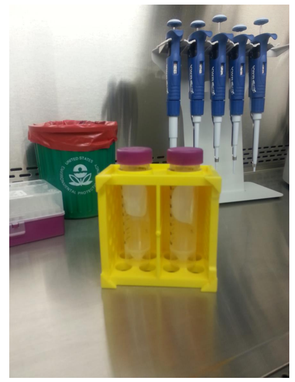
Due to the increase in ricin toxin events, there is a need to establish a capability within the EPA that can be used to detect ricin toxin in environmental samples. Using electrochemiluminescence (ECL) technology developed by MesoScale Defense and assays evaluated by the Department of Defense, the CMAD Bioanalytical Laboratory will test multiple sampling devices and examine sample recovery in the presence of environmental inhibitors to ensure that this laboratory will be ready to accept environmental samples for analysis in the event of a ricin toxin incident.

DHS S&T/EPA CMAD Collaborations:

NYC Subway UTR Project:

The CMAD Bioanalytical Laboratory provided support for two Department of Homeland Security Science and Technology Directorate/EPA collaborations. Both collaborations were a part of the Underground Transport Restoration (UTR) project, a four-year long project intended to develop sample collection techniques, improve pathogen detection methods, and optimize rapid decontamination procedures to return a subway system to normal operation following contamination with a human pathogen such as anthrax, at the same time minimizing the impact on humans and the environment.

In May of 2016, led by MIT Lincoln Lab, DNATrax molecules (developed at LLNL), small maltodextrin spheres containing barcoded DNA, were released in the NYC Subway system. EPA On Scene Coordinators collected more than 8000 samples over the week-long study. Prior to this field study, the CMAD Bioanalytical Laboratory worked with researchers at MIT LL and LLNL to improve detection methods and optimize the qPCR analytical protocol. Following the sample collection, the laboratory processed air filters, swabs, gauze wipes, and cloth wipes by DNA extraction and qPCR analysis, resulting in over 250 total samples analyzed for the entire project.



UTR-OTD Project:

The Underground Transportation Restoration (UTR) Operational Technology Demonstration (OTD) was conducted at Fort A.P. Hill's Asymmetric Warfare Training Center (AWTC) to evaluate decontamination techniques that could be used in the event of an anthrax incident in a subway system. Using an anthrax surrogate, the study consisted of two rounds of background sampling, surrogate release, decontamination, sampling, waste removal decontamination, and post-decontamination sampling.

The CMAD Bioanalytical Laboratory received over 300 samples of surface material and gravel ballast wash during the five-week study. Each sample was evaluated for the presence of the anthrax surrogate using microbial culture, resulting in over 4000 bacterial media plates analyzed by the laboratory. The laboratory was able to return results rapidly within the week that the samples were received providing near real-time results.

Acknowledgements:

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