



CMAD's Biosafety Level 2 Enhanced Laboratory

In support of EPA's Homeland Security Mission, CMAD maintains a certified Biosafety Level 2 Enhanced Laboratory (BSL-2E). The CMAD laboratory operations and capabilities have been incorporated into the contract supporting EPA's mobile PHILIS laboratory to allow a well-developed technical and facility management team to support laboratory staff and operations.



Gene expression analysis can be performed using the laboratory's two real-time PCR thermal cyclers.

- CDC/NIH Biosafety and Biomedical Laboratories Guidelines (5th Edition) compliant for BSL-2E laboratory operations.
- 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.

The CMAD BSL-2E laboratory is supplied with molecular- and micro-biological equipment including:

<ul style="list-style-type: none"> • Two ABI 7500 fast real-time PCR machines <ul style="list-style-type: none"> ✓ Specific gene expression analysis ✓ 96-well plate analysis in under an hour
<ul style="list-style-type: none"> • Janus automated workstation <ul style="list-style-type: none"> ✓ High throughput screening ✓ Automated plating/pipetting
<ul style="list-style-type: none"> • AirClean PCR workstation <ul style="list-style-type: none"> ✓ Separate "clean" station to minimize DNA contamination
<ul style="list-style-type: none"> • IKA microbial culture shaker <ul style="list-style-type: none"> ✓ Self-contained and environmentally controlled
<ul style="list-style-type: none"> • Bead beater and Sonicator <ul style="list-style-type: none"> ✓ Used for sample extraction of environmentally resistant pathogens
<ul style="list-style-type: none"> • Qubit fluorometer <ul style="list-style-type: none"> ✓ Instant quantification of DNA, RNA and protein samples
<ul style="list-style-type: none"> • Class II biosafety cabinet <ul style="list-style-type: none"> ✓ HEPA filtration with self-contained exhaust
<ul style="list-style-type: none"> • Pass-through two door autoclave <ul style="list-style-type: none"> ✓ In-laboratory waste sterilization to prevent facility contamination ✓ Secondary waste decontamination to ensure "cradle to grave" disposal
<ul style="list-style-type: none"> • Microbiological incubator <ul style="list-style-type: none"> ✓ Large capacity for high throughput analysis of bacterial media plates
<ul style="list-style-type: none"> • Large Cell Culture Incubator <ul style="list-style-type: none"> ✓ Water-jacketed, can accommodate CO2 tank addition for cell culture needs.
<ul style="list-style-type: none"> • MSD PR2 1800 Electrochemiluminescence (ECL) machine <ul style="list-style-type: none"> ✓ Biological toxin and protein detection ✓ Compatibility with DoD detection methods for ricin toxin



Certified Biosafety Cabinet and Chemical Fume Hood that houses a Janus Automated Workstation for high throughput sample processing.



ECL machine capable of detecting biological toxins and proteins.

Currently, the CMAD BSL-2E laboratory can test for the presence of BSL-1 and BSL-2 pathogens by traditional microbial culture and/or DNA extraction followed by real-time PCR analysis. Recent studies that the laboratory supported included the EPA/DHS UTR-OTD and the DHS NYC Subway Tracer Study. Future capabilities include the RV-PCR detection method for live *B. anthracis* as well as the ECL detection method for ricin toxin.

Located at the Denver Federal Center (within EPA's NEIC) in Lakewood, Colorado
 For additional information contact the PHILIS Contract Project Officer
 David Bright, USEPA – CMAD OEM, (913) 551-7897



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Current Analytical Capabilities

Pathogen	BSL	Detection Method	Samples Analyzed/24 hours ¹
Cryptosporidium	2	qPCR ²	~40
Giardia	2	qPCR	~40
Toxoplasma	2	qPCR	~40
<i>E. coli</i>	1	qPCR/culture	~40
<i>E. coli</i> OH:157	2	qPCR	~40
Legionella spp.	2	qPCR	~40
<i>Listeria monocytogenes</i>	2	qPCR	~40
<i>B. globigii</i> (anthrax surrogate)	1	qPCR/culture	~40/60
<i>B. thuringiensis</i> (anthrax surrogate)	1	qPCR/culture	~40/60
Microbial Source Tracking	1	qPCR	~40

¹ PCR reagents/DNA extraction kits for some pathogens would have to be acquired prior to testing
typical lead time 7-10 days for reagent acquisition

² qPCR detection indicates presence of pathogen DNA, cannot distinguish between live/dead organisms

BSL - biosafety level

qPCR - qualitative real-time PCR

Note: If commercially available DNA extraction kits and known qPCR primers/probes exist for a BSL-1 or BSL-2 organism, the CMAD BSL-2E Laboratory can accept and test environmental samples for that organism.

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