BioBriefcase

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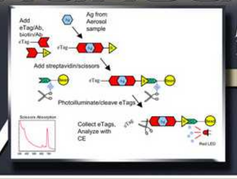
The BioBriefcase is an autonomous, broad-spectrum bioagent detector developed jointly by Lawrence Livermore and Sandia National Laboratories for the detection of aerosolized biological agents in the environment.

The system incorporates an aerosol collector to concentrate sub-10 µm particles into a 1 ml volume, which is distributed to a sample archive and three automated sample analysis trains. All three analysis trains—immunoassay, PCR and reverse-transcriptase PCR—utilize an eTag reporter-based multiplex assay to detect a broad range of threat agents.

Automated decontamination of the system between analyses permits continuous autonomous operation. Tests of the prototype instrument, performed as part of the BAND Phase II program at Edgewood Chemical and Biological Center, indicate excellent performance in a series of blind challenges with both liquid and aerosolized gamma killed Bacillus anthracis.

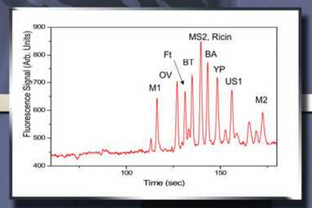
Immunoassay



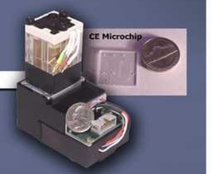
Monogram eTag Immunoassay

The immunoassay train is based on a microfluidic assay that releases eTags for indicated agents upon binding and photoactivated cleavage, then detects the released eTags by capillary electrophoresis with laser-induced fluorescence detection (488 nm excitation).

Flow-Through Immunoassay Reactor



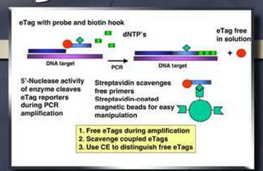
eTag detection of OV, BA, BG, FT, MS2, YP



µChemLab CE platform

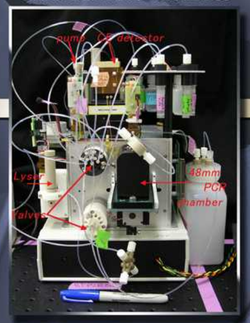
PCR Assay

The nucleic acid assay train is based on a microfluidic assay that releases eTags for indicated agents upon PCR, then detects the released eTags by capillary electrophoresis with laser-induced fluorescence detection (488 nm excitation).

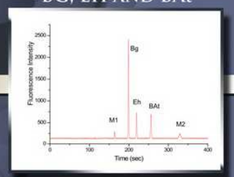


eTag Reporter Assay for Nucleic Acids

Lyser & Flow-through PCR chamber and fluidics



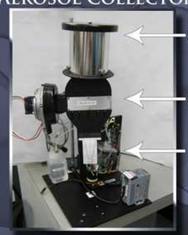
eTag PCR analysis of BG, EH and BAt



µChemLab CE platform

Aerosol

Aerosol Collector



BAND Design Requirements

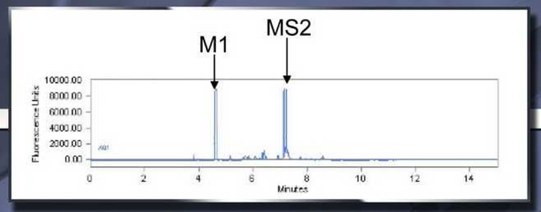
Performance Targets	Cost Targets
Continuous autonomous operation.	≤$25k (1k units)
Sample every four hours.	≤$10K/Y
Ability to simultaniously analyze for a minimum of 20 agents.	
LOD 100 organisms 10 ng toxin.	
False positive 10^{-7} – 10^{-8}.	
Archive capability (5d).	
One month maintenance interval.	
Indoor and outdoor operation.	
2-8 ft³	

RT-PCR Assay

Reverse transcriptase PCR (RT-PCR) occurs on the same platform as DNA PCR. Samples are purified, concentrated, and amplified on a packed bed with eTags analyzed on CE. A one step RT-PCR mix with an initial incubation step for the RT enzyme are implemented on the flow through PCR platform.

Flow-through RT-PCR system

MS2 amplification with eTag



µChemLab CE platform

HOMELAND SECURITY

BAND System Testing

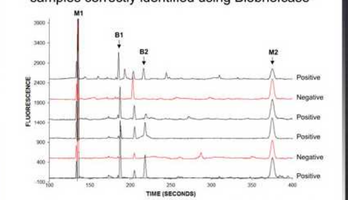
Testing conducted at US Army Edgewood Chemical and Biological Center

- Laboratory testing (20 blind samples) –
  - Liquid sample (400 µl) was drawn automatically into the instrument and analyzed
  - Data analysis software generated the detection calls (positive or negative)
- Aerosol chamber testing (18 blind samples) –
  - Aerosolized sample was produced using an ink-jet aerosol generator directly into the BBC aerosol collector
  - Collection continued for 5 additional minutes, then the collection bowl fluid was pumped into the instrument and analyzed
- LLNL and SNL staff performed initial instrument set-up and system maintenance
- ECBC personnel performed all system operations associated with the testing protocols

Summary of testing results

- Correctly identified 33 of 38 samples (87%) over the two series of blind challenge tests
- Reasons for incorrect calls were immediately identified and correctable
  - False negatives: Mechanical failure (PCR heater) and reagent lot issue
  - False positives: Random noise peak assigned as B1 peak
    - Signal averaging, use of B2 give correct call
- Ambient temperature variations shift CE peak times; accounted for using internal standards
  - Independent study of temperature effects indicates a minimum of two internal standards are required for accurate peak calling
  - Fielded instrument will be environmentally controlled

Aerosol chamber testing results for 6 sequential samples correctly identified using Biobriefcase



Samples contained two eTag reporters for B. anthracis: B1 and B2. M1 and M2 are electrophoretic markers used to correlate migration times with database species.

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