Design of a Novel, Multi-port-addressable Bioaerosol Collection System\textsuperscript{1,2}

2004 Joint Service Scientific Conference on Chemical & Biological Defense Research

Trina Vian
MIT Lincoln Laboratory
tvian@ll.mit.edu

\textsuperscript{1}This work was sponsored by the Department of the Air Force under Air Force Contract #F19628-00-C-0002. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the United States Government.

\textsuperscript{2}This work was sponsored by the Department of Homeland Security – Homeland Security Advanced Research Projects Agency. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the United States Government.
**Report Documentation Page**

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

<table>
<thead>
<tr>
<th>1. REPORT DATE</th>
<th>2. REPORT TYPE</th>
<th>3. DATES COVERED</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 NOV 2004</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4. TITLE AND SUBTITLE</th>
<th>5a. CONTRACT NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Design of a Novel, Multi-portaddressable Bioaerosol Collection System</td>
<td></td>
</tr>
<tr>
<td>1,2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>6. AUTHOR(S)</th>
<th>5b. GRANT NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</th>
<th>5c. PROGRAM ELEMENT NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIT Lincoln Laboratory</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>8. PERFORMING ORGANIZATION REPORT NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>10. SPONSOR/MONITOR’S ACRONYM(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>11. SPONSOR/MONITOR’S REPORT NUMBER(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>12. DISTRIBUTION/AVAILABILITY STATEMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approved for public release, distribution unlimited</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>13. SUPPLEMENTARY NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>See also ADM001849, 2004 Scientific Conference on Chemical and Biological Defense Research. Held in Hunt Valley, Maryland on 15-17 November 2004., The original document contains color images.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>14. ABSTRACT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>15. SUBJECT TERMS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>16. SECURITY CLASSIFICATION OF:</th>
<th>17. LIMITATION OF ABSTRACT</th>
<th>18. NUMBER OF PAGES</th>
<th>19a. NAME OF RESPONSIBLE PERSON</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. REPORT unclassified</td>
<td>UU</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>b. ABSTRACT unclassified</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. THIS PAGE unclassified</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

 prescribed by ANSI Std Z39-18
Difficulties with Current Samplers

Filter-based Sampling

- Limitations:
  - Target organisms desiccated during collection
  - Suboptimal recovery efficiency
  - Labor-intensive post-collection processing

Other Methods

- Limitations:
  - Sample still needs to be transferred
  - Suboptimal recovery efficiency
  - Possibly labor-intensive post-collection processing

Viable Sampling

- Limitations:
  - Short sampling duration
  - Culture results may take days to weeks
  - Growth media selection bias
Air Collection System Requirements

• High collection and extraction efficiency in the 1 – 10 micron particle-size range

• A robust collection scheme that offers reconfigurable sample collections at any time interval from nominally 3 to 12 hours

• A collection scheme that preserves viability, for even fragile vegetative cells and viruses

• A self-sealing mechanism that renders the collected samples safe for retrieval, handling and transport

• A collection format optimized for compatibility with standard assays such as PCR while reducing subsequent laboratory processing requirements
Aerosol Collector Concept

- Use impaction collection into coated 96-well PCR plates
  - PCR plate can be directly assayed at processing laboratory
  - Sample does not require elution, sample preparation or transfer
  - Mineral oil coating enhances capture efficiency, maintains sample viability and serves as an evaporation barrier for PCR
  - Time resolution of sample is achieved through serial activation of the rows of the plate
Impaction into Uncoated PCR Tubes

**Design features**

- > 50% collection/extraction efficiency for 1 - 10 µm particles based on particle counts and bio-assays
- no sample transfer required (particles immobilized in reaction well)
- expect mineral-oil coating to increase collection efficiency

**Graph**

- X-axis: Aerodynamic Diameter (µm)
- Y-axis: Collection Efficiency

**Legend**

- red line: fit
- square: avg collection efficiency

**Diagram**

- Impaction nozzle protrudes deep into the PCR tube
Preliminary Design Parameters

• Aerosol Inlet Design
  – Disperse particles evenly over 1 x 12 impaction manifold

• Flow Rate for Impaction Nozzles
  – Design for flow rate of 3 lpm/nozzle
  – Collection efficiency greater than 50%

• Sealing Mechanism
  – Integrate plate sealer into overall design
  – Facilitate ease of handling and transport for the operator

• Communication and Control
  – Remotely readable and modifiable user parameters such as sampling interval, sampler location and system status
  – Valve actuation by controller
PCR Plate Impaction Test Fixture

- 1 x 8 nozzle array test fixture
  - 1 row of plate used at a time
  - Manifold and plate holder have registration features to aid alignment

aerosol in

inlet array

PCR plate

impactor

\[ d_{\text{nozzle}} = 1.6\text{mm} \]

\[ s = 1.6\text{mm} \]
Notional System Design

- **Air collector subsystems***
  - Inlet stack
  - Impaction nozzle manifold *(8 x 12 array)*
  - PCR plate and receptacle
  - Valve bank
  - Exhaust manifold
  - Pump (not shown)
  - Viable sampler (not shown)

- **Mechanical subsystems**
  - Drawer assembly
    - Drawer, plate shuttle, pneumatic spring, cam, lock
  - Valve bank

- **Electrical**
  - Communication and control
    - User interface, pump and valve switching, power supplies
  - Environmental conditioning (not shown)

- **Plate Sealing**
  - Adhesive film roller (not shown)

*All tubing omitted for clarity*
Plate Loading
Example PCR Plate Layout

Agent tested: A, B, etc

Time-resolved air collects into each well

In lab, add agent specific DNA as positive controls to give a PCR $C_T$ of 25

No air sample

PCR controls (neg. and pos.) for each primer set
Standard PCR Reaction – No Agent Present

Agent tested: A, B, etc

Time-resolved air collects into each well

No air sample

PCR controls (neg. and pos.) for each primer set

In lab, add agent specific DNA as positive controls to give a PCR C<sub>T</sub> of 25

PCR Cycle Threshold Key

- Red
- Orange
- Yellow
- Green
- White

22, 25, 28, 31, 34, ND
Other Possibilities and Interpretations

- Daily knowledge of system performance
- Effect of inhibitors (with time resolution)
- False positive and negative rates
- Confirmation of true positives in control wells
- Simple plate layout for lab-based error reduction

**Normal PCR?**

**Attack during T2**

- Correlation in positive controls, above $C_T=25$

**PCR Cycle Threshold Key**

- Red: Inhibited
- Orange: False positive
- Yellow: False negative
- Green: No air control
- Black: Normal PCR
- 22, 25, 28, 31, 34, ND: Cycle Thresholds
Summary

High collection and extraction efficiency in the 1 – 10 micron particle-size range

\[ \varepsilon_{\text{collection}} > 50\% \]
\[ \varepsilon_{\text{extraction}} \sim 100\% \]

A robust collection scheme that offers reconfigurable sample collections at any time interval from ~ 3 to 12 hours

Timed valving allows for user-defined collection interval

A collection scheme that preserves viability, for even fragile vegetative cells

Mineral oil coated impaction substrate keeps organisms viable

A self-sealing mechanism that renders the collected samples safe for handling, retrieval and transport

Film seal isolates sample

A collection format optimized for compatibility with PCR and designed to minimize the subsequent laboratory processing requirements

PCR plate usage allows for easy pipetting of reagents

MIT Lincoln Laboratory
This work is currently being funded by the US Department of Homeland Security – Homeland Security Advanced Research Projects Agency
Collector Close Up

- Impaction nozzle manifold (with luer slip-fit hubs)
- Inlet manifold
- Gasket
- PCR plate (wells coated with mineral oil)
- Impaction nozzles (COTS dispensing needles)
- Exhaust ports