Design of a Novel, Multi-port-addressable Bioaerosol Collection System[1,2]

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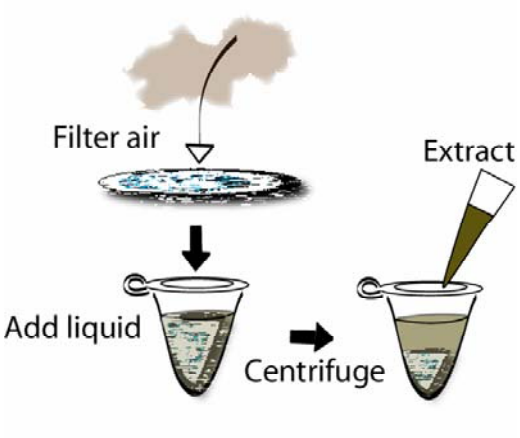
Difficulties with Current Samplers

Filter-based Sampling



Limitations:

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

Viable Sampling

Limitations:

- Short sampling duration
- Culture results may take days to weeks
- Growth media selection bias

Other Methods

Limitations:

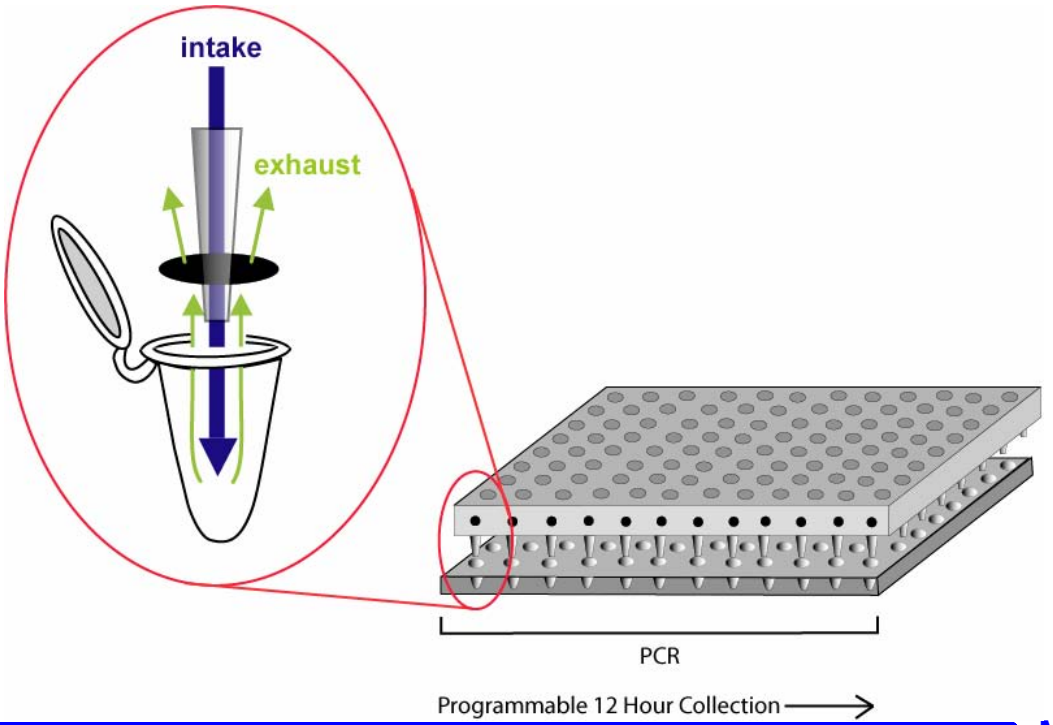
- Sample still needs to be transferred
- Suboptimal recovery efficiency
- Possibly labor-intensive post-collection processing

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

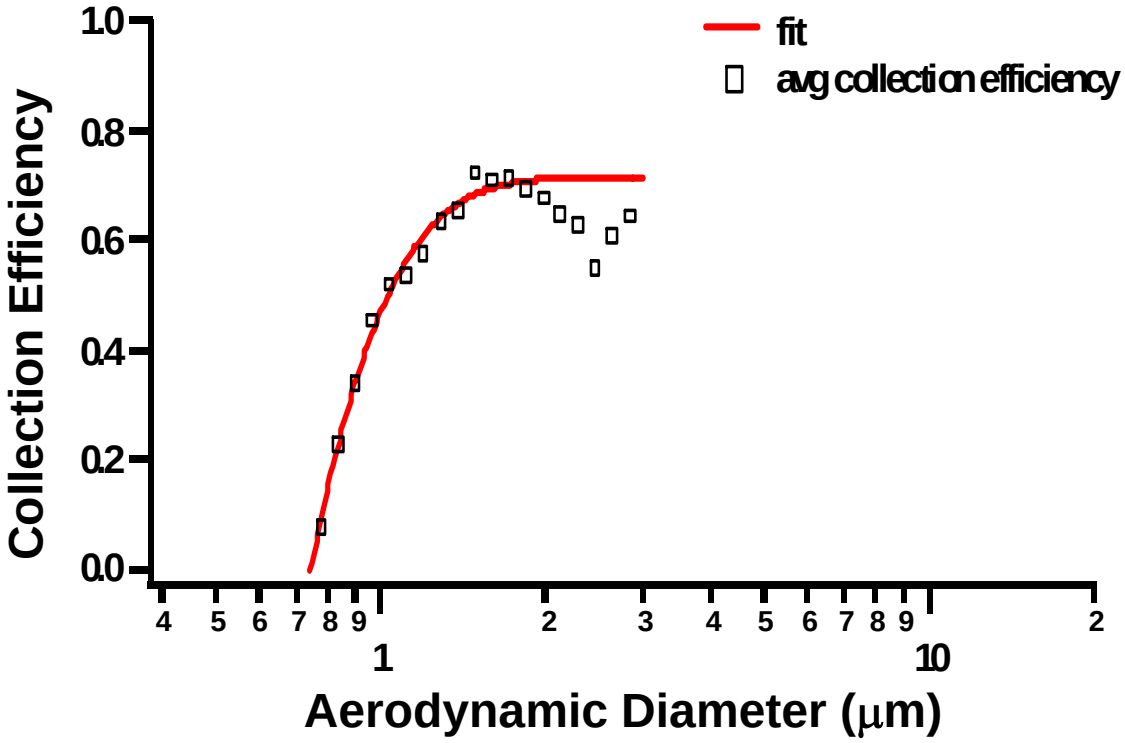
- **Use impaction collection into coated 96-well PCR plates**
  - **PCR plate can be directly assayed at processing laboratory**
  - **Sample does not require elution, sample preparation or transfer**
  - **Mineral oil coating enhances capture efficiency, maintains sample viability and serves as an evaporation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

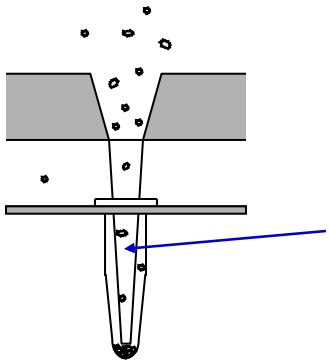


Impaction into Uncoated PCR Tubes

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





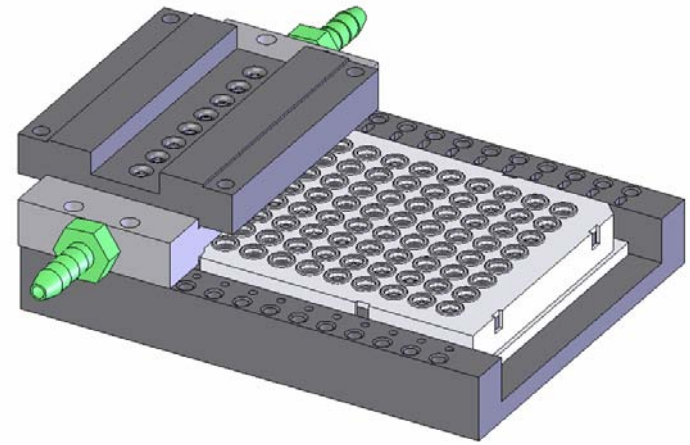
Impaction nozzle protrudes deep into the PCR tube

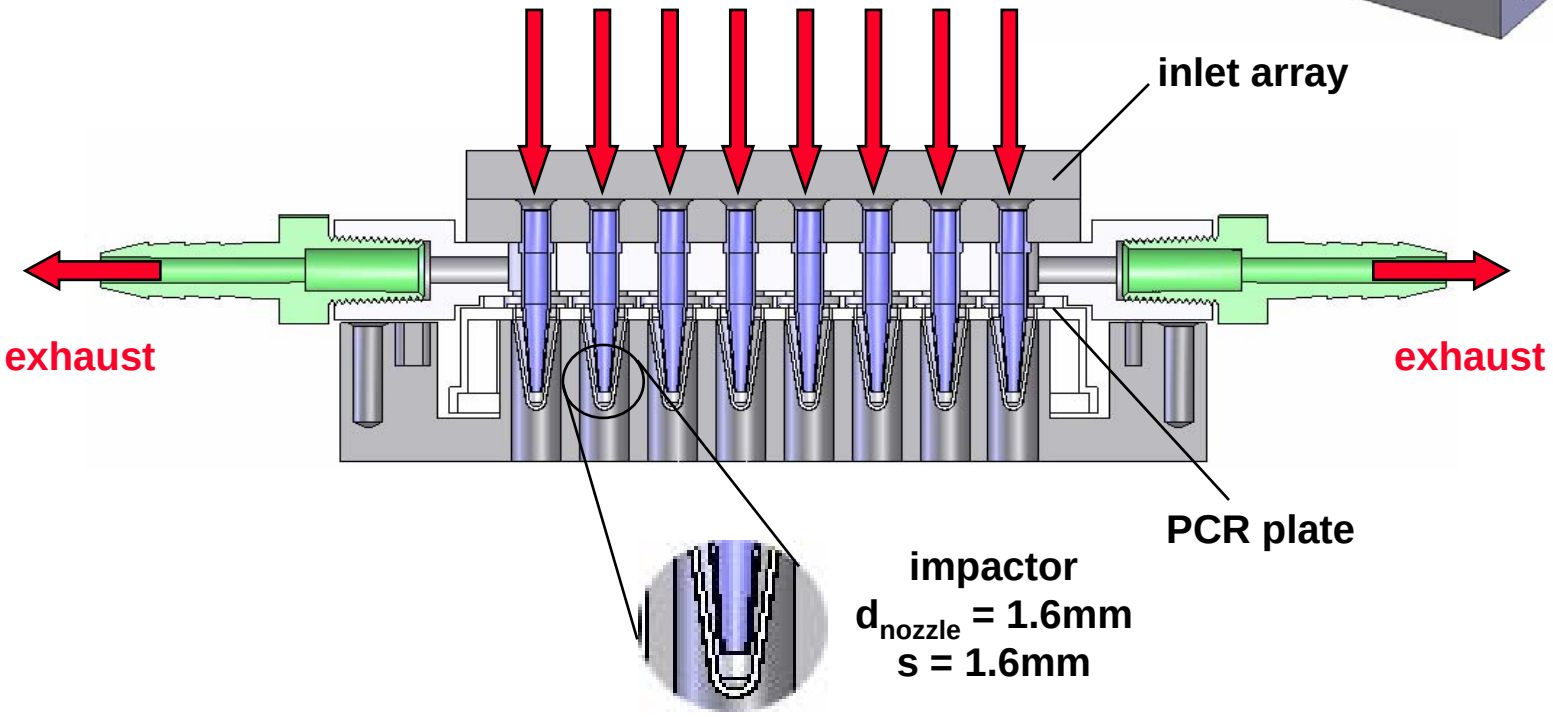
Preliminary Design Parameters

- **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

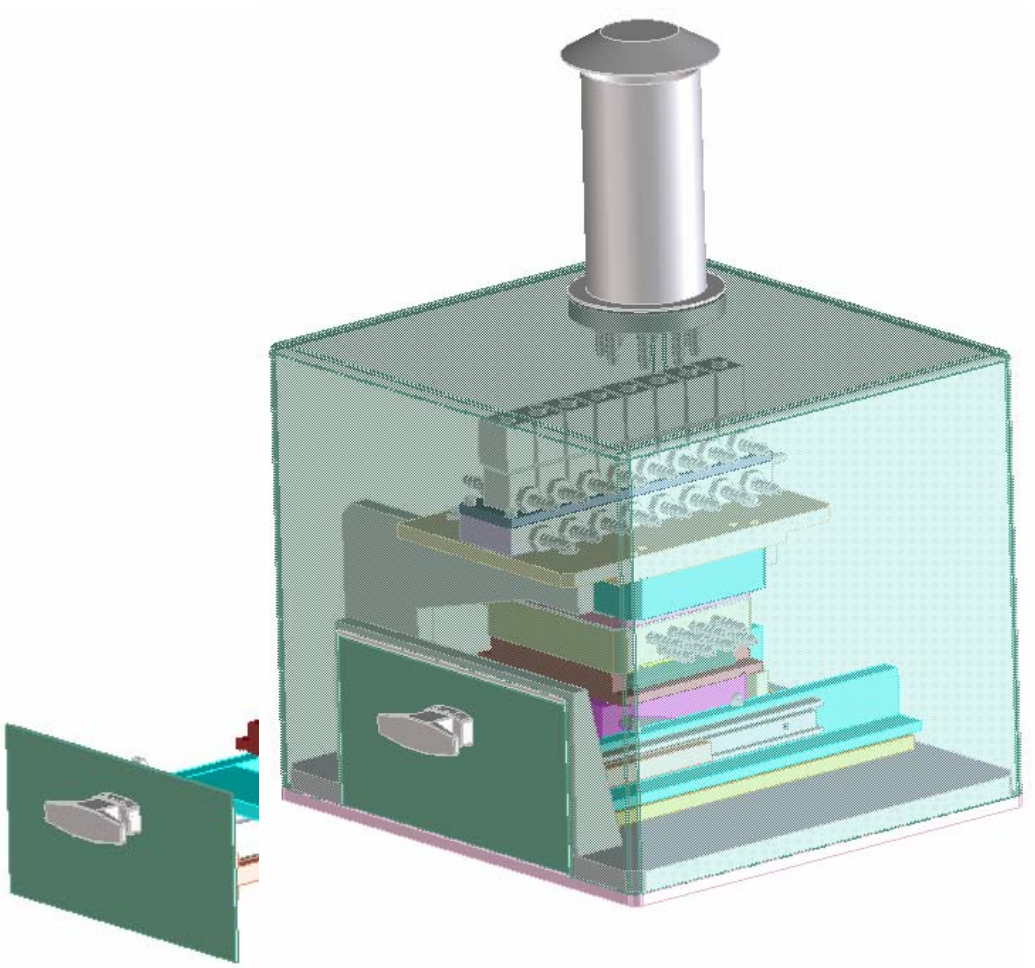
- 1 x 8 nozzle array test fixture
  - 1 row of plate used at a time
  - Manifold and plate holder have registration features to aid alignment





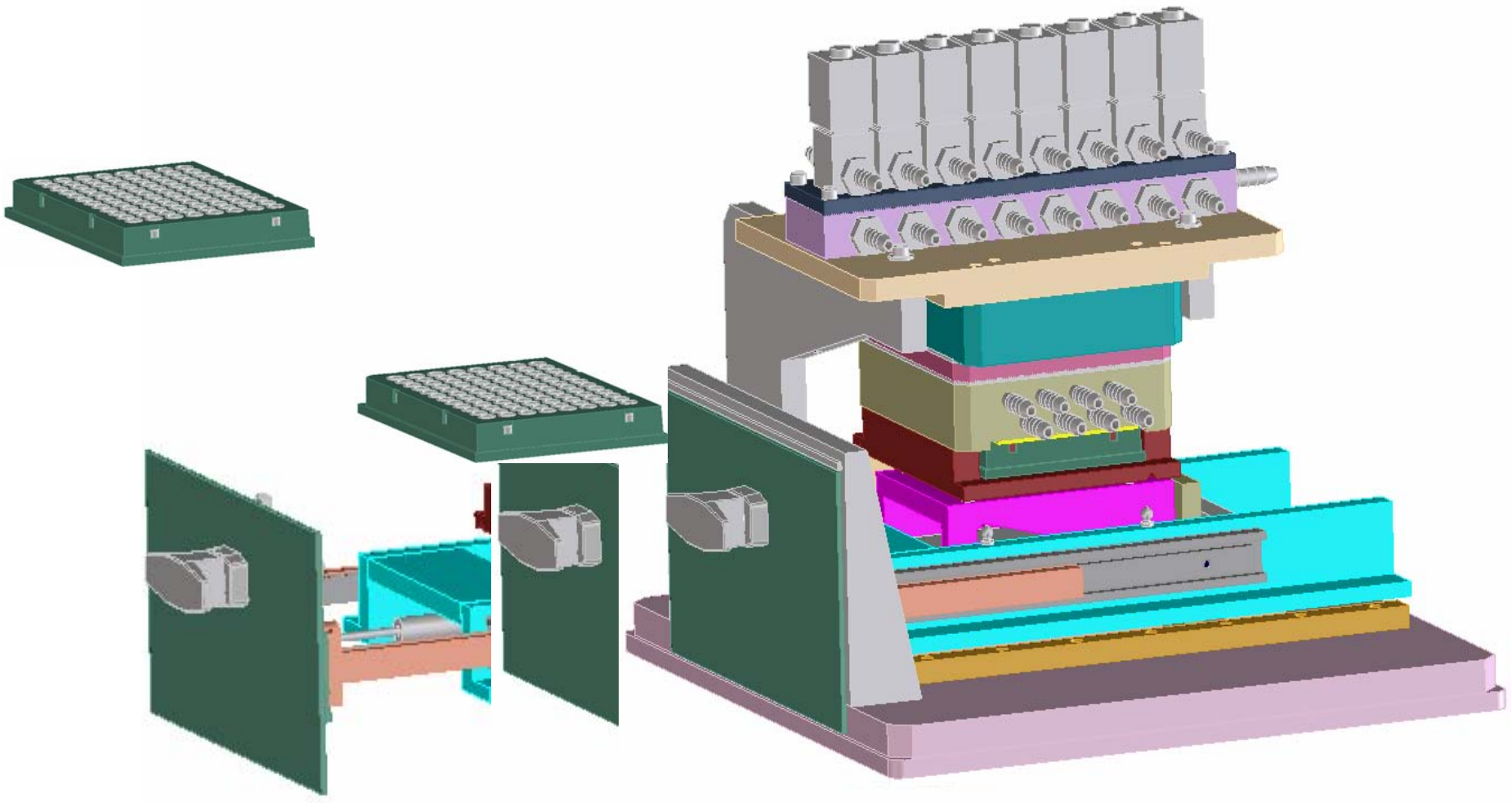
Notional System Design

- **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

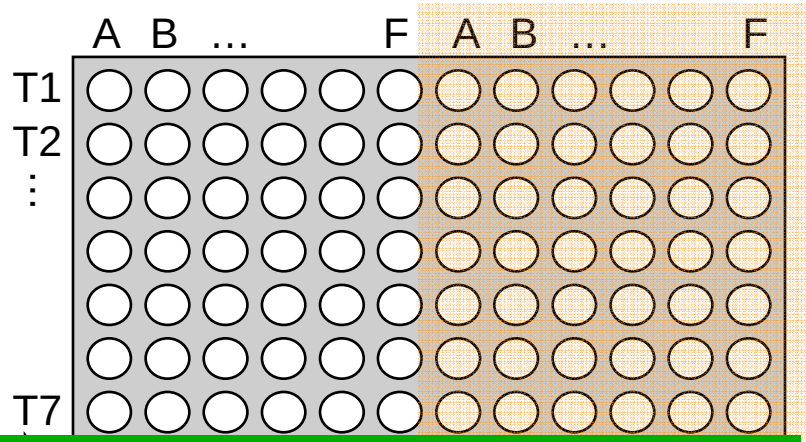
Plate Loading



Processing and Interpretation Benefits

Example PCR Plate Layout

Agent tested: A, B, etc



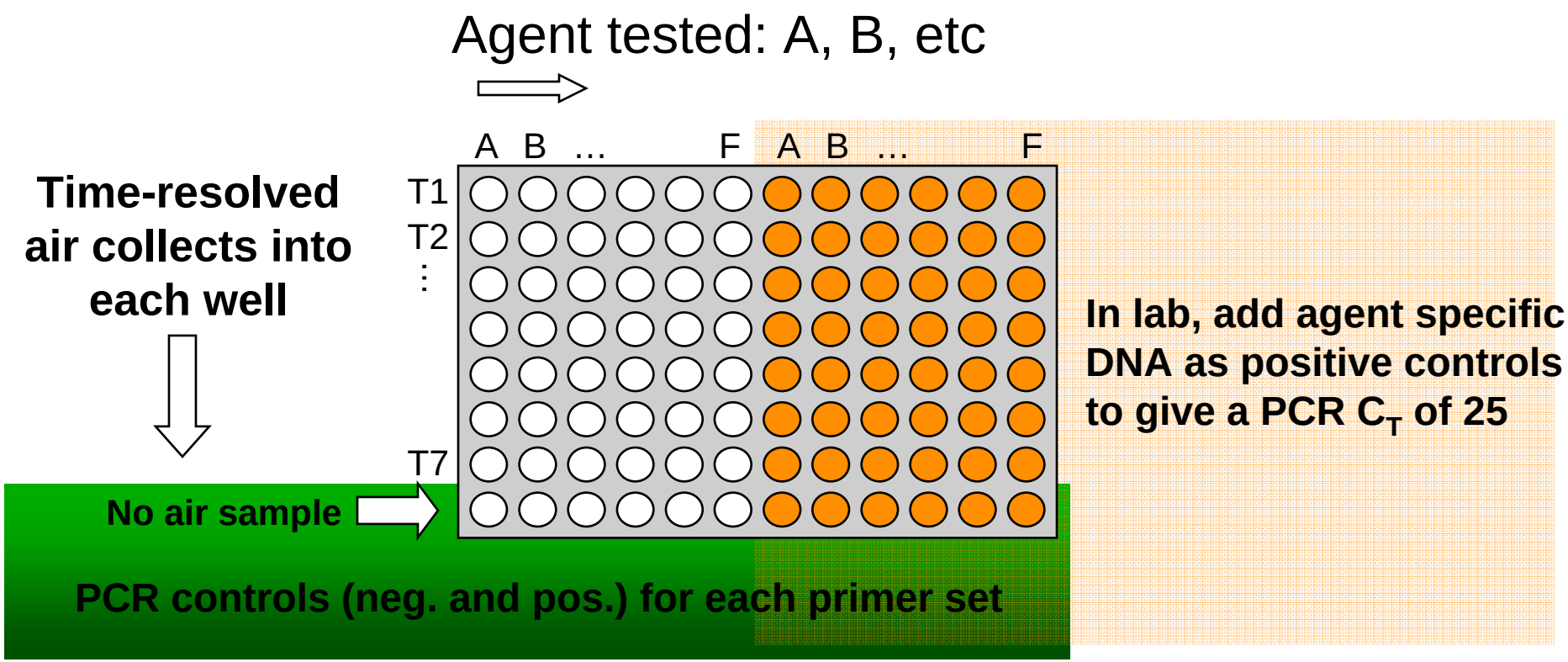
Time-resolved air collects into each well

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_T of 25

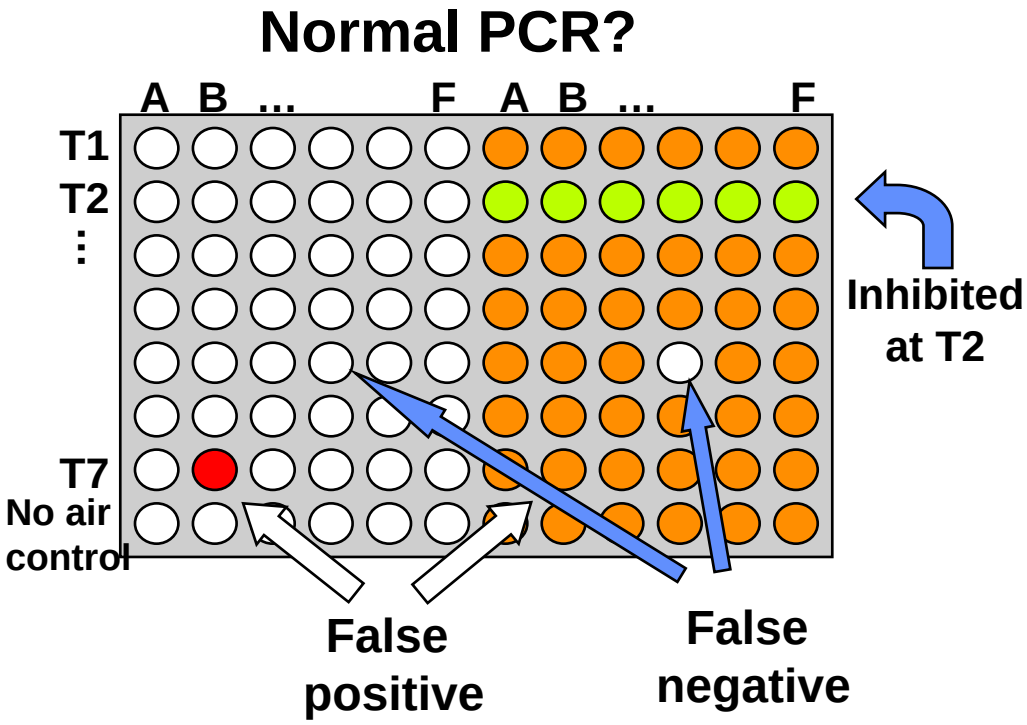
Standard PCR Reaction – No Agent Present

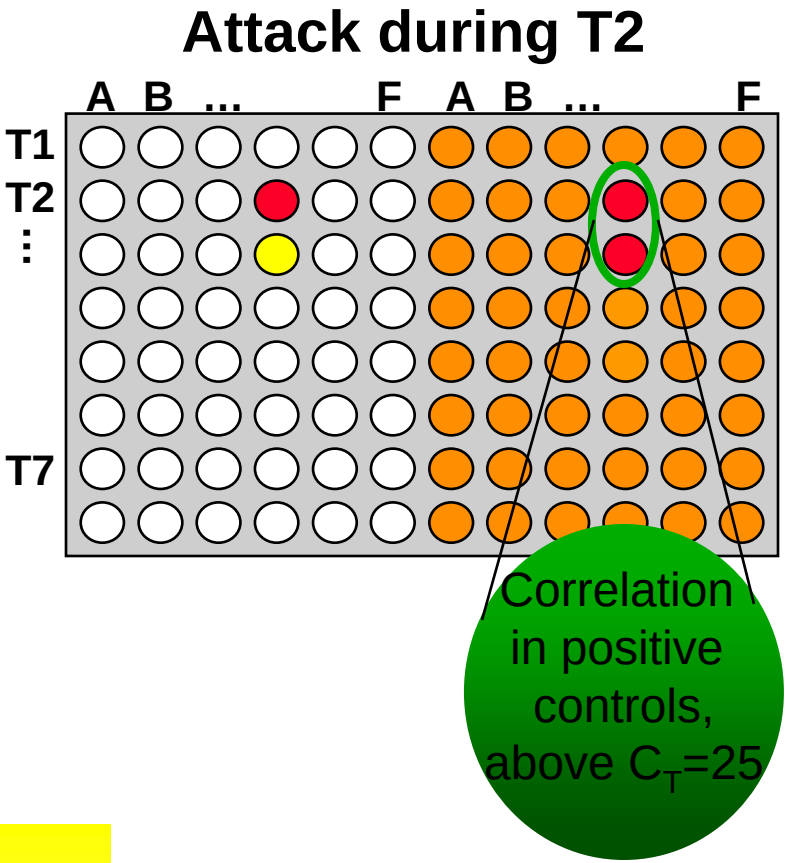


PCR Cycle Threshold Key

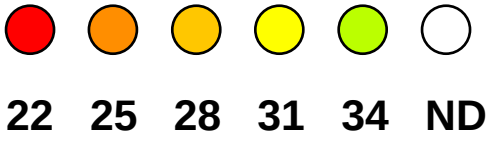
22 25 28 31 34 ND

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



Summary

High collection and extraction efficiency in the 1 – 10 micron particle-size range	$\varepsilon_{collection}$ > 50% $\varepsilon_{extraction}$ ~ 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

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Collector Close Up