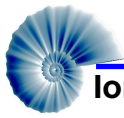


Ionian Technologies, Inc.

Rapid Isothermal Nucleic Acid Assays for the Detection of Pathogens

Lab Automation 2008
January 30th, 2007
Palm Springs, CA

Rich Roth, Ph.D., Principal Investigator



Outline

- Background on Ionian Technologies, Inc.
- Core technologies
- Market opportunities
- Detection system components



Ionian Technologies, Inc.

- Development-stage company located in San Diego, CA
 - 15 employees
- Founded by Dr. David Galas in 2000
 - Keck Graduate Institute (KGI, Claremont, CA)
- Initial funding provided by government contracts
 - For biothreat detection (DARPA & Homeland Security)
- Rapid nucleic acid detection technologies
 - DNA and RNA detection
- Current focus
 - Healthcare, agriculture (GMOs), and biodefense



Core Technology

- Technology based on 2003 PNAS paper by Galas et al.
- Isothermal nucleic acid amplification technology
 - Can amplify DNA or RNA
 - Uses a DNA polymerase and a nicking endonuclease
- Extension of short oligos along a template at a reaction temperature that is significantly above the T_m of the oligo/template duplex
 - Polymerase stabilizes the duplex
 - Reiterative cycles of polymerase extension and DNA nicking provide rapid exponential amplification



Amplification & Detection Details

- Rapid and specific amplification of DNA or RNA targets in 5 minutes
- Sensitivity down to 10's of copies
- True isothermal assay; does not require initial heat denaturation
- Reaction products are complementary oligos of 21-28 nts
 - Generate final product concentrations of approximately 1 μ M
- Single-base resolution
- Multiplex capabilities (currently up to 5-plex)
- High sensitivity and specificity
 - Extensive false alarm testing under government contracting
 - Tolerant to excess exogenous nucleic acid
- Multiple readout options
 - Real-time fluorescence, end-point fluorescence, colorimetric, gel, mass spec



Core Technology Portfolio

Amplification

NEA Assay

2-5 minutes, 65°C
LOD: 100 copies

NEAR Assay

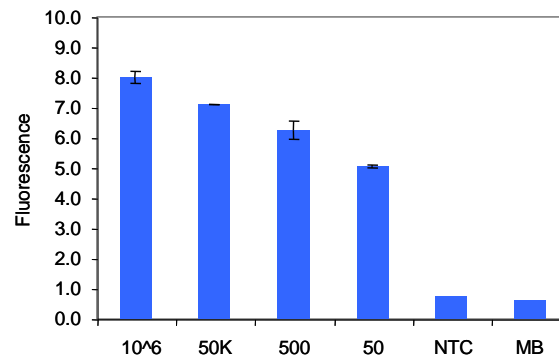
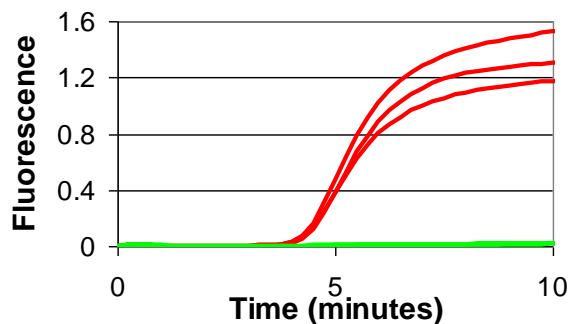
3-5 minutes, 55°C
LOD: 10 copies

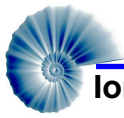
Detection

Real-time Fluorescence
SYBR Green

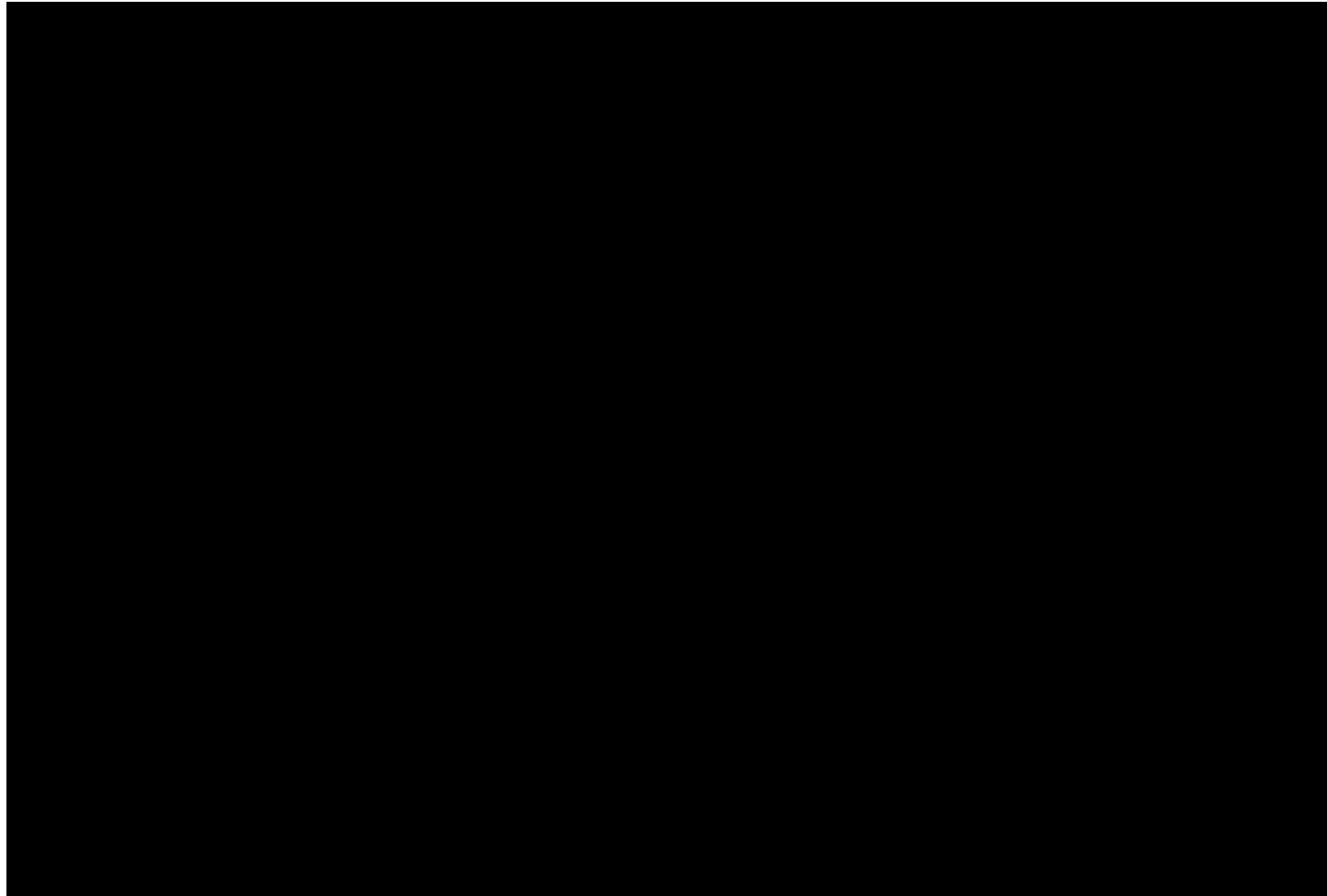
Endpoint Fluorescence
Molecular Beacons

Lateral Flow Device
Capture & Detection



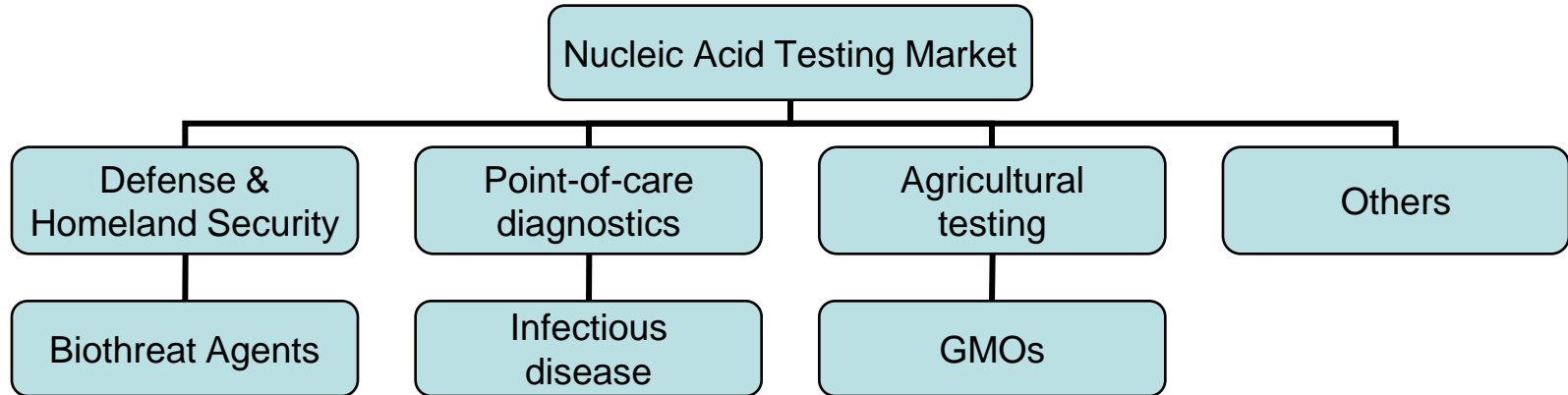


NEAR Assay





Rapid Assay Market Opportunities

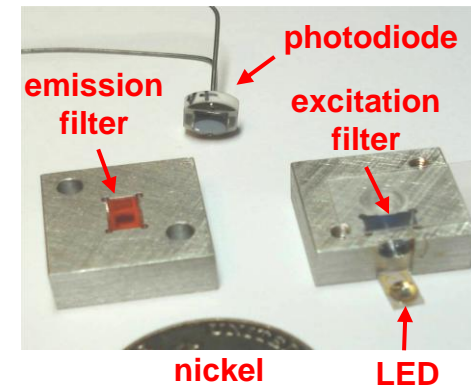


- Ionian's technologies can be leveraged in markets where there is a need for rapid results (~10 minutes or less)
- Rapid results allow for immediate, specific action

Rapid Assay Market Opportunities

Biothreat Detection

- Require rapid results
 - Less than 10 minutes for complete analysis
 - ≤ 5 minutes for assay
- Fixed detectors (buildings, airports, etc.)
- Portable detectors
 - Isothermal conditions reduce need for heating/cooling
 - Resistive or cartridge heaters
 - Battery operated
 - LED excitation & photodiode detection
- Stabilized reagents allow for field-based operation
- Low cost for high-volume or continuous testing





Biodefense Programs

- Dept. of Defense: DARPA
 - HISSS programs
 - Handheld Isothermal Silver Standard Sensor
 - Partnered with Northrop Grumman (prime contractor)
 - Spore lysis, amplification & readout
- Dept. of Homeland Security: Science & Technology
 - Detect-to-Protect (RABIS) (Subcontractors: SAIC, KGI)
 - Rapid Automated Biothreat Identification Sensor
 - Air collection, sample prep, amplification and detection
 - IBADS (GHC Technologies, La Jolla, CA)
 - FBADS (MesoScale Diagnostics, Gaithersburg, MD)
 - BAND (Northrop Grumman)
 - BIAD (SAIC, San Diego)



DHS Detect-to-Protect Program

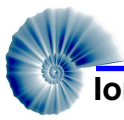
Goal: Rapid detection of biothreat agents in collected air samples

System Characteristics

- Operating modes:
 - ✓ Continuous
 - ✓ Time triggered
 - ✓ Optically triggered
 - ✓ Remote operation
- System volume under 10 cubic feet
 - ✓ 2.5 ft W × 1.8 ft D × 1.9 ft H , + air inlet
- One month stand-alone operation
- Operates on 120 VAC
- Sensitivity of 100 bacteria or viruses per liter of air
- Detection time in under 10 minutes
 - ✓ Rapid sample processing
 - ✓ Fast, proprietary isothermal amplification
- Detects 20 bacterial and viral threats
- Direct detection of threat DNA and RNA

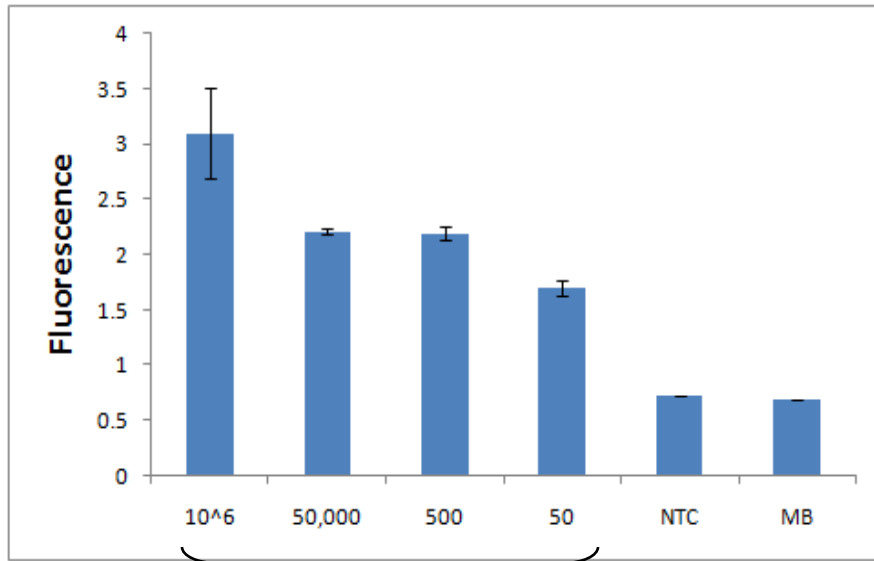
Threats Currently Detected (CDC Class A and B threats)

- *Bacillus anthracis* (anthrax)
- *Yersinia pestis* (plague)
- *Francisella tularensis* (tularemia)
- Variola major virus (smallpox)
- Ebola virus (a hemorrhagic fever)
- Marburg virus (a hemorrhagic fever)
- Lassa virus (a hemorrhagic fever)
- Machupo virus (a hemorrhagic fever)
- *Clostridium botulinum* (botulism toxin)
- *Brucella melitensis* (brucellosis)
- *Burkholderia pseudomallei* (melioidosis)
- *Chlamydophila psittaci* (psittacosis)
- *Coxiella burnetii* (Q fever)
- *Rickettsia prowazekii* (Typhus fever)
- Venezuelan Equine Encephalitis virus (VEE)
- Eastern Equine Encephalitis virus (EEE)
- Western Equine Encephalitis virus (WEE)
- *Staphylococcus aureus* (SEB toxin)
- *Bacillus subtilis* (bacterial simulant)
- MS2 bacteriophage (RNA viral simulant)



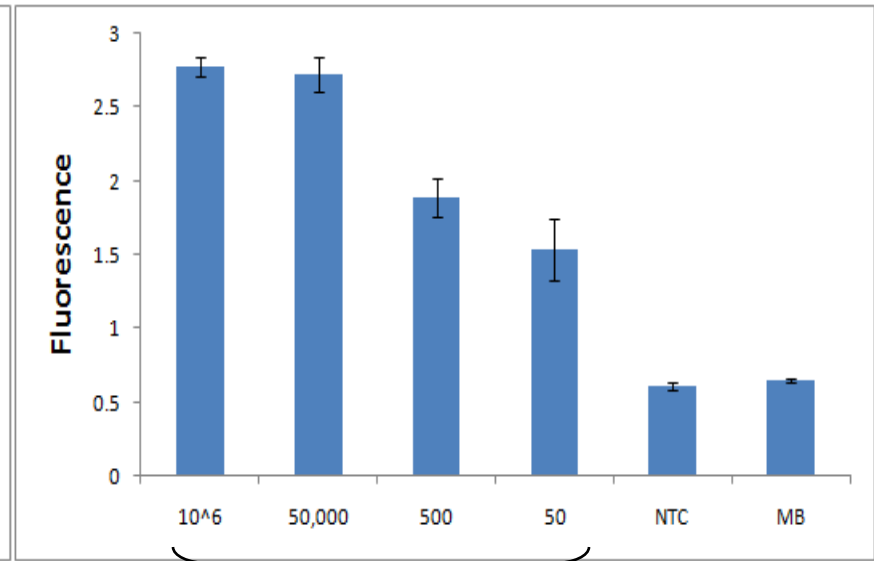
NEAR Assay for Biothreat Agents

Y. pestis



Copies Genomic DNA

F. tularensis



Copies Genomic DNA

5 minute amplification reactions

Detection down to 50 copies of genomic DNA

Molecular beacon readout



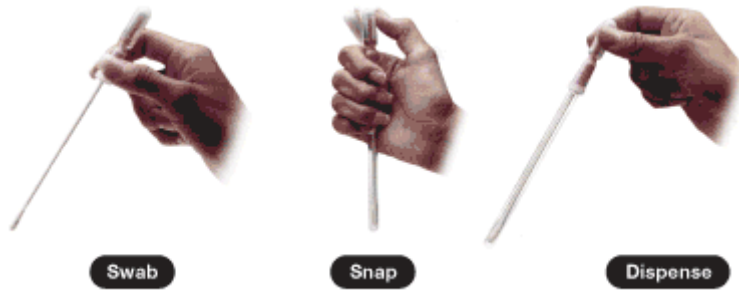
Rapid Assay Market Opportunities

Point-of-Care Diagnostics

- Rapid test results allow for immediate treatment in point-of-care setting
- Stabilized reagents allow for easy storage in clinics or physician offices
- Small, inexpensive equipment facilitates acceptance by point-of-care personnel
- Physicians are comfortable with dipstick-type formats for immunoassays
 - Extend assay format to nucleic acid testing
- **Amenable to non-blood based samples without purification**
 - Nasopharyngeal or genitourinary swabs
 - Urine samples (up to ~10% of reaction volume)
- Blood can be assayed with minimal sample processing

Sample Preparation

- Sample collection must be compatible with downstream analysis
 - Elution & Lysis
 - Non-ionic detergents are compatible with assay chemistry
- NEAR Assay is tolerant to many interferents
 - Minimal sample purification allows for simple, rapid approaches

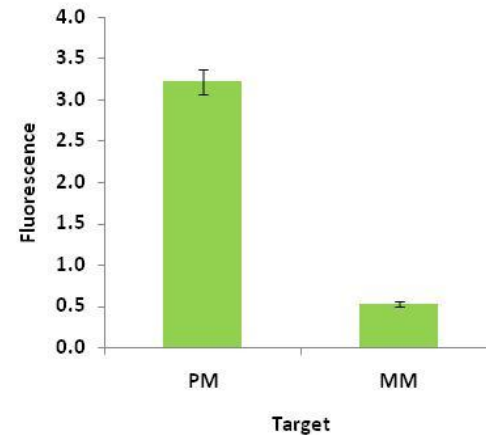
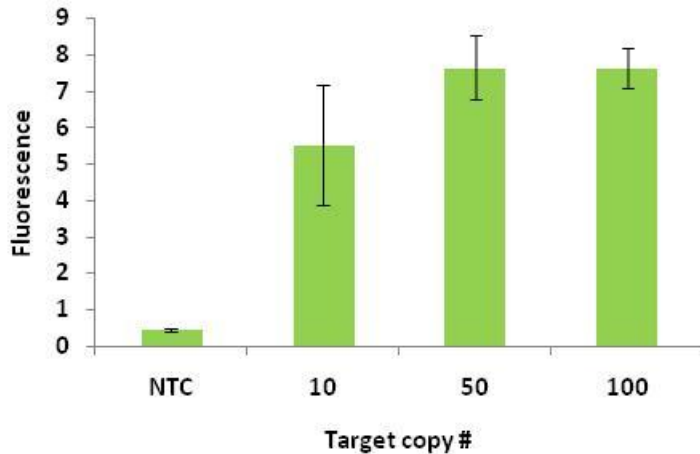


“Snap-Valve” Technology – Medical Packaging Corp.

Detect-to-protect program
Rapid sample purification

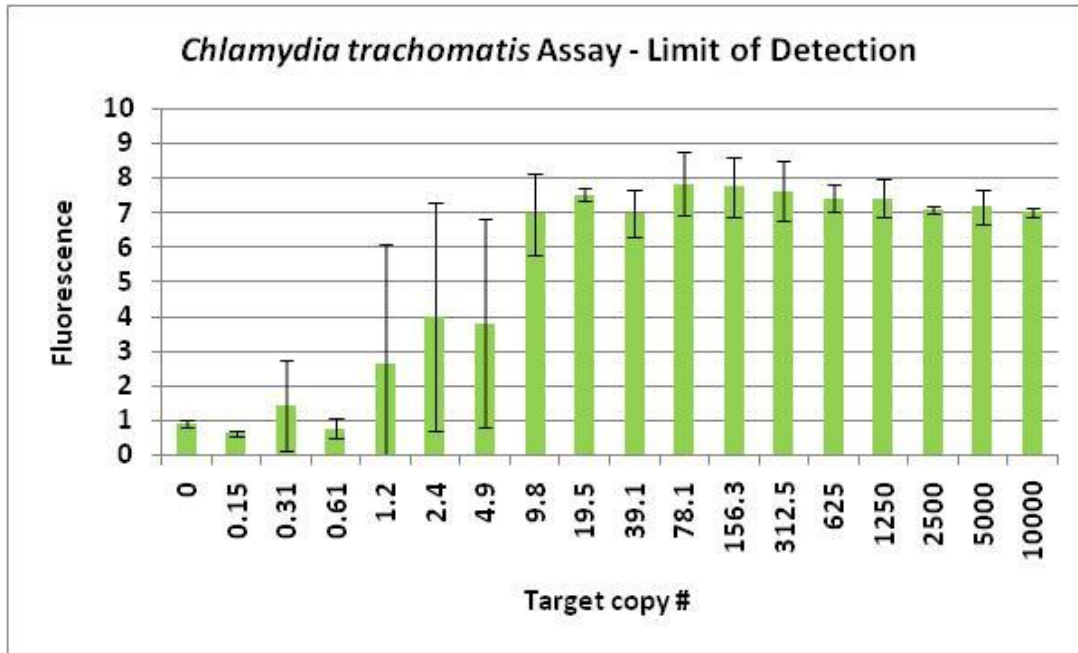
Rapid STD Assays

- 5 minute NEAR assay for *Gc* detection (*Neisseria gonorrhoeae*)

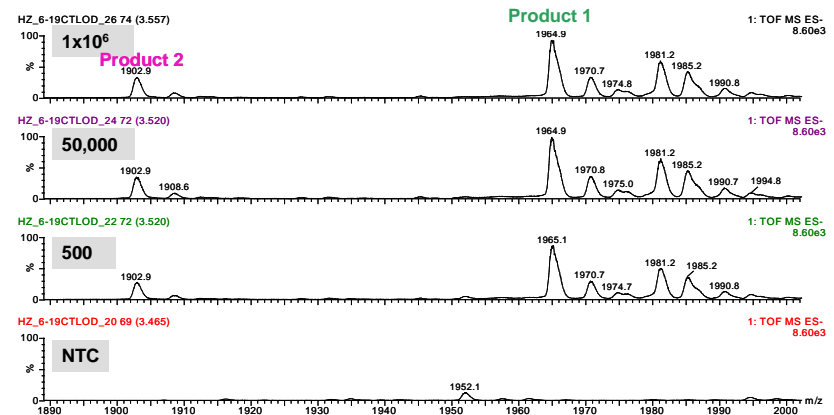
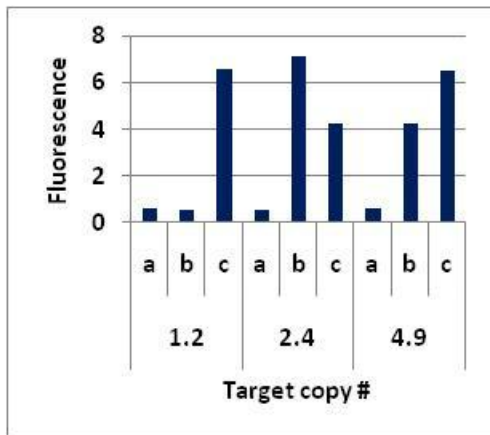


- *Gc* LOD – 10 copies
- Single nucleotide resolution

Chlamydia Assay: LOD



- 2-fold dilutions from 10^4 copies to <1 copy
- Ability to detect ≤ 10 copies
 - Single-copy detection is possible (?)





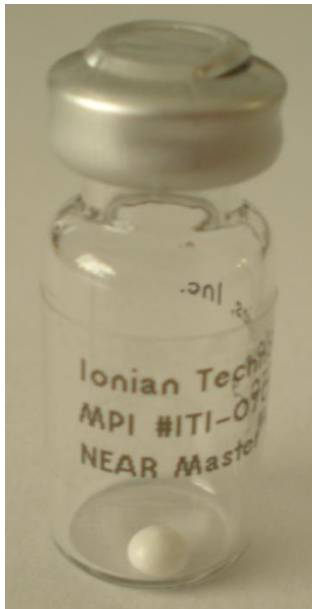
Rapid Assay Market Opportunities

Point-of-Use Agriculture Testing

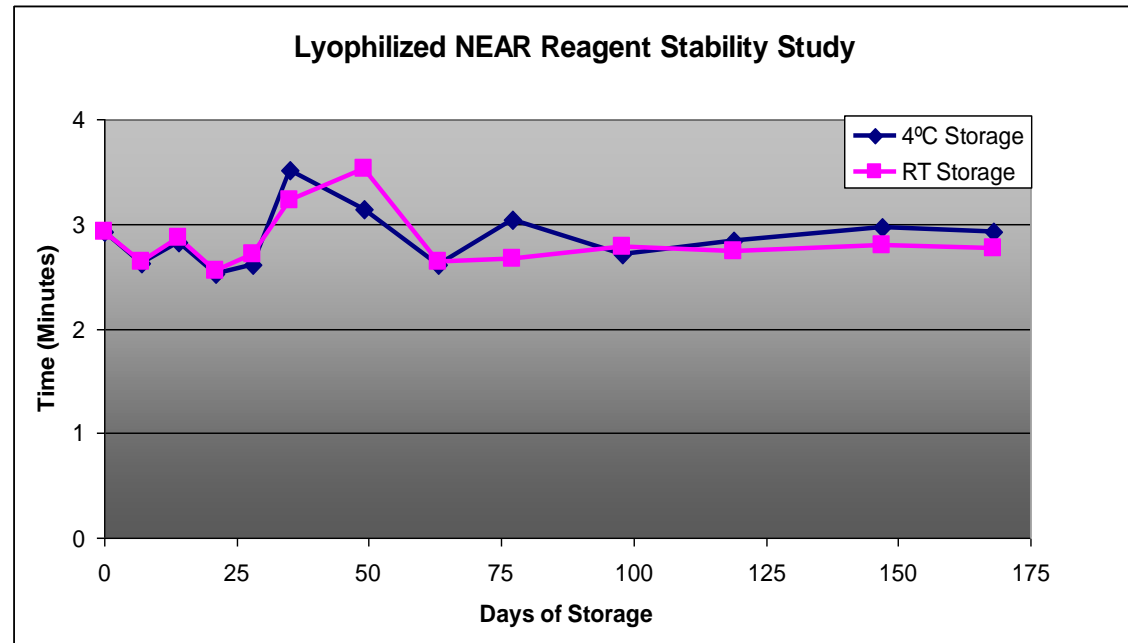
- Genetically modified crop testing
 - Ensure segregation of GM from non-GM crops
 - Required for import/export
 - License fee recovery
 - Surveillance
- No FDA or regulatory requirements
 - Certificate of Performance from USDA's Grain Inspection, Packers and Stockyards Administration (GIPSA)
- Some current GM traits are amenable to immunoassays
- Next generation of traits require DNA testing
- Potential customers
 - Seed companies and grain handlers

Reagent Systems

- Stable reagent systems are essential for POC/POU market
- Lyophilized reagents provide sufficient stability
 - Room temperature storage demonstrated for 6 months with no detectable loss of activity
 - Limited testing at 37°C and 42°C with no loss of activity

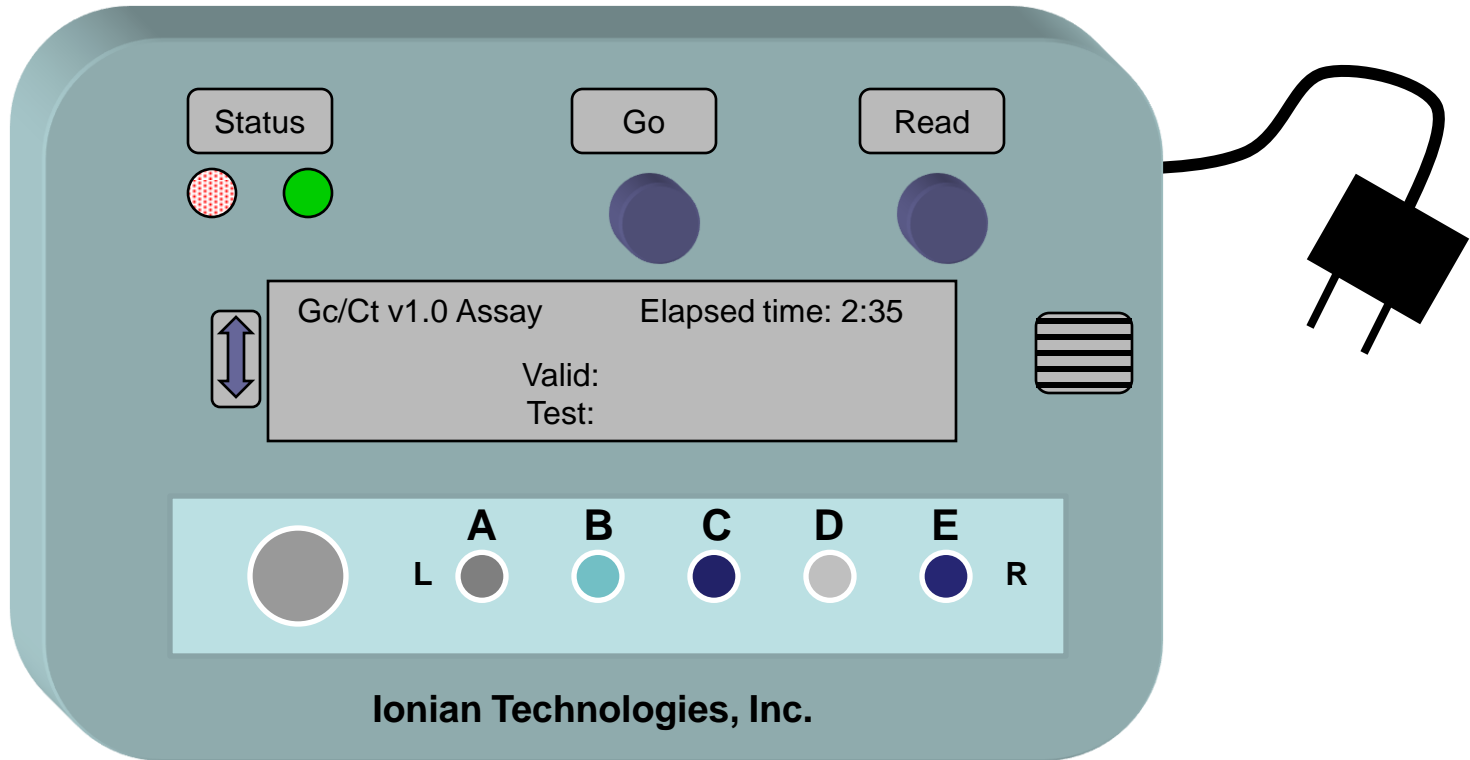


4 mm diameter



Amplification & Detection Device

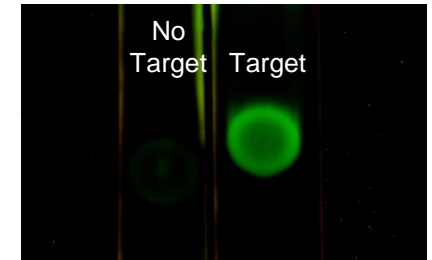
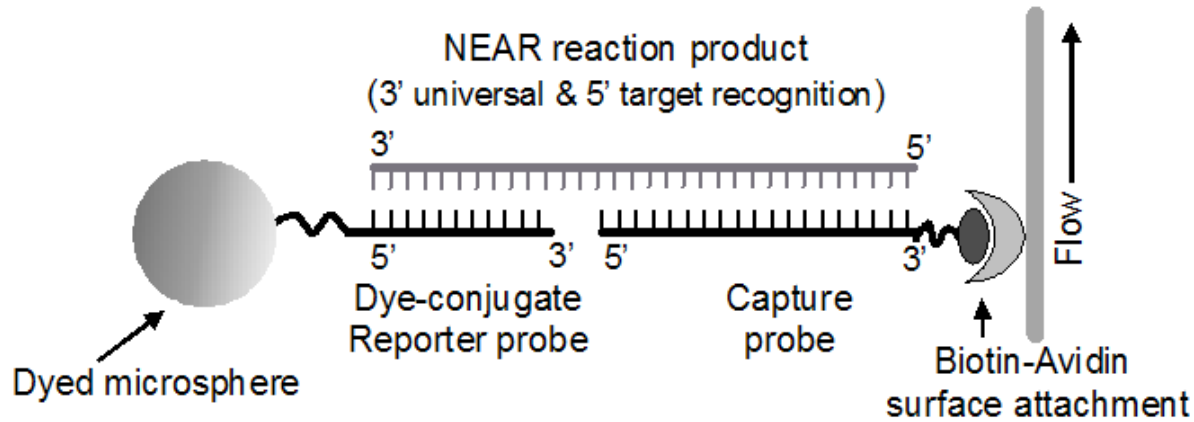
- Device development is in progress
 - Schematic of initial prototype



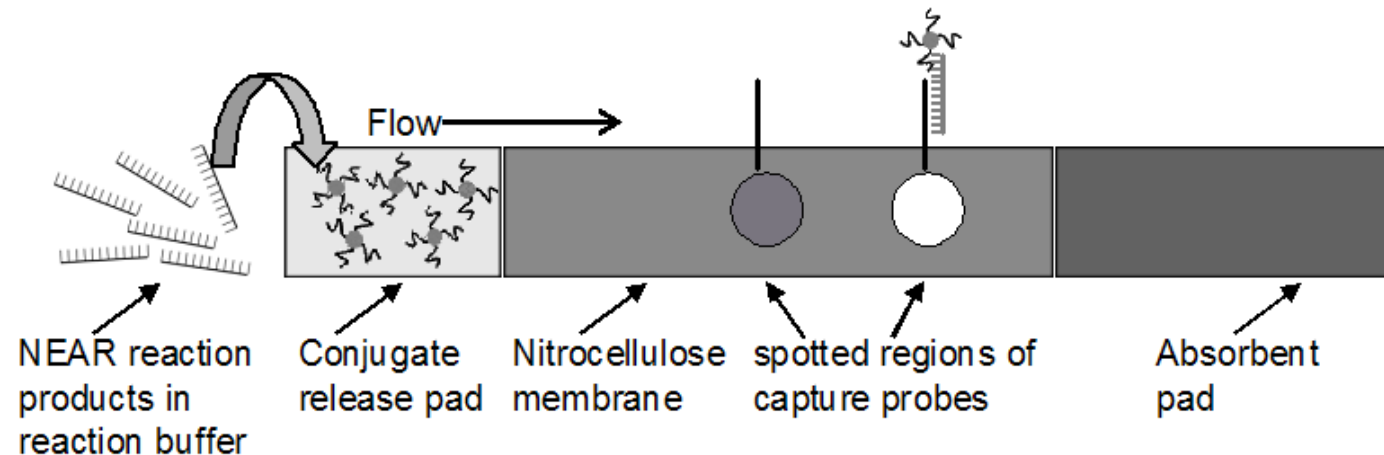
USB, printer ports

Solid-Surface Readout

Lateral flow detection



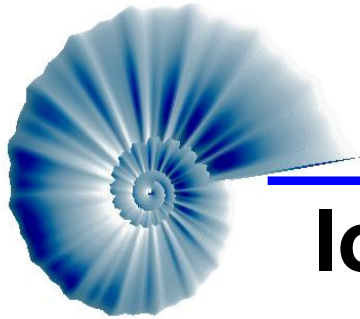
B. subtilis assay
4 minute NEAR reaction





Summary

- Developed highly effective assays for a number of biothreat agents
 - DARPA, DHS S&T
- Demonstrated excellent performance on clinical samples with *Ct* and *Gc* assays
 - Minimal sample prep/purification
- Entering agriculture-GMO test market
 - Minimal regulatory requirements
- Stable reagent system
- Rapid sample prep methods
- Developing amplification & detection device
- Multiple other readout options
 - Solid phase, lateral flow
- Robust, flexible assay system for rapid nucleic acid testing



Ionian Technologies, Inc.

Contact Information:

Andrew Miller, PhD (VP of R&D)

amiller@ionian-tech.com

Rich Roth, PhD (Principal Investigator)

rroth@ionian-tech.com