Biosensors and Diagnostics for Pandemic Response

PRESENTED BY

Robert Meagher – Sandia National Laboratories
About myself and Sandia Biosciences

Robert Meagher, Ph.D.
- Principal Member of Technical Staff
- 15 years at Sandia, starting as a postdoc
- Research interests: Microfluidics, nucleic acid amplification, virus detection

Sandia Biosciences
- Interdisciplinary research groups located both in Livermore, CA and Albuquerque, NM
- Broad mission areas are Biodefense and Emerging Infectious Disease and Bioenergy
- Traditionally has leveraged Sandia capabilities in device & systems engineering for applications in Biosensors, Diagnostics, and Detection, including early foundational work in microfluidics
- The Biosciences groups collaborates broadly with other programs in Sandia, including programs in systems analysis, homeland security, global biosecurity, and other areas
• Starting in March, Sandia as well as DOE Office of Science saw an urgent need to respond to the growing COVID-19 pandemic in the USA.
• Numerous efforts within Sandia and across the DOE Labs were rapidly initiated, leveraging multiple capabilities within the national labs (not just diagnostics).
• Two specific technologies that I have been involved with:
  • Viral RNA detection - QUASR / Smart LAMP
  • Viral protein / antibody detection - SpinDx

“QUASR” and Smart LAMP – Rapid Viral RNA detection

- From previous research (LDRD, DTRA, and NIH funding), we had been working with isothermal diagnostics (LAMP), especially focused on arbovirus detection, and worked to advance:
  - “Direct” amplification from viruses (circumventing extraction)
  - “QUASR” detection chemistry that enabled much higher specificity as well as very bright signals that could be discerned with simple instrumentation, e.g. smartphone.
- We pivoted to SARS-CoV-2 detection, and are currently seeking commercialization opportunities.
- But we’re also interested in advancing the basic science of isothermal amplifications.

Smart Trap, Smart LAMP, QUASR for arboviruses (2013-2017)

SARS-CoV-2 Detection & Diagnostics (saliva, swabs, environmental)
SpinDx – Portable instrument for multiplexed immunoassays

- Sandia began developing “SpinDx”, a portable bead-based sedimentation immunoassay, around 2009
  - Numerous applications in biodefense, emerging infectious disease, bioenergy
  - Mostly direct antigen detection, but also serology and nucleic acid detection
- SARS-CoV-2 pandemic has brought serology “test strips” into common use
  - Challenges include high variability from manufacturer to manufacturer, and unclear significance of antibody results
- Sandia SpinDx enables quantitative, multi-analyte immunoassays and even parallel immunoassay + nucleic acid assay
Other Sandia Biosensing/Diagnostic technologies (Slide 1 of 2)

Wearable Micro-Needle Array based sensor for bioagent exposure
• Real-time multianalyte sensing
• Proteins, RNA, small molecule biomarkers in dermal interstitial fluid
• Early warning capabilities across populations for viral infections

POC(s): Ronen Polsky (rpolsky@sandia.gov), Phil Miller (prmille@sandia.gov)

VOC Biomarker Discovery
• Sampling methods for breath, skin volatiles, chyme
• State of the art laboratory equipment and expertise for biomarker discovery & ID
• Novel modality for rapid diagnostics

POC: Joshua Whiting (jjwhiti@sandia.gov)

Portable VOC Diagnostics technologies
• 2D Micro GC system
• Custom ion mobility spectrometer
• Custom low-SWaP electronics
• Chemical collector to improve selectivity

POC: Matthew Moorman (mmoorma@sandia.gov)
Other Sandia Biosensing/Diagnostic technologies (Slide 2 of 2)

**BaDx**
- No-power cartridge for culture-based anthrax detection & ID
- Self-sterilizing
- Adaptable to other pathogens
POC: Jason Harper (jcharpe@sandia.gov)

**Surface Acoustic Wave Biosensors**
- Sensitive, scalable, multi-mode detection (proteins, nucleic acids)
- Miniature low-power electronics
- Integrated sample prep
POC: Darren Branch (dwbranc@sandia.gov)
Collaborating with Sandia

Sandia IS NOT a funding agency!
- Sandia is a federally funded research and development center (FFRDC) owned by the US Department of Energy
- (Almost) all of Sandia’s work is funded by federal government (DOE and non-DOE)
- Sandia works on full cost recovery

- Sandia has limited “internal funding” - Laboratory Directed Research & Development (LDRD)
  - LDRD is a congressionally-mandated program that allows National Labs to use a small percentage of funds from federal sponsors for internally directed R&D efforts
- Sandia dedicates some portion of these LDRD funds to seed collaborative projects with Academic Alliance partners

Academic Alliance LDRD funding is not an end in itself!
- The goal is to catalyze enduring, sustainable collaborations with external sponsorship, as well as talent development
Strategizing pathways to externally funded collaborations

**University leads**
- Work may be basic science, but relies on a unique Sandia capability
- Certain sponsors and mechanisms that Sandia can’t be lead institution for (e.g. NSF, non-profits)

**Sandia leads**
- Basic or applied science
- Good fit to national lab mission and capabilities or existing program
- Sandia may rely on university partner for unique capabilities, e.g. BSL-3, hospital collaboration

**Sponsor ecosystem** - some are more suitable than others for national lab involvement
Rapid Point-of-Care Testing and Monitoring

Jacqueline Linnes, PhD

Marta E. Gross Assistant Professor
Weldon School of Biomedical Engineering
Purdue University
jlinnes@purdue.edu

DIAGNOSTICS: SAMPLE-T0-RESULT DETECTION

Sample Preparation → Signal Amplification → Signal Detection → Result
LABORATORY PCR-BASED TESTS

- Automated Detection
  - Multiplex samples and sample pooling
  - High throughput

- Cons:
  - Samples must be shipped to lab
  - Results not immediate

RAPID DIAGNOSTIC TESTS (ANTIGEN/ANTIBODY TESTS)

Sample Preparation

Antigen separation/capture

Antibody binding

Visual particle aggregation

Result
SIGNAL ENHANCED ANTIGEN/ANTIBODY TESTS
Molecular diagnostics

- Sample
  - Cell lysis
  - Nucleic acid purification
  - Nucleic acid amplification
  - Fluorescence / Colorimetric
  - Result

Steps:
- Sample
- Lyse
- Filter
- Amplify
- Detect

Infected
Combine simplicity and portability of handheld rapid tests with sensitivity and specificity of lab-based nucleic acid diagnostics.
 USER STEPS

Phillips and Moehling et al. Lab on a Chip 2019
PAPER-BASED DIAGNOSTICS FOR RESPIRATORY INFECTIONS

COVID-19 Test: Purdue University, Raytheon BBN Technologies, a Raytheon Technologies company, PortaScience Inc., Cortex Design Inc., and LaDuca LLC

PORTABLE SMARTPHONE PLATFORM

Add Water  Start Device  Get Result

OmniVis

Katherine Clayton  Tamara Kinzer-Ursem

Steven Wereley

Conflict of Interest Warning: I am a Co-Founder of OmniVis, Inc.
VISCOSITY CHANGES WITH AMPLIFIED NUCLEIC ACID

Low Diffusivity: Pathogen Detected

High Diffusivity: No Pathogen
SMARTPHONE DIFFUSOMETER

I: Add sample
II: Heat for 30 min
III: Add nanoparticles
IV: Deposit into chip
V: Image & analyze

Taylor Moehling and DongHoon Lee et al. Biosensors and Bioelectronics 2020
WEARABLE ELECTROPHYSIOLOGY DEVICES

Sample

Hyperspectral imaging  Image processing  Spectral calculations → Result
M-HEALTH SPECTROSCOPY – NONINVASIVE BLOOD ANALYSIS

Young Kim
Natural and Biological Photonics Laboratory
WEARABLE ELECTROPHYSIOLOGY DEVICES

Sample

EKG / PPG Signal → Algorithm Corrections → Heart Rate, Respiration, SpO₂ Monitoring → Result
WEARABLES FOR INFECTION MONITORING

Craig Goergen
CardioVascular Imaging Research Laboratory
Accurate, Accessible Testing for the Whole Community

Jacqueline Linnes, PhD
jlinnes@purdue.edu

https://www.elmhurst.edu/blog/what-is-community-health/
Thoughts on Pathogen Detection for Emerging Threats

Michael Shannon, Ph.D.
Mike Farrell, Ph.D.
True Merrill, Ph.D.
Nick Speller, Ph.D.
Miles Paca

05NOV2020
GTRI developed a saliva-based COVID-19 test

GOAL: develop a Georgia-based, supply chain-stable, affordable, molecular real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 virus in saliva specimens

Technical Pedigree

Validation Plan

Clinical Validation with Georgia DPH

Reverse Transcription Real-Time PCR

Collected samples from patients

Seeking 30 negative samples and 30 positive samples for comparison

Kit Production Capability Development

Bridging Study Development – Substitute RNA-Lock for Viral Transport Medium (VTM)

FDA Emergency Use Authorization (pending final approval):
Georgia Tech Research Institute Saliva Collection Kit for the SARS-CoV-2 Virus
Georgia Tech SARS-CoV-2 Virus Molecular Diagnostic Test (GT COVID-19 Saliva Test)
Our Interests – Coupling Speed with Gold Standard Assays for Pathogen Detection

Emerging Threats

Bacteriology

Microbiology

Virology

Focus across pathogen families

Attacking current gaps by creating capabilities to improve testing speed, scale and fidelity

Novel Specimen Collection

Extractionless PCR

Asymptomatic Testing at Scale

Innovation in Point of Care

Extract from Georgia Tech Asymptomatic Gold Standard Data

Aug 14

Aug 15

Aug 16

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Aug 28
Isothermal Amplification for the Detection of SARS-CoV-2

Andrew D. Ellington
Center for Systems and Synthetic Biology
University of Texas at Austin

Sandia, November 5, 2020
How to make computers with carbon, rather than silicon

Zhang et al. (2007) Science 318:1121
DNA/RNA amplification
LAMP

Signal processing
Toehold-mediated strand exchange computation probes
DNA/RNA amplification

**LAMP**

**Stage I**
- B3
- B2
- B1
- SP
- F1c
- F2c
- F3c
- F3
- Template

**LAMP initiation by BIP**

**Stage II**
- B3c
- B2c
- B1c
- F3
- F2
- F1
- Bst
- BIP amplicon displaced by F3 extension

**Stage III**
- Hairpin formation and self-priming

**Stage IV**
- Bst
- FIP

**Stage V**
- FIP

Signal processing

Toehold-mediated strand exchange computation probes

RFU vs. time/min graph

2x10^4 copies
2x10^5 copies
2x10^6 copies
20 copies
200 copies
0 copies

100bp
300bp
500bp
1000bp
1500bp
2000bp
2500bp
DNA/RNA amplification

**LAMP**

- **Stage I**: B3, B2, B1, SP, F1c, F2c, F3c, FIP, F3
- **Stage II**: BIP initiation by BIP, B3c, B2c, B1c, BIP ampiclon displaced by B3 extension, F1, F2, F1c
- **Stage III**: Hairpin formation and self-priming
- **Stage IV**: Bst
- **Stage V**: FIP

Signal processing

**Toehold-mediated strand exchange computation probes**

One or multiple amplicon processing
**DNA/RNA amplification**

**LAMP**

**Signal processing**

**Toehold-mediated strand exchange computation probes**

**One or multiple amplicon processing**

**Visual readout**

- **Cellphone**
- **Dipstick**

**DNA/RNA amplification**

LAMP

- LAMP initiation by BIP
- BIP ampiclon displaced by B3 extension
- B3c B2c B1c
- Stage II
- F1 F2 F3c
- FIP ampiclon displaced by F3 extension
- LAMP pathway
- Hairpin formation and self-priming

Stage I

- Template
- B1 B2 B3 SP
- Bst

Stage II

- F1c F2c F3c
- FIP
- LAMP initiation by FIP
- LAMP initiation by BIP

Stage III

- B3c B2c B1c
- F1 F2 F3c
- FIP ampiclon displaced by B3 extension

Stage IV

- FIP
- Bst

Stage V

- FIP

**Signal processing**

**Toehold-mediated strand exchange computation probes**

**One or multiple amplicon processing**

**Visual readout**

- **Cellphone**
- **Dipstick**
Detection of human fecal contamination in recreational waters

We nonetheless had some confidence we could make a pretty good mousetrap.
Direct detection from mosquito guts

Or-gated probe captures multiple amplicons into one signal

Use of degenerate primers captures phylogenetically wide range of Zika viruses

Bhadra et al. (2018), *Viruses* 10:714;

Cell phone image detection of individual infected mosquitoes

Results align with qPCR

Especially a good mousetrap for viruses!
In involving undergraduates in the crisis that besets us all

- Feb 24, DIY Diagnostics Mentors and Students vote to change spring curriculum
  - New spring students implement COVID-19 CDC qPCR Dx
  - Experienced students form development team for COVID-19 LAMP-OSD Dx
- Feb 29, Existing COVID LAMP assays pulled from literature and ordered
- March 2, First successful demonstration of CDC qPCR
- March 4, screening of known primer sets begin
- March 11, OSDs designed and ordered
- March 13, campus closed, project transferred to Ellington Lab
As usual, primer choice in LAMP is key

We also have newer primer sets that reduce detection time to 20’ - 30’

Figure 2. SARS-CoV-2 LAMP-OSD assays. OSD fluorescence measured in real-time during LAMP amplification for NB (A), 5 primer Lamb (B), and Tholoth (C) LAMP-OSD assays are depicted as amplification curves. Presence or absence of OSD fluorescence visually observed at assay endpoint for NB (D), Lamb (E), and Tholoth (F) LAMP-OSD assays are depicted as images of reaction tubes. NB LAMP-OSD assays were seeded with indicated copies of SARS-CoV-2 N RNA or MERS-CoV N RNA or no templates. Lamb and Tholoth LAMP-OSD assays were seeded with indicated copies of gBlock DNA templates.

Working towards a true POC assay

Figure 6. LAMP-OSD analysis of human saliva containing SARS-CoV-2 virions. Indicated virion amounts were spiked in TE buffer or human saliva and added to individual or multiplex (Mx) LAMP-OSD assays. Endpoint images of OSD fluorescence are depicted for Tholoth (A), 6 primer LAMB (B), and NB (C) individual LAMP-OSD assays and Tholoth+NB Mx LAMP-OSD assays (D).

- Spike inactivated virions into saliva
- Add to LAMP-OSD assay
- That’s it. Really
- Sensitivity could be better ... but keep this Figure in mind going forward

https://www.biorxiv.org/content/10.1101/2020.04.13.039941v3
Direct saliva analysis within 30 to 40 min

1. 3 µL of irradiated SARS-CoV-2 virions and saliva directly added to a total of 25 µL multiplex LAMP-OSD reactions
2. Reactions incubated at 65 °C for 30 to 40 min
3. OSD fluorescence imaged at endpoint

The addition of in-house engineered enzymes improves performance

Commercial RT-LAMP enzymes

<table>
<thead>
<tr>
<th>Irradiated virions in saliva</th>
<th>Irradiated virions in saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>5000 500 50 5 0</td>
<td>5000 500 50 5 0</td>
</tr>
</tbody>
</table>

SARS-2 Mx LAMP-OSD 1
SARS-2 Mx LAMP-OSD 2
Bst2.0+RTX (30 min)
Bst2.0+RTX (40 min)

In-house engineered RT-LAMP enzymes

<table>
<thead>
<tr>
<th>Irradiated virions in saliva</th>
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<td>5000 500 50 5 0</td>
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</tr>
</tbody>
</table>

SARS-2 Mx LAMP-OSD 1
SARS-2 Mx LAMP-OSD 2
BR512+VG125 (30 min)
BR512+VG125 (40 min)

Mx LAMP-OSD 1 = one tube NB and Lamb6 multiplex assay
Mx LAMP-OSD 2 = one tube NB and Tholoth multiplex assay
<table>
<thead>
<tr>
<th></th>
<th>SARS-CoV-2 DETECTR</th>
<th>SARS-CoV-2 SHERLOCK</th>
<th>CDC SARS-CoV2 qRT-PCR</th>
<th>RT-LAMP-OSD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target</strong></td>
<td>N gene &amp; E gene</td>
<td>S gene &amp; Orf1ab gene</td>
<td>N-gene (3 amplicons)</td>
<td>Multiplex: ORF1ab, N gene</td>
</tr>
<tr>
<td></td>
<td>(N gene gRNA compatible with CDC N2 amplicon, E gene compatible with WHO protocol)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sample control</strong></td>
<td>RNase P</td>
<td>None</td>
<td>RNase P</td>
<td>Gapd</td>
</tr>
<tr>
<td><strong>Limit of Detection</strong></td>
<td>10-50 copies/µL input</td>
<td>10-100 copies/µL input</td>
<td>1-3.16 copies/µL input</td>
<td>2-20 copies/µL input (total 5 µL)</td>
</tr>
<tr>
<td><strong>Assay reaction time</strong></td>
<td>~40 min</td>
<td>~60 min</td>
<td>~120 minutes</td>
<td>60-90 min</td>
</tr>
<tr>
<td><strong>Assay components</strong></td>
<td>RT-LAMP (62°C, 30 min) Cas12 (37°C, 10 min) Lateral flow (RT, 2 min)</td>
<td>RT-RPA (42°C, 25 min) IVT + Cas13 (37°C, 30 min) Lateral flow (RT, 2 min)</td>
<td>UDG digestion (25°C, 2 min), reverse transcription (50°C, 15 min), denature (95°C, 2 min) amplification, (95°C, 3 sec; 55°C 30 sec; 45 cycles)</td>
<td>One pot RT-LAMP-OSD using either commercial enzymes Bst2.0 + RTx or using open source enzymes Bst-LF + FeRT</td>
</tr>
<tr>
<td><strong>Heavy instrumentation required</strong></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Cost estimate for SARS-CoV-2 test

Rough estimates based on market cost of small batch reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Cost per tube ($)</th>
<th>Cost ($) per test (4 tubes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAMP Primers</td>
<td>0.11</td>
<td>0.44</td>
</tr>
<tr>
<td>OSD probe</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>Bst 2.0</td>
<td>0.57</td>
<td>2.28</td>
</tr>
<tr>
<td>RT</td>
<td>1.1</td>
<td>4.4</td>
</tr>
<tr>
<td>Betaine</td>
<td>0.04</td>
<td>0.16</td>
</tr>
<tr>
<td>dNTP</td>
<td>0.24</td>
<td>0.96</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2.1</strong></td>
<td><strong>8.32</strong></td>
</tr>
</tbody>
</table>

- Enzymes are the most expensive ingredient.
- This cost might be reduced by using engineered enzyme alternatives.

1. SARS-2 Mx RT-LAMP-OSD no primers (control)
2. SARS-2 Mx RT-LAMP-OSD with primers (test)
3. Human *gapd* no primers (control)
4. Human *gapd* with primers (test)
We have a total of 306 cases of plasmid distribution (overall)
All thanks to Dr. Jenny Molloy at Cambridge for spearheading these efforts!
• Just about everything: Sanchita Bhadra

• Enzyme engineering: Andre Maranhao, David Walker, Inyup Paik

• Environmental screening: Dalton Towers, David Brown

Funding: NSF RAPID, NIBIB Supplement, Welch Foundation