Real-time Autonomous Field Surveillance for Vector-Borne Pathogens

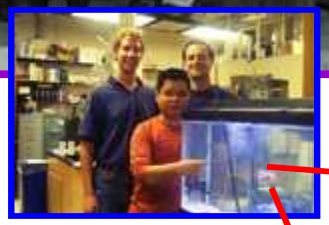
Robert Meagher, Ph.D.
Biotechnology and Bioengineering Department
Sandia National Laboratories
Livermore, CA

Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under Contract DE-AC04-94AL85000!

Outline

- Why am I here – Prehistory
- Biosurveillance for Vectorborne Diseases
  - Assay chemistry
  - Microfluidics
  - System integration
  - Modeling and visualization
- Future work and side lights

Who am I? Why am I here?



Puffy (RIP)

Barron research group
Chemical Engineering Dept.
Northwestern University
Evanston, IL, ~2001

INTERNET ARCHIVE
WayBackMachine

BIOLOGICAL & ENGINEERING Sciences

8600

Sandia National Laboratories

Anal. Chem. **2007,** *79,* 1848−1854

Multiplexed p53 Mutation Detection by Free-Solution Conjugate Microchannel Electrophoresis with Polyamide Drag-Tags

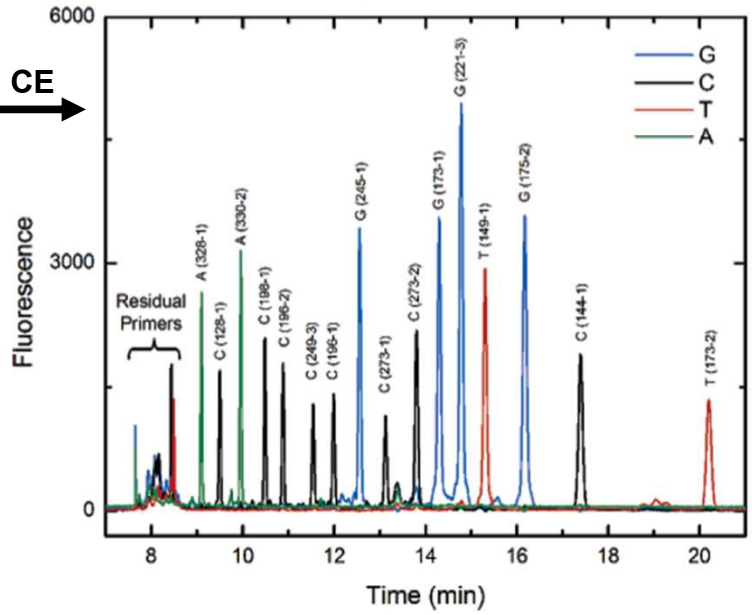
Robert J. Meagher,[†,§] Jennifer A. Coyne,[†] Christa N. Hestekin,[†] Thomas N. Chiesl,[†] Russell D. Haynes,[‡] Jong-In Won,[†,||] and Annelise E. Barron*[,†,‡]

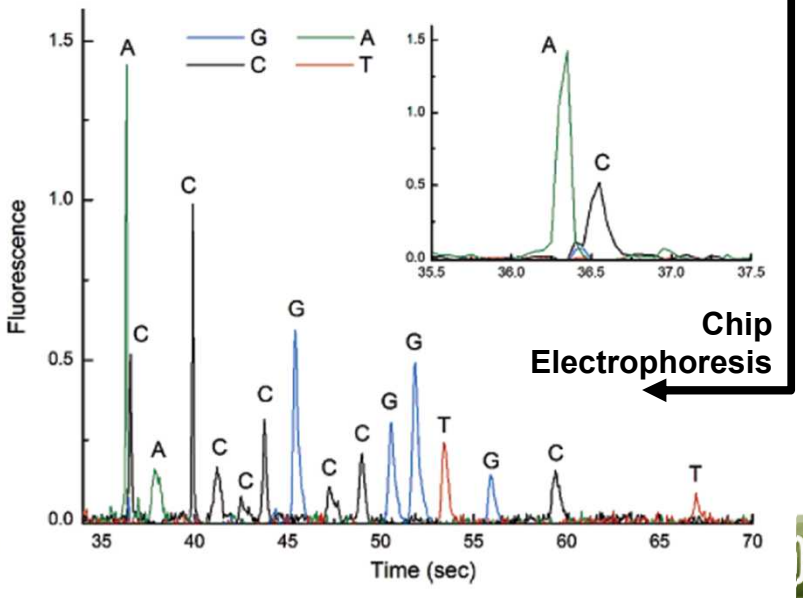
Department of Chemical and Biological Engineering and Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208

We report a new, bioconjugate approach to performing highly multiplexed single-base extension (SBE) assays, which we demonstrate by genotyping a large panel of point mutants in exons 5−9 of the p53 gene. A series of monodisperse polyamide "drag-tags" was created using both chemical and biological synthesis and used to

specific SNPs have been found to predispose individuals to certain diseases, including sickle cell anemia and Alzheimer's disease.[3,4] For example, mutations in the p53 gene have been implicated in a wide variety of human cancers, with missense mutations comprising a large majority of deleterious p53 sequence alterations.[5–] Furthermore, sequence polymorphisms in a variety of interacting

CE

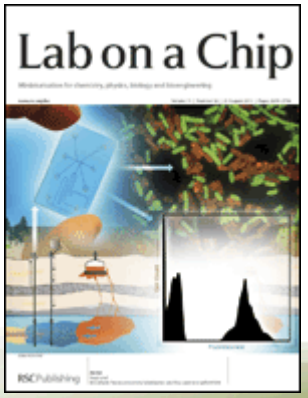




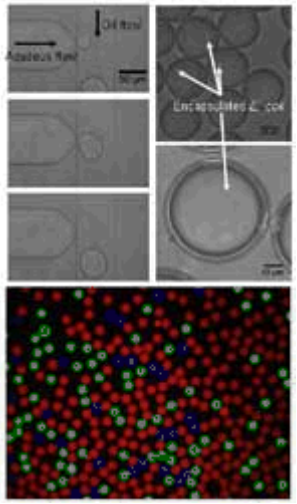
Microfluidics @ Sandia National Labs

Portable instrumentation for biothreat detection

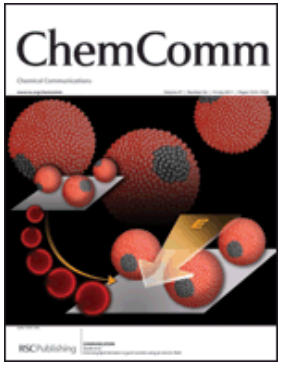
Chip-based liquid-liquid extraction for protein purification

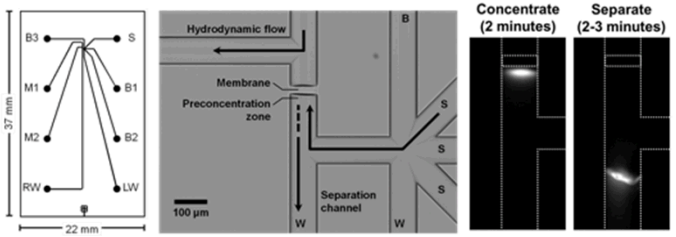


Microfluidic Flow-FISH for environmental microbiology

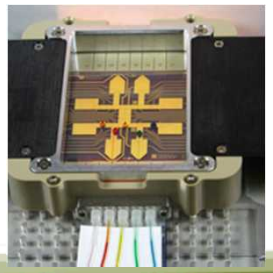


Microfluidic emulsions and droplets for single-bacteria applications



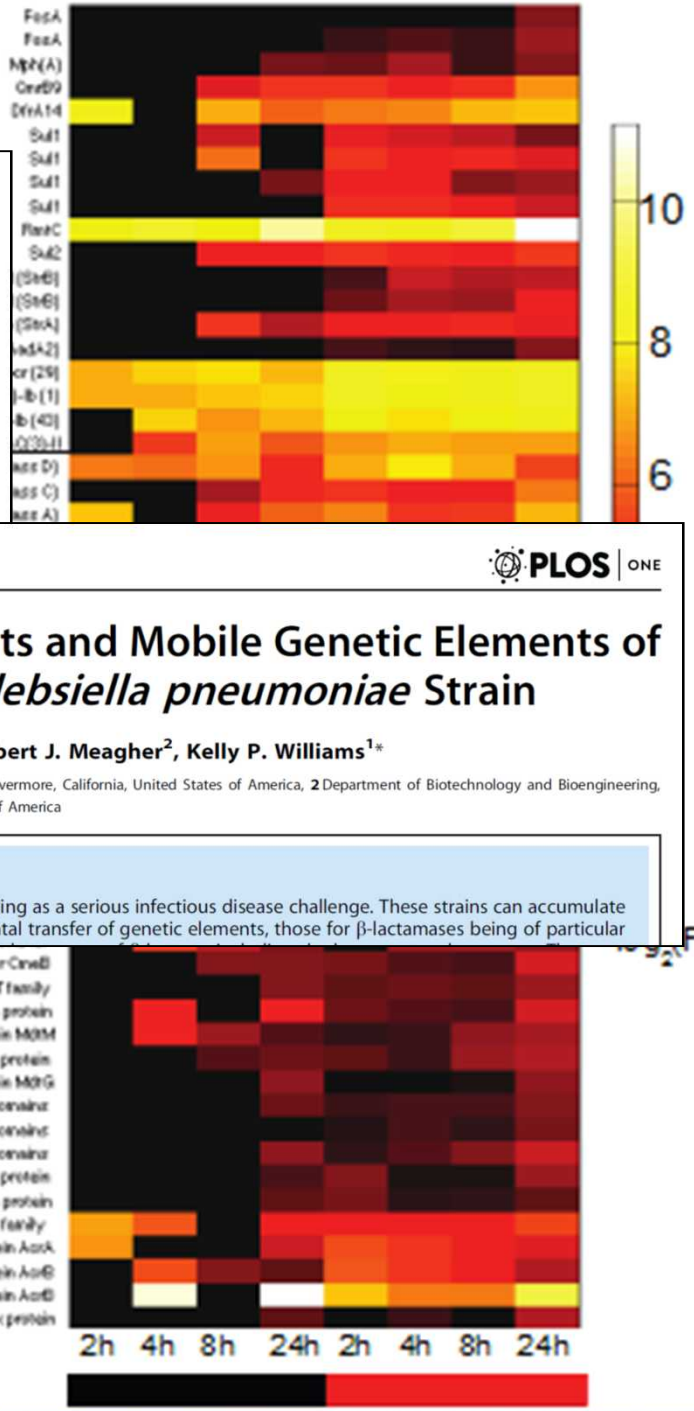


Microchannel electrophoresis and sample preconcentration



Microfluidic sequencing library prep and sample prep

Infectious disease @ Sandia



IAI
Journals.ASM.org

Transcriptomic Analysis of *Yersinia enterocolitica* Biovar 1B Infecting Murine Macrophages Reveals New Mechanisms of Extracellular and Intracellular Survival

Zachary W. Bent,[a*] Kunal Poorey,[a] David M. Brazel,[a*] Annette E. LaBauve,[b] Anupama Sinha,[a] Deanna J. Curtis,[a] Samantha E. House,[a] Karen E. Tew,[a] Rachelle Y. Hamblin,[a] Kelly P. Williams,[a] Steven S. Branda,[b] Glenn M. Young,[c] Robert J. Meagher[b]

Departments of Systems Biology[a] and Biotechnology and Bioengineering,[b] Sandia National Laboratories, Technology, University of California, Davis, California, USA[c]

Yersinia enterocolitica is typically considered an extracellular pathogen; howev number of bacteria are stably maintained within host cell vacuoles. Little is kn during an infection. To address this question and to elucidate the spatially and *Y. enterocolitica* biovar 1B through the course of an *in vitro* infection, transcri

OPEN ACCESS Freely available online

PLOS ONE

Resistance Determinants and Mobile Genetic Elements of an NDM-1-Encoding *Klebsiella pneumoniae* Strain

Corey M. Hudson[1], Zachary W. Bent[1], Robert J. Meagher[2], Kelly P. Williams[1]*

s, Livermore, California, United States of America, 2 Department of Biotechnology and Bioengineering, es of America

erging as a serious infectious disease challenge. These strains can accumulate zontal transfer of genetic elements, those for β-lactamases being of particular

PLOS ONE

RESEARCH ARTICLE

Surveillance for Western Equine Encephalitis, St. Louis Encephalitis, and West Nile Viruses Using Reverse Transcription Loop-Mediated Isothermal Amplification

Sarah S. Wheeler[1¤a], Cameron S. Ball[2], Stanley A. Langevin[2¤b], Ying Fang[1], Lark L. Coffey[1], Robert J. Meagher[2]*

1 University of California Davis, School of Veterinary Medicine, Department of Pathology, Microbiology and Immunology, Davis, California, United States of America, 2 Sandia National Laboratories, Biotechnology and Bioengineering Department, Livermore, California, United States of America

¤a Current address: Sacramento-Yolo Mosquito and Vector Control District, Elk Grove, California, United States of America
¤b Current address: University of Washington, Department of Microbiology, Seattle, Washington, United States of America
* rmeaghe@sandia.gov

CrossMark
click for updates

BIOLOGICAL & ENGINEERING

genomeweb

Jan 13, 2016

Sandia Researchers Developing Multiplex Assay Differentiating Viral Febrile Illnesses, Malaria

Jan 13, 2016 | Madeleine Johnson

Premium

NEW YORK (GenomeWeb) – Researchers at Sandia National Laboratories have been awarded a grant to develop a field-deployable assay for differential diagnosis of malaria and viral febrile illness.

The two-year grant from the National Institute of Allergy and Infectious Disease totals $188,759 and is expected to fund the proof-of-concept phase of the project. The Sandia research team will also be collaborating with researchers at the Institute for Human Health and Immunity at the University of Texas Medical Branch in Galveston.

The point-of-care molecular diagnostic device will potentially discriminate infection from a panel of viruses causing febrile illness as well as the parasite causing malaria.

Specifically, the proposed technology will use closed-tube isothermal nucleic acid amplification to detect and discriminate viruses endemic in West Africa — Ebola, Lassa, yellow fever, chikungunya, dengue, and West Nile viruses — as well as *Plasmodium falciparum*.

The amplification method of choice is reverse-transcription loop-mediated isothermal amplification (RT-LAMP), Robert Meagher, lead researcher on the project at Sandia, told GenomeWeb in an interview.

Although multiplex LAMP has been reported in the literature — a *BioTechniques* study in 2012, for example — Meagher said the method has not yet been widely adopted. The Sandia technique is also different, and has some unique advantages, particularly in that the multiplexing is enabled by improvements the group has made in visualization.

While many using LAMP rely on turbidity or add an intercalating dye like SYBR Green, Meagher said his group's strategy is more akin to a fluorophore and quencher-based approach.

"That adds an element of target specificity and allows us to do multiplex

- **NIAID R21-R33 award**
- **UTMB / Scott Weaver**
- **Ebola, Lassa, yellow fever, chiknungunya, dengue, and West Nile viruses plus *Plasmodium falciparum***
- **Shortcut sample prep**
- **Multiplex LAMP with target specificity**
- **Smart phone for control and detection of assay**

So what led up to this?

- Started working on RT-LAMP for RNA virus detection for the “Smart Trap” – an autonomous, field-based detector for mosquito-borne pathogens
- Funded by DTRA as a demonstration technology for the “Biosurveillance Ecosystem”
- Currently finishing year 2 of 3.
- Developments in LAMP and microfluidics along the way were spun into the NIH febrile illness diagnostic.

Who cares about arboviruses?

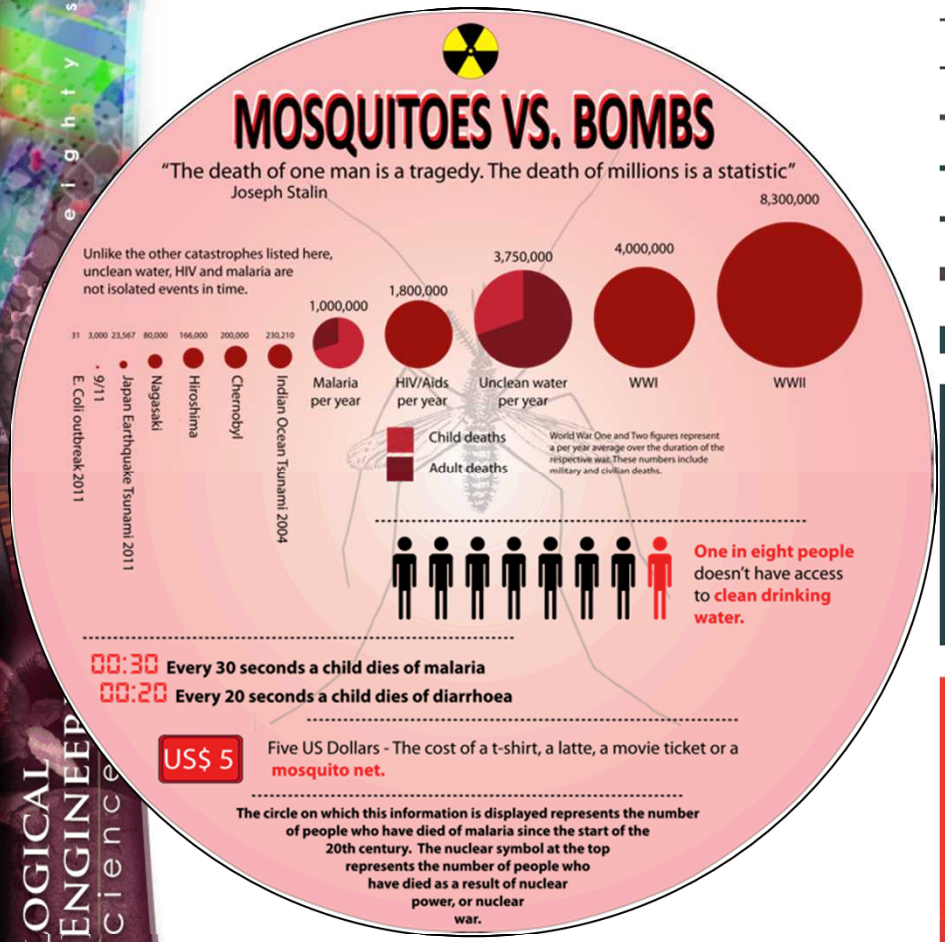
- "Arbovirus" = Arthropod-borne virus
  - Mostly RNA viruses; carried by mosquitoes, ticks, flies, *etc*
- West Nile, Dengue, Chikungunya, and now Zika exemplify how fast these viruses can emerge, re-emerge, or change boundaries.

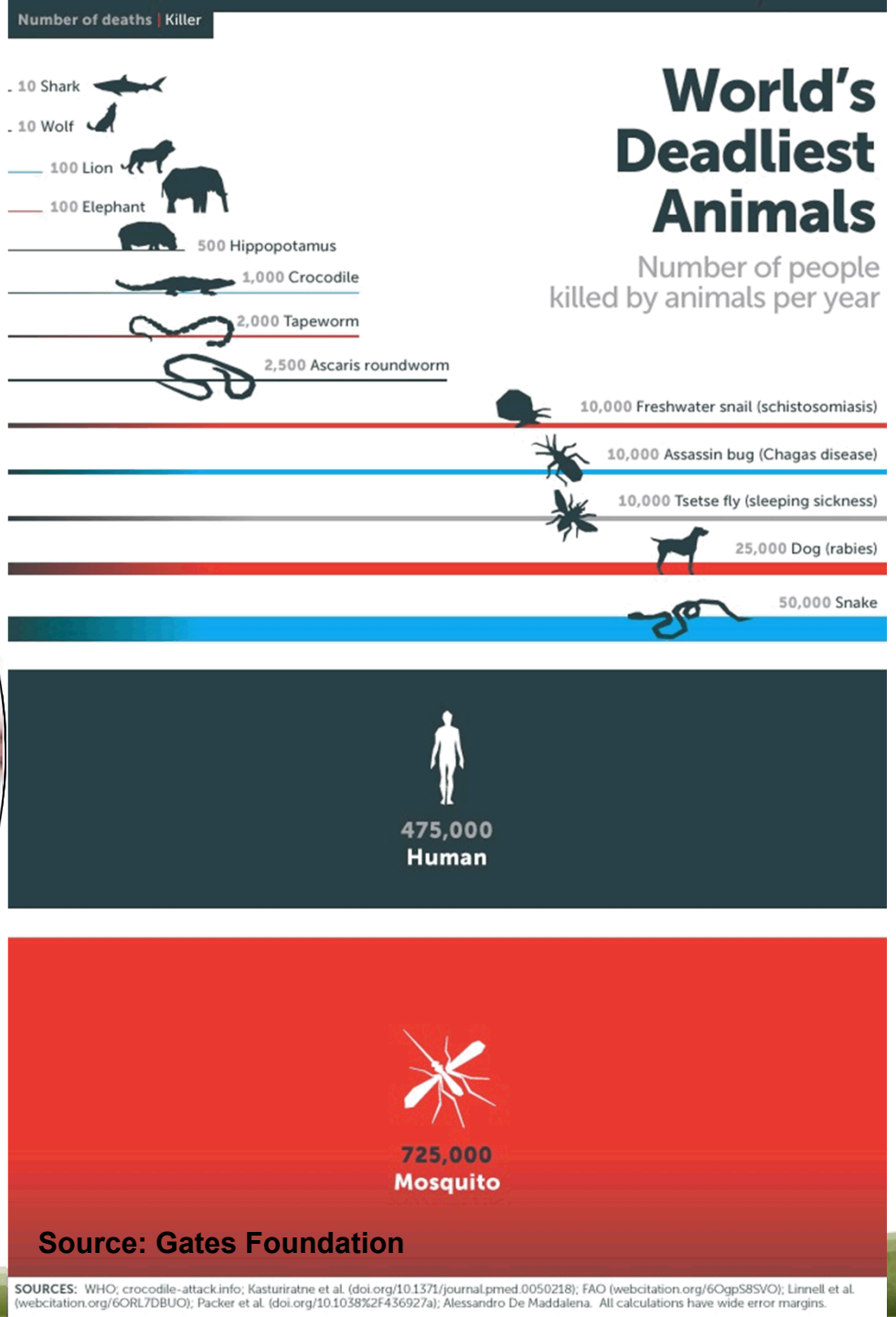
Dengue viruses
Yellow fever virus
Chikungunya virus
Zika virus

West Nile virus*
St. Louis encephalitis virus*
Japanese encephalitis virus
Rift Valley fever virus
Equine encephalitis viruses
(WEE*, VEE, EEE)

Transmits malaria
(not a virus!)

*** Historically found in California**


MOSQUITOES VS. BOMBS
"The death of one man is a tragedy. The death of millions is a statistic"
Joseph Stalin
Unlike the other catastrophes listed here, unclean water, HIV and malaria are not isolated events in time.
31 E. Coli outbreak 2011
3,000 9/11
23,567 Japan Earthquake Tsunami 2011
80,000 Nagasaki
166,000 Hiroshima
200,000 Chernobyl
230,210 Indian Ocean Tsunami 2004
1,000,000 Malaria per year
1,800,000 HIV/Aids per year
3,750,000 Unclean water per year
4,000,000 WWI
8,300,000 WWII
Child deaths
Adult deaths
World War One and Two figures represent a per year average over the duration of the respective war. These numbers include military and civilian deaths.
One in eight people doesn't have access to clean drinking water.
00:30 Every 30 seconds a child dies of malaria
00:20 Every 20 seconds a child dies of diarrhoea
US$ 5
Five US Dollars - The cost of a t-shirt, a latte, a movie ticket or a mosquito net.
The circle on which this information is displayed represents the number of people who have died of malaria since the start of the 20th century. The nuclear symbol at the top represents the number of people who have died as a result of nuclear power, or nuclear war.


Number of deaths | Killer
World's Deadliest Animals
Number of people killed by animals per year
10 Shark
10 Wolf
100 Lion
100 Elephant
500 Hippopotamus
1,000 Crocodile
2,000 Tapeworm
2,500 Ascaris roundworm
10,000 Freshwater snail (schistosomiasis)
10,000 Assassin bug (Chagas disease)
10,000 Tsetse fly (sleeping sickness)
25,000 Dog (rabies)
50,000 Snake
475,000
Human
725,000
Mosquito
Source: Gates Foundation
SOURCES: WHO; crocodile-attack.info; Kasturiratne et al. (doi.org/10.1371/journal.pmed.0050218); FAO (webcitation.org/6OgpS8SVO); Linnell et al. (webcitation.org/6ORL7DBUO); Packer et al. (doi.org/10.1038%2F436927a); Alessandro De Maddalena. All calculations have wide error margins.

Vector-borne pathogens are a GLOBAL problem

Pakistani homeless sleeping on the street under a mosquito net

Photo credit: AP Photo/Muhammed Muheisen

Vector-borne pathogens are a local problem

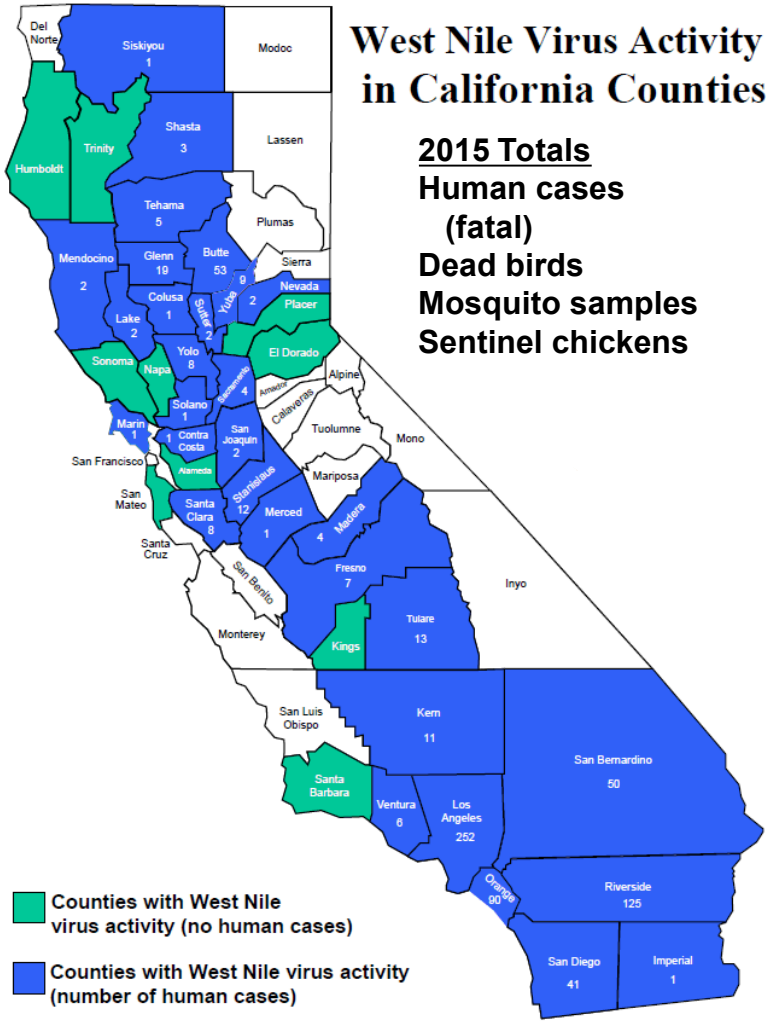
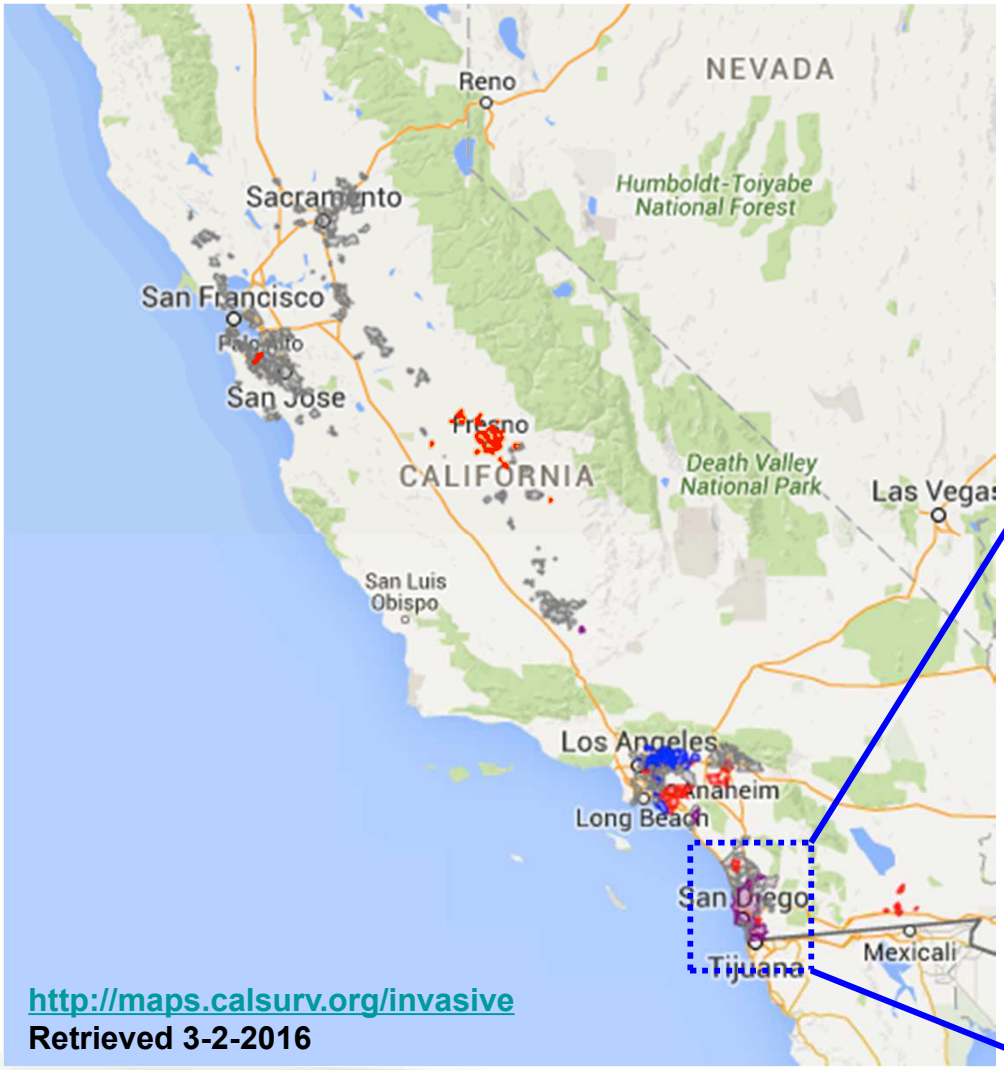


Image retrieved from: www.westnile.ca.gov

45 Human deaths (YTD) in CA from West Nile virus infection in 2015

In San Diego County, there were 41 human cases and 5 deaths from West Nile in 2005.

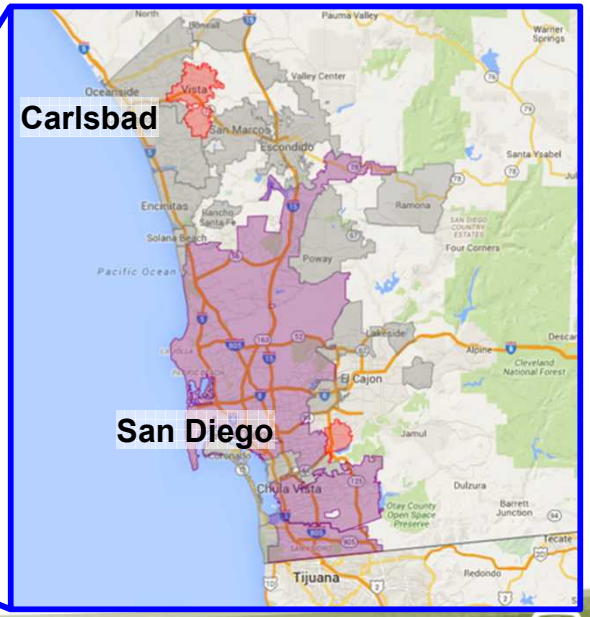
West Nile isn't the only threat



http://maps.calsurv.org/invasive
Retrieved 3-2-2016

Invasive vector species

- ***Aedes aegypti***
- ***Aedes albopictus***
- ***A. aegypti* + *A. albopictus***
- **Surveillance areas with no detection**



Current methods for arbovirus surveillance

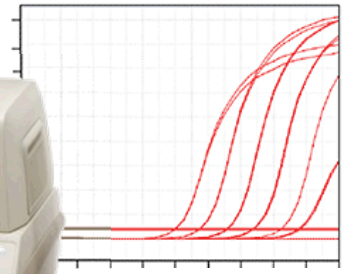
- Low-tech sample collection
- Manual skilled labor (mosquito sorting, etc)
- Sophisticated molecular assays

} 1-2 week turnaround
>$20/sample

Mosquito collection

Mosquito sorting

Sentinel animals

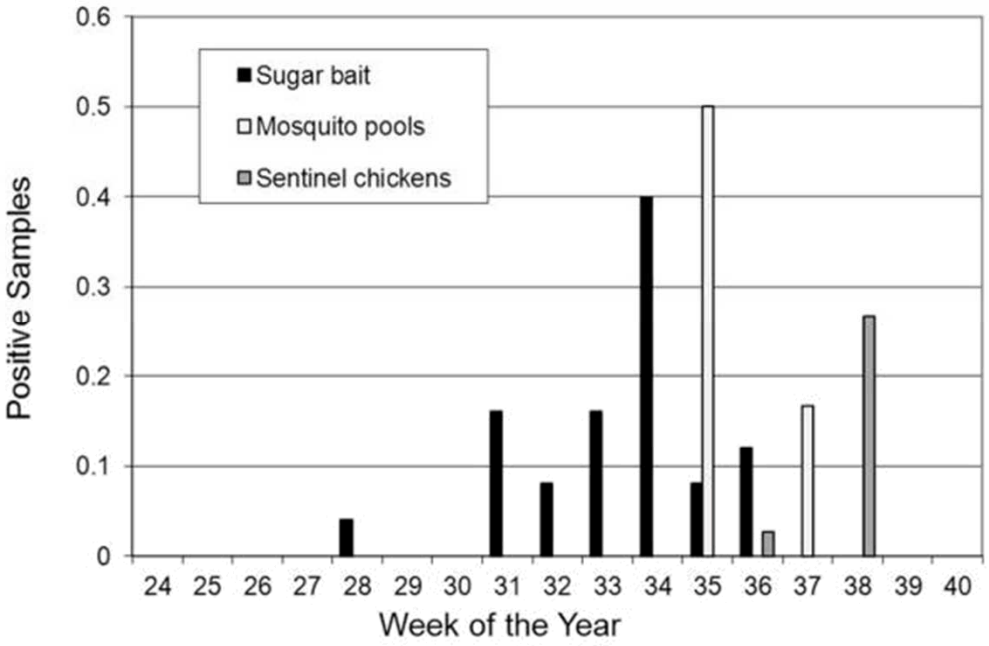


Mosquito sorting is tedious

- Thousands of mosquitoes sorted by hand

- Is it a female? Which species?
  - People who know how to do this are in short supply!

Sugar baiting is a field-tested alternative to mosquito trapping



A sugar bait, made from a cryovial and dental wick with blue-colored syrup and a floral attractant

Baits detected WNV weeks before mosquito pools or sentinel chickens

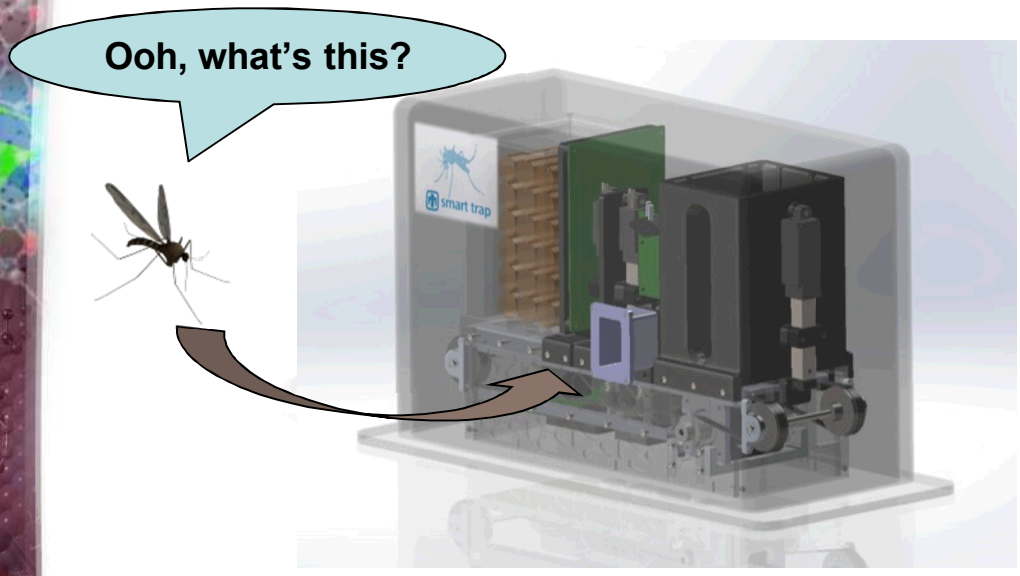
Lothrop et al. (2012)

But sugar baits still require a human to schlep to the field

line positive results. The small quantity of virus present on the cards is not surprising, given that mosquitoes expectorate approximately 4.7 nL of saliva during feeding (Devine et al. 1965, Hurlbut 1966) and that the cards can remain in the field for periods of up to 2 weeks before being dispatched to a diagnostic laboratory.

van den Hurk et al. (2014)

We propose that sugar-bait testing enables autonomous vector surveillance



Data transmission and predictive modeling

Viral incidence map

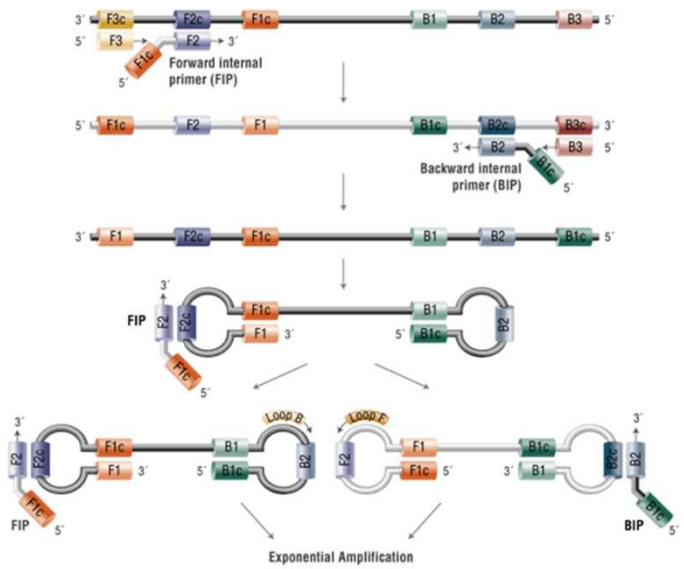
Robert Meagher

Outline

- Why am I here – Prehistory
- Biosurveillance for Vectorborne Diseases
  - **Assay chemistry**
  - Microfluidics
  - System integration
  - Modeling and visualization
- Future work and side lights

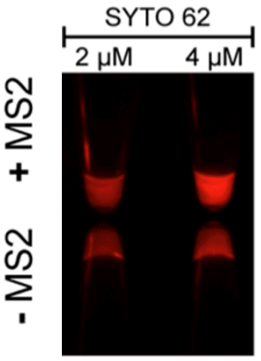
LAMP is a PCR alternative well suited to low resource settings (such as Smart Trap)

- Isothermal technique, uses 6 “primers” to locate and amplify target DNA



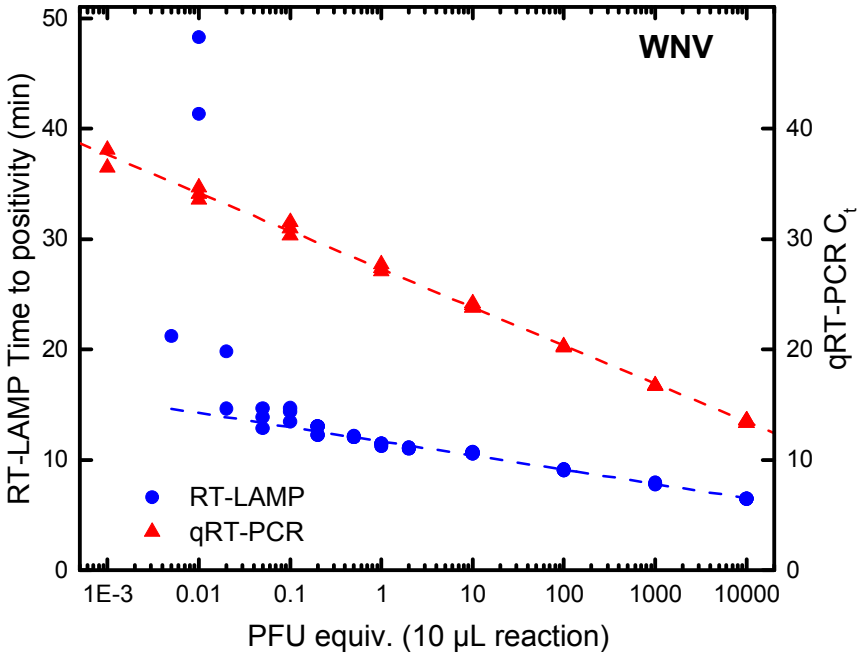
- Fast (5-20 min), robust, simple, sensitive
- Low capital expense
- Add RT for RNA targets

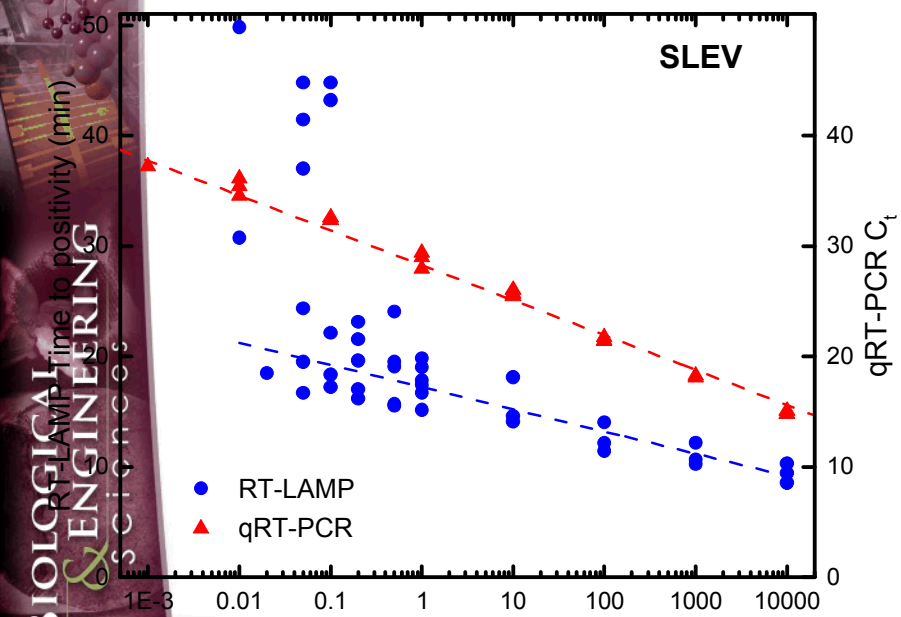
- Can’t easily multiplex
- Endpoint signals with dyes are not easily interpreted by eye
- Prone to false positives
- Less quantitative than qPCR

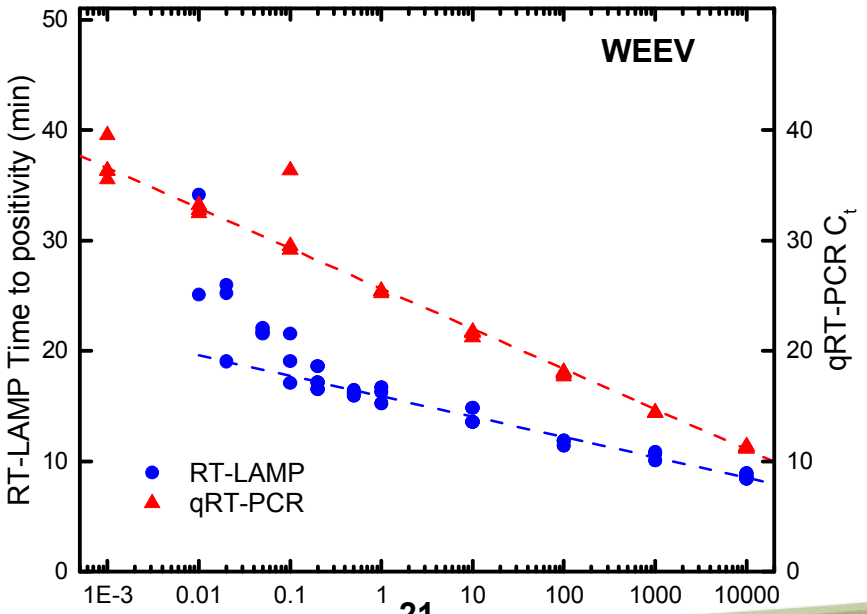


RT-LAMP *vs* qRT-PCR

- In all cases tried, qRT-PCR is ~1 log more sensitive than RT-LAMP
- RT-LAMP for WNV, WEEV, SLEV all detects down to 0.01 PFU equiv.
- qRT-PCR is detecting 0.001 PFU equiv.
- RT-LAMP time-to-positivity is non-quantitative at lower end of sensitivity (and not great at high end either)
- RT-LAMP usually takes <30 minutes.





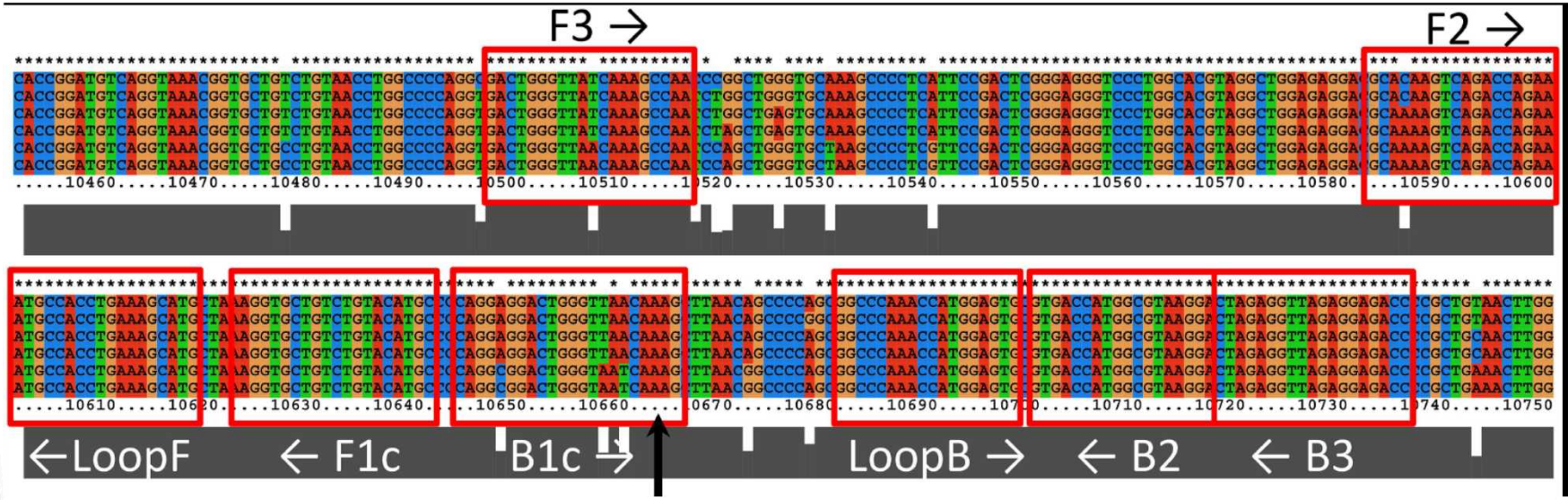


Wheeler *et al*, PLOS One 2016

Challenges of RT-LAMP primer design

- Primers can cover >60% of a target region (200-250 bp)
- Tight constraints on spacing of primers and ΔG for ends
- RNA viruses often have high sequence variability

SLEV 3'-UTR alignment & primer location



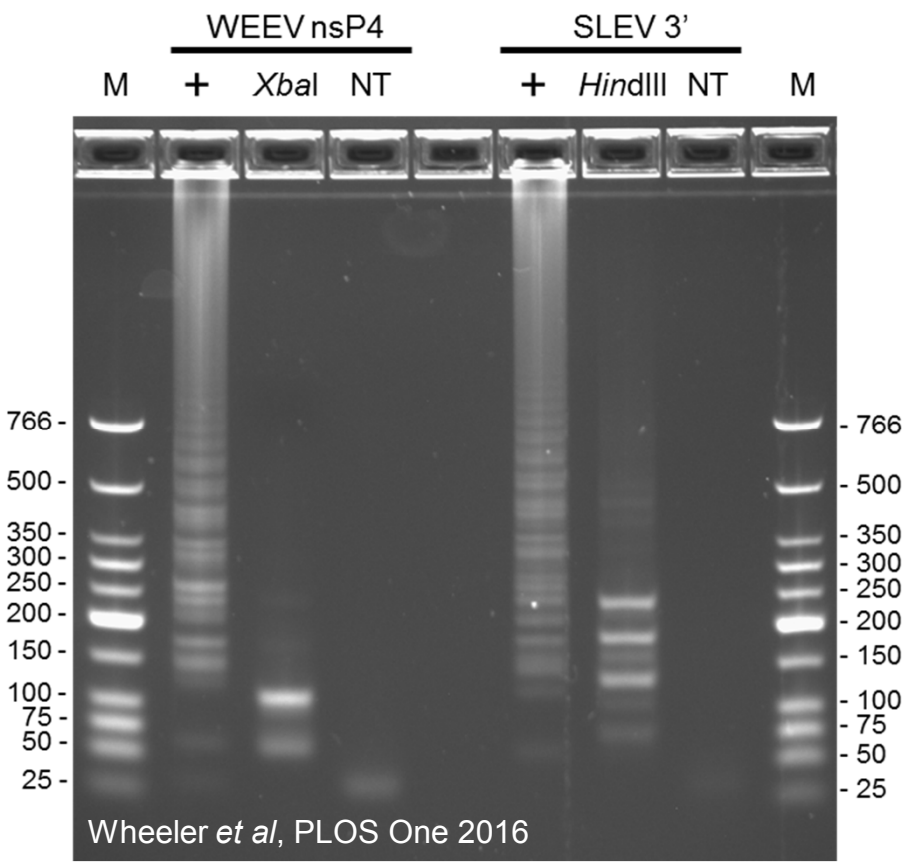
Wheeler *et al*, PLOS One 2016

For a deployed LAMP diagnostic:

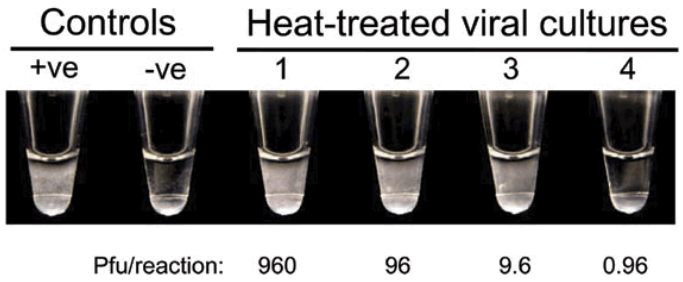
- *Closed tube* detection – don't want to open the tube after the reaction
- *Large discrimination* between positive and negative samples
- *Bright signals* – naked eye or simple detector
- *Endpoint* is good enough for yes/no answer (LAMP is semi-quantitative at best anyway!)
- *Target-specific*, vs detecting total DNA

How to know if LAMP worked? (old school)

A. Run product on a gel, with optional target-specific restriction digest

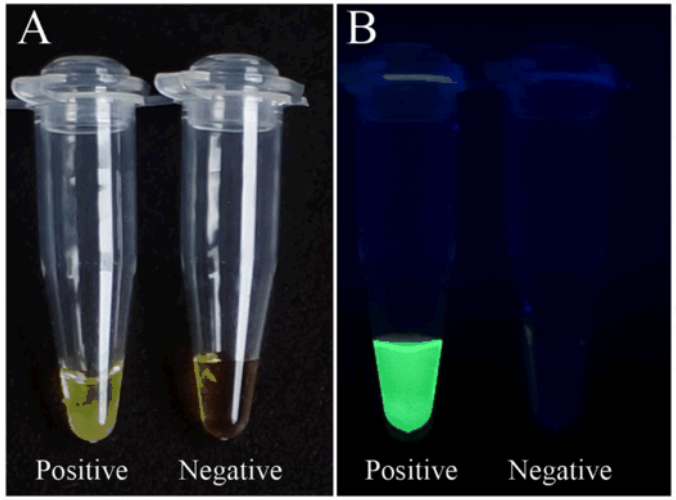


B. Turbidity (precipitation of Mg pyro-phosphate, from making a ton of DNA)



Jayawardena, *Emerg. Inf. Dis.* 2007

C. Post-reaction, open the tube and add a ton of SYBR Green

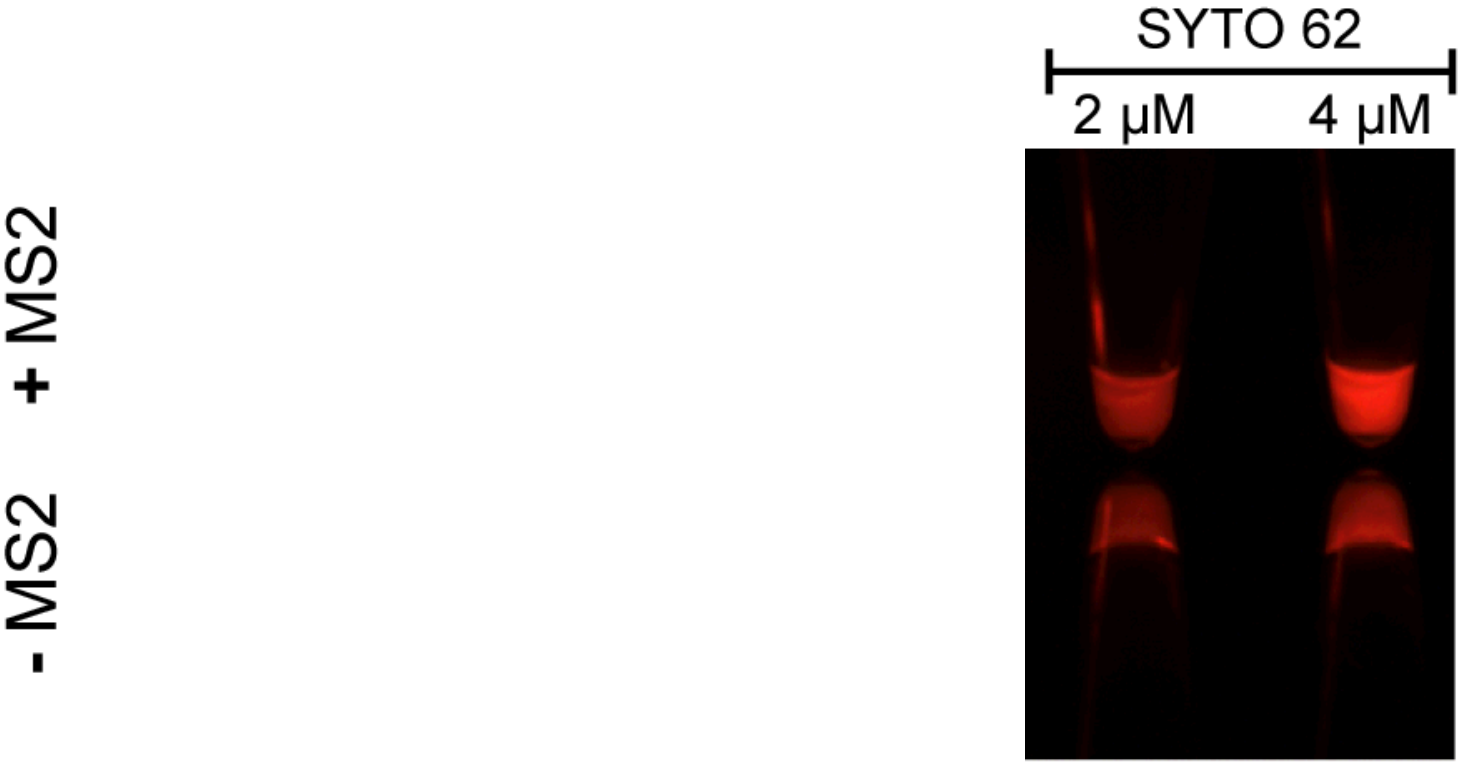


Nie *PLoS One* 2012

D, E, F, G... Other nonspecific indicators of total DNA synthesis…

QUASR improves discrimination between +/- results

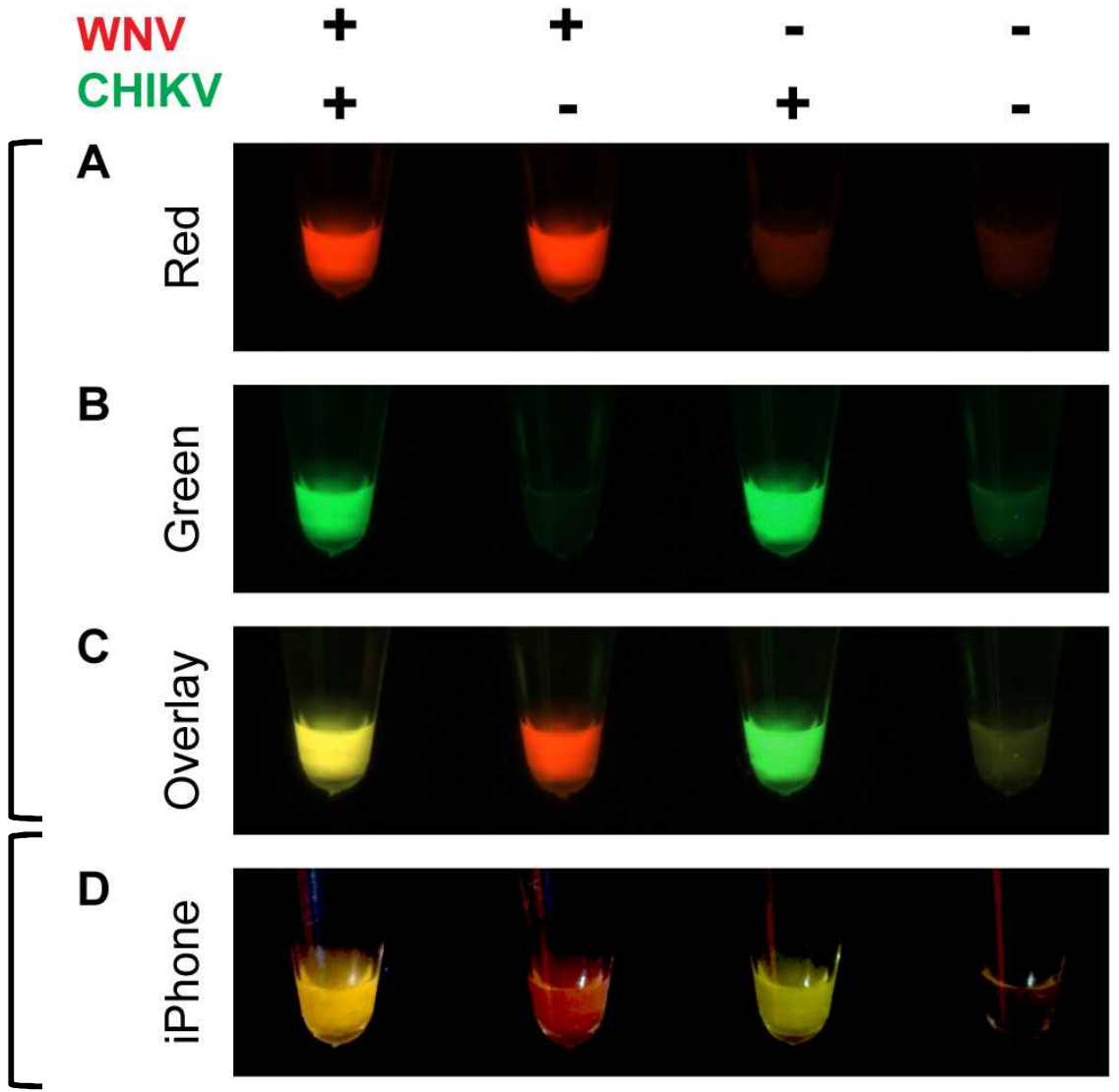
- New detection chemistry for LAMP based on interaction between a dye-labeled primer and quencher
- QUASR stands for… details coming soon (Ball *et al* minor revisions requested for *Anal Chem*)



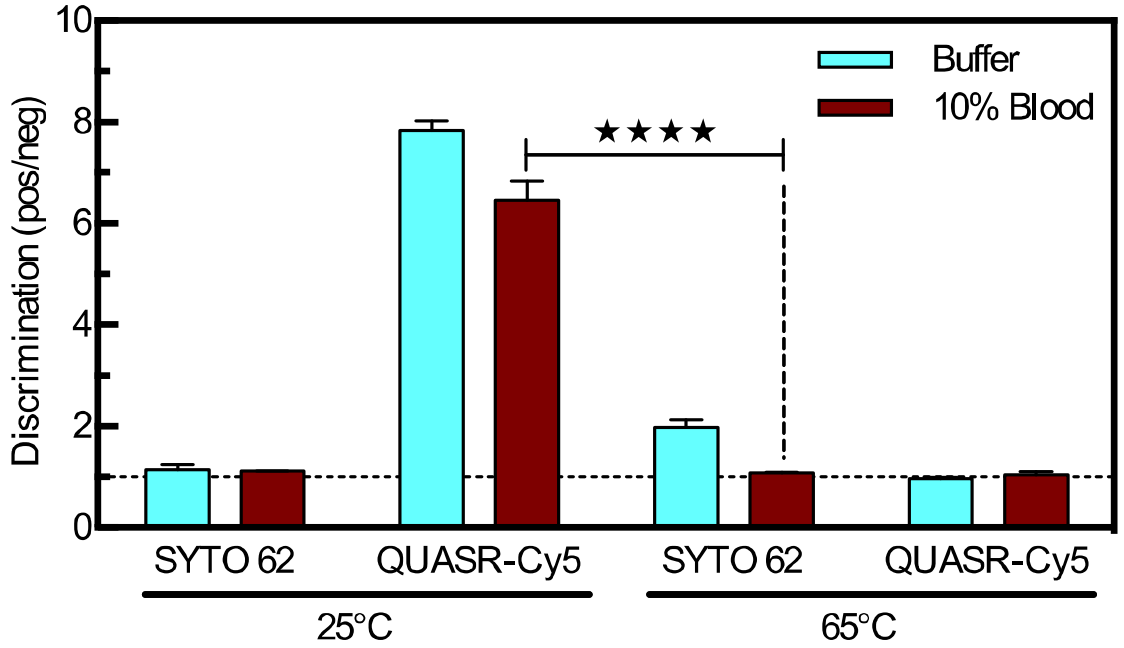
QUASR enables multiplexed LAMP

- High-powered LED excitation
- Hard-coated glass excitaiton and emission filters
- Machine vision camera

- LED flashlight
- Red plastic filter (theatre lighting gel)
- iPhone camera with white balance card for exposure



QUASR enables direct detection from complex samples



- Detection of MS2 phage spiked into whole blood, diluted at 10% into RT-LAMP reaction, with either SYTO 62, or QUASR-Cy5 for detection

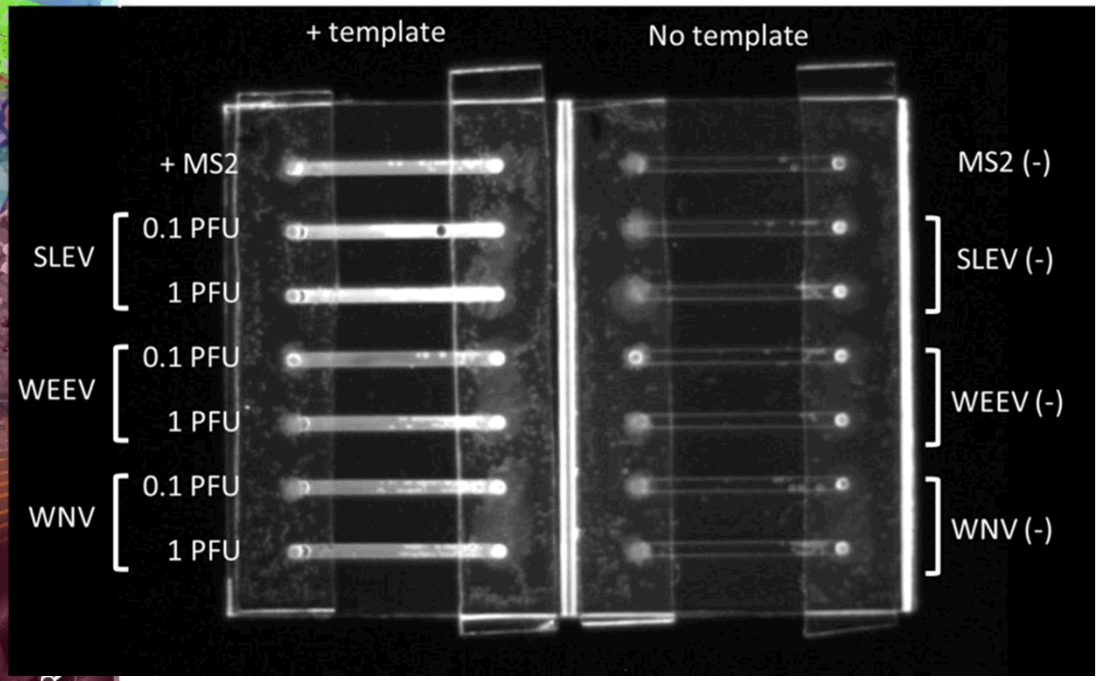
QUASR eliminates false positives

Reporter Mixture	Decision fluorophore	False Positives, X/Y (%)
SYTO only	SYTO 62	25/28 (89%)
SYTO + QUASR (all)	SYTO 62	42/117 (36%)
	QUASR (all)	0/117 (0%)
QUASR only (all)	QUASR (all)	1/80 (1%)*

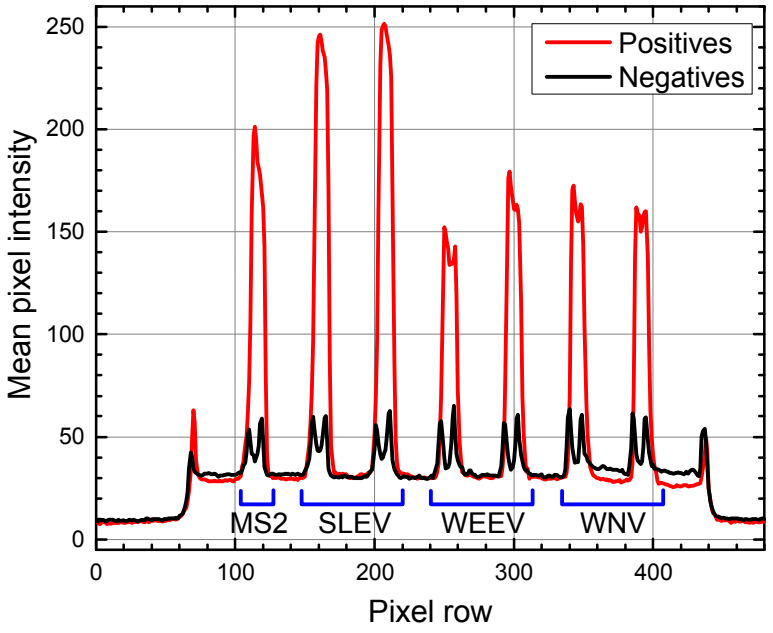
- RT-LAMP reaction conditions setup to intentionally encourage false positives
- QUASR showed 1% false positives
- SYTO showed 89% false positives
- Handy for detecting rare viruses
  - Lothrop et al. (2012) found 6.8% baits pos. for WNV in Coachella Valley

QUASR in Microfluidic Device

Polycarbonate test channels, sealed with tape



Integrating along length of channels

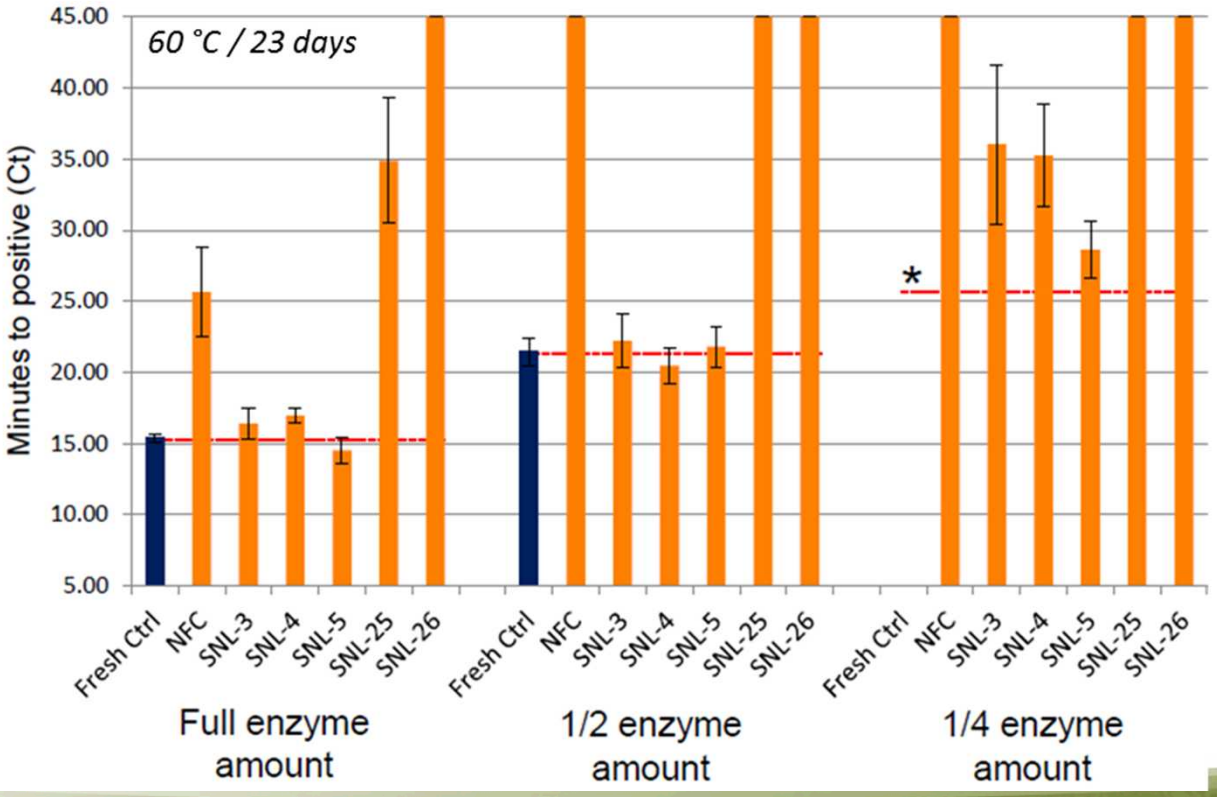


Works better in our chip than the SYTO dyes.

- **Better discrimination between positive and negative**
- **Detection at ambient temperature**
- **Reduced problem with chip-associated false-positives**

Field stabilized reagents

- Partnered with Biomatrica to formulate RT-LAMP assay reagents for long-term stability in dry form; rehydrate with “bait fluid” to run assay.
- Minimal loss in activity after >3 weeks at 60 C, even with reduced enzyme



Outline

- Why am I here – Prehistory
- Biosurveillance for Vectorborne Diseases
  - Assay chemistry
  - **Microfluidics**
  - System integration
  - Modeling and visualization
- Future work and side lights

Daily assay chip for Smart Trap

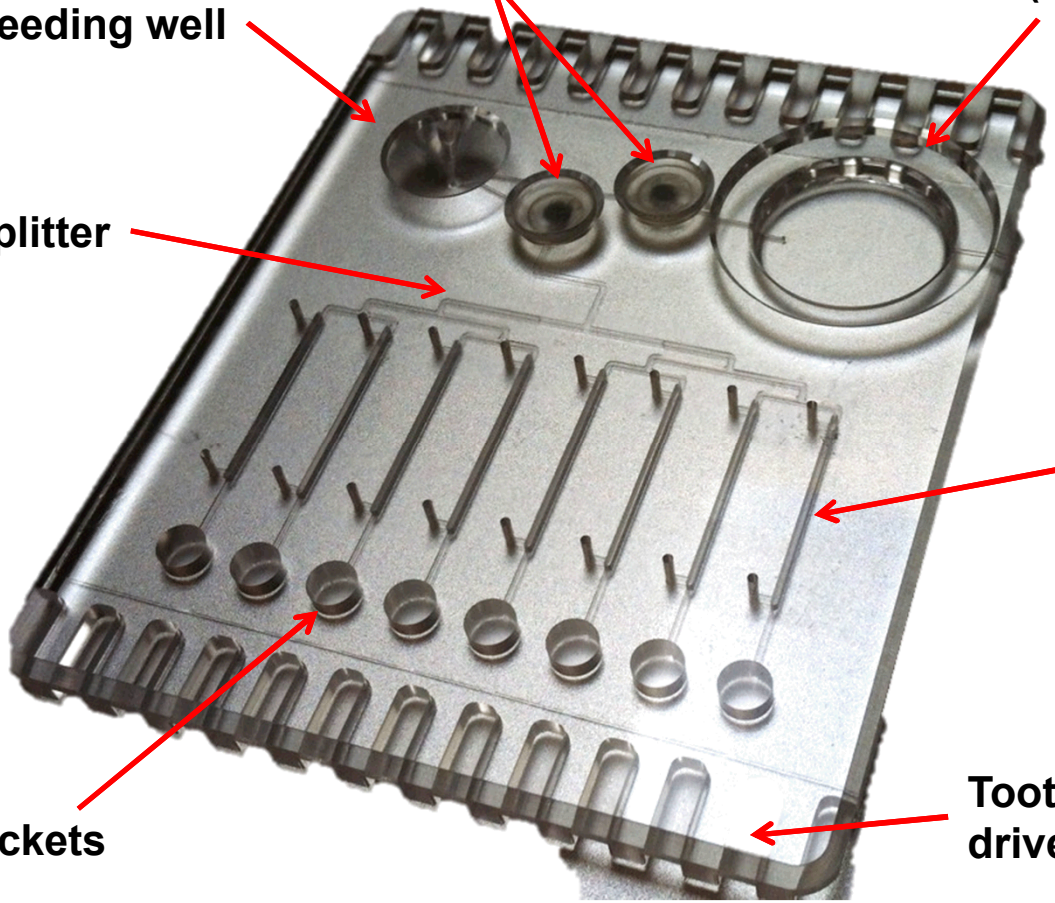
Production/durability requirements

- Amenable to scale up manufacturing (injection mold)
  - In 2014, CA detected WNV in ~3,500 mosquito pools, which means many more were tested
  - We will need thousands of chips at low cost
- Withstand reaction conditions (high temp + water)
- Sit in a field/marsh/desert for a month

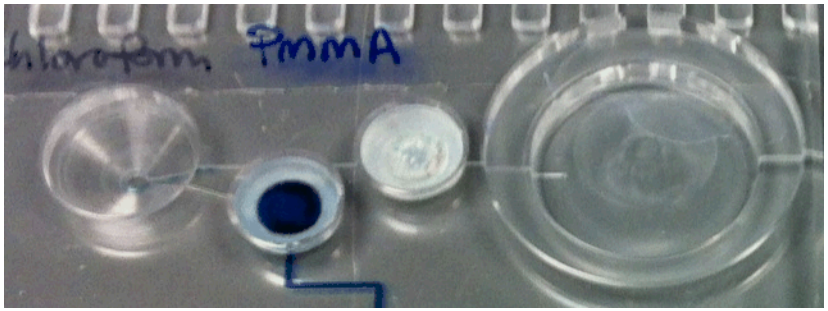
Functionality

- Test for a panel of viruses in parallel
- Minimize external instrumentation with passive fluidic control
- Guarantee sealing to prevent evaporation or contamination
- Store all reagents on-chip to minimize operator intervention
- Isolate sensitive reagents for maximum stability

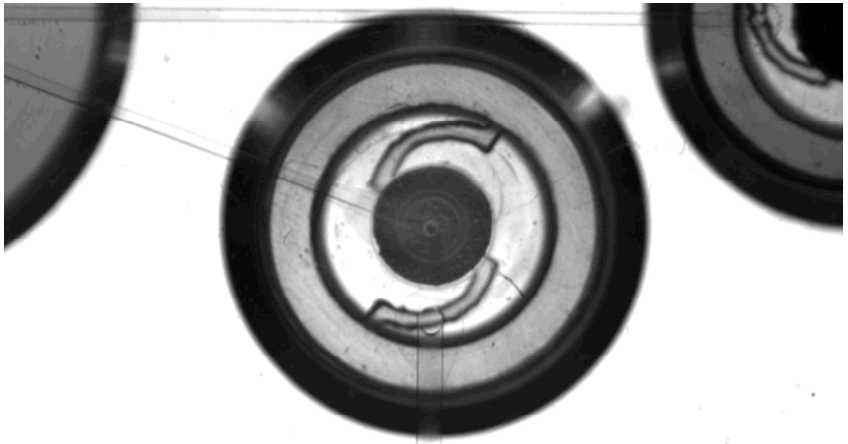
Smart Trap Daily Consumable Chip



Check valves seal reagents from the environment and stage fluid delivery



Top angled view
(actual)



Bottom view
(actual)

- Passive
- Normally closed
- Planar spring, low-profile for microfluidics
- Cracking pressure can be tuned by spring design and material
- Robust sealing against back pressure
- Pick-and-place spring installation
- Low-volume rapid prototyping for R&D, but scalable for higher volume manufacturing.

Feeding well, aka “the bait cave”

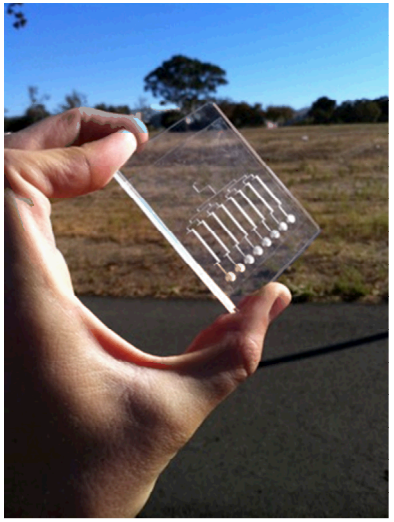
3D printed bait cave prototype covered in *Culex tarsalis* mosquitoes

Mosquitoes that take the bait turn blue from the food coloring

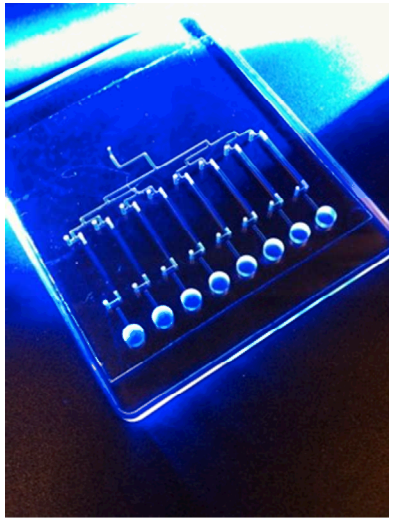
- The only part of the smart chip or smart trap exposed to mosquitoes for feeding
- Presents sugar bait in conical well
- Size prevents drowning

Assay reagents were pre-dried into the chip

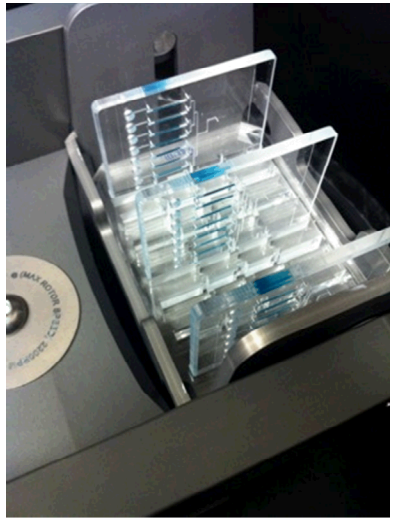
Bonded chip



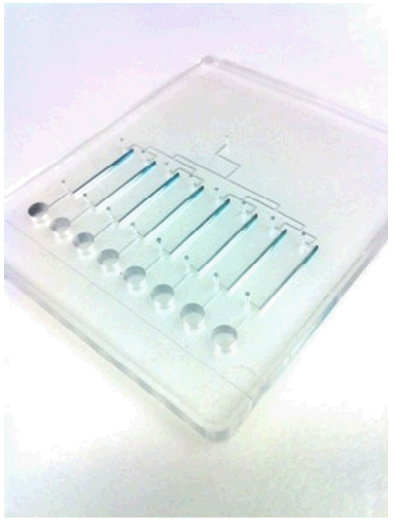
Lanes filled



Centrifuged under vacuum



Dried reagents in lanes



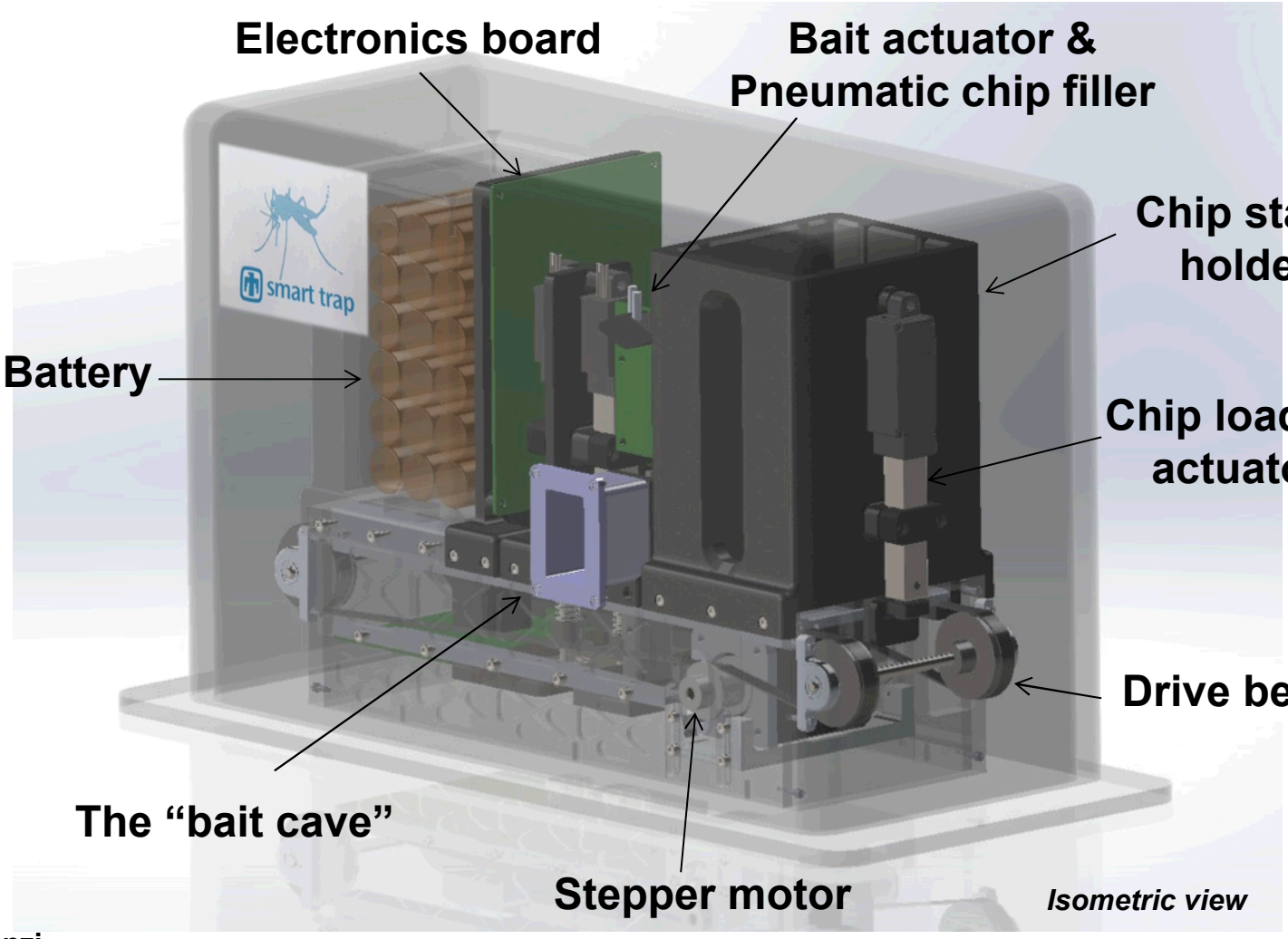
30-40 min to dry reagents into all 8 channels of 14 smart chips

Clear epoxy seals fill holes after drying

Outline

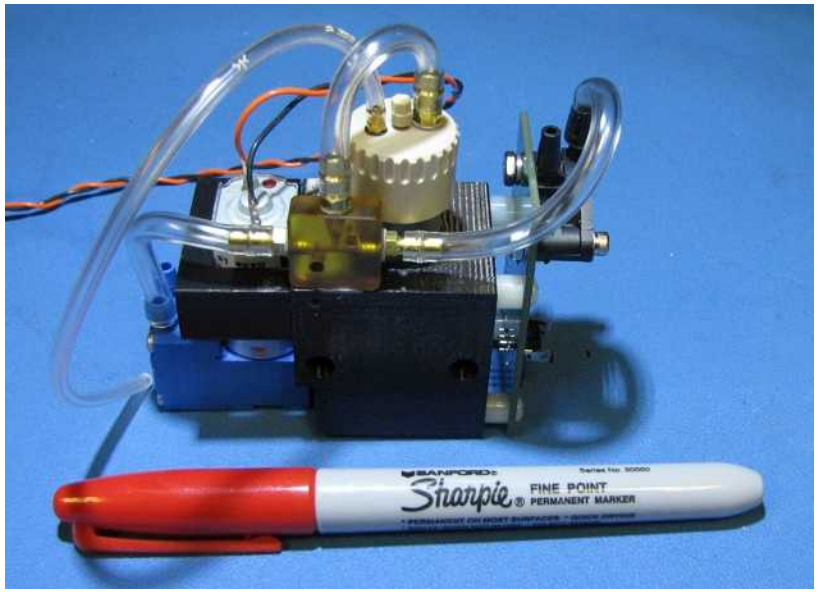
- Why am I here – Prehistory
- Biosurveillance for Vectorborne Diseases
  - Assay chemistry
  - Microfluidics
  - **System integration**
  - Modeling and visualization
- Future work and side lights

The smart trap is a portable vector biology lab the size of a shoebox



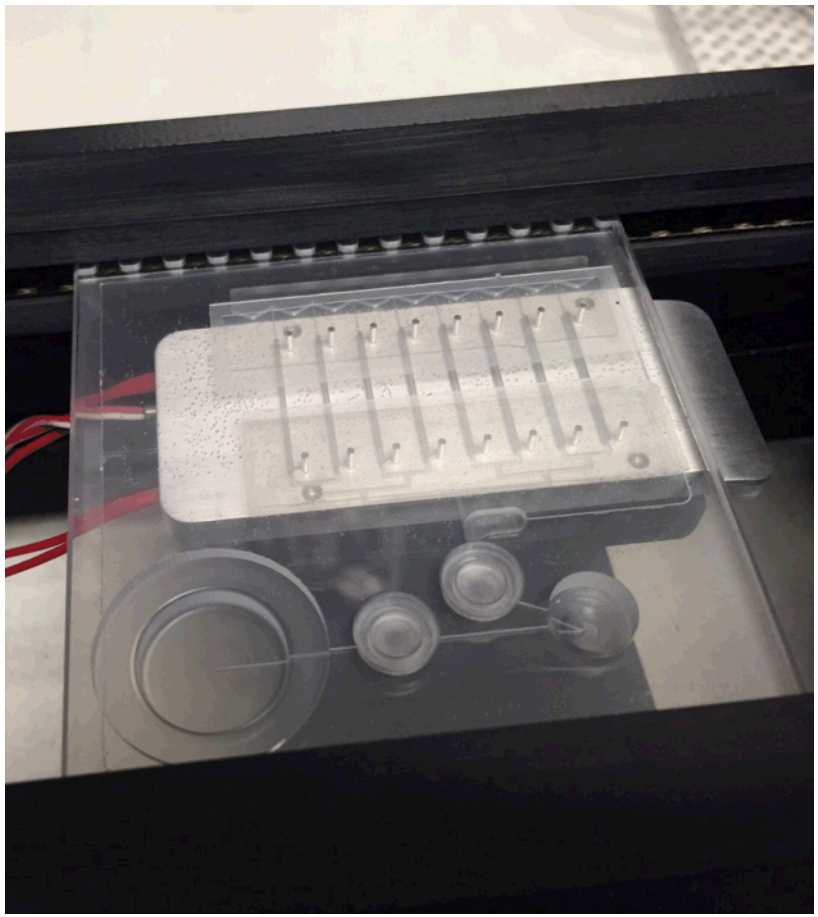
Ron Renzi

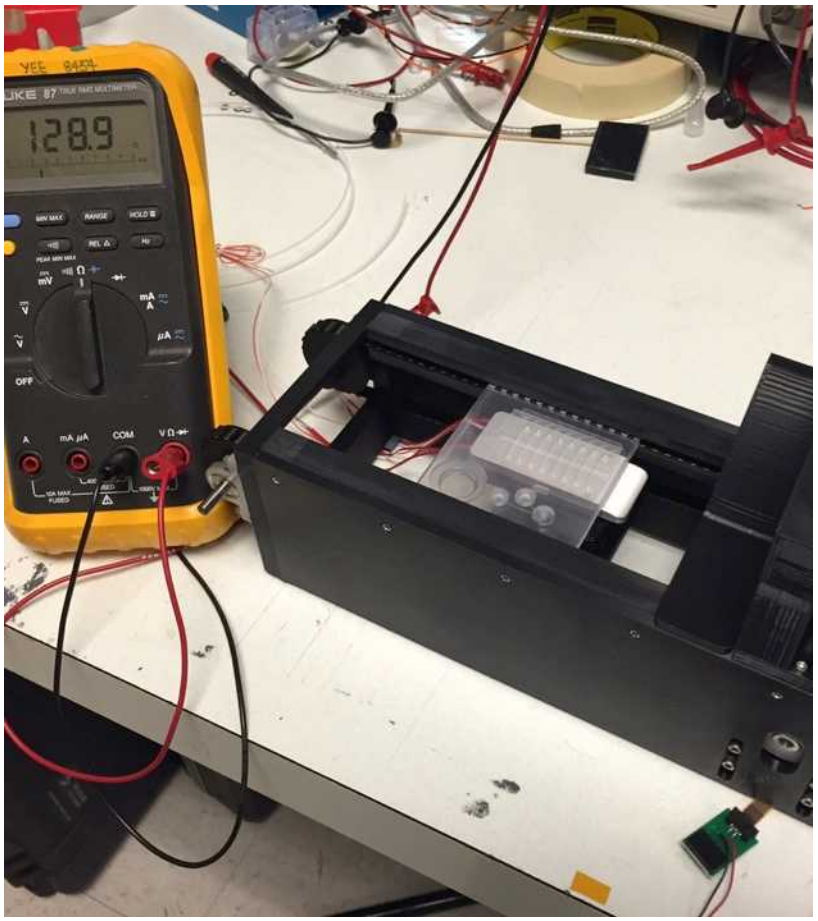
Pneumatic system fills chip



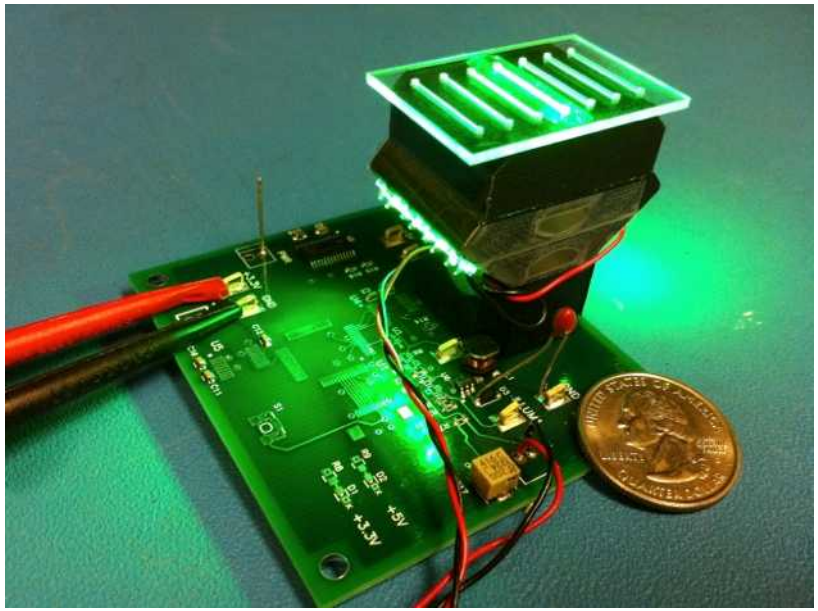
- Miniature air compressor
- Seals over reservoir to pressurize fluids into assay channels

Spring-mounted heater controls reaction channel temperature

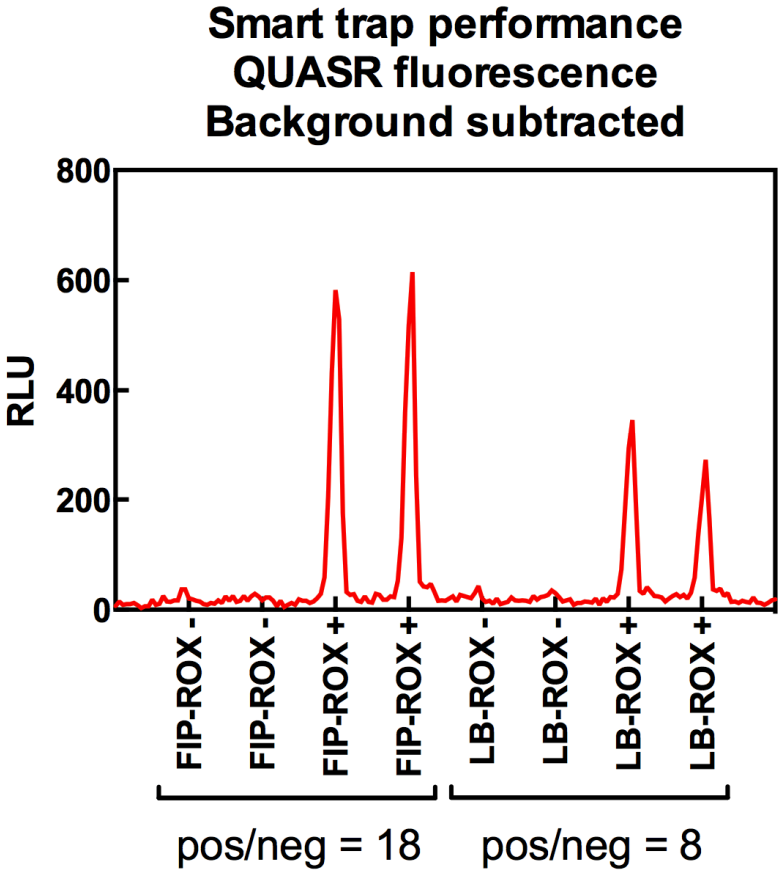




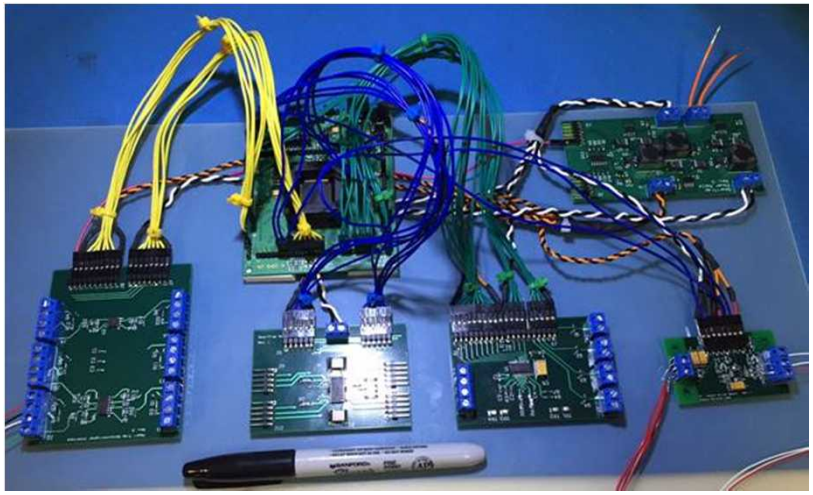
Detector module



Photodiode detector, equipped with green LEDs and theater gel emission filter. Inexpensive optics integrated into 3D printed part.



Networked electronics run trap and communicate with the cloud

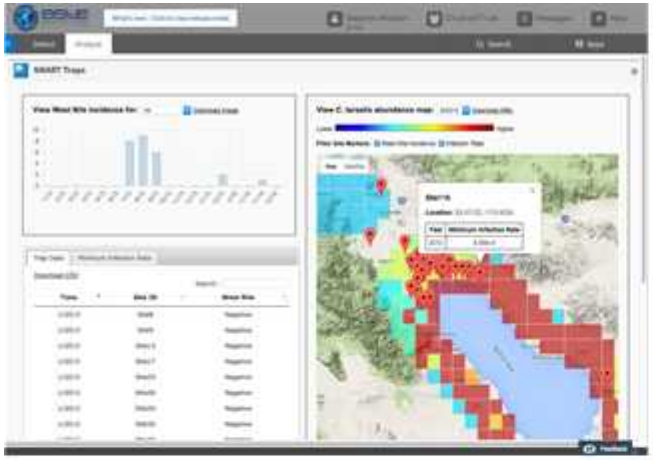


- Modular electronics design
- Onboard system state of health diagnostics
- XBee communication among smart trap neighbors
- Master trap enabled with 4G smartphone
- Data to Amazon web services and DTRA biosurveillance ecosystem

Outline

- Why am I here – Prehistory
- Biosurveillance for Vectorborne Diseases
  - Assay chemistry
  - Microfluidics
  - System integration
  - **Networking and visualization**
- Future work and side lights

Communication Architecture

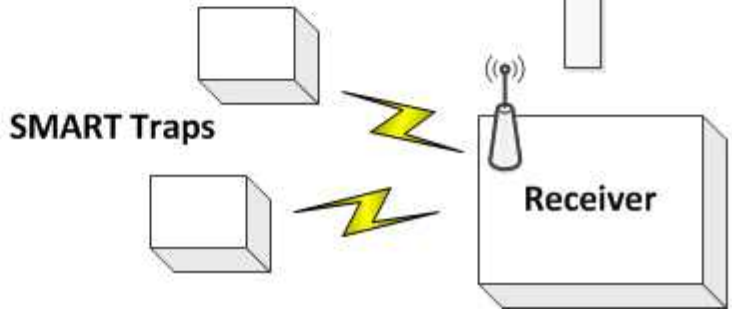


SMART Traps App
Running within BSVE as 3rd Party Application
HTML5, Javascript, Google Maps API

Download data via REST

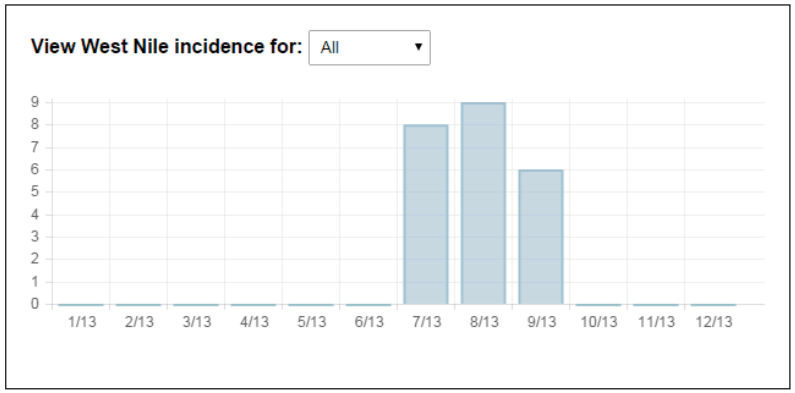
SMART Traps Web Server and Datastore
Running on Amazon AMI
Spring Framework, Java, R

Upload data via REST



Cloud-based mapping and modeling

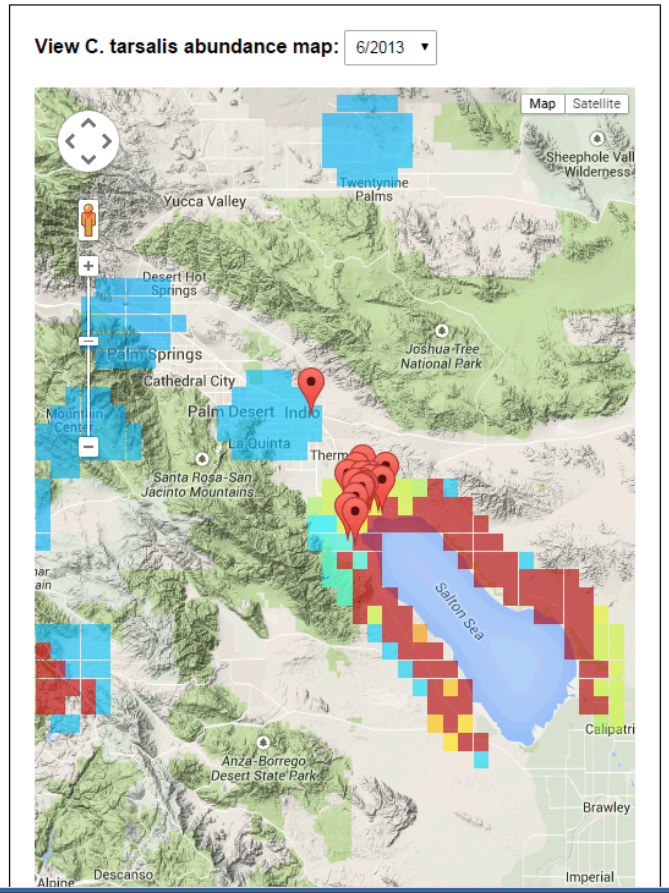
- 3rd party app: data stored on Amazon cloud, "private" data (from CA vector control) used to generate model visualizations for BSVE
- Daily viral incidence data, combined with physical data and models of vector abundance lead to prediction of disease transmission risk.



Trap Data

Search:

Time	Site ID	West Nile
1/2013	Site121	Negative
1/2013	Site13	Negative
1/2013	Site17	Negative
1/2013	Site204	Negative
1/2013	Site30	Negative
1/2013	Site33	Negative
1/2013	Site34	Negative
1/2013	Site35	Negative

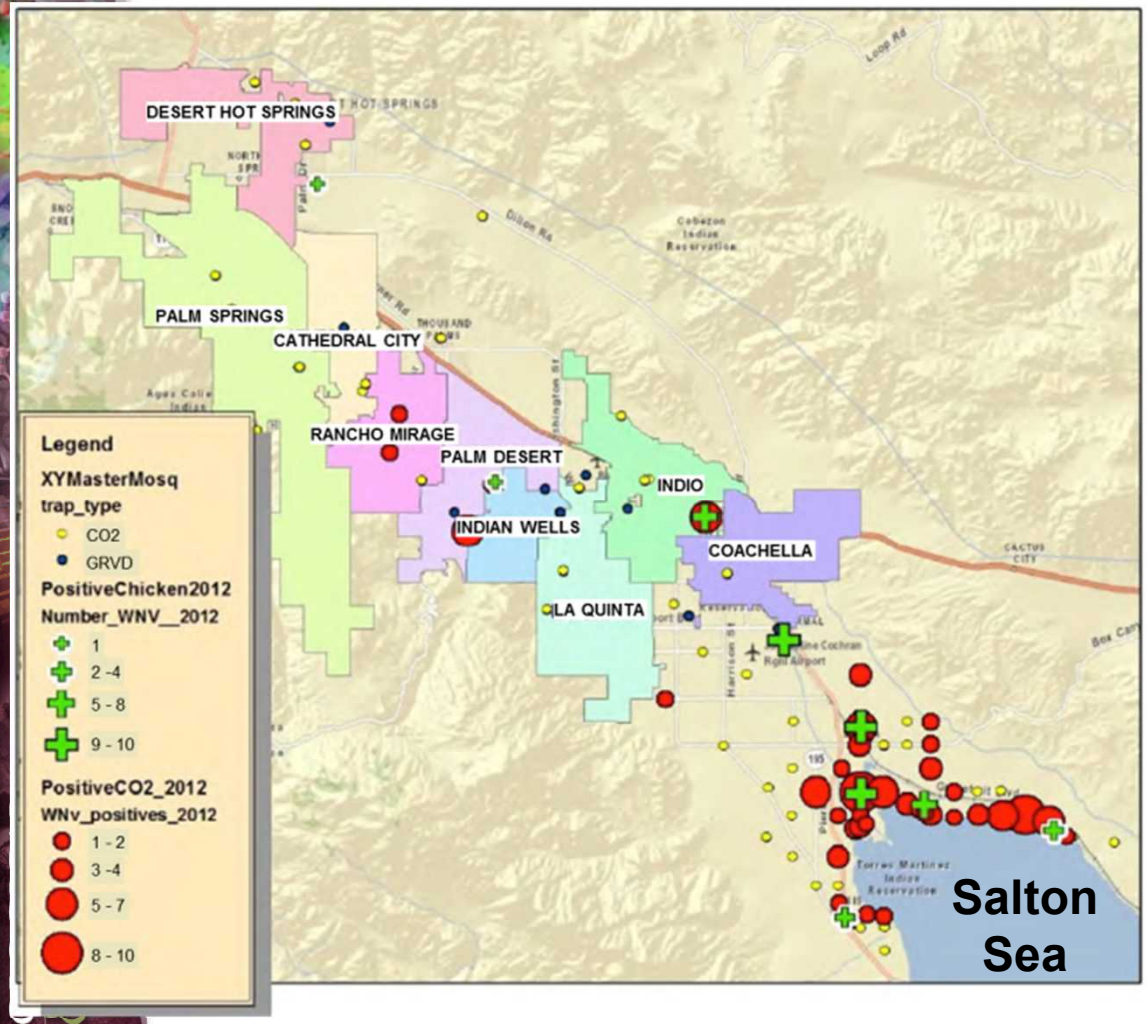


BIOLOGICAL & ENGINEERING Sciences

8600

Sandia National Laboratories

Field test for Smart Trap planned 2016



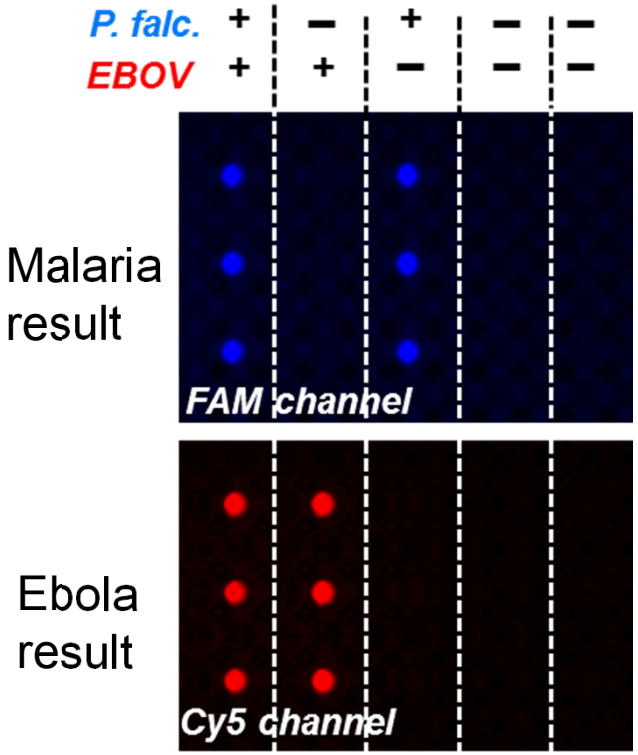
- We will deploy a network of Smart Trap prototypes near the Salton Sea in southern California.
- Irrigation, warm summers, and abundant birds lead to ideal conditions for West Nile virus
- We will perform a field test of the Smart Trap concurrently with conventional vector surveillance for WNV (traps & sentinel chickens)

Outline

- Why am I here – Prehistory
- Biosurveillance for Vectorborne Diseases
  - Assay chemistry
  - Microfluidics
  - System integration
  - Networking and visualization
- **Future work and side lights**

Potential for clinical diagnostic (NIH grant)

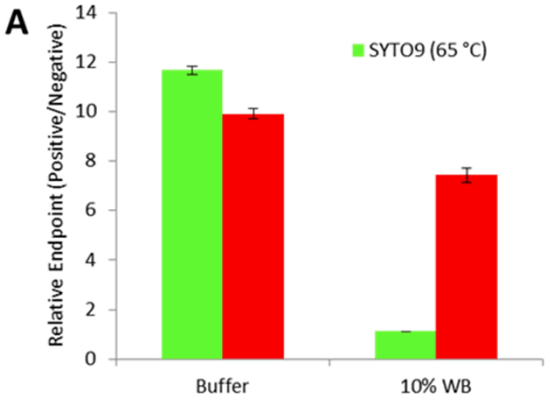
Single-tube multiplexing Ebola and *Plasmodium*



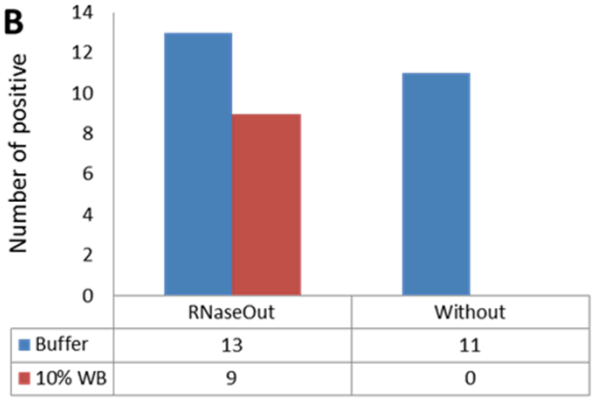
Adaptable to bright visual readout

Newly designed RT-LAMP primers for Ebola GP gene target both historic (1976) and recent (2014) isolates; detect 200 copies in about 20 minutes

RT-LAMP with new detection technique enables detection of Ebola RNA directly in whole blood (10% of total reaction volume, no RNA extraction)

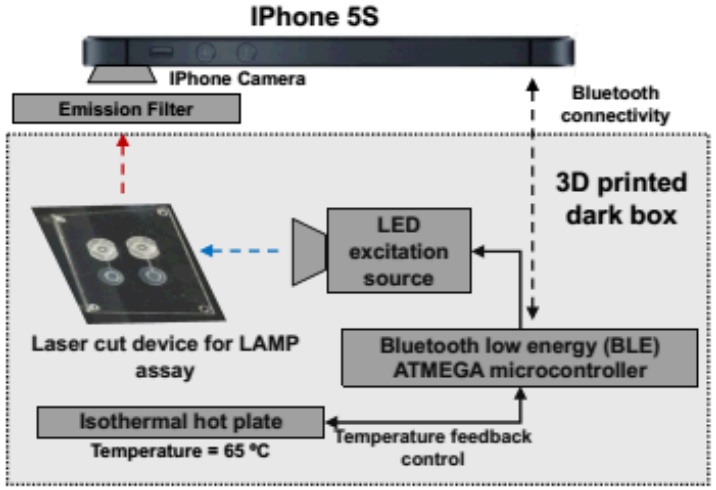


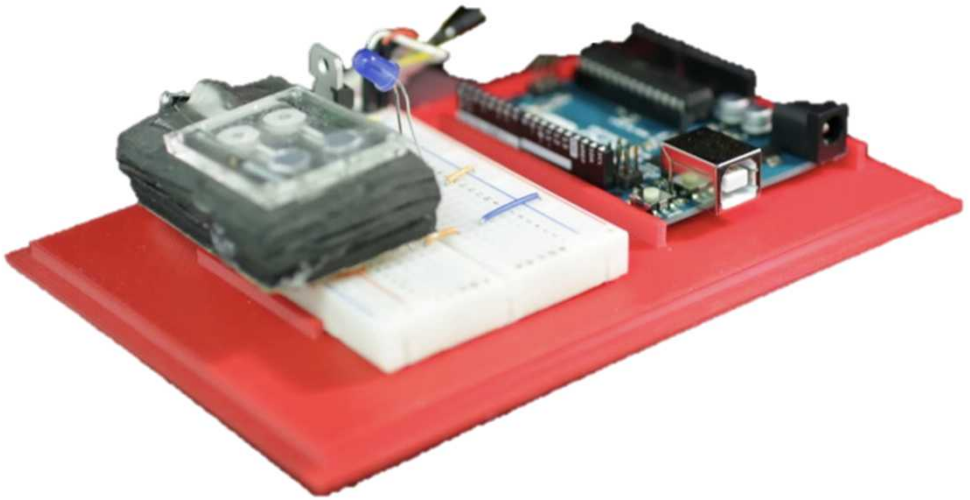
1000 copies EBOV RNA (100% detection rate)



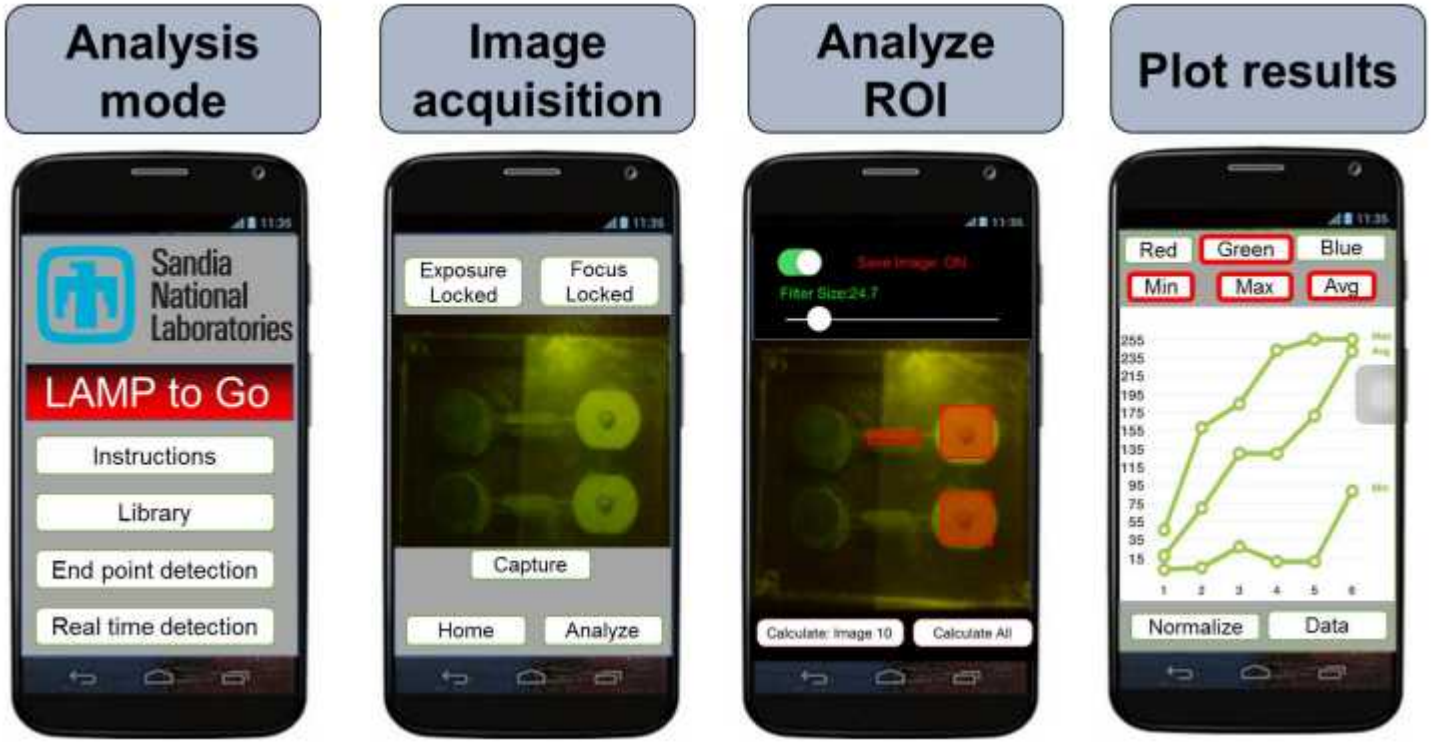
200 copies EBOV RNA × 20 replicates per condition ~50% detection rate

Smartphone enabled “Portable LAMP box”

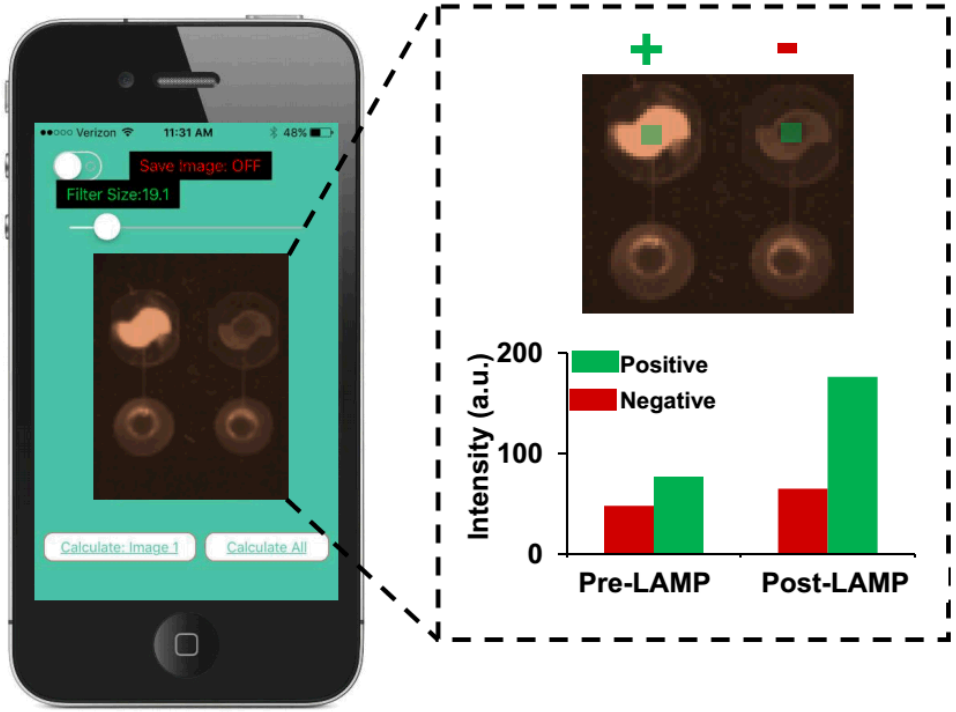




Portable LAMP app

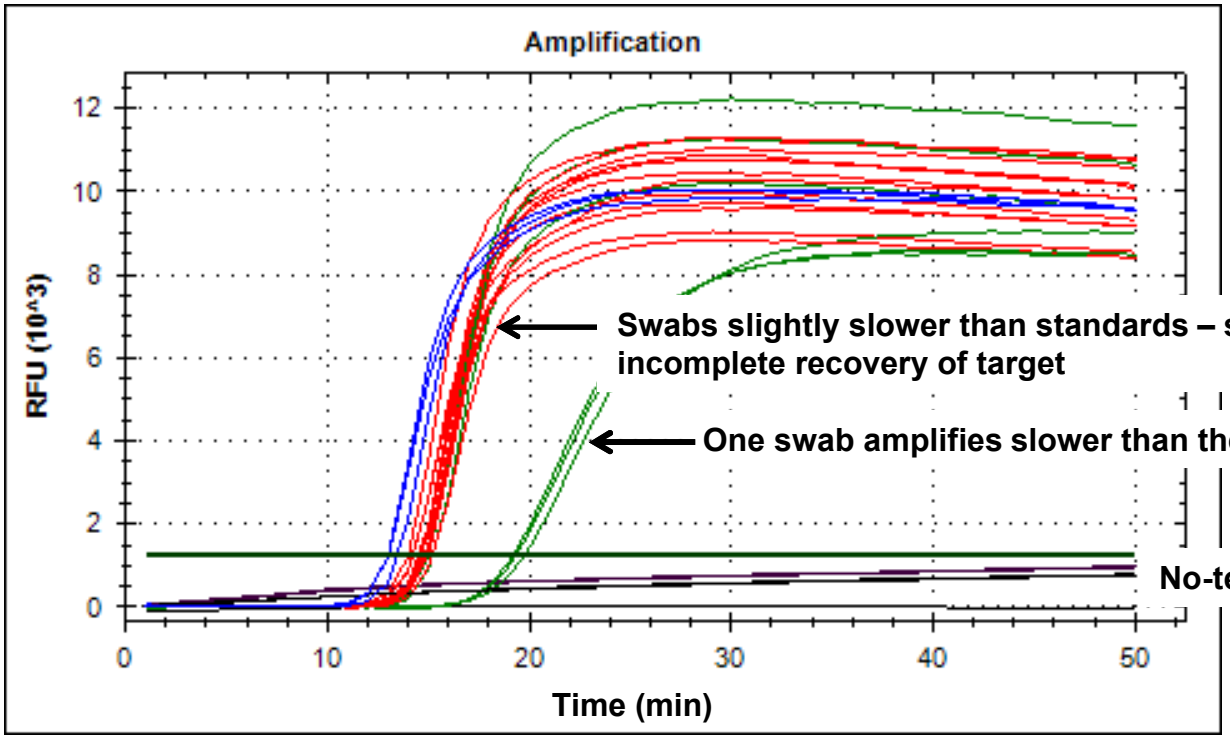


Detection of WNV virus on passive check valve chip



- Similar to valve described for Smart trap
- Easy integration with no external actuation
- Tunable opening pressure

RT-LAMP amplification with crow oral swabs*



Red = MS2 phage instilled on swab, swab instilled in 250 µL H_2O
Green = Swab instilled into 250 µL MS2 phage suspension
Blue = MS2, no swab (1/10,000 or 1/20,000 in H_2O)
Purple, Black = no template control (with or without swab)

***2 µL swab eluent per 10 µL reaction**

RT-LAMP could work for "direct" (extraction-free) detection of virus from swab samples
Also (not shown): Direct amplification of WNV instilled on RNA Sound (treated filter paper)

Who we are (acknowledgements)

Cameron Ball

Ron Renzi

Aashish Priye

Lark Coffey

Jaideep Ray

Jonathan Helm

Mark Claudnic

Stephen Mueller

Who we are (acknowledgements)

Yooli Light (not pictured)

Lark Coffey, Sarah Wheeler, Chris Barker, Bill Reisen, et al.
from UC Davis Center for Vectorborne Diseases

Sandia National Laboratories

UC DAVIS
UNIVERSITY OF CALIFORNIA

U.S. DEPARTMENT OF ENERGY

DTRA
Defense Threat Reduction Agency

BSVE
BIOSURVEILLANCE ECOSYSTEM

(Not actually my building...)

Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under Contract DE-AC04-94AL85000!