SAND2016-5984C

Real-time, Autonomous Biosurveillance for Vector-borne Viral Pathogens (SMART Traps)

Sandia National Laboratories
Robert Meagher (PI)
Jaideep Ray
Ron Renzi
Cameron Ball
Aashish Priye
Stephen Mueller

UC Davis Center for Vectorborne Diseases (CVEC)
Cody Steiner
Sarah Wheeler
Lark Coffey
Chris Barker
William Reisen

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!

Sandia National Laboratories UCDAVIS UNIVERSITY OF CALIFORNIA U.S. DEPARTMENT OF ENERGY BSVE BIOSURVEILLANCE ECOSYSTEM

Project Overview

- Overall goal is to develop and field-test an *autonomous sensor* to detect presence of mosquito-borne viruses with daily reporting capabilities.
- Data from sensors will be integrated into BSVE along with mapping & visualization software and predictive models.
- Partnership between Sandia National Laboratories
  - Systems engineering, assays, statistical modeling
- …and UC Davis Center for Vectorborne Diseases (CVEC)
  - Virology, entomology, and ecology of vectorborne disease
  - Integrated with public health and vector control districts in CA

BIOSURVEILLANCE ECOSYSTEM

Current approaches to 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

Laboratory processing

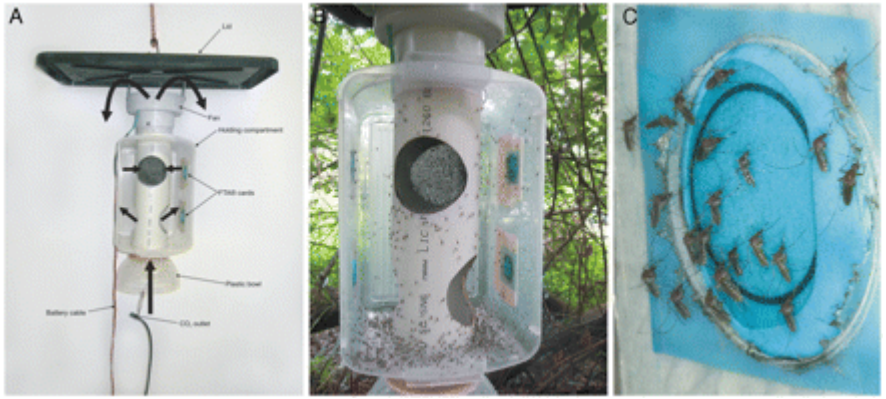
Molecular assays

BSVE
BIOSURVEILLANCE
ECOSYSTEM

Sugar-feeding as an alternative for viral detection: Hall-Mendelin et al (2010)

- Developed a novel system that:
  - Captured mosquitoes with updraft technology
  - Presented honey-soaked nucleic acid preservation cards for sugar feeding
- Result
  - WNV was detected on 83% (25/30) of the sugar substrates fed on by WNV+ mosquitoes.
  - The viral RNA remained stable on the substrate for up to 1 week.
  - Still requires extensive laboratory processing & qRT-PCR to detect virus

Figure 1

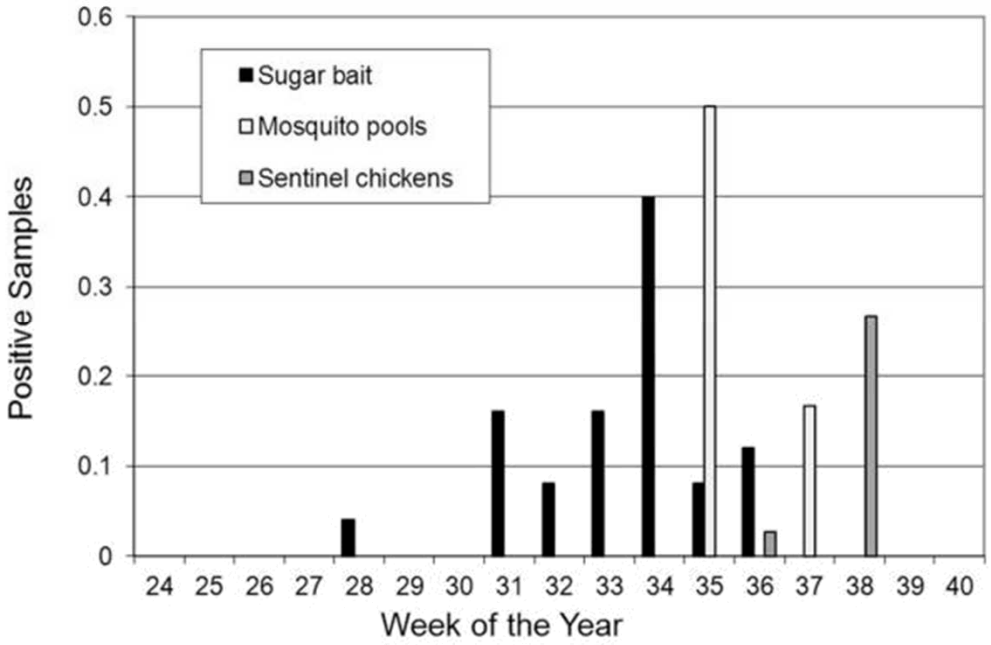


BSVE
BIOSURVEILLANCE
ECOSYSTEM

Slide 4

2 This data, while nice, is unnecessary. Summarize their results in 1 bullet
Lark Coffey, 11/2/2015

Sugar baiting tested for WNV detection in California (Coachella valley; arid)



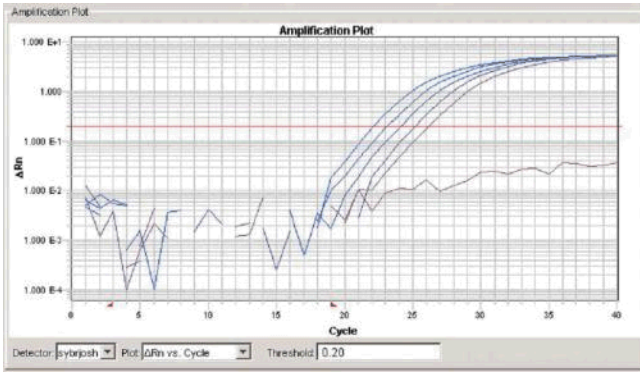
- A passive sugar bait, made from a cryovial and dental wick with blue-colored syrup and a floral attractant
- tested by UC Davis for WNV surveillance in southern California
- Requires laboratory processing to recover viral RNA for qRT-PCR testing

- Sugar baits were positive for WNV *before* mosquito pools or sentinel chickens
- Research is ongoing by UC Davis to compare passive sugar baits to CO_2 baited traps for WNV surveillance in California.

BSVE
BIOSURVEILLANCE ECOSYSTEM

Ongoing Sugar bait testing at UC Davis (Sacramento/Yolo County, suburban/rural)

- Baits comprising sugar-soaked cotton wick scented with Plumeria spray
- Placed in field in locations near (but not too near) to CO_2 baited EVS traps
- Returned to lab for processing (RNA extraction/ qRT-PCR) to evaluate coincidence of WNV detection between sugar baits and traps



Cody Steiner & Lark Coffey, UC Davis 2015

BSVE
BIOSURVEILLANCE
ECOSYSTEM

Slide 6

4 As before, write only "Aim 1" and then a methodology heading.
Lark Coffey, 11/2/2015

5 You need to state the lower limit of detection not a range.
Here you need to add Sarah's data from Lothrop showing that mosquitoes expectorate low doses.
Lark Coffey, 11/2/2015

Aim 1: Comparing sugar baits and EVS traps

	Sugar Bait	EVS Traps
Requires CO_2 and/or "smelly" water	No ✓	Yes
Requires Fans / Batteries	No ✓	Yes
Positives / $1000 cost	4.18 ✓	0.8
Field personnel training requirements	Minimal (< 5 min.) ✓	Extensive
Site visits per week	1 ✓	2
Duration of active detection	1 week ✓	1 day
Estimates transmission	Yes ✓	No
Estimates abundance	No	Yes ✓
Quantification of test results	No	Yes (with MIA) ✓
Species identification	No	Yes ✓

Cody Steiner & Lark Coffey, UC Davis 2015

BSVE BIOSURVEILLANCE ECOSYSTEM

Aim 2: Do sugar baits and EVS traps simultaneously detect WNV activity?

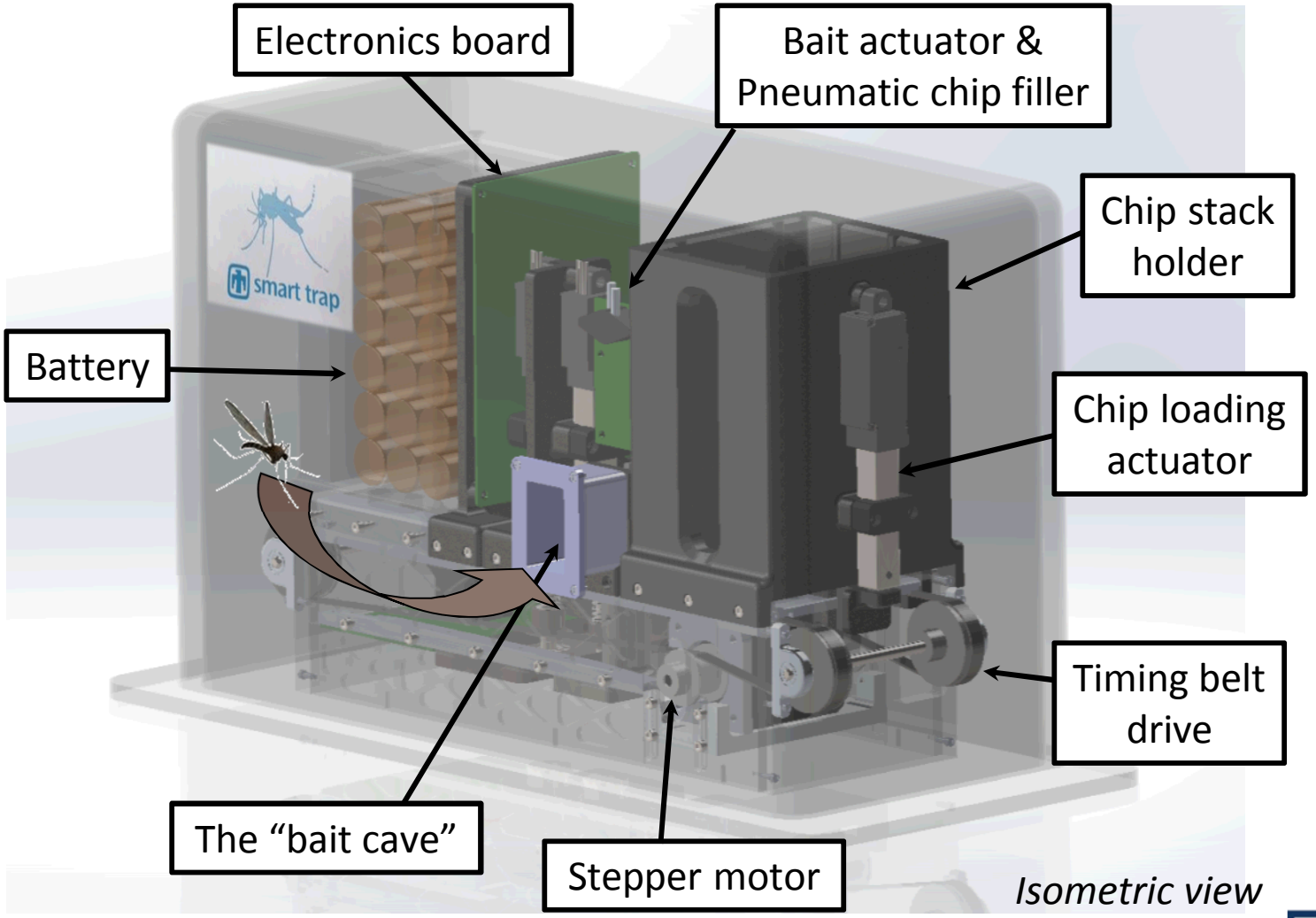
	EVS Trap WNV+	EVS Trap WNV-	Total
Sugar Bait WNV+	12	21	33
Sugar Bait WNV-	43	67	110
Total	55	88	143

- Cohen's Kappa statistic
  - Range: -1 (complete disagreement) to 1 (complete agreement))
  - k = -0.022
  - Instances of simultaneous detection occurred by chance.
  - Early season and arid site detection disagree most.

Cody Steiner & Lark Coffey, UC Davis 2015

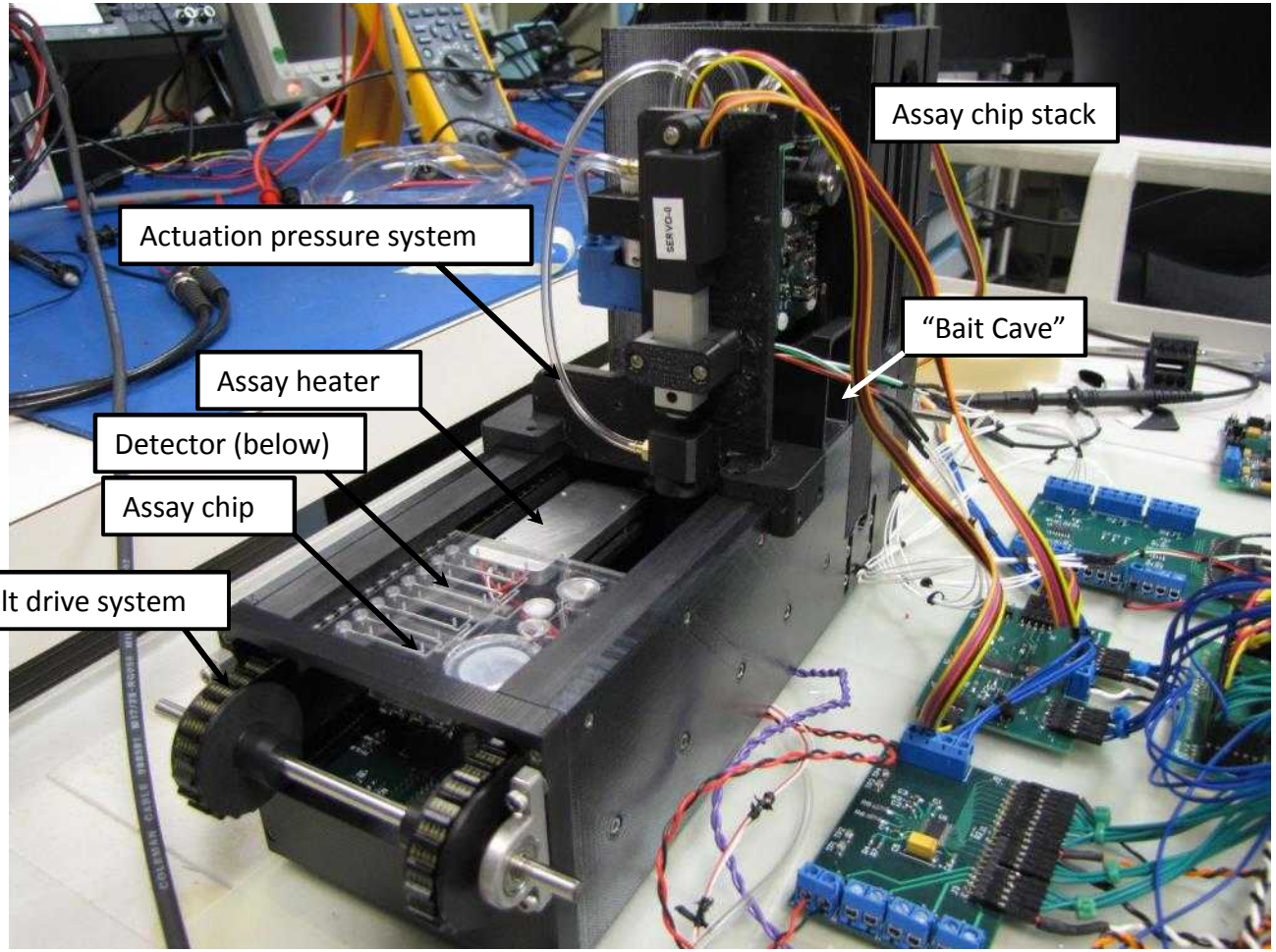
BSVE BIOSURVEILLANCE ECOSYSTEM

The smart trap automates sugar baiting and molecular assay for viral RNA



BSVE BIOSURVEILLANCE ECOSYSTEM

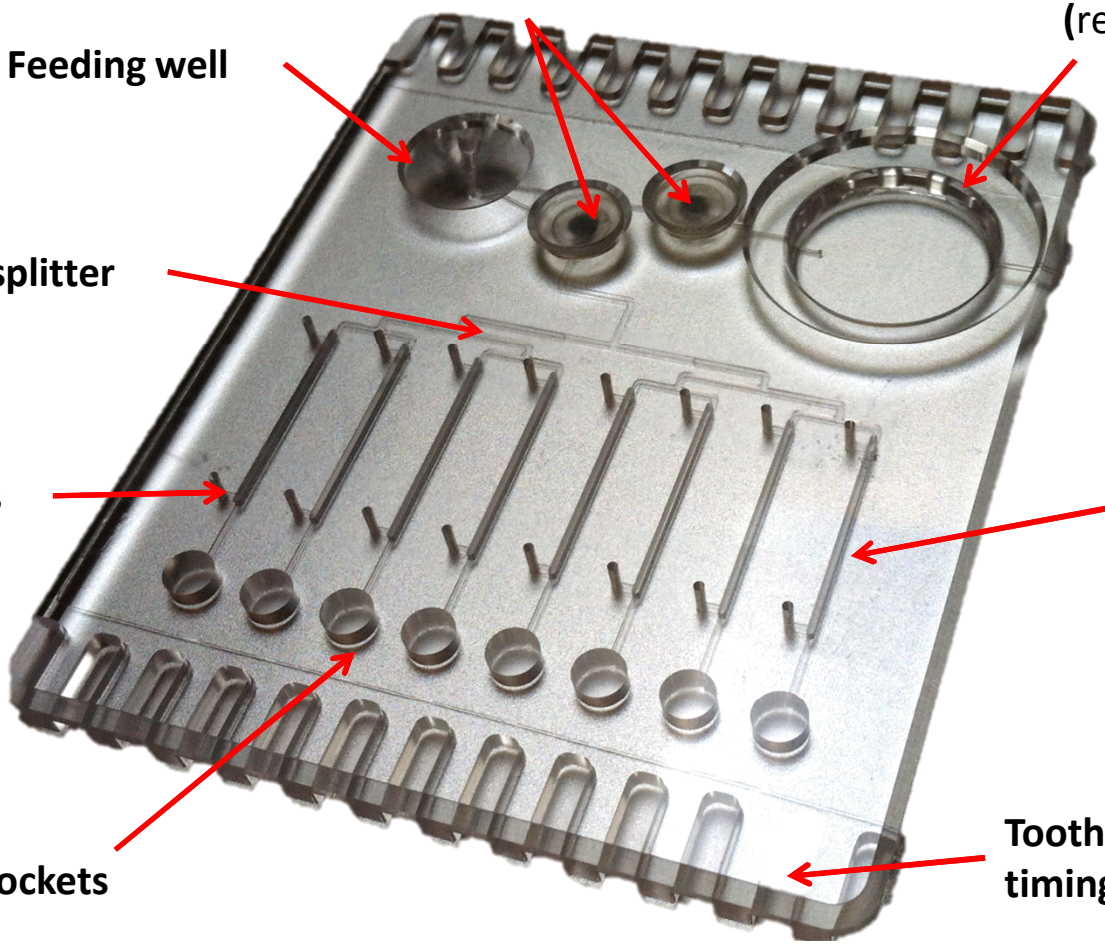
Smart Trap hardware



Electronics – now on a single board, but not shown here to allow better view of components

Not shown: system case and battery pack, normally positioned above where assay chip is situated

Smart Trap Assay Chip



Check valves

Fluid reservoir (reaction buffer)

Feeding well

Flow splitter

Fill holes

Assay channels for multiple viral targets + controls (stabilized, dried RT-LAMP reagents)

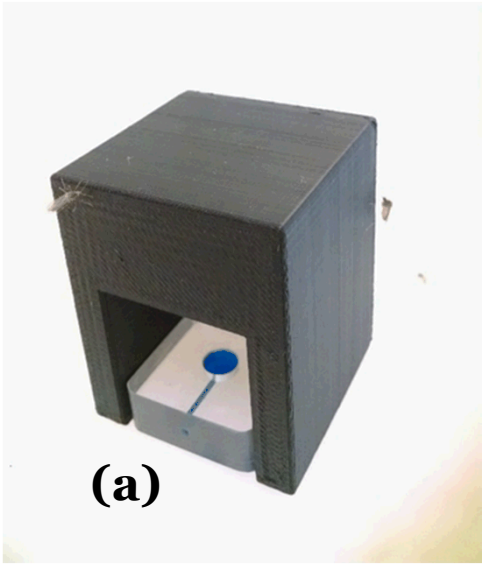
Air pockets

Toothed track to engage timing belt

BSVE
BIOSURVEILLANCE ECOSYSTEM

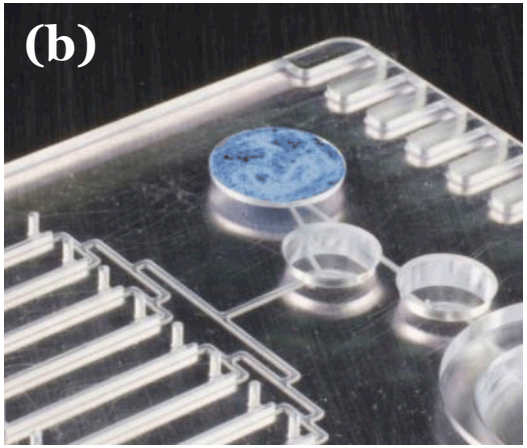
Mosquito feeding from baits

Previous sugar baiting: sugar-soaked cotton balls/wicks (with poor recovery of virus)
Smart Trap achieves total recovery from either (a) liquid-phase baits, or
(b) dried sugar films/spun sugar



"Bait cave" with blue-colored sugar bait

Blue food coloring allows identification of mosquitoes that fed on bait

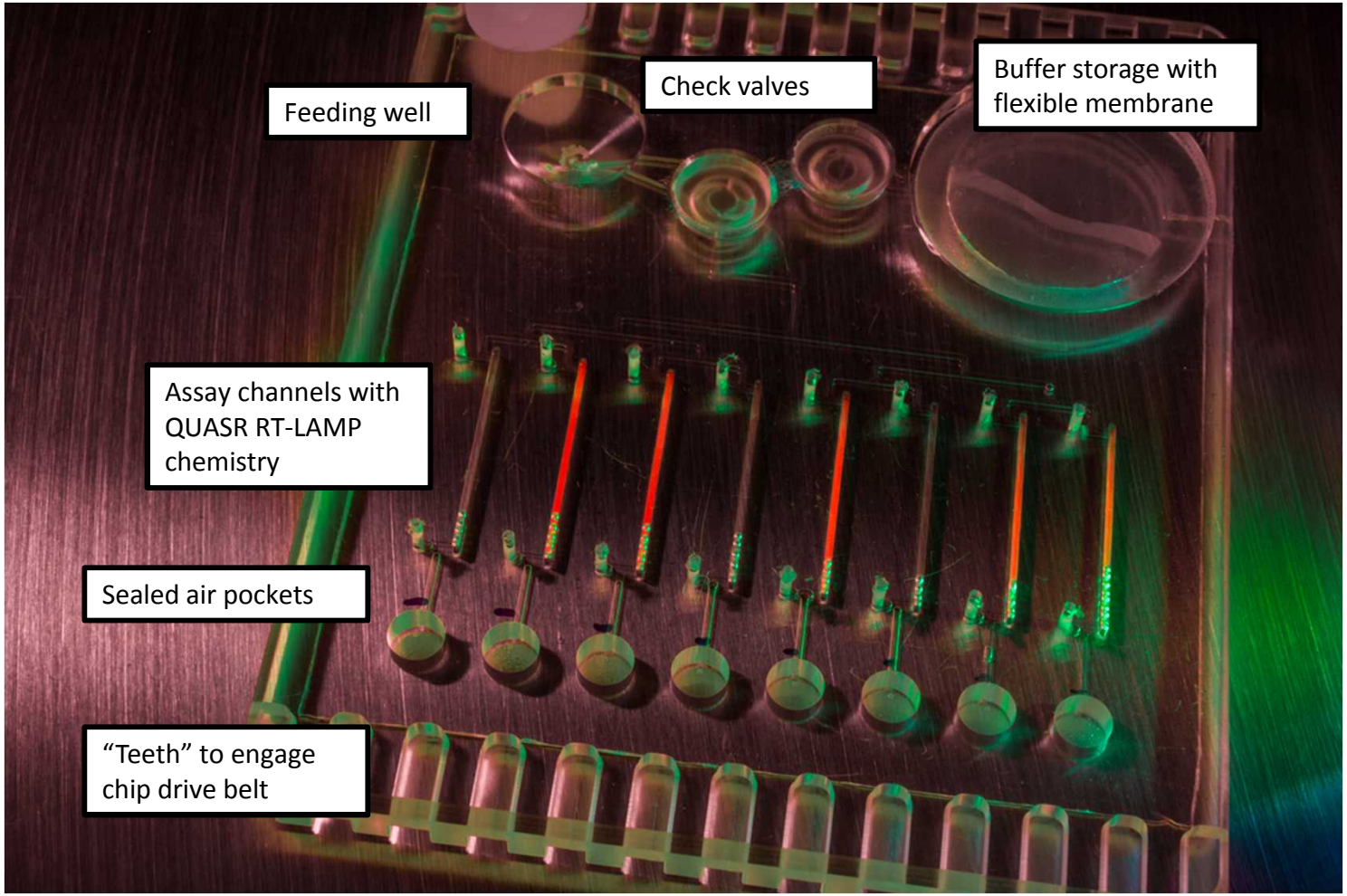


Scented, polymer-modified spun sugar as a stable attractive bait for mosquitoes

BSVE
BIOSURVEILLANCE
ECOSYSTEM

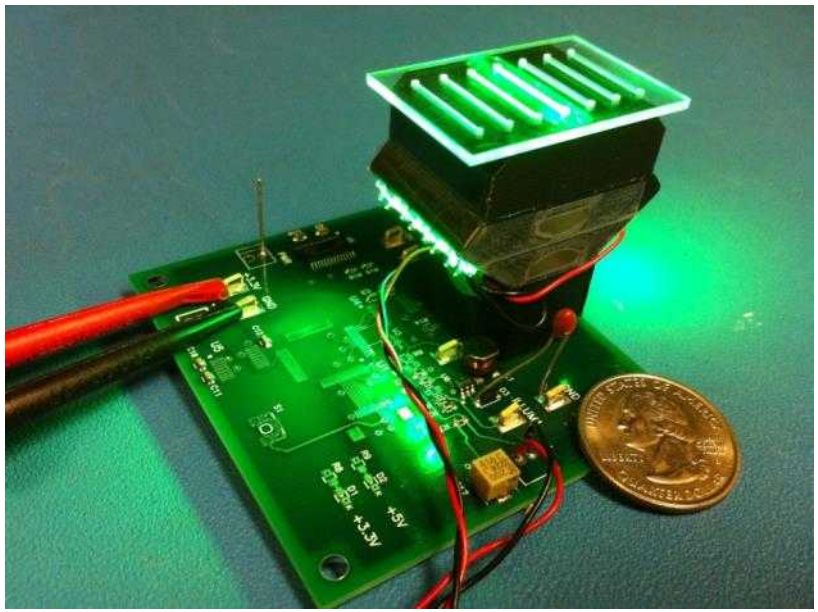
Mosquito sugar feeding assay chip

RT-LAMP amplification is performed dried-down reagents, stabilized with reagent from Biomatrica

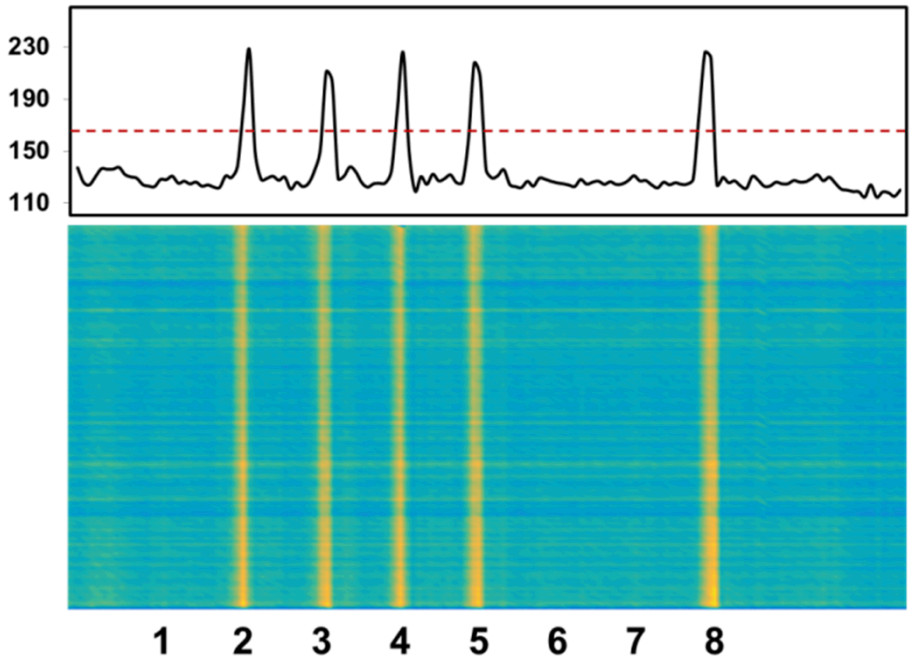


BSVE
BIOSURVEILLANCE ECOSYSTEM

Reading the assay chip



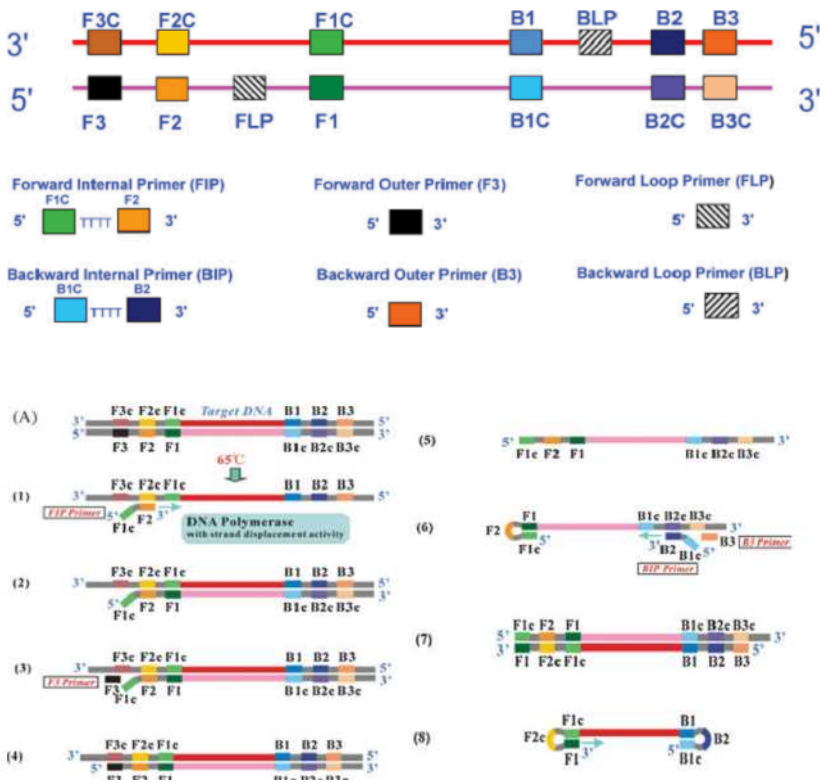
Photodiode detector module, equipped with green LEDs and colored plastic gel filters. Inexpensive optics integrated into 3D printed part.



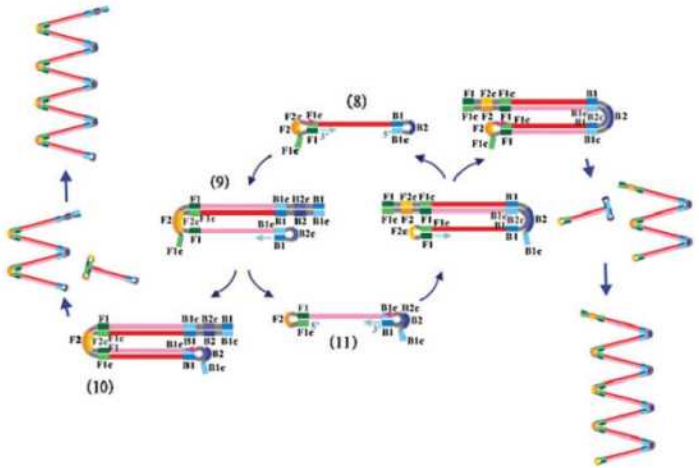
Detector scan of an 8-channel assay chip, illustrating discrimination between positive and negative channels and comparison to a threshhold (red dashed line).

BSVE
BIOSURVEILLANCE
ECOSYSTEM

RT-LAMP viral assays



- Isothermal (65 °C) “alternative” to RT-PCR for point-of-care or low-resource settings
- Complex reaction scheme, but high sensitivity (<0.1 PFU virus in 20-30 minutes) and high specificity
- Many RNA viruses including WNV can be detected “directly” by RT-LAMP, without purification, lysis, or RNA extraction.

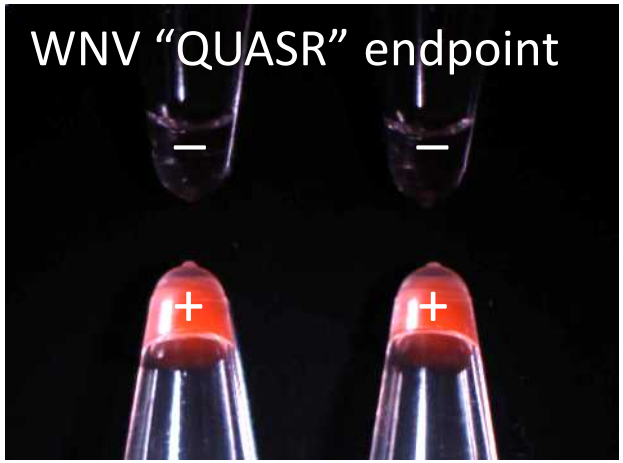


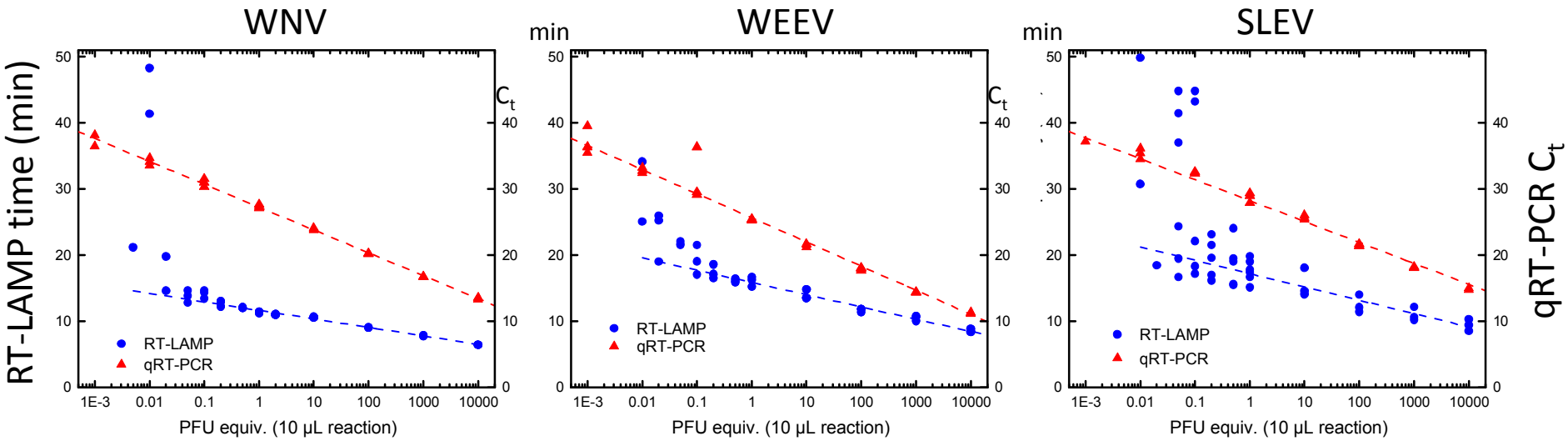
LAMP reaction scheme proposed by Notomi *et al* (2000).

BSVE
BIOSURVEILLANCE
ECOSYSTEM

Viral assays by RT-LAMP

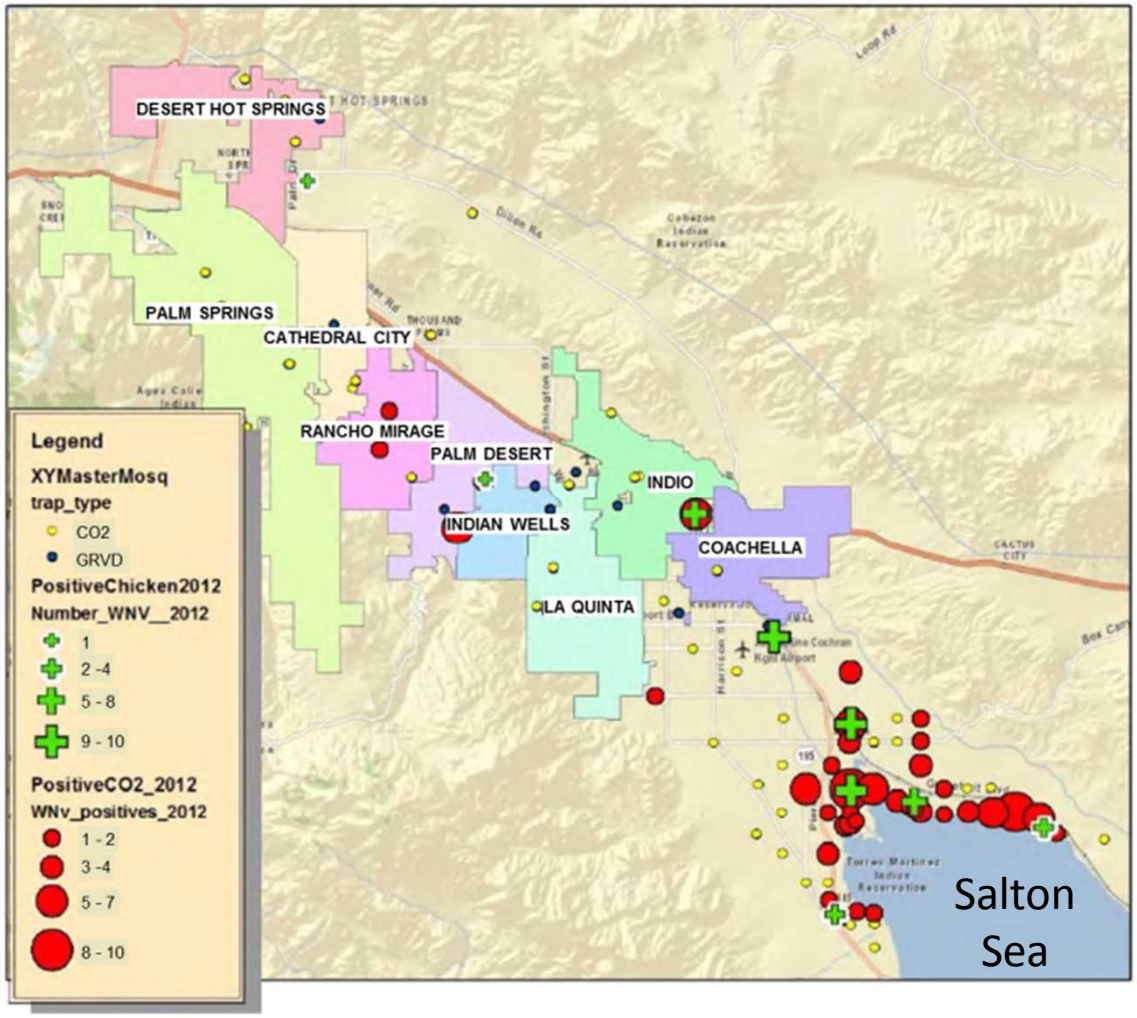
- Novel detection chemistry “QUASR” gives bright and distinctive fluorescence endpoint signal, with multiplexing capability and reduced false positives compared to “traditional” LAMP
- RT-LAMP quantitative precision and sensitivity is usually less than qRT-PCR





Wheeler *et al*, PLOS One 2016

Field test for Smart Trap planned 2016

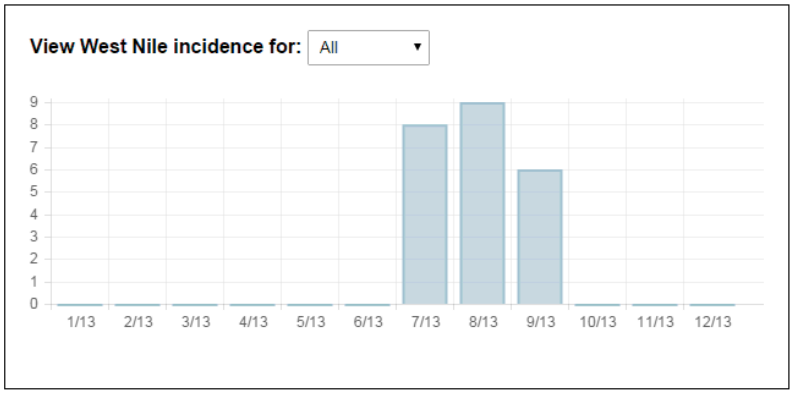


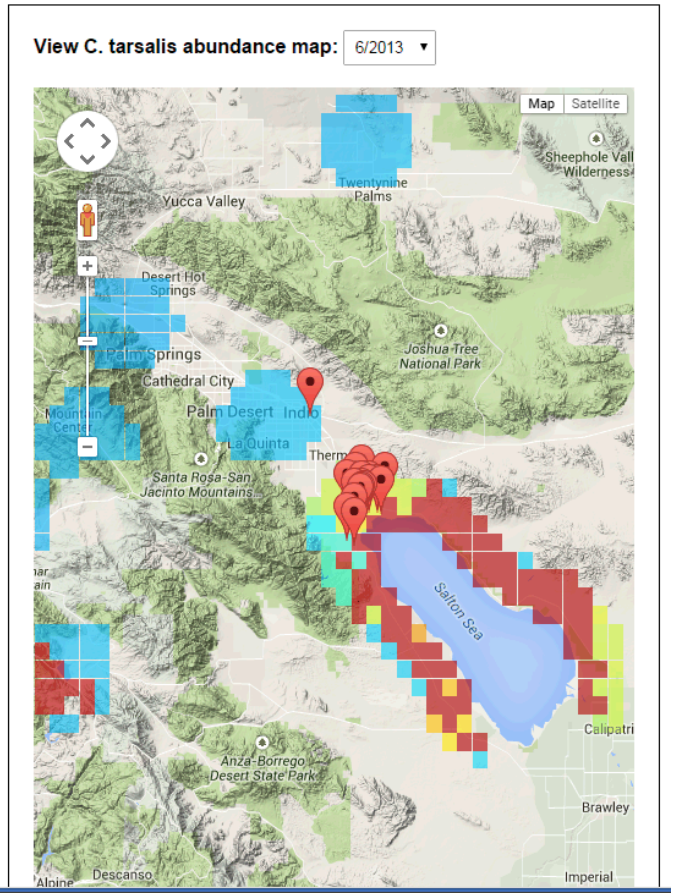
- We will deploy a network of Smart Trap prototypes near the Salton Sea in southern California.
- Irrigation, warm summers, and abundant birds lead to ideal conditions for West Nile virus
- We will perform a field test of the Smart Trap concurrently with conventional vector surveillance for WNV (traps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

BSVE
BIOSURVEILLANCE ECOSYSTEM

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.





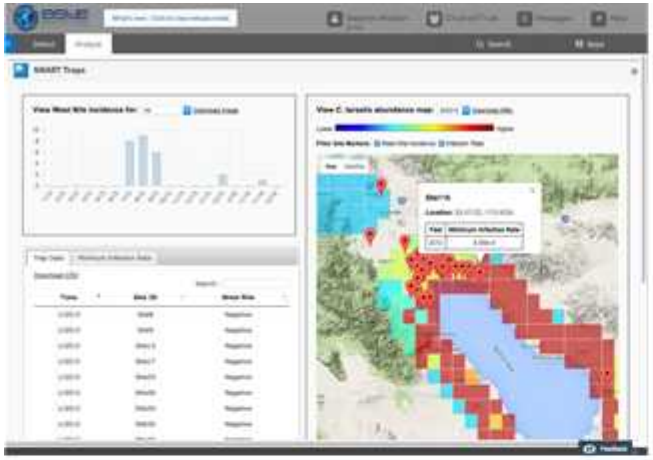
Trap Data

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

BSVE
BIOSURVEILLANCE ECOSYSTEM

SMART Trap communication with BSVE

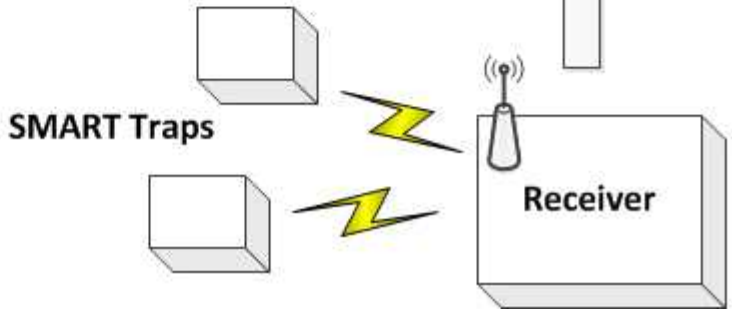


SMART Traps App
Running within BSVE as 3rd Party Application
HTML5, Javascript, Google Maps API

Download data via REST

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

Upload data via REST



BIOSURVEILLANCE ECOSYSTEM

Extending Smart Trap to *Aedes*

- What (if anything) needs to be changed to get *Aedes aegypti* to sugar-feed on Smart trap sugar baits
  - Different scents?
  - Add CO_2?
  - Locate smart trap apparatus within a larger enclosure designed to collect *A. aegypti?*

BSVE
BIOSURVEILLANCE
ECOSYSTEM

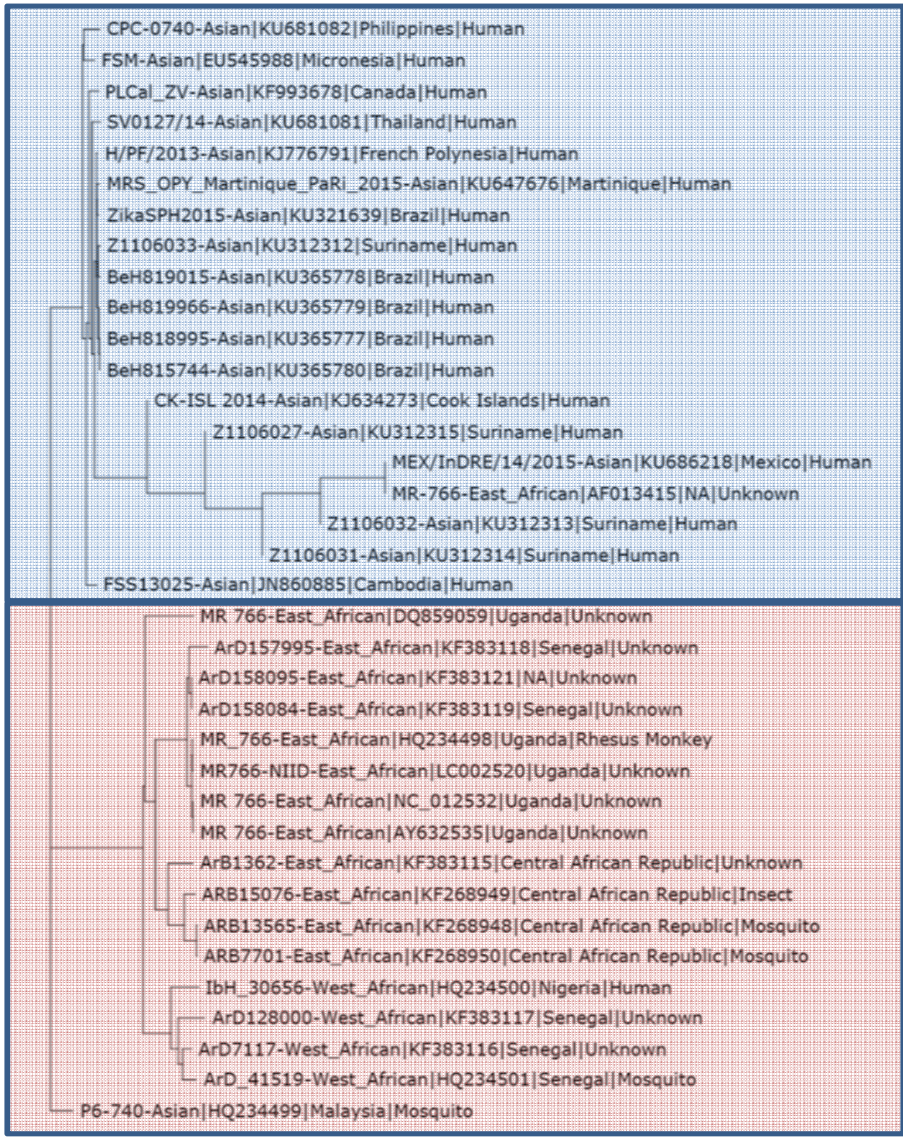
RT-LAMP for DENV, CHIKV, ZIKV

- We have tested serotype-specific and pan--DENV primers from literature
  - Mixed success, don't have a large collection
  - Probably need geographic-specific primers
- CHIKV – primers from literature have worked well, but only 1-2 isolates
- ZIKV – testing underway

BSVE
BIOSURVEILLANCE
ECOSYSTEM

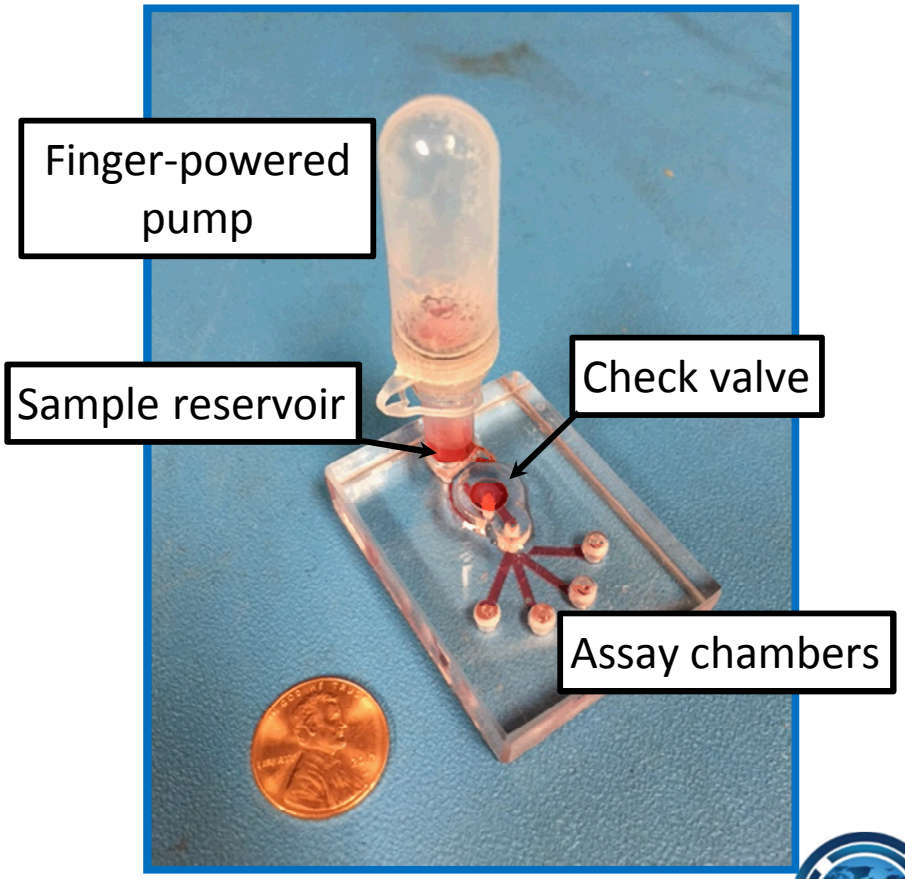
ZIKV RT-LAMP

- Seven candidate primer sets screened so far, targeting Envelope, NS1, NS3, NS5, 3’-UTR
  - Designed to match most/all Asian/American lineage strains; coverage of African strains not certain
- Detailed characterization for sensitivity, specificity, *etc.* using 2015-2016 isolates from Puerto Rico, Honduras, Brazil (courtesy CDC and UCSF)



Portable devices for viral screening

- Development underway; Intended to be cheap, disposable, easy
- Dehydrated RT-LAMP master mix and primers: just add sample, squeeze, incubate, and read with smart phone app.
- Adaptable for different sample types and targets
  - Swabs
  - Sugar baits
  - Mosquito slurry
  - Blood/urine/saliva
  - Dried blood spots

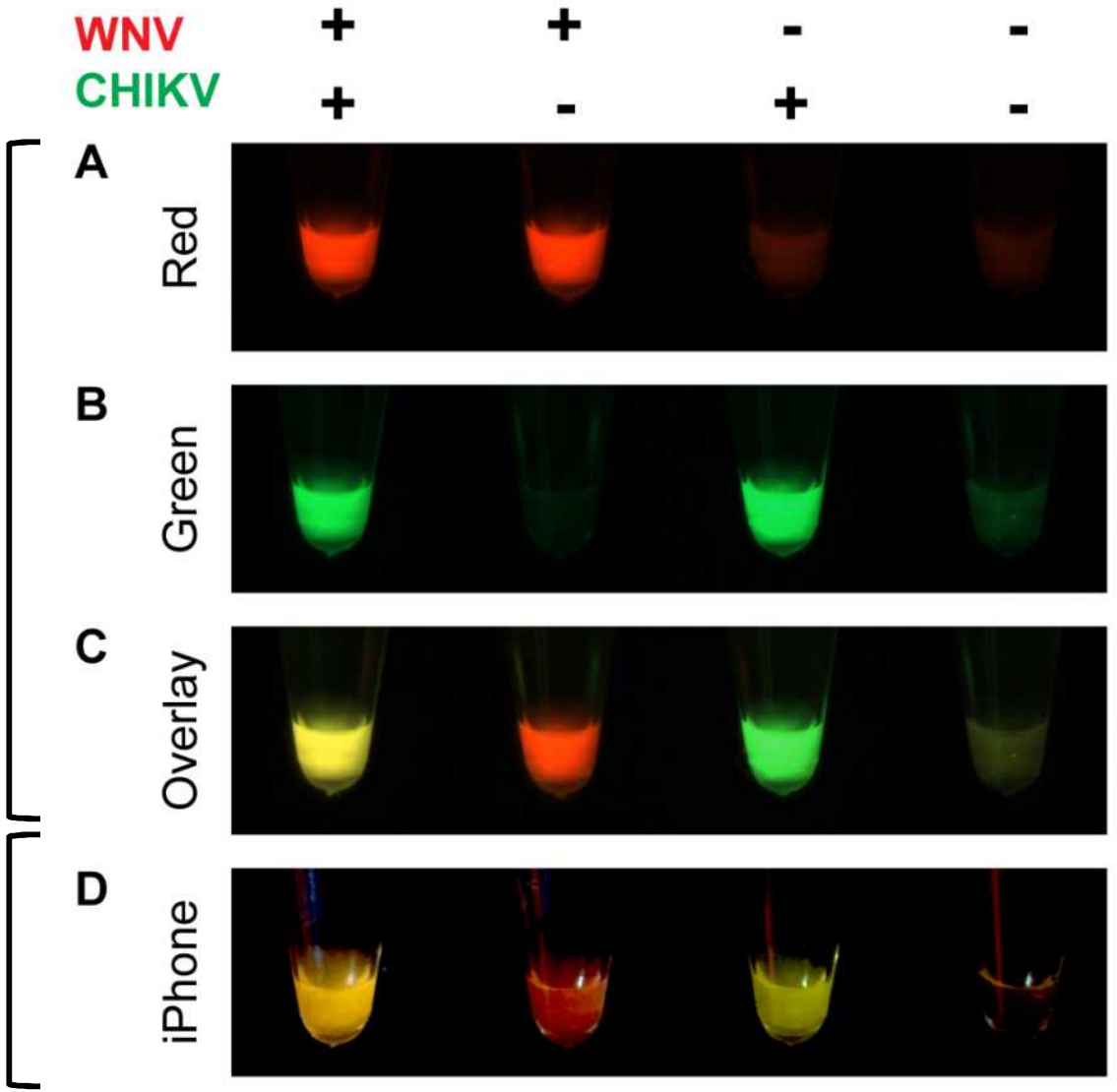


BSVE
BIOSURVEILLANCE ECOSYSTEM

Multi-color RT-LAMP with “visual” detection

High-quality fluorescence photography setup (~$1000 equipment)

Cheap fluorescence photography (iPhone, LED flashlight and colored plastic films)



BIOSURVEILLANCE ECOSYSTEM

Acknowledgments

- Funding
  - DTRA BSVE / Kathleen Quinn & Chris Kiley (Smart Trap)
  - Sandia LDRD (Zika, QUASR RT-LAMP, smart phone assays)
  - ISDS (for this trip)
- UC Davis Center for Vectorborne Diseases (CVEC) / Davis Arbovirus Research & Training (DART)
  - Lark Coffey, Cody Steiner, Chris Barker, Sarah Wheeler, Bill Reisen
- Sandia staff & postdocs
  - Cameron Ball, Aashish Priye, Ron Renzi, Jaideep Ray, Stephen Mueller, Yooli Light, Jonathan Helm, Mark Claudinc

BSVE
BIOSURVEILLANCE
ECOSYSTEM