SAND2015-1369PE

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

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U.S. DEPARTMENT OF ENERGY

UC DAVIS UNIVERSITY OF CALIFORNIA

Project Overview

- Overall goal is to develop and field-test an *autonomous sensor* to detect presence of mosquito-borne viruses (**WNV**, WEEV, SLEV) with daily reporting capabilities.
- Data from sensors will be integrated into BSVE along with mapping & visualization software and predictive models.
- 3-year effort began 2014; year one ends Feb 28, 2015
- Partnership between Sandia National Laboratories, Livermore, CA…
  - Systems engineering, microfluidic assays, statistical modeling expertise
- …and UC Davis Center for Vectorborne Diseases (CVEC)
  - Virology, entomology, and ecology of vectorborne disease
  - BSL-3 laboratory facility and insectary
  - Viral modeling and forecasting expertise
  - Integrated with public health and vector control districts in CA

Background on Arboviruses

- "Arbovirus" = Arthropod-Borne Virus, *i.e.* viruses transmitted by mosquitoes, fleas, ticks, flies, *etc*.
- Significant emerging & re-emerging pathogens worldwide
- Major burden on public health & agriculture worldwide
  - Dengue virus is the most significant globally (up to 400 million infections/yr)
  - West Nile virus is currently the most prevalent in United States
  - Chikungunya is emerging as a worldwide threat, and now in the southeastern US.

- Some of these (like WEEV, VEEV, EEEV, RVFV) have potential for weaponization/bioterrorism
- Military entomologists routinely monitor vector-borne disease threats to protect personnel overseas.

Aedes aegypti, the vector of Dengue, yellow fever, chikungunya, and other arboviruses

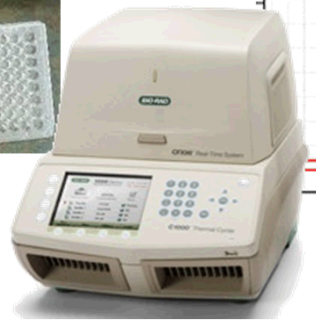
Field surveillance for arboviruses

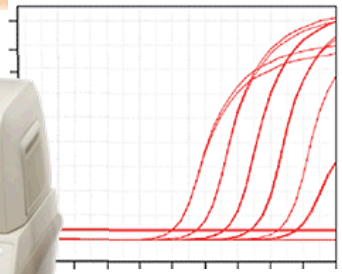
- Mosquito collection is the primary source of physical data on viral incidence
- Low-tech collection of mosquitoes, skilled manual labor to sort & pool insects, and sensitive laboratory tests for viral RNA (RT-PCR, $20/pool)

Vector surveillance

Photo: Fairfax County, VA via Flickr


Photo: Palm Beach Post





1-2 weeks later

Adulticide spraying

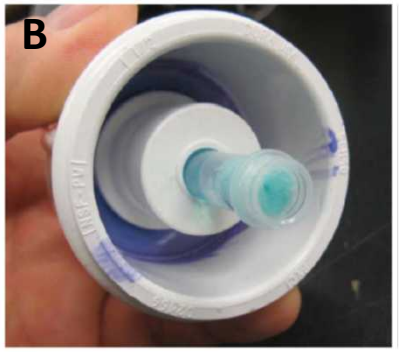

Photo: Huffington Post Alamy


Photo: Lafayette City Parish Gov

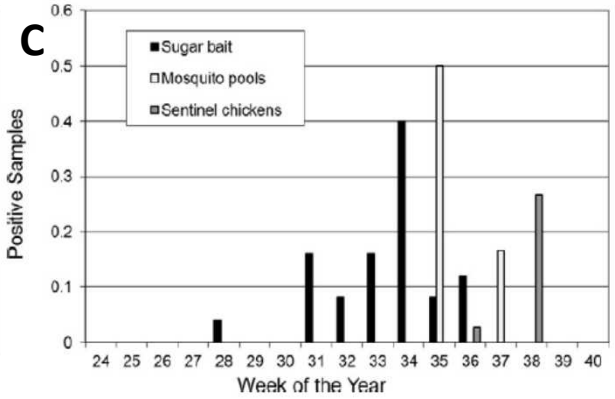
Sugar-feeding for detecting arboviruses

- Alternative approach to trapping bugs: attract mosquitoes to feed on a sugar bait, and leave behind "samples" of saliva containing viruses
- Demonstrated in field for detection of viral RNA in Australia and California.
  - Passive baits placed in field for 1 week, collected, and returned to the laboratory for RT-PCR detection of viruses.
  - Open questions include stability of viral RNA in field, recovery of RNA from baits, and best method of attracting mosquitoes to baits.
  - But sugar baits in Coachella Valley showed positive results *earlier* than trapped mosquitoes or sentinel chickens in same locations.

CO_2 baited



Floral scent / No CO_2



Previous trials of viral RNA detection by sugar feeding mosquitoes (B,C by our collaborator William Reisen at UC Davis for WNV surveillance in Coachella Valley)

Our proposed enhancement

- Automate the sugar-feeding surveillance technique *via* a "smart trap" that sits in the field for up to 1 month and autonomously:
  - Presents a fresh bait to attract mosquitoes each evening…
  - and performs assays the following morning for viral RNA
  - scores the assays as positive or negative for a panel of viruses
  - And wirelessly transmits the data back to a monitoring station.
- Data on viral incidence as function of time and place can be a data source into the BSVE.

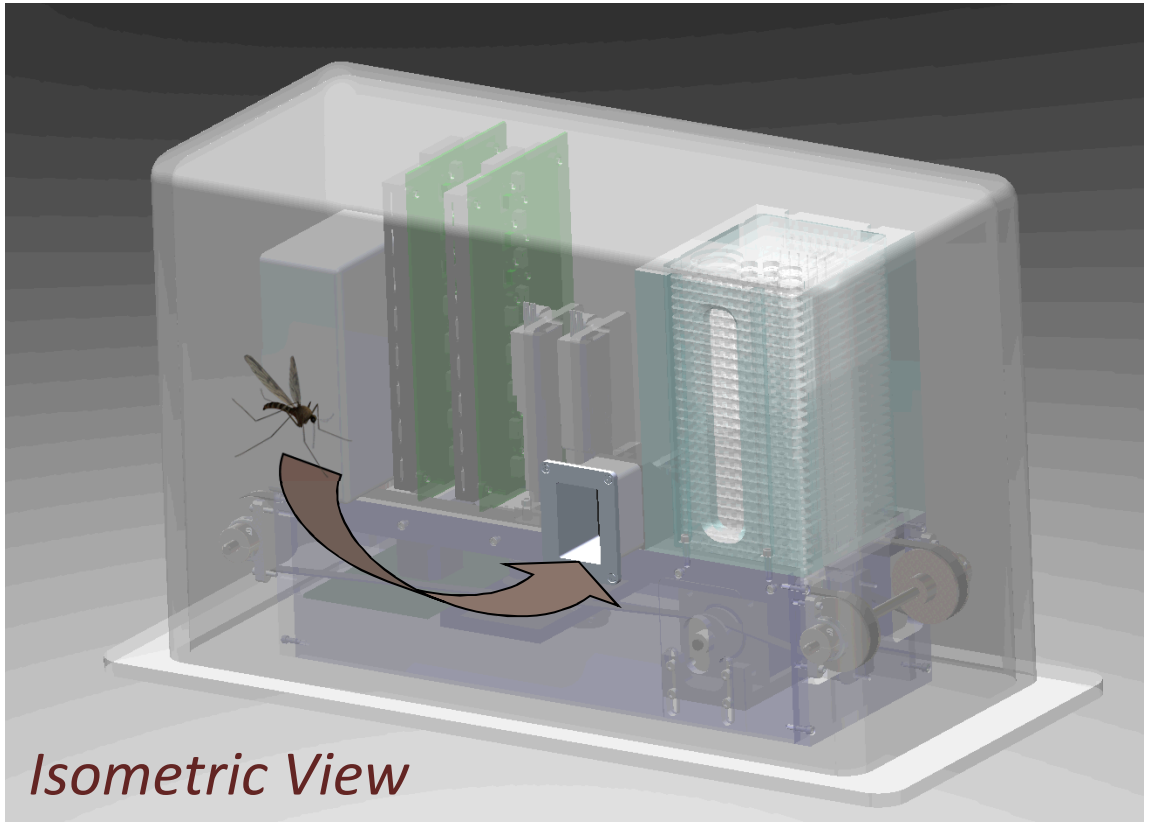
To make an impact…

- Smart traps need to be cheap, reliable, and rugged
  - Deploy a spatial grid of traps to create viral incidence maps
  - Easily portable with low footprint and low power requirements – shouldn't require a car battery, or a laboratory presence
- Assays need to be cheap, reliable, sensitive
- Sugar-feeding data needs to be understood to provide risk estimate.

Project Objectives / Tasks

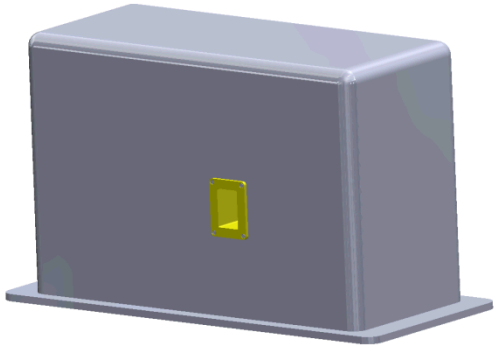
1. Develop single-use assay cartridges to detect a panel of arboviruses historically endemic in California: West Nile Virus (WNV), St. Louis Encephalitis Virus (SLEV), Western Equine Encephalitis Virus (WEEV)
2. Build fieldable integrated trap system to perform sugar-feeding and viral detection, for up to one month of autonomous operation in the field
3. Test complete system (trap hardware and assays) in Coachella Valley, CA concurrent with conventional vector surveillance (traps & sentinel chickens)
4. Develop strategies/designs for scaling up trap and assay device manufacturing
5. Develop protocols and architecture to archive assay data and integrate into BSVE
6. Develop protocols to map and model viral incidence from integrated data sets (trap data plus environmental data).

Smart Trap Prototype Design- Feb 2015



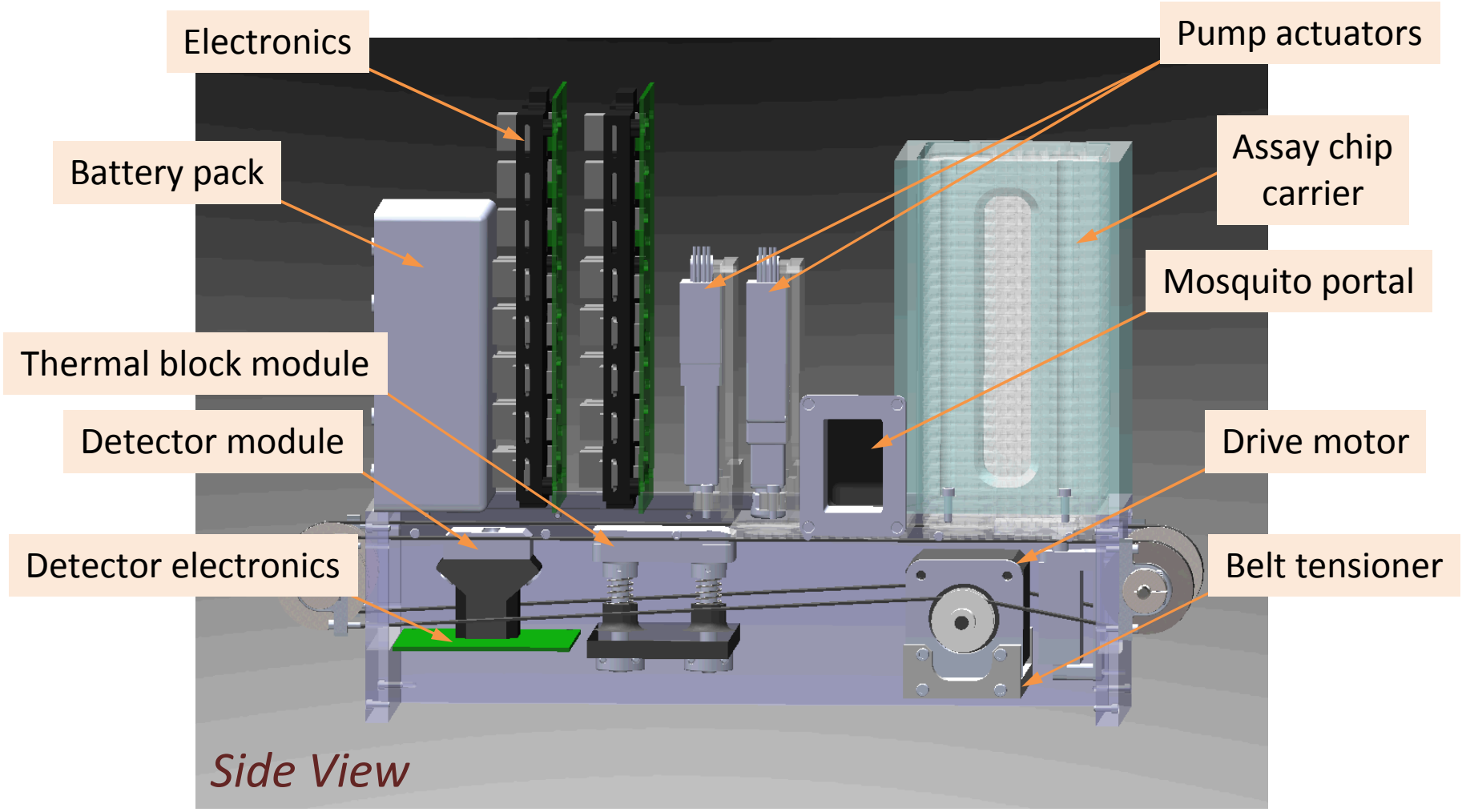
- *Microfluidic chip based*
- *Battery operated autonomous operation*
- *Low-cost design approach*
- *Mass manufacturable chip design*
- *Multiple assays from common sample*
- *Isothermal amplification*
- *Wireless-enabled*

System cover shown translucent for illustration purposes



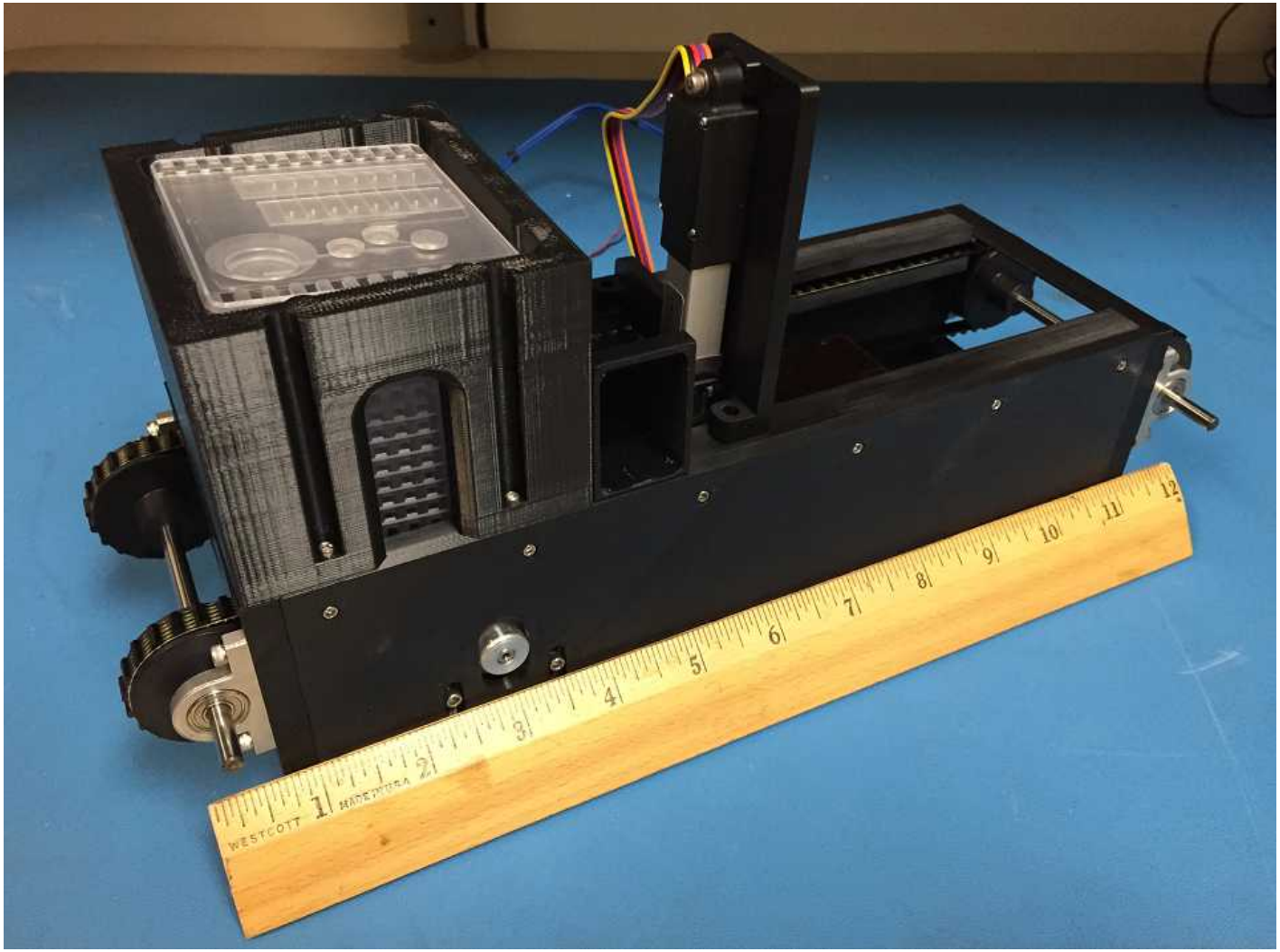
Approximate size : 14 x 6 x 8 inches

Smart Trap Prototype Design



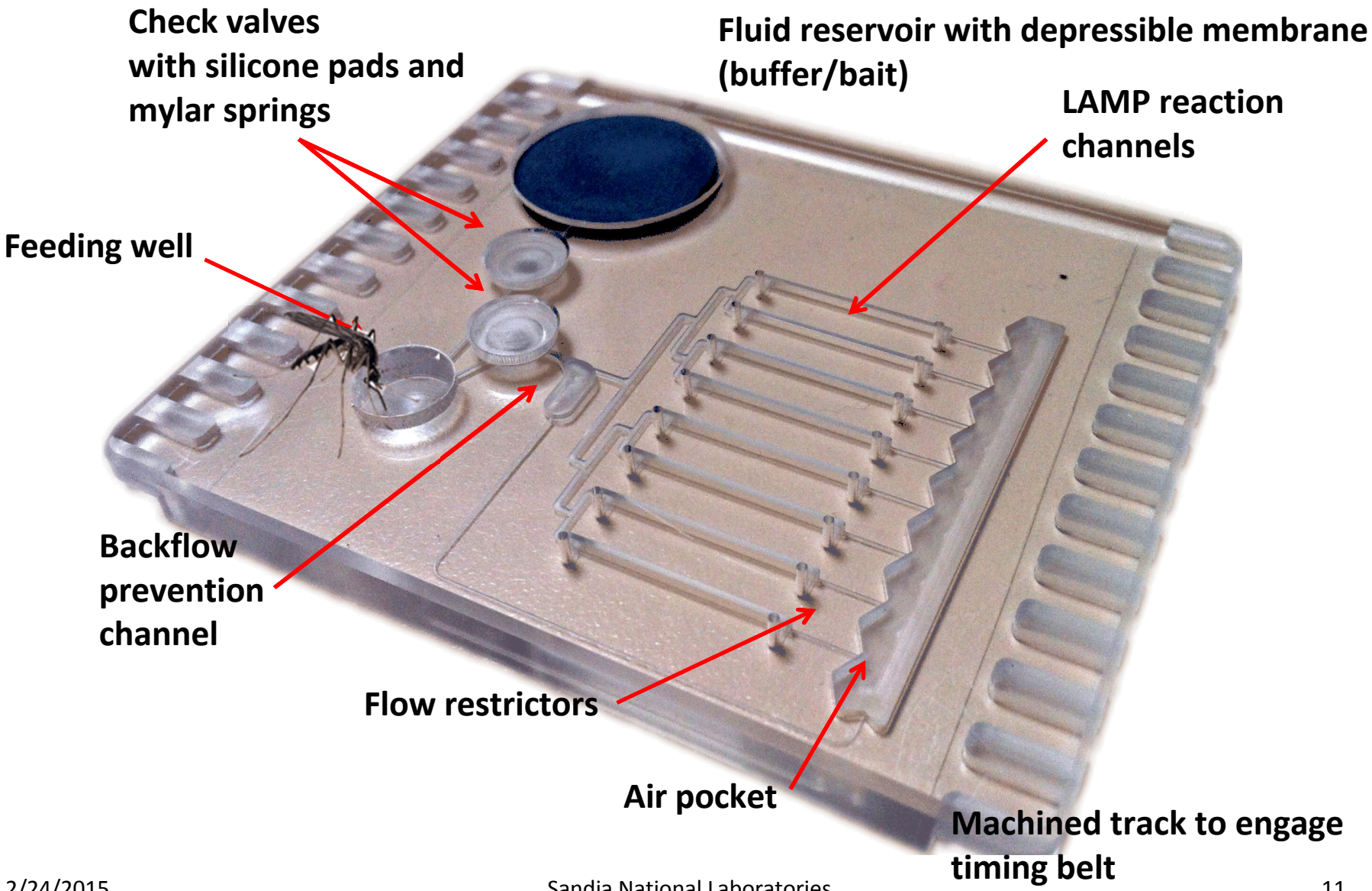
System cover and side covers removed for illustration purposes

Smart Trap Prototype Design (Actual)



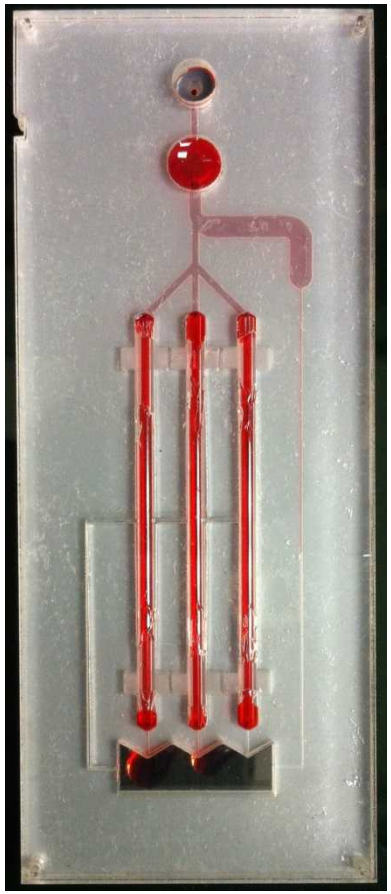
Approximate size : 14 x 6 x 8 inches

Polycarbonate chip prototype, assembled

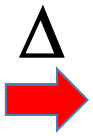


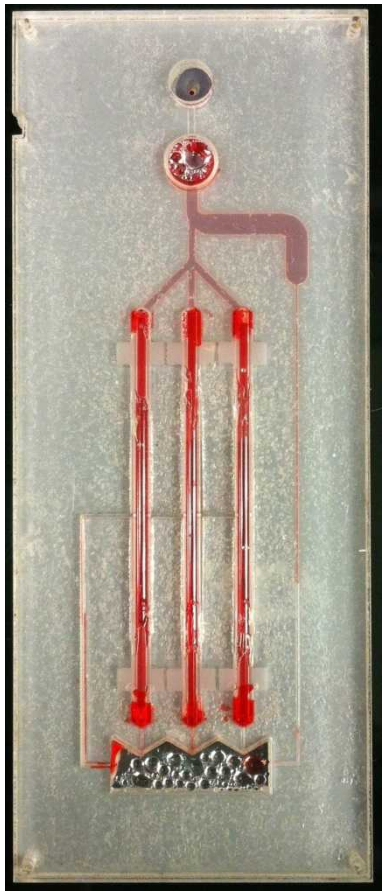
Passive check valve + air pocket

- dead-end fill channels under pressure
- Reduces evaporation, bubbling, and backflow during heating

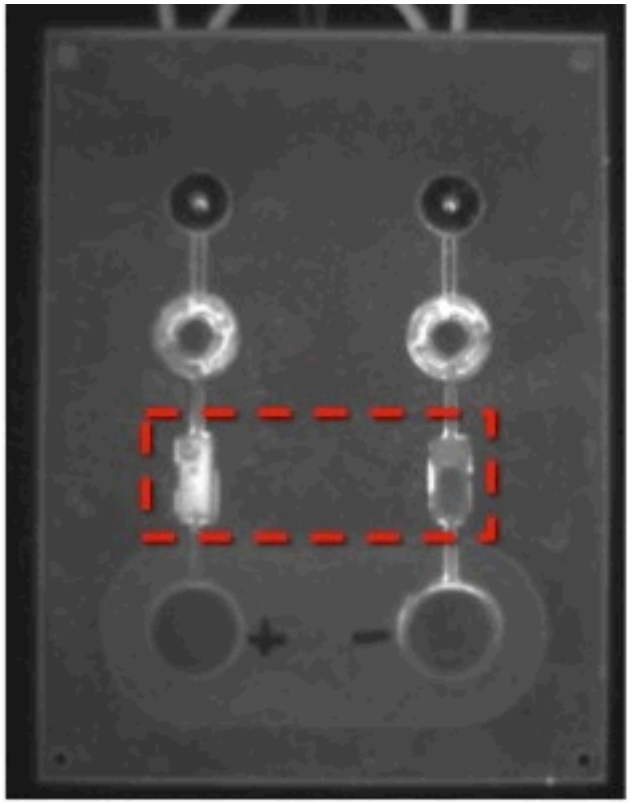


Dead-end fill
under pressure



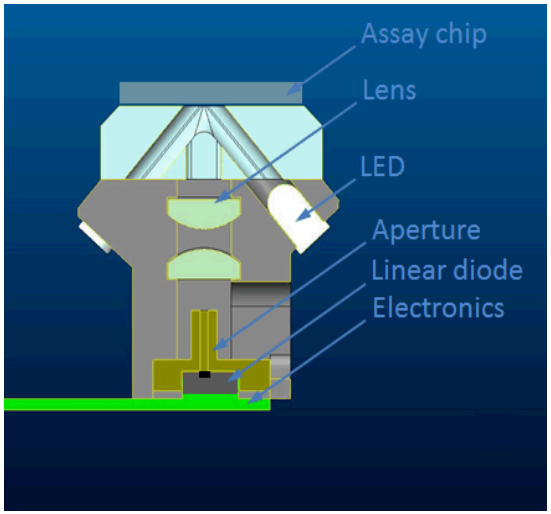


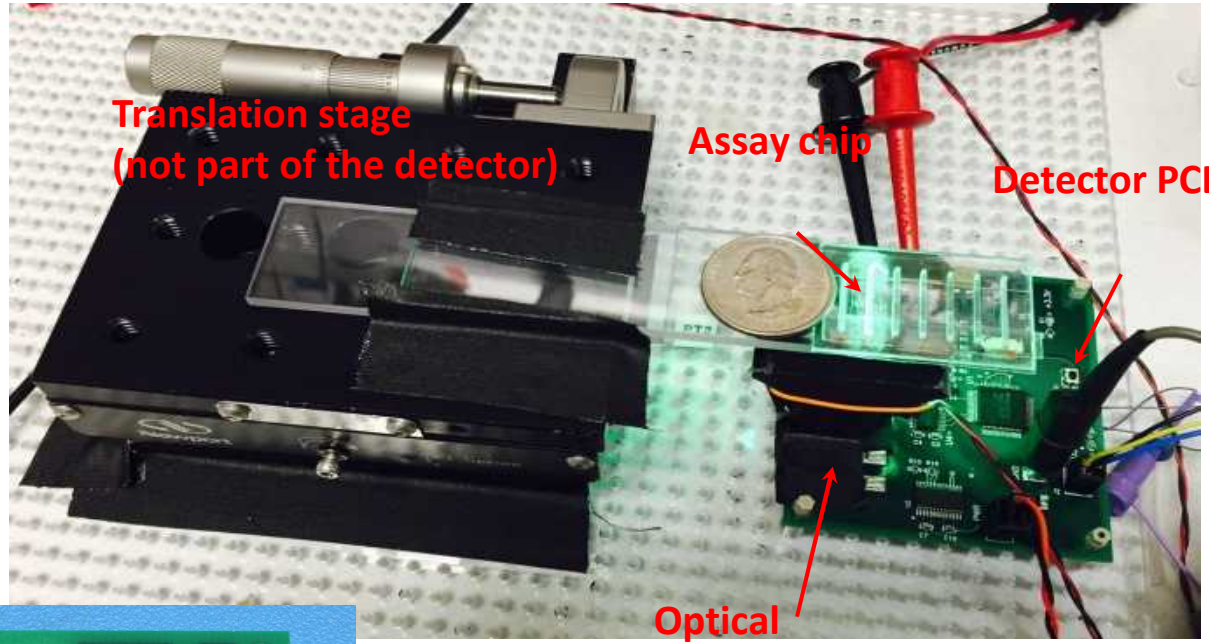
Heated
1 hr @ 90 °C

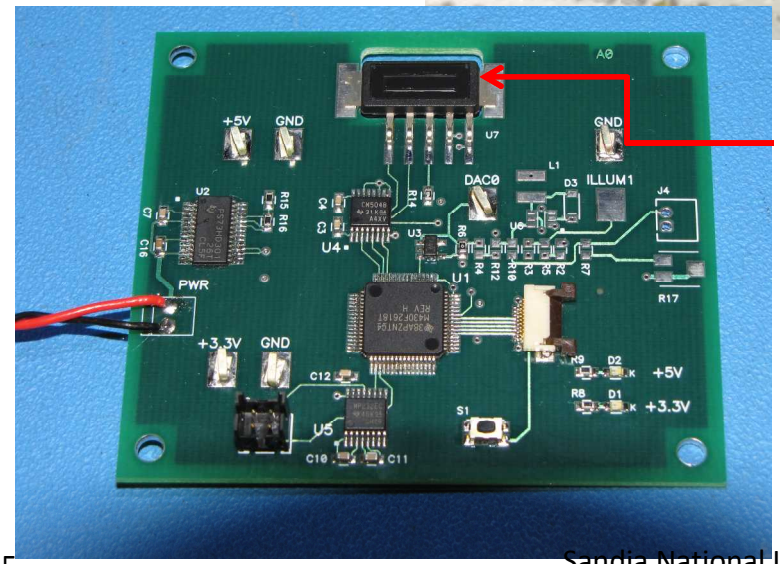


WNV RT-LAMP in
check valve devices

LED/photodiode fluorescence detector







- Linear photodiode array matches size of channel
- Uses colored theater lighting gels as cheap excitation and emission filters
- >10:1 signal:background on positive assay channels

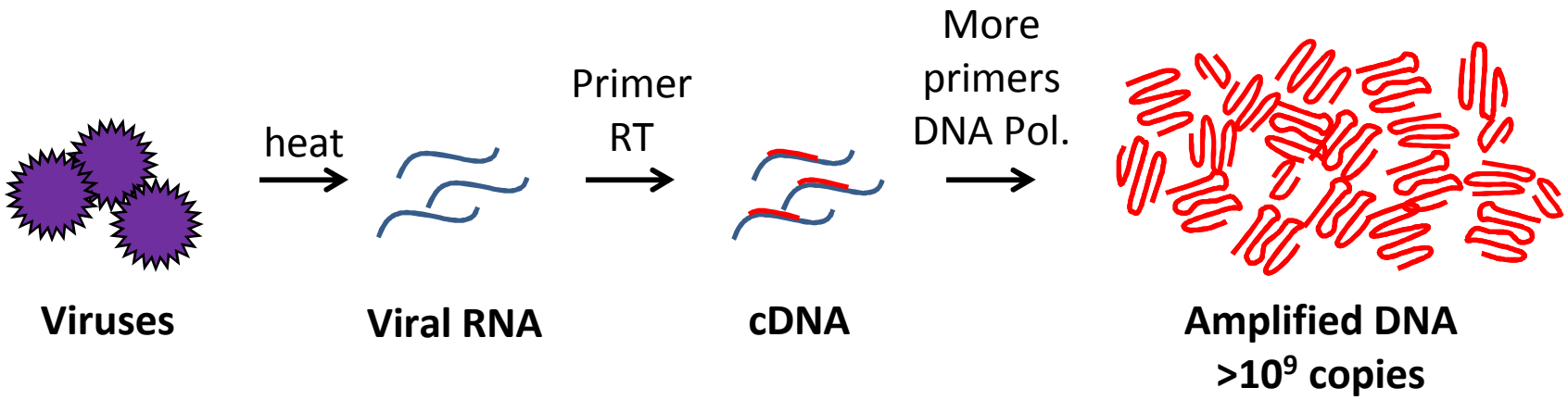
Viral detection assay

- In a single feeding, an infected mosquito may deposit 10^2-10^4 PFU of virus
  - For RNA viruses: 100-1000 genome copies per PFU.
- This is too little to detect directly or by immunoassay (e.g. “dipstick”/LFA)
- Nucleic acid amplification is the only practical way to detect such small amounts.
- Need to transduce a large signal from a small amount of viral RNA.

- In the laboratory: RT-qPCR is “gold standard” technique with best sensitivity
  - But generally requires a well-equipped lab, or rigorous on-board sample prep for an automated system (*e.g.* Cepheid, BioFire)

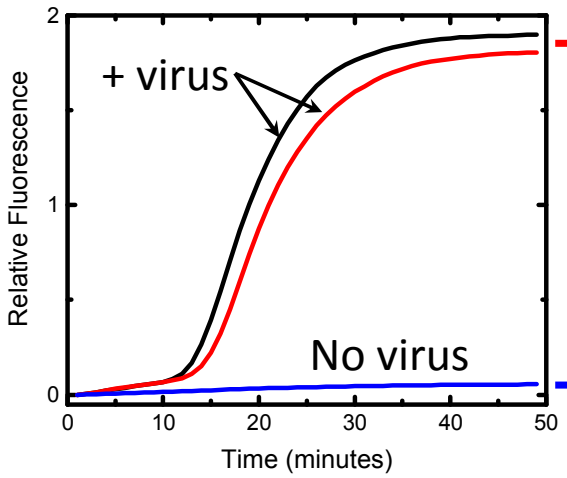
- In the field / low-resource settings: RT-LAMP is an alternative
  - Isothermal nucleic acid amplification chemistry
  - May be slightly less sensitive than RT-PCR, but straightforward and robust
  - Tolerant to crude samples / little or no sample prep required

Task 1 - RT- LAMP RNA detection overview

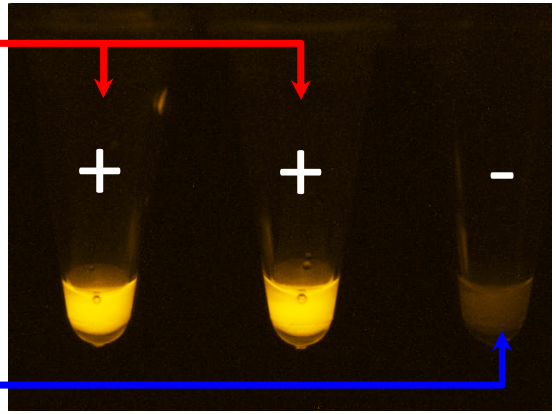


- 2 enzymes, single tube, single continuous process, single temperature (63 °C)
- Viruses become "leaky" at 63 °C – don't absolutely need to extract RNA

"Real time" fluorescence

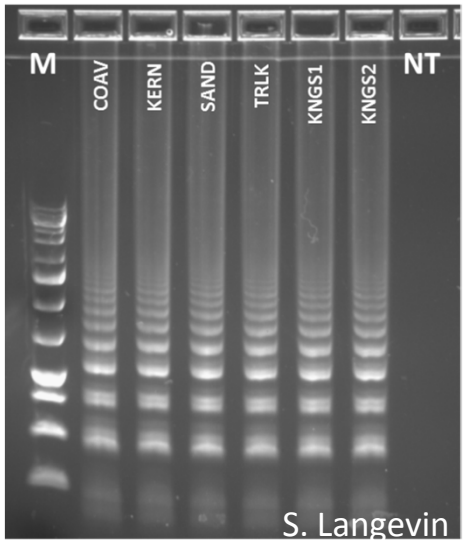


Endpoint fluorescence



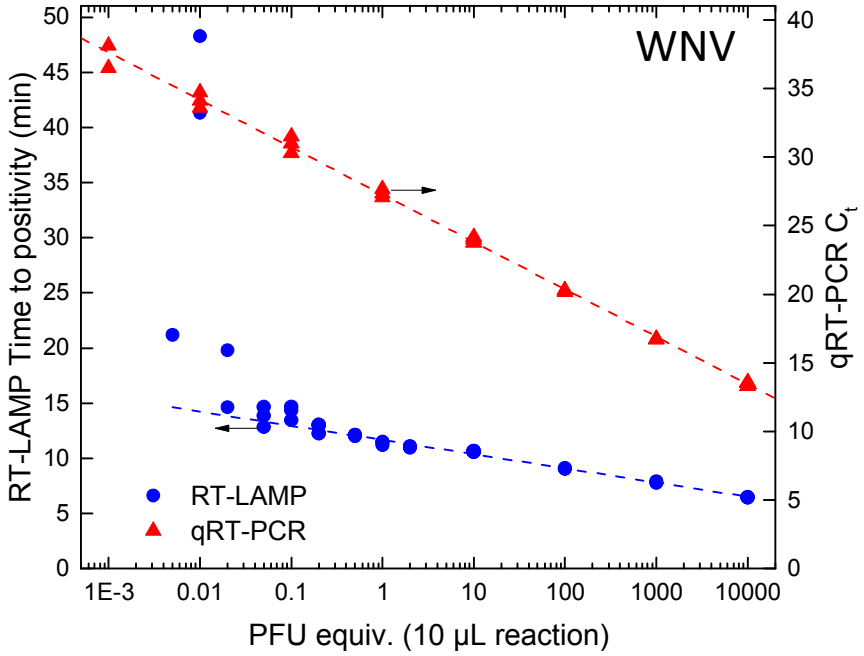
or

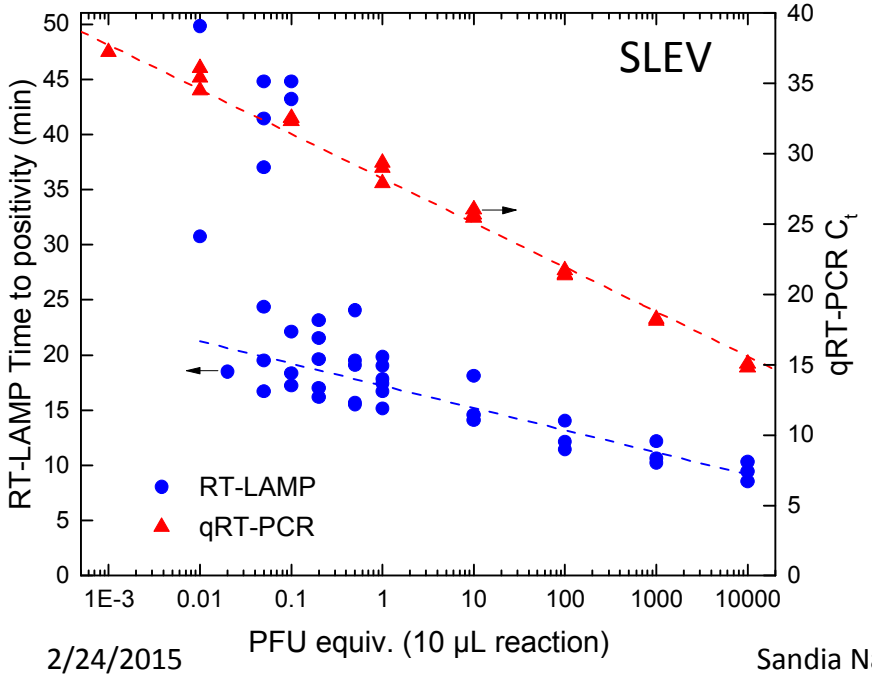
RT-LAMP for WNV in mosquito extracts
Endpoint gel analysis

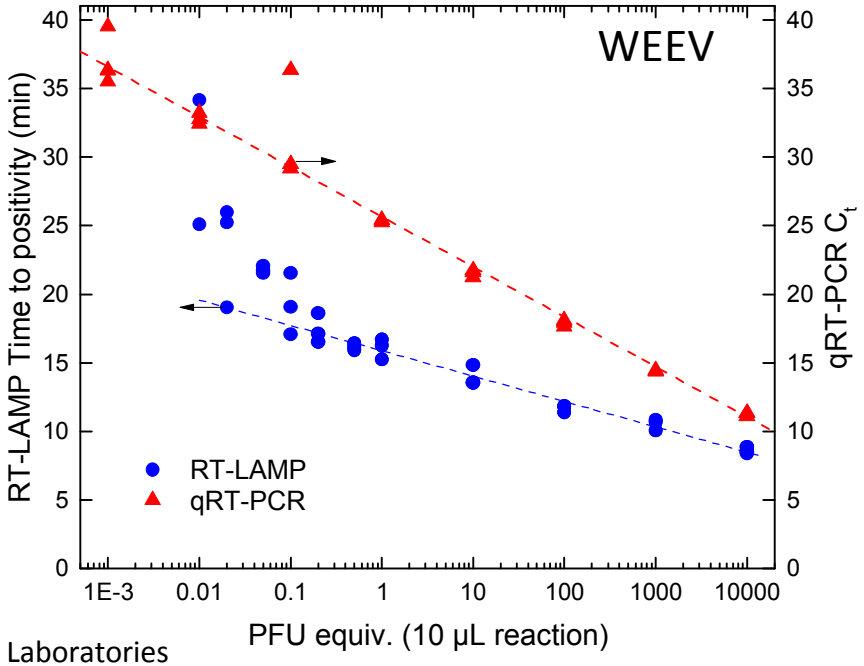


RT-LAMP *vs* qRT-PCR

- 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.







WNV, WEEV, SLEV LAMP specificity

		RT-LAMP detection (100 PFU target)		
Virus	Strain Designation	SLEV	WNV	WNV
Flaviviruses				
St. Louis Encephalitis Virus (SLEV)	Kern217	+	–	–
	1750	+	–	–
	Ruls	+	–	–
West Nile Virus (WNV)	CA2004	–	+	–
Yellow Fever Virus (YFV)	17D	–	–	–
Rocio Virus (ROCV)	SP H 34675	–	–	–
Usutu Virus (USV)	SA AR 1776	–	–	–
Ilheus Virus (ILHV)	Ilheus (?)	–	–	–
Dengue Virus serotype 1 (DENV-1)	BC-796	–	–	–
Dengue Virus serotype 2 (DENV-2)	BC-122-94	–	–	–
Dengue Virus serotype 3 (DENV-3)	BC 156-97	–	–	–
Alphaviruses				
Western Equine Encephalitis Virus (WEEV)		–	–	+
Sindbis Virus (SINV)	EDS-14	–	–	–
Ross River Virus (RRV)	SW 38457	–	–	–
Chikungunya Virus (CHIKV)	Ross	–	–	–
Venezuelan Equine Encephalitis Virus (VEEV)	TC-83	–[1]	–	–
Barmah Forest Virus (BFJ)	Barmah Forest (?)	–	–	–
Highlands J Virus (HJV)	WC-431	–	–	–
Eastern Equine Encephalitis Virus (EEEV) (*in vitro* transcribed nsP4 RNA fragment, 10^6 copies)	EEEV/X/USA/A15072/2003	–	–	–

[1] VEEV TC-83, 1 of 18 reactions with SLEV primers became positive, probably a cross-contamination

Mosquito pool samples tested by LAMP

	WEEV Ct	SLEV Ct
2002 COAV 490	22.8 ☑	
2002 COAV 512	26.5 ☑	
2002 COAV 513	25.1 ☑	
2002 COAV 584	23 ☑	
2002 COAV 942	24.5 ☑	
2002 COAV 862		27.7 ☑
2002 IMPR 136		24.4 ☑
2002 IMPR 139		26.5 ☑
2002 IMPR 145	32 ☒	27.3 ☑
SLE 1750 (Bakersfield 1953)		17.7 ☑
SLE KERN 217(1989)		11.6 ☑
SLE RULS		12.9 ☑

LAMP missed one WEEV with high Ct (near lower end of sensitivity for WEEV LAMP)

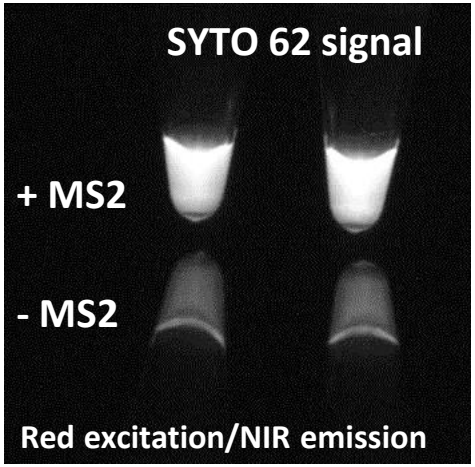
No off-target amplifications (WNV primers don't amplify SLEV, *etc*).

West Nile mosquito pools
KERN 14-133 Ct = 23.3, LAMP ☑
MARN 14-300 Ct = 25.0, LAMP ☑
SUYA 14-144 qRT-PCR ☒, LAMP ☒

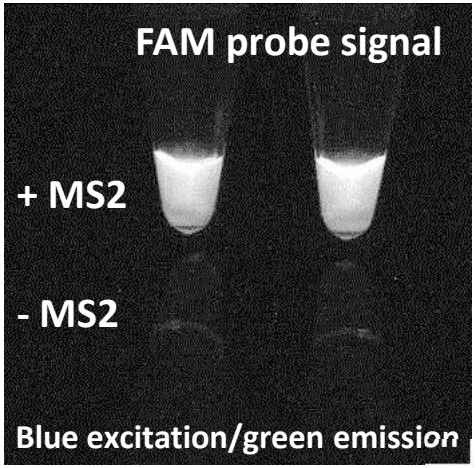
Improved LAMP detection chemistry

- Closed tube, doesn't inhibit amplification
- Detection works at room temperature
- Strong discrimination between positives & negatives
- Multiplexable, but not doing that for smart trap

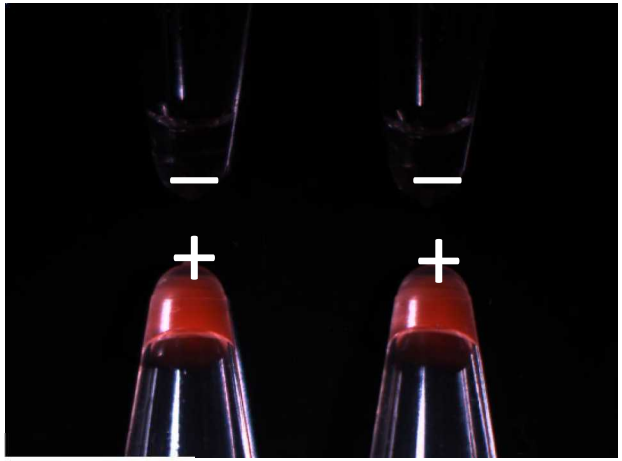
Old: Intercalating dye



New: Probe/quencher



WNV-ROX visual detection



$\frac{\text{positive}}{\text{negative}}$ **3:1** **10:1**

Strong signal with a cheap LED light and colored plastic film – ideal for Smart trap

Detection of intact viruses

- Can we really detect viruses without lysis and extraction?
- Yes, for cultured WNV, WEEV, and SLEV. Seemingly <u>better</u> for WEEV *without* extraction.
- An initial heat treatment before LAMP is *not helpful*.
- No sample prep necessary – perfect for the Smart trap.

	RT-LAMP Result					
	WNV		WEEV		SLEV	
PFU/rxn	Extracted RNA	Infectious virus	Extracted RNA	Infectious virus	Extracted RNA	Infectious virus
100000	NT	neg*	pos	neg*	NT	NT
10000	NT	pos	pos	pos	NT	NT
1000	pos	pos	pos	pos	pos	neg*
100	pos	pos/pos	pos	pos	pos	pos
10	pos	pos/pos	pos	pos	pos/pos	neg**
1	pos	pos/pos	pos/pos	pos	pos/pos	pos/pos
0.1	pos	pos/pos	pos/pos	pos	neg/pos	pos/pos
0.01	pos	neg	neg/neg	pos/pos	neg/neg	neg/neg
0.001	neg	neg	neg/neg	pos/neg	neg	neg/neg
0.0001	NT	neg	neg	neg/neg	neg	neg
0.00001	NT	neg	neg	neg/neg	neg	neg
water	neg	neg	neg	neg	neg	neg

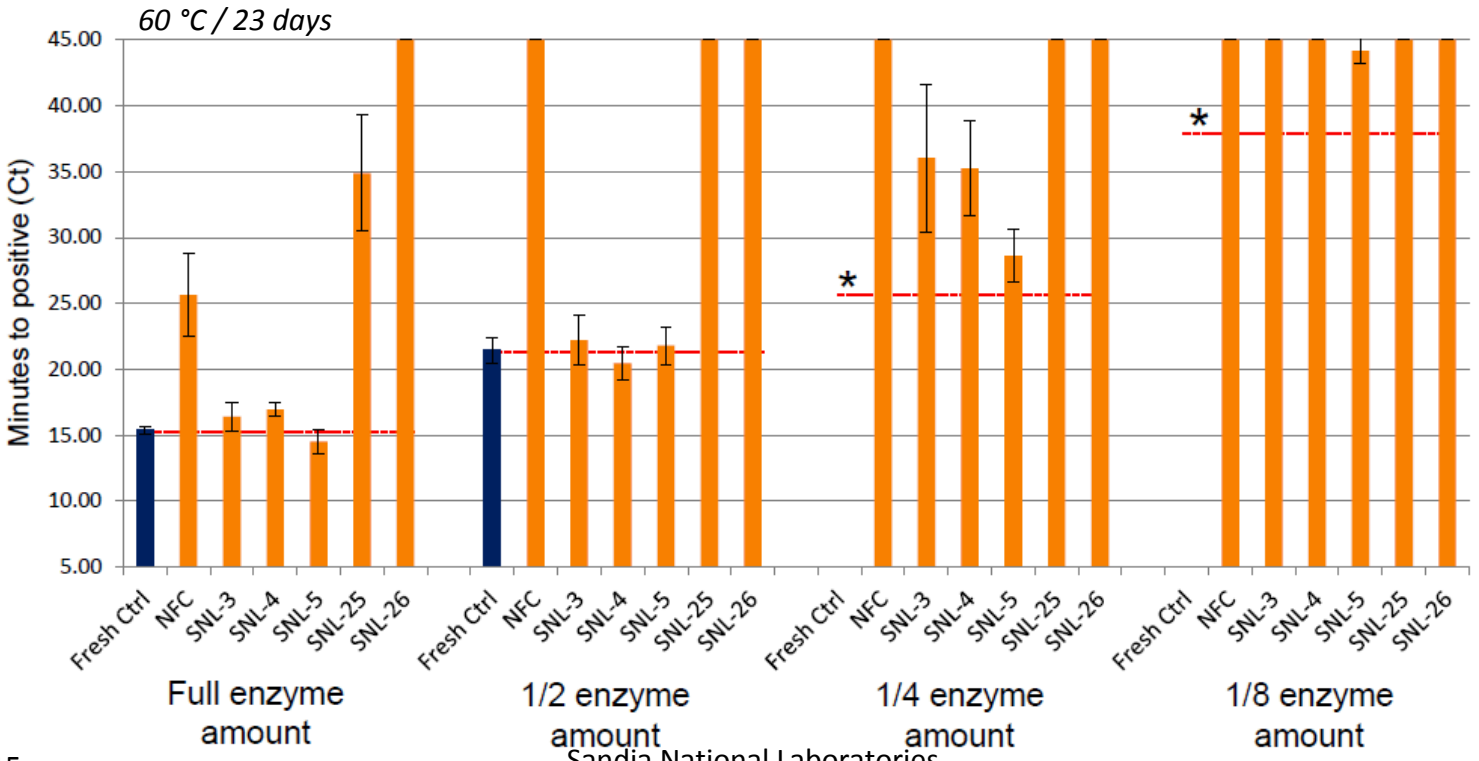
LAMP for detecting viruses in mosquito saliva

- Mosquito expectorate from lab-infected mosquitoes collected into viral media.
- Quantitated by RT-qPCR and plaque assay.
- LAMP detects 0.1 PFU or higher *intact virus* (qRT-PCR goes lower, but requires RNA extraction)
- Still to do… test infected mosquitoes feeding on our sugar baits with LAMP

Expectorant Sample #	RT-qPCR ct score	Starting titer by Plaque Assay (log^{10}pfu/mL)	Approx. titer after 1:10 dilution (pfu/mL)	Approx. pfu/rxn	RT-LAMP result
1	28	1.3	1	0.001	neg
5	25	>4	1000	1	pos
6	29	3.1	100	0.1	pos
17	31	2.2	10	0.01	neg
22	23	>4	1000	1	pos
26	28	3	100	0.1	pos
7	NT	water	NT	NT	neg
8	NT	Pos control	NT	NT	pos

Stabilizing LAMP assays for field use

- Freeze-drying was promising, but we are now pursuing air-drying using a contract vendor (Biomatrica) with a library of stabilizers.
- Achieved >3 weeks of stability stored at 60 °C, even with reduced amount of enzyme (to make the assay fail faster). Expect to translate to several months of stability at 40-45 °C (testing of daily temperature cycling is planned).



Smart trap network

Smart traps in field communicate to relay trap

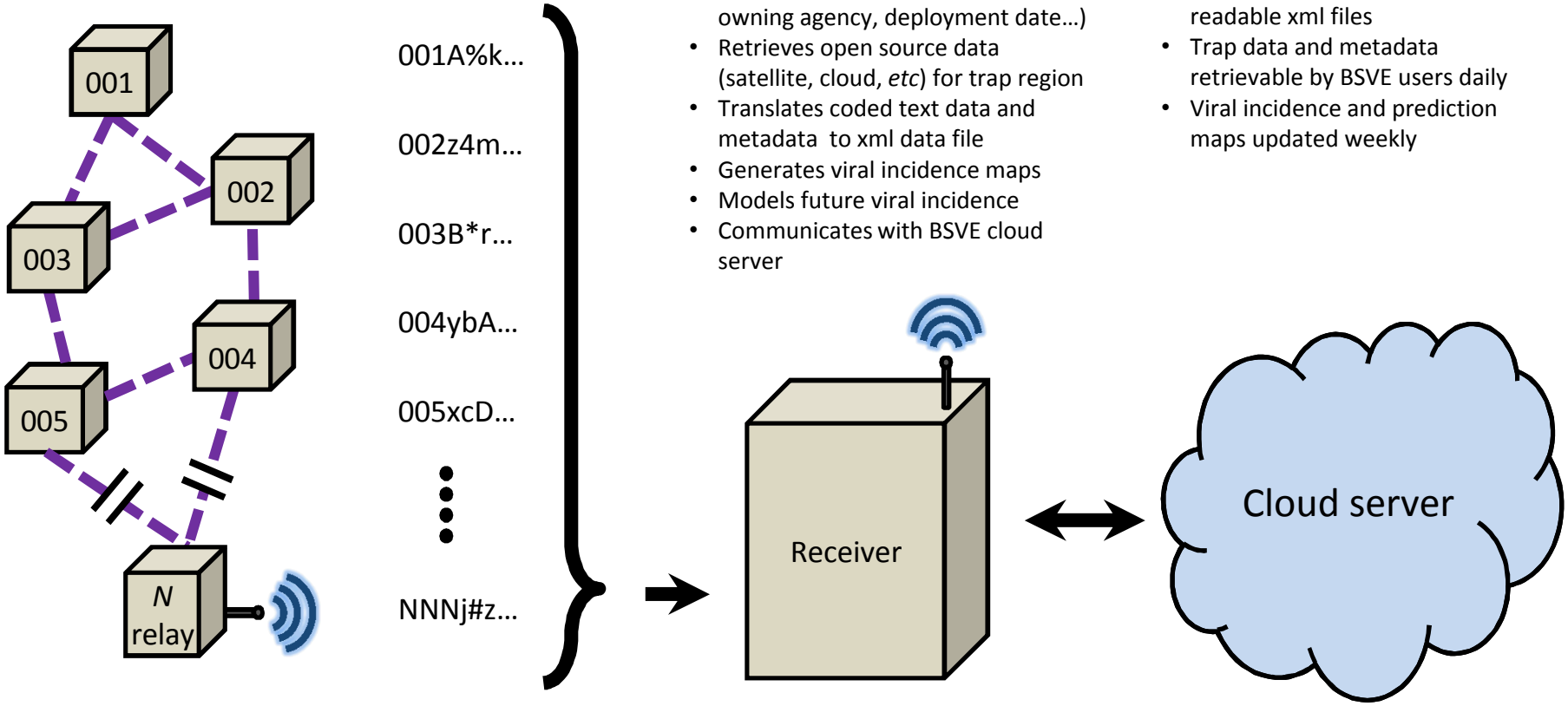
Relay compiles and sends trap data and metadata as SMS text messages

Receiver machine

- Retrieves additional trap metadata (GPS coordinates, assay panel, owning agency, deployment date…)
- Retrieves open source data (satellite, cloud, *etc*) for trap region
- Translates coded text data and metadata to xml data file
- Generates viral incidence maps
- Models future viral incidence
- Communicates with BSVE cloud server

BSVE

- Accepts data from receiver in the form of human- and machine-readable xml files
- Trap data and metadata retrievable by BSVE users daily
- Viral incidence and prediction maps updated weekly



Integration of smart trap network into BSVE. Communication between the traps and the cloud is mediated by an intermediate "receiver".

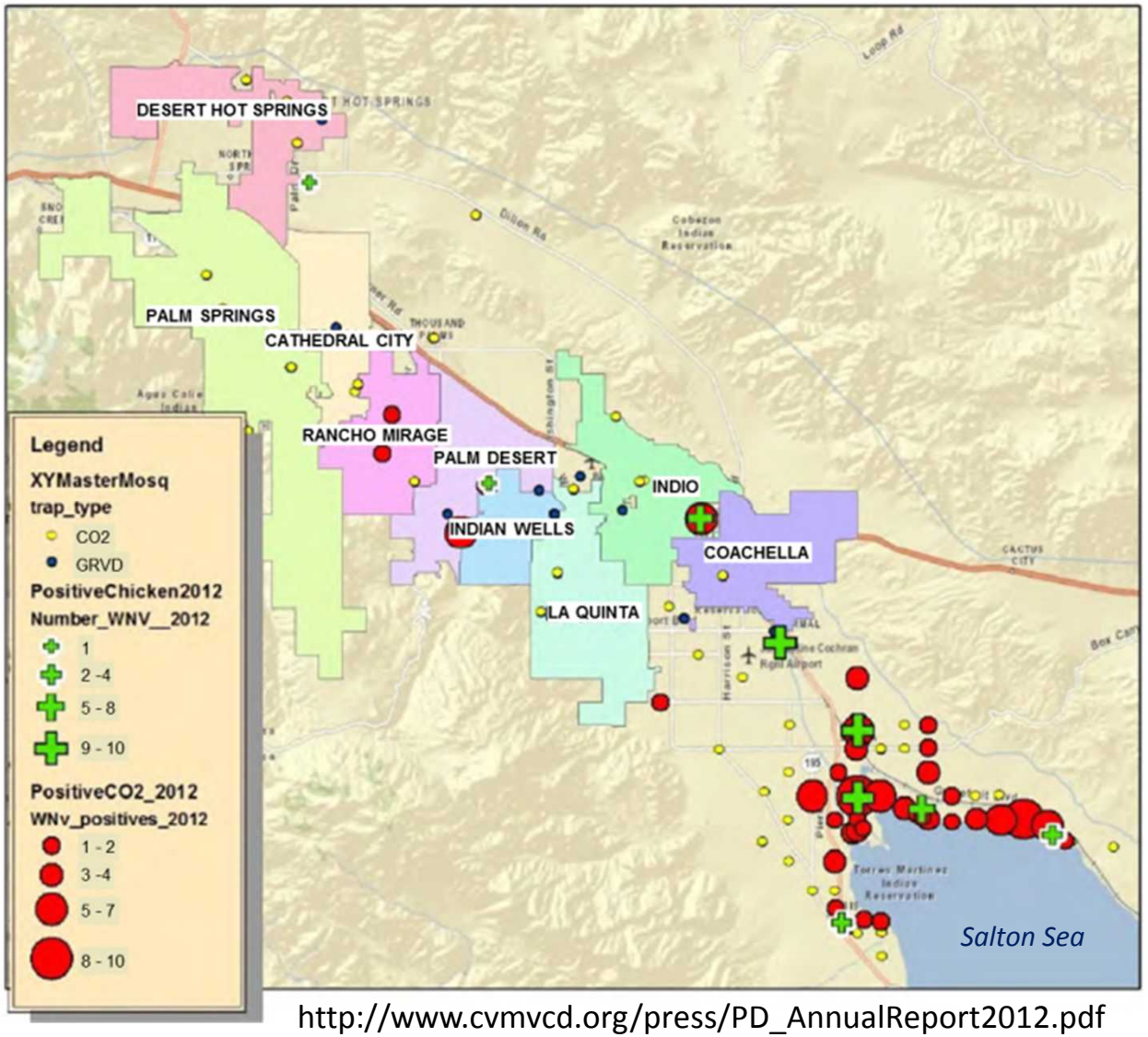
BSVE Integration - Introduction

- Aim: To map the data collected by our SMART traps deployed around Salton Sea, Southern California
  - Develop an AWS site where trap data can be uploaded
  - Develop statistical models to map the abundance of C. tarsalis (the dominant mosquito around Salton Sea)
  - Plot data regarding West Nile Virus (WNV) detection by our traps
  - Plot maps of minimum infection rate
- Data supplied by us to BSVE
  - Model predictions of C. tarsalis abundance in the Salton Sea area
  - XML detection messages uploaded to our database by our traps
  - Interactive data analysis at our site on the AWS
- Data used by us (but not supplied to BSVE)
  - CA Vector Control data from 81 sites regarding C. tarsalis abundance and WNV detection
  - Uploaded every 15 days into AWS, manually, as Excel spreadsheets

Background

- Our traps will trap C. tarsalis mosquitoes, detect the presence of WNV and upload the detection data every night into the AWS
- The success of the detection approach depends on (1) the abundance of C. tarsalis and (2) their infection rate
- In the Salton Sea area, mosquitoes are found in artificial wetlands around the shores, in urban areas and in irrigated farmlands
  - Their abundance is weakly dependent on the temperature and precipitation during winter and spring (controls how many C. tarsalis females survive and lay eggs in spring)
  - Peak abundance in July & August
- Most of the region around Salton Sea is desert scrub
  - C. tarsalis distribution very localised
  - CA Vector Control sites & our traps will be collocated
    - But we need to decide where we will deploy our 10-16 sensors

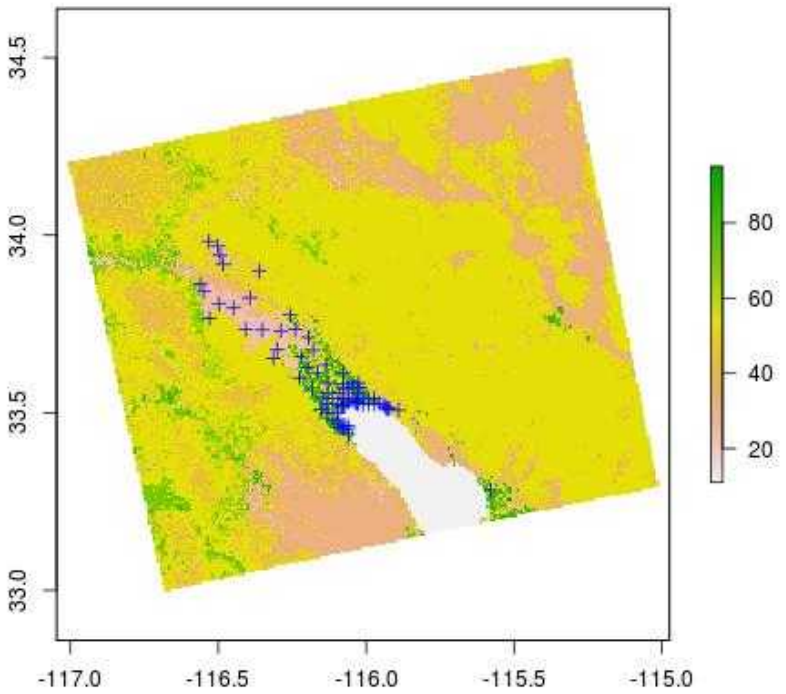
Coachella Valley WNV Surveillance, 2012



http://www.cvmvcd.org/press/PD_AnnualReport2012.pdf

Technical approach – mapping abundance

- We need C. tarsalis abundance spatially so that we can map them
  - CA Vector control supplies data only at a limited set of sites
  - Develop regression models that predict female C. tarsalis abundance as a function of local meteorology, time and land-use pattern (NDVI-based)
    - Use climatologically averaged MERRA reanalysis products for meteorology, separate model
    - The model, called the “mean model”, is approximate
- Use CA Vector Control data to compute “deviance” from mean model at the 81 sites



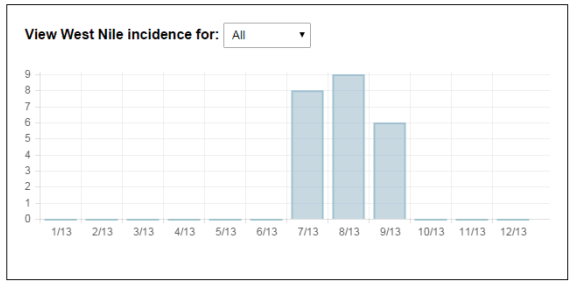
- Interpolate deviance from CA Vector Control sites to anywhere using Kernel Smoothing

Technical approach – tracking WNV infection

- CA Vector control also supplies WNV detection data from its sites
  - Painstaking and manual analysis of pools of trapped mosquitoes
  - Provides (biweekly) data on WNV detection at each of its sites
  - But the data is sufficiently detailed to compute an infection rate
- Our 10-16 traps will be deployed at a subset of these 81 sites
  - The sites will provide validation data
  - Our traps will detect and upload data nightly
  - Infection rate, as estimated from our trap detections and abundance maps, should be similar that computed from CA Vector Control data
    - Or we calculate a "detection" efficiency – the price paid for an autonomous detector
- CA Vector Control data is only for learning models & validation
- BSVE gets the data/information gleaned from our traps and models

Our AWS site

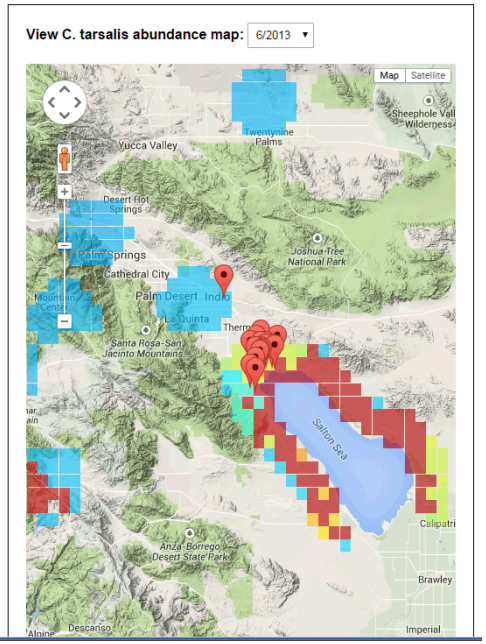
- Provides a platform where the traps can upload data
  - Traps encode their message using the BSVE PON diagnostic XML schema
- CA Vector Control data uploaded every month, as .xlsx files



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



- R scripts convert meteorology, land-use and Vector Control data into abundance maps and min. infection rates
- AWS site plots these derived data
- Also allows interactive manipulation of archived data and time-series of detections by each archived trap

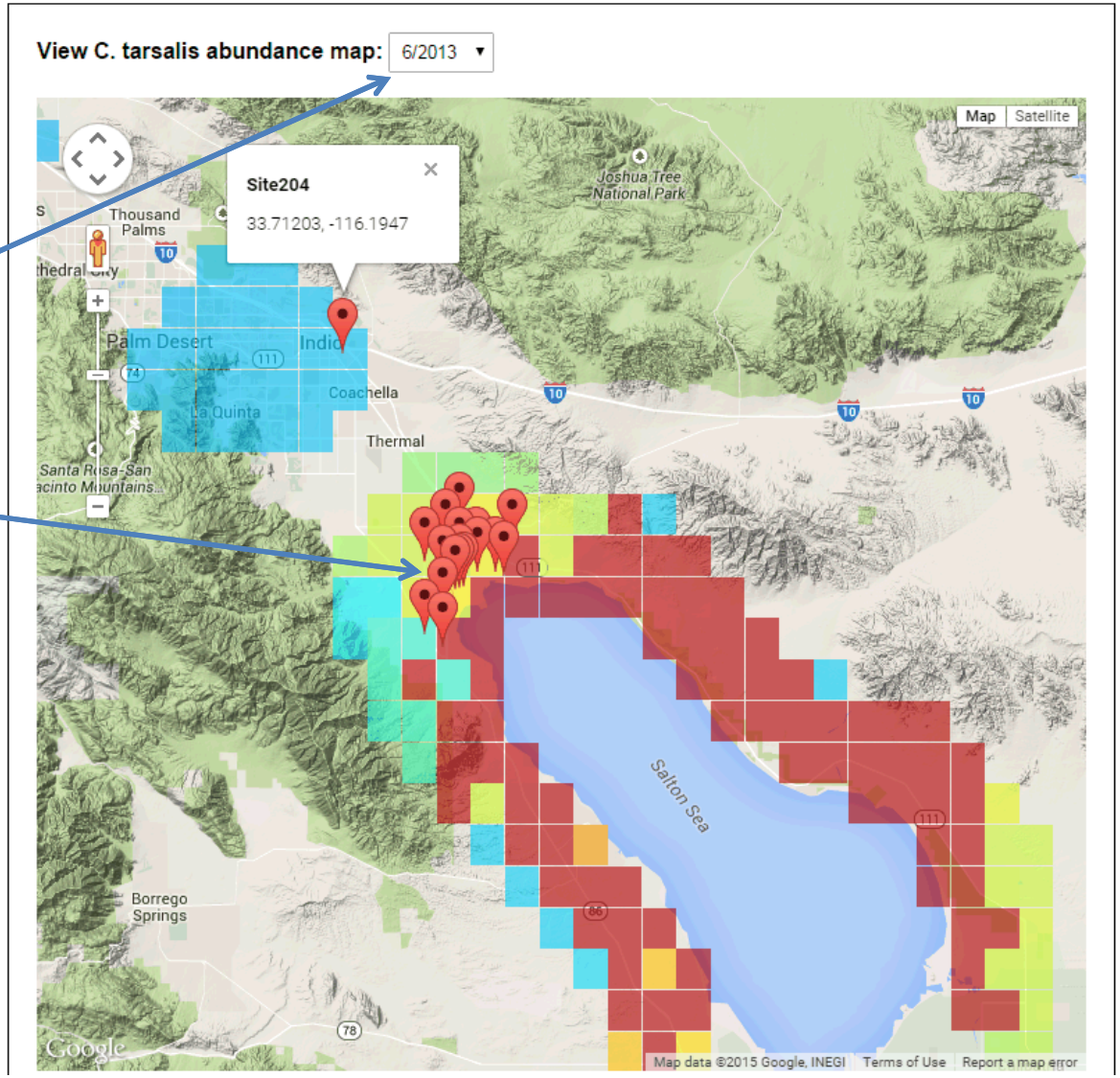
AWS architecture and implementation

- Web application currently running on Amazon Machine Instance (AMI)
  - m3.2xlarge: Intel Xeon E5-2670 (Ivy Bridge) processors, 8 CPUs, 30GB memory
  - Current application is not computationally expensive and should be able to run on a lower end AMI if necessary
- HTTP/REST interface for handling upload of data
  - Trap data uploaded as XML messages
  - CA vector control abundance data uploaded as *.xlxs files
  - In future, REST interface can be used by BSVE to download trap data and model prediction files (not yet implemented)
- Security
  - Website and REST calls (trap data and abundance data uploads) go through HTTP/SSL with Basic authentication (username/password)
- Backend Framework
  - Technologies used: Java, Spring MVC, Hibernate/H2
  - Trap data stored in H2 database
  - CA vector control data and model predictions stored as flat files
- Frontend Website
  - Technologies used: HTML, Javascript, Jquery, DataTables, Chart.js, Google Maps API

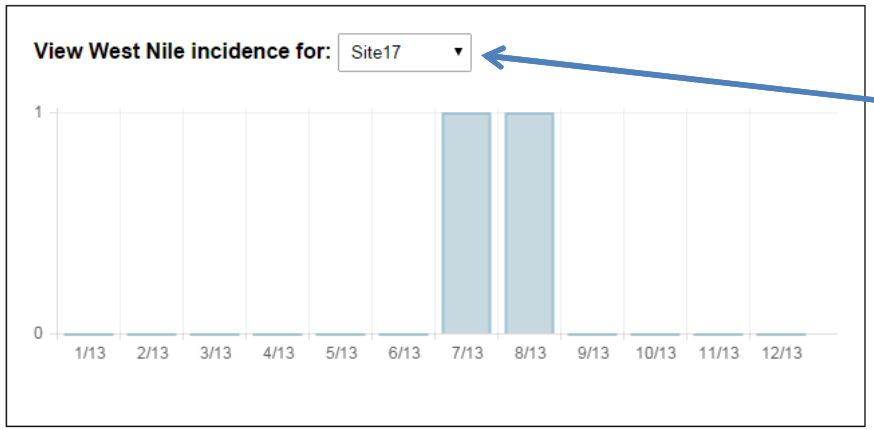
Abundance visualization

Select map by date

Site locations



Detector visualization



View time series of detection by site

Trap Data

Search:

Time	Site ID	West Nile
7/2013	Site17	Positive
7/2013	Site204	Negative
7/2013	Site30	Positive
7/2013	Site33	Negative
7/2013	Site34	Positive
7/2013	Site35	Positive
7/2013	Site37	Negative
7/2013	Site40	Negative

View/search SMART trap data

Come see our demo on Wednesday afternoon!

Summary

- Tasks accomplished
  - A preliminary abundance model is working (R scripts)
  - Algorithm for computing infection rate available; not yet ported to AWS
  - http interface on AWS for uploading trap messages and vector control abundance data working in AWS
  - Website for processing mosquito, meteorology etc. data up and working; ported to AWS
- Tasks for Year 2
  - Improve abundance model; exploit temporal correlations; complete porting of infection rate computations to AWS
  - Implement automated downloading capability (for BSVE)
  - Develop smartphone app that traps can use to upload their messages into AWS
  - Re-implement using SDK

Smart Trap – BSVE integration

- Proposed effort is mostly development of novel hardware/assay
- Field test data comes online in year 3
- Will work with BSVE partners & SDK for best fit into Harbinger system