SAND2017-12803C

Smart trap for autonomous monitoring of mosquito-borne viruses

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Sandia National Laboratories

U.S. DEPARTMENT OF ENERGY

DTRA Defense Threat Reduction Agency

Outline

- Background
- Purpose & Objective
- Rationale
- Relation to other areas of study
- Methods
- Results
- Impact on mission
- Conclusions

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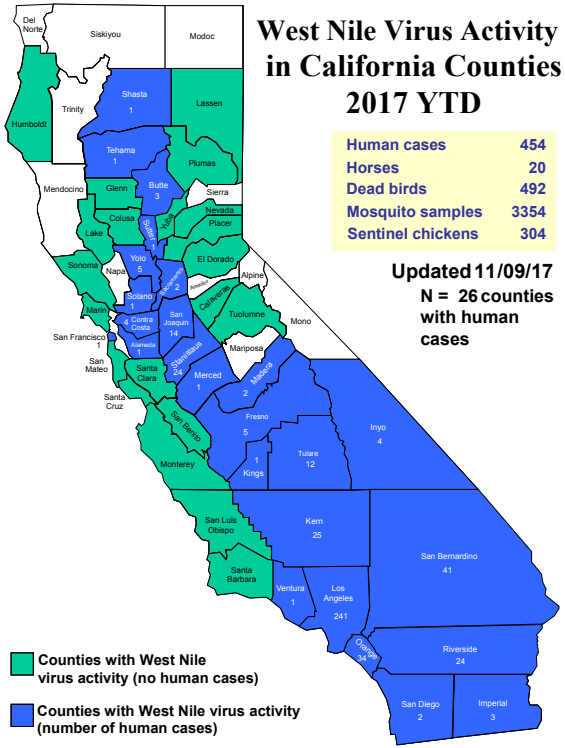
Ah, Livermore!

AAAAAGGH!!! Livermore!

*There aren't that many mosquitoes in Livermore, actually. Come visit!

Vector-borne pathogens are a local problem

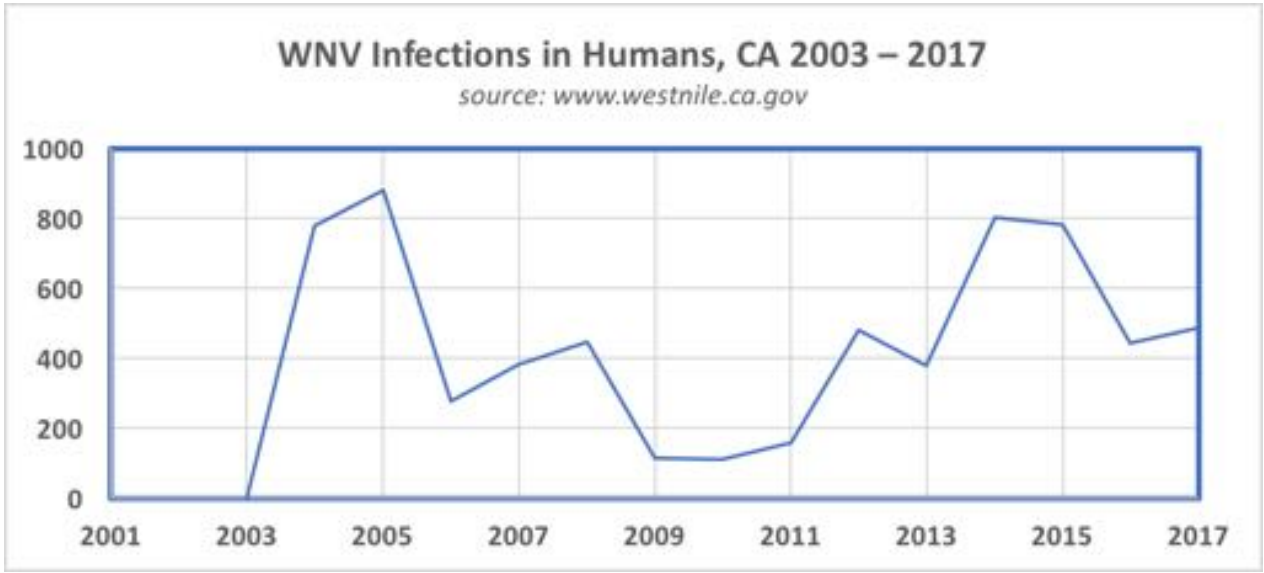


454 Reported human cases (YTD) in CA from West Nile

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

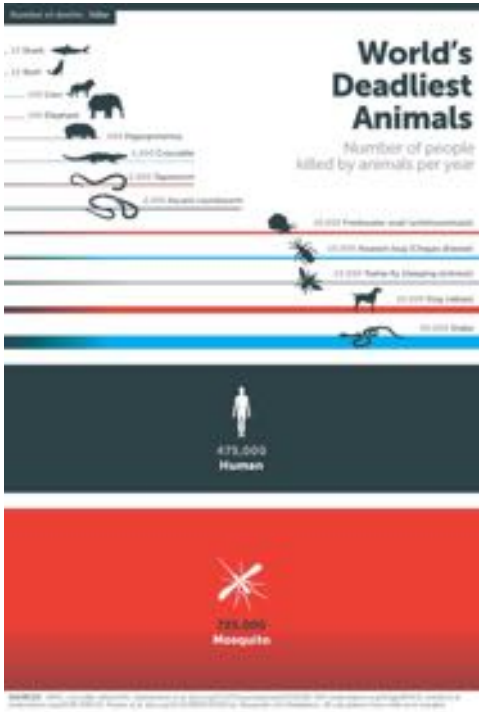
Image retrieved from: www.westnile.ca.gov on 11/10/17

Vector-borne pathogens are a local problem



Data retrieved from: www.westnile.ca.gov on 11/10/17

Vector-borne pathogens are an even greater GLOBAL problem



Source: Gates Foundation

Homeless sleeping on the street under a mosquito net

Photo credit: AP Photo/Muhammed Muheisen

Mosquitoes can also be *weaponized*

Table 3. (U) Resource Cost Summary for a Yellow Fever-Infected Mosquito Attack on a City.

Item	Cost (1976 $)
Planning	547
Agent Production	9,066
Munition Acquisition	500
Weapon Employment	360
TOTAL:	10,473

THIS TABLE IS UNCLASSIFIED

$45k USD (2017)

Operation Big Buzz—in 1955, US army dropped mosquito “bombs” over civilian populations in Georgia, dispersing 300,000 blood-hungry female *Aedes aegypti* mosquitoes

Mosquitoes can also be ***weaponized***



- Infections are incapacitating (dengue, West Nile, chikungunya, Rift Valley fever, etc.) and sometimes fatal
- Potential for bioterrorism targeting civilians or overseas military personnel

Surveillance of field-caught mosquitoes provides an early warning

- No specific treatments or vaccines for many arboviruses
  - West Nile virus, St. Louis encephalitis virus, western equine encephalitis virus (local concerns)
  - Dengue virus, Zika virus (global concerns)
- Prevention is the only option
- Surveillance ***focuses*** vector control efforts and informs epidemiological investigations of human transmission.

However, current surveillance methods are labor-intensive and slow

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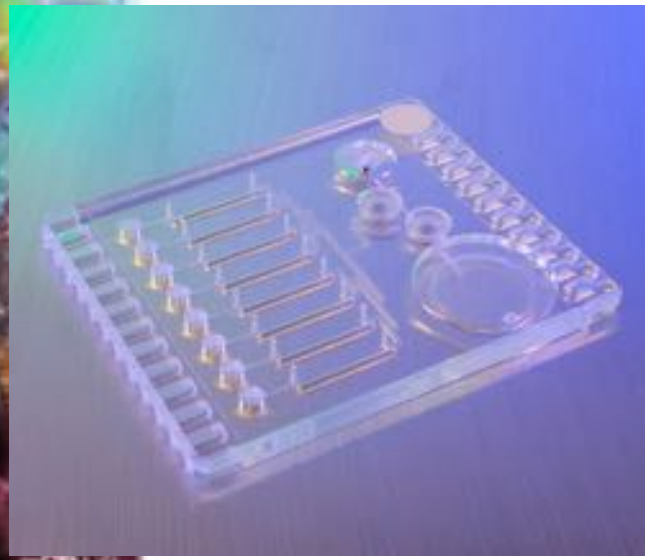
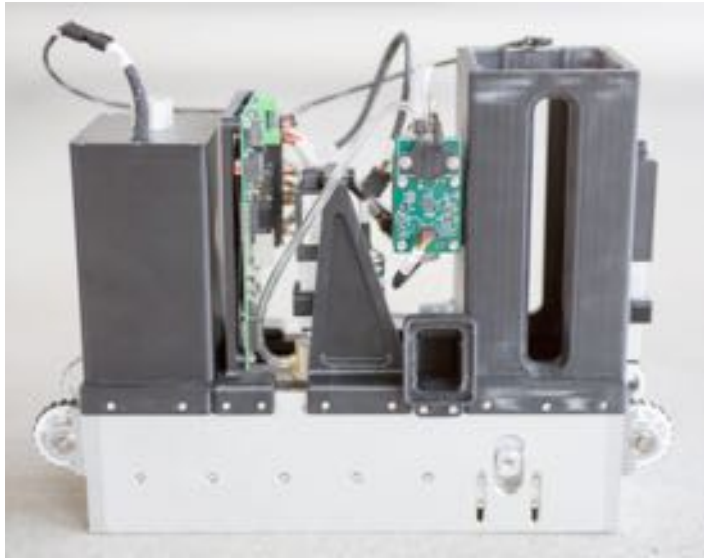
Goal: Automate viral surveillance



µfluidic assay



Assay automation system

Field deployment

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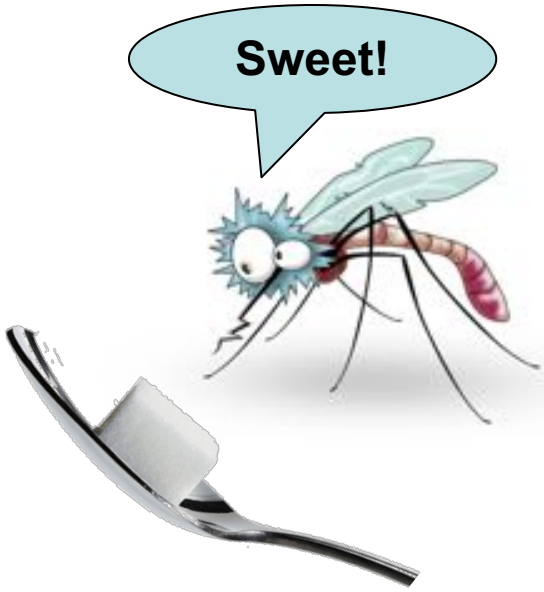
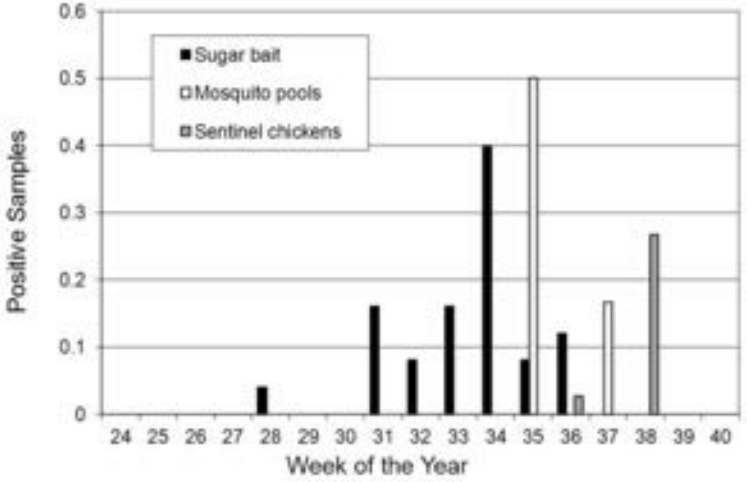
Mosquitoes like sugar



- Provides energy for flight
- Mosquitoes drawn to sources of nectar (but don’t pollinate)
- Not picky, will also bite plants directly
- Salivate while feeding, releasing virus

Sugar baits are less labor intensive and yield earlier results

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)

Sugar baiting is an alternative solution to trapping whole mosquitoes

Converting mosquito surveillance to arbovirus surveillance with honey-baited nucleic acid preservation cards
EJ Flies, C Toi, P Weinstein... - Vector-Borne and ..., 2015 - online.liebertpub.com
... The recently developed techniques of testing **mosquito** expectorate using honey-**baited** nucleic acid preservation cards or **sugar bait** stations allows a sensitive method of testing for infectious, rather than infected, **mosquito** vectors. ...
Cited by 1 Cite Save

Applications of a sugar-based surveillance system to track arboviruses in wild mosquito populations
AF van den Hurk, S Hall-Mendelin... - Vector-Borne and ..., 2014 - online.liebertpub.com
... the application of this system for detecting flaviviruses and alphaviruses in wild **mosquito** populations in ... passive box traps (PBTs) that were designed to house cards **baited** with honey ... a template for gene sequencing, enhancing the utility of the **sugar-bait surveillance** system for ...
Cited by 8 Related articles All 6 versions Cite Save

Use of scented sugar bait stations to track mosquito-borne arbovirus transmission in California
HD Lothrop, SS Wheeler, Y Fang... - Journal of medical ..., 2012 - jme.oxfordjournals.org
... 2006), so deployed **sugar bait** stations would not likely serve as a source of **mosquito** infection. ... in that uninfected **mosquitoes** were not likely to become infected by **sugar** feeding on ... **Mosquitoes** frequently feed on **sugars** throughout their lifetime (Foster 1995) and are attracted to ...
Cited by 7 Related articles All 8 versions Cite Save

[PDF] **Evolution of mosquito-based arbovirus surveillance systems in Australia**
AF van den Hurk, S Hall-Mendelin... - BioMed Research ..., 2012 - downloads.hindawi.com
... species identification and infection rates in **mosquito** populations cannot be determined using the honey-**bait** system, it ... [14] D. Rohe and RP Fall, "A miniature battery powered CO2 **baited** light trap ... [41] RC Smallegange, WH Schmied, KJ Van Roey et al., "**Sugar**-fermenting yeast ...
Cited by 17 Related articles All 14 versions Cite Save More

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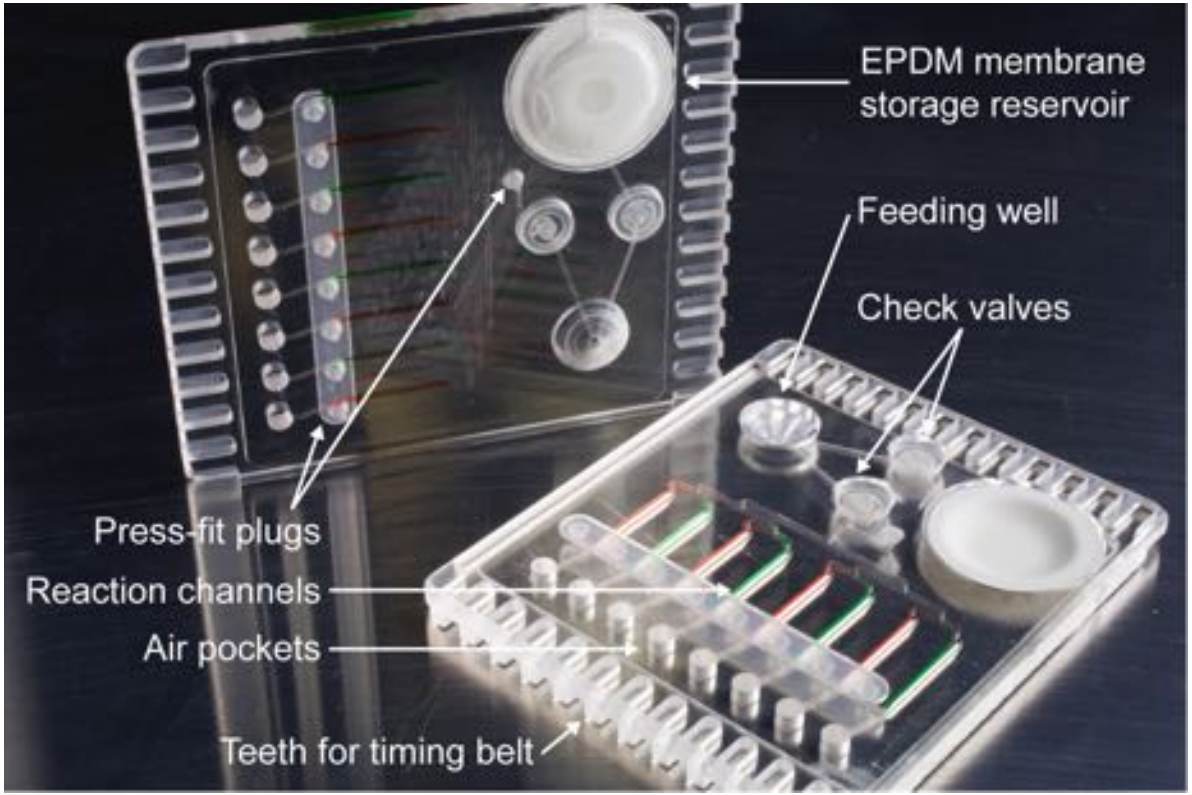
Smart trap's microfluidic cartridge is designed to facilitate sugar feeding and analyze saliva



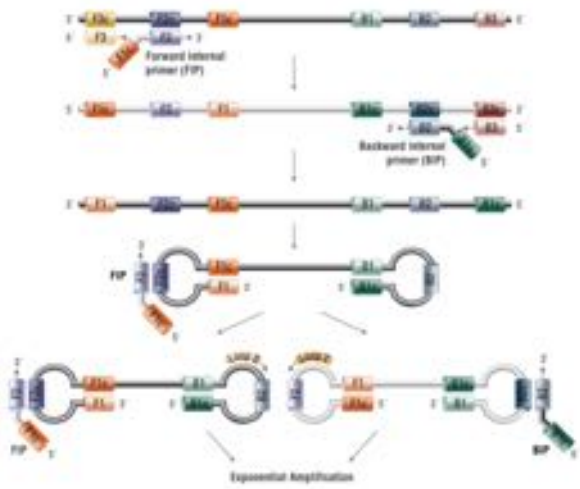
Feeding well (above) contains dried enzyme pellet, magnetic ball mixer, and honey bait

LAMP is a PCR alternative well suited to low resource settings

- Loop Mediated Isothermal Amplification: primer-based amplification of DNA/RNA targets

- Fast (5-20 min), robust, simple, sensitive
- Low capital expense/Low power
- Can work with minimal/no sample pretreatment

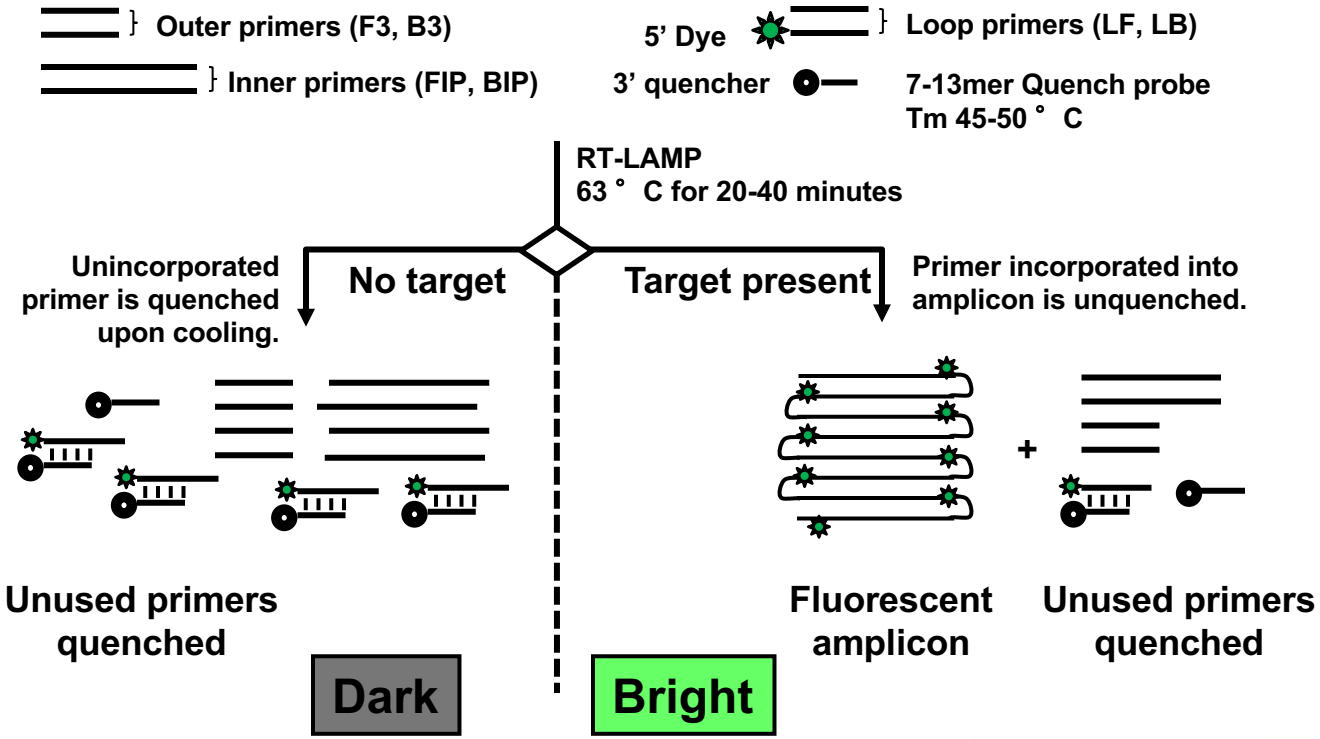
- Can't easily multiplex
- Most detection techniques are non-specific (turbidity, colorimetric, etc)
- Prone to false positives
- Less quantitative than qPCR



www.neb.com

Complex reaction scheme involves strand displacement instead of thermal denaturation

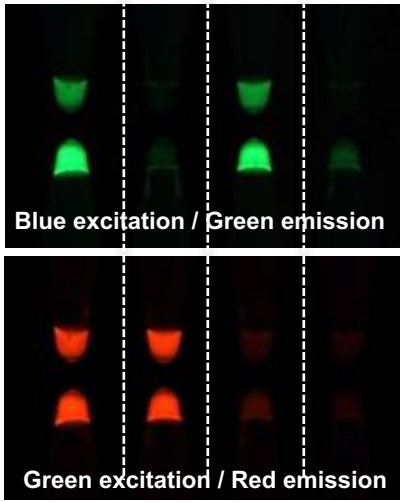
QUASR: Quenching of Unincorporated Amplification Signal Reporters



QUASR yields bright endpoints and reduces false positives

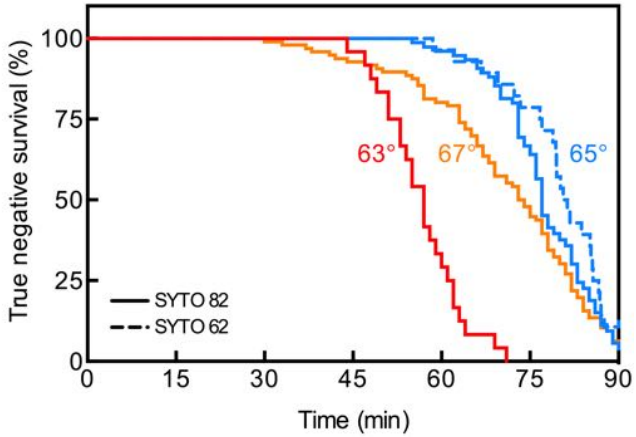
Chikungunya virus + West Nile virus

CHIKV	+	–	+	–
WNV	+	+	–	–



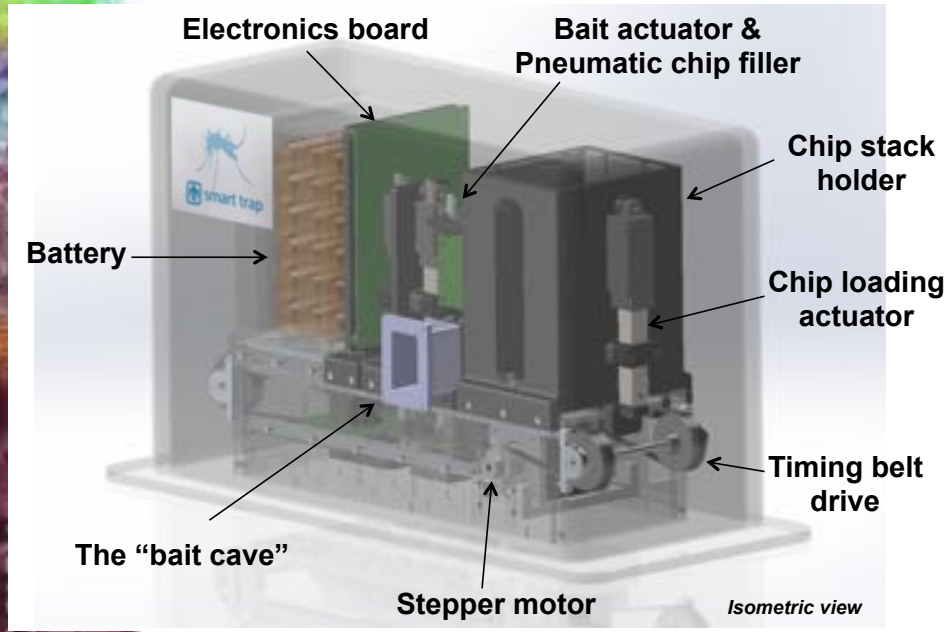
Yooli Light

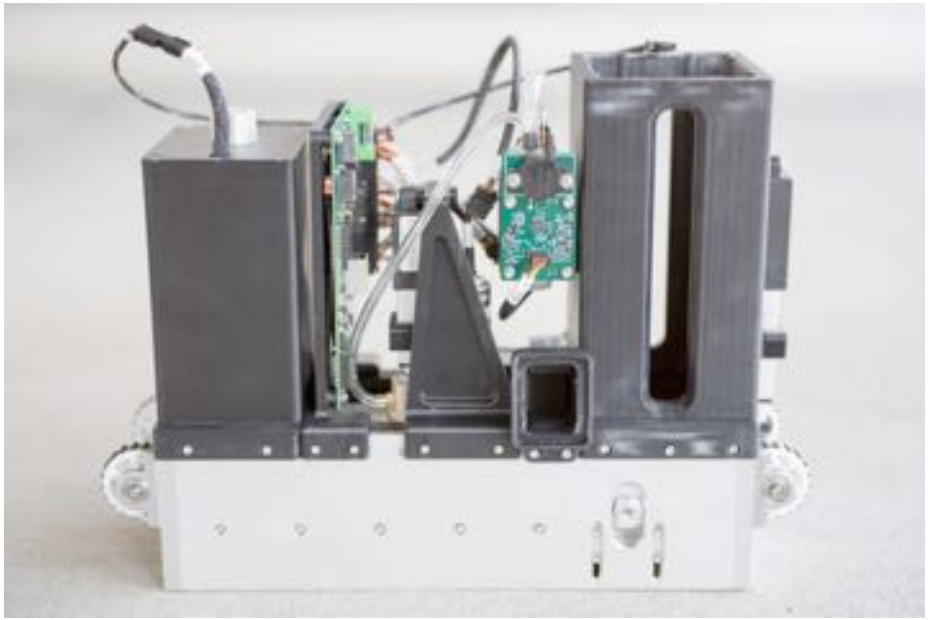
- ☑ **Target specific**
- ☑ **Muliplexable**



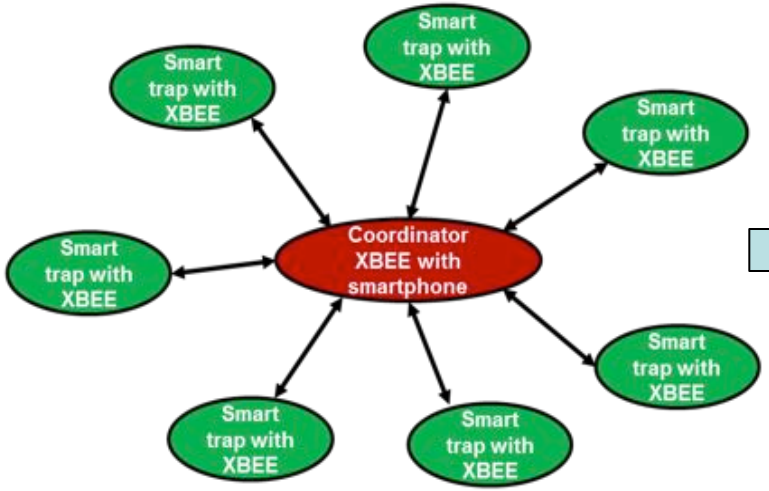
Reporter Mixture	Decision fluorophore	False Positives, X/Y (%)
SYTO only	SYTO 62	25/28 (89%)
SYTO + QUASR (all)	SYTO 62	42/117 (36%)
	QUASR (all)	0/117 (0%)
QUASR only (all)	QUASR (all)	1/80 (1%)*

Smart trap automates assay with small motors





Wireless networking and remote power enable 30 day deployments

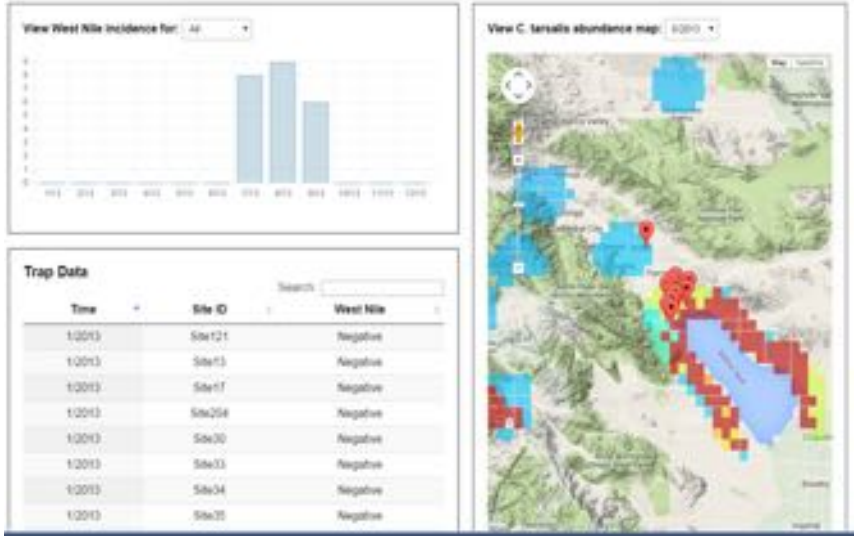


Zigbee star network topology

SMART traps web server and data store running on Amazon AMI
Spring Framework, Java, R

Wireless networking and remote power enable 30 day deployments

SMART traps web server and data store running on Amazon AMI
Spring Framework, Java, R



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

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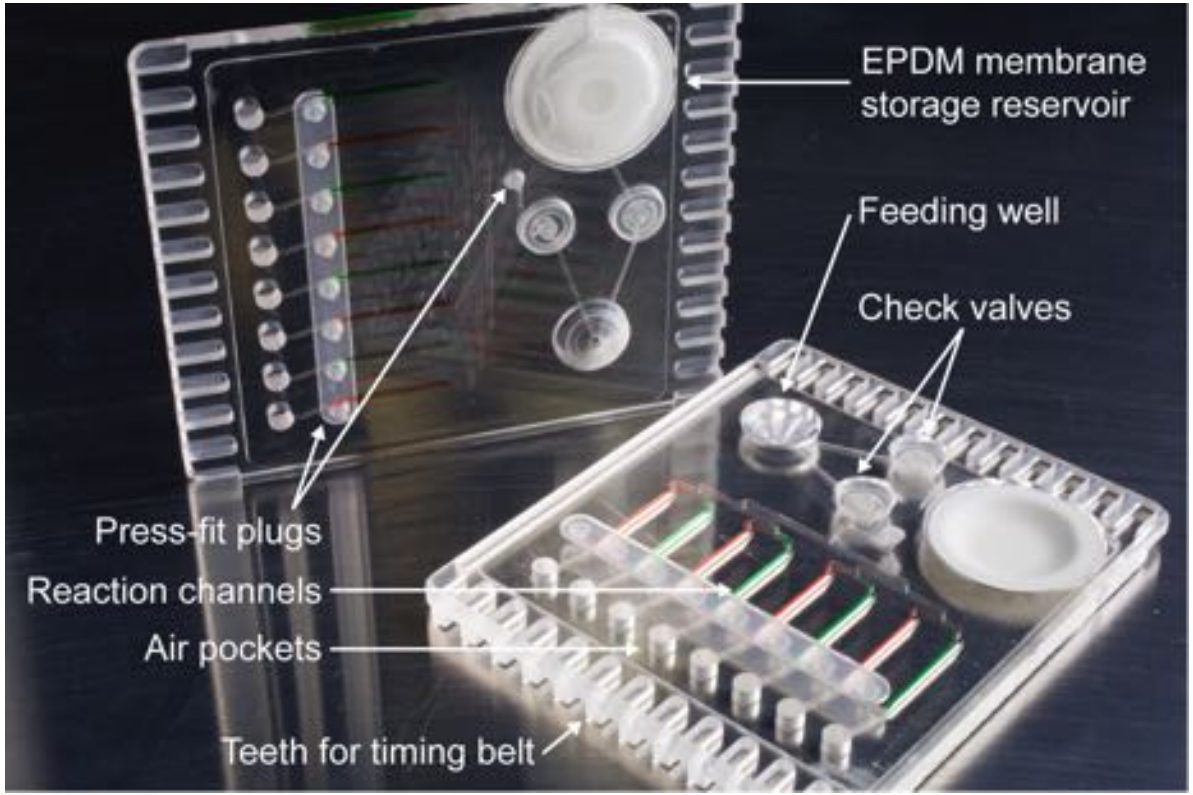
Smart trap function

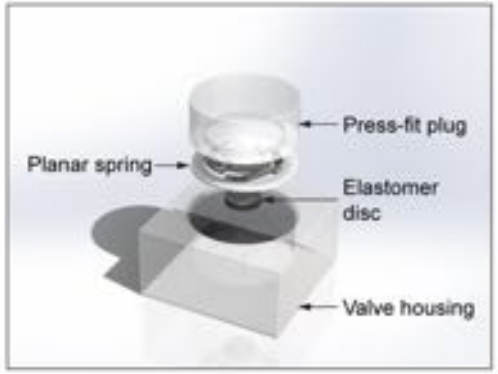
- Video of operation

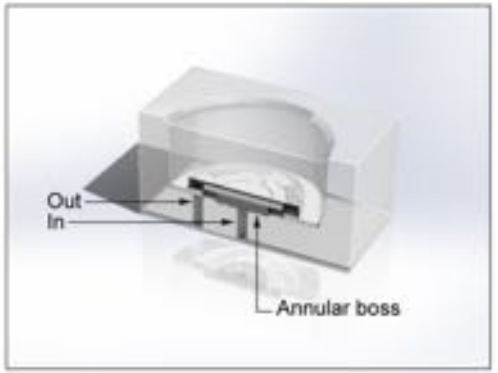
Chip bonding and assembly worked but revealed room for improvement

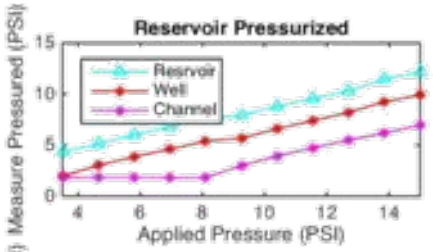


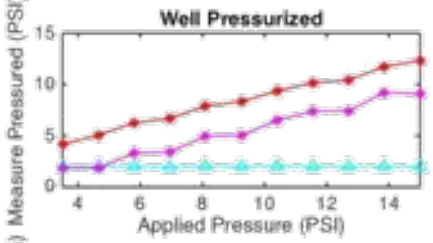
- 300 final chips produced for field testing
- ~10% scrap rate from bonding
- Machining tolerance issues
- Cutting fluid lubricants may impact assay stability
- Switch to injection molding early on and vacuum bond

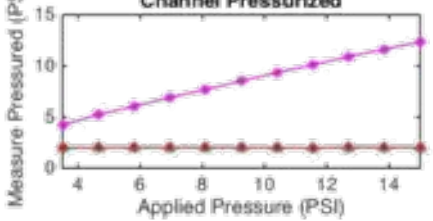
Check valves operated as anticipated, >95% success

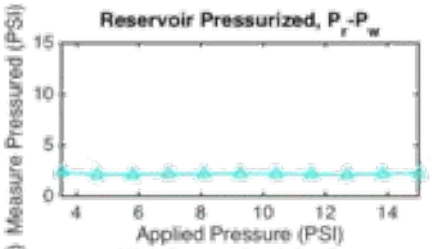


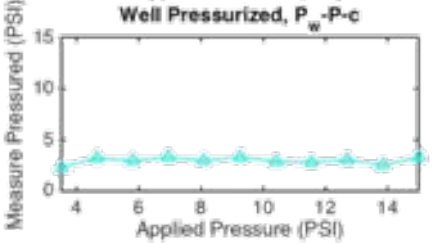


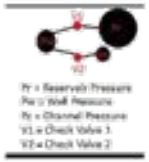




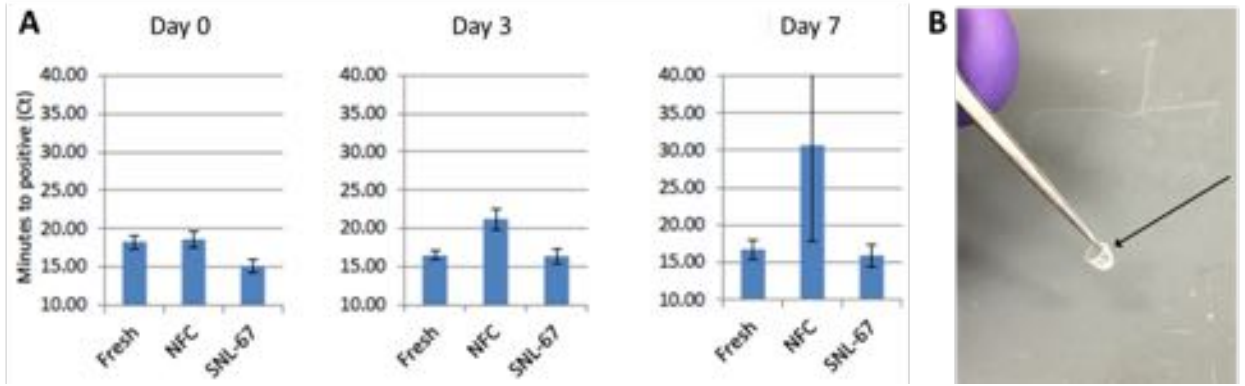








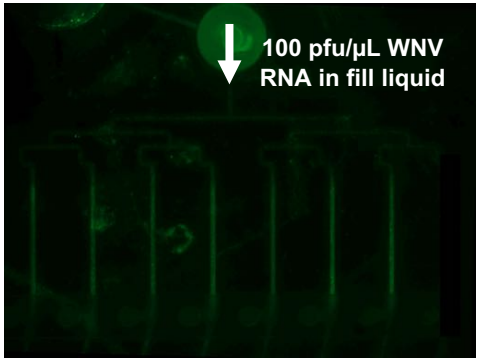
Dried enzyme pellets stabilize assays in ambient (lab) conditions

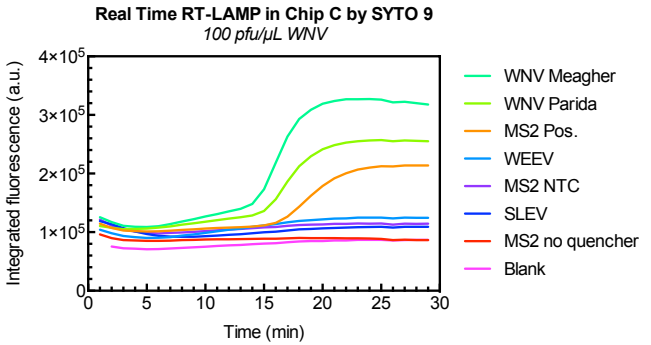


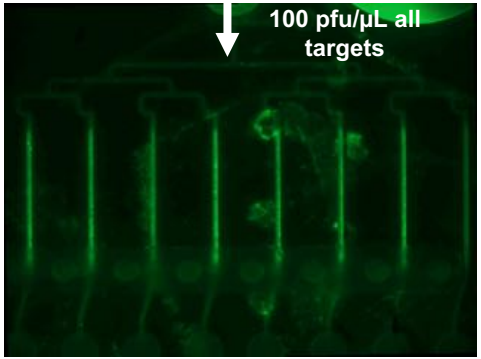
Previous tests demonstrated 7 day stability at 60ºC

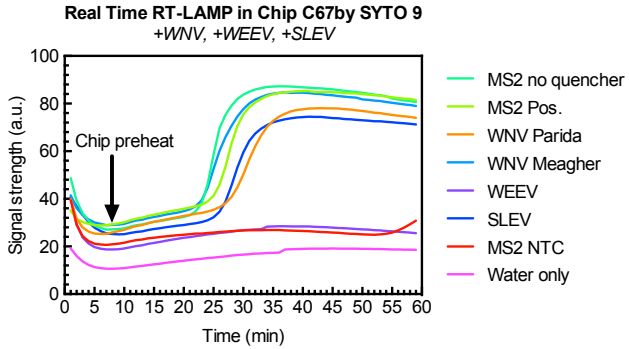
In our field trial, we found loss of activity beyond 1 month, possibly from uncontrolled humidity, cycling temperatures, and leaching from PMMA materials

SYTO 9 shows positive amplification in < 15 min in tubes, < 40 min in chips. Detection limit 160 cp RNA

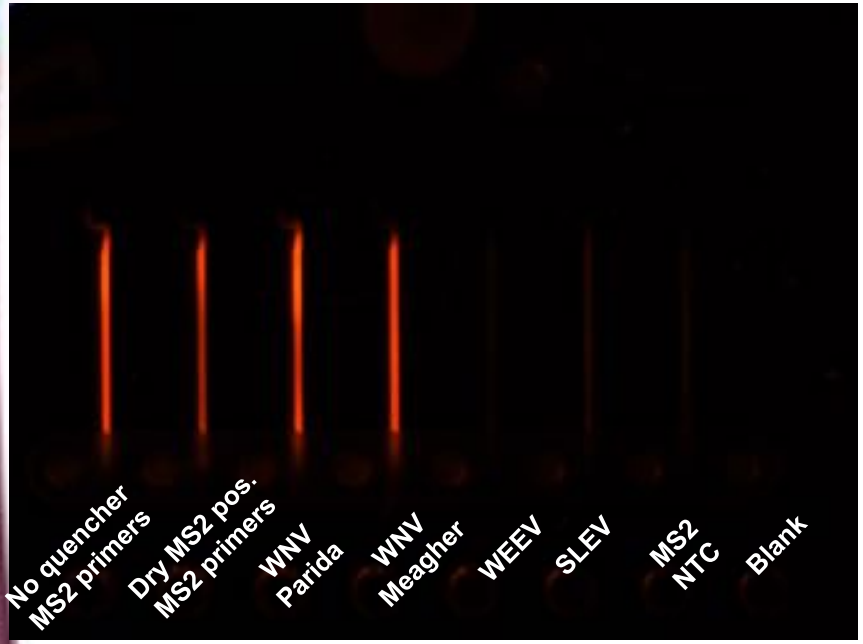


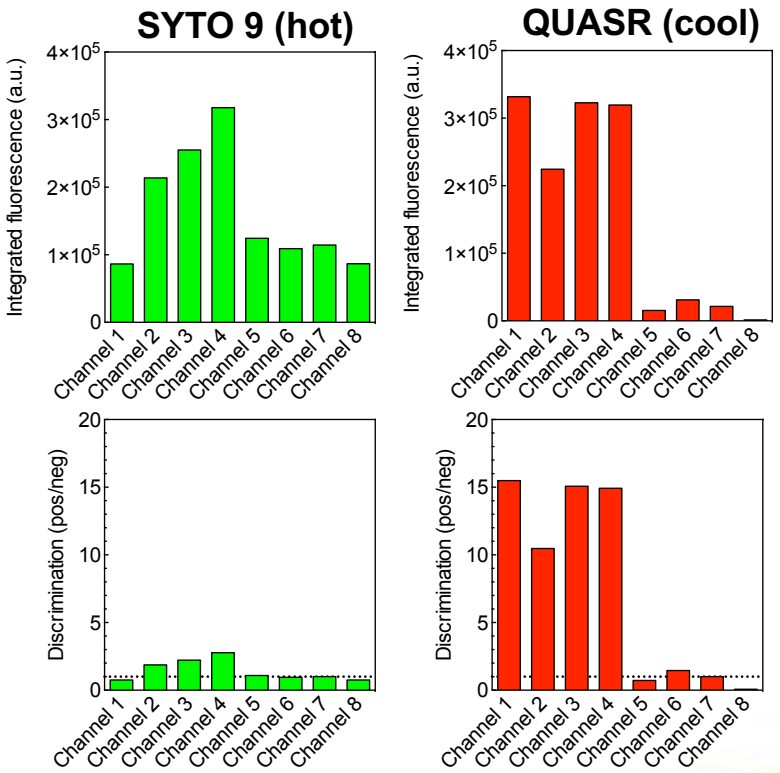






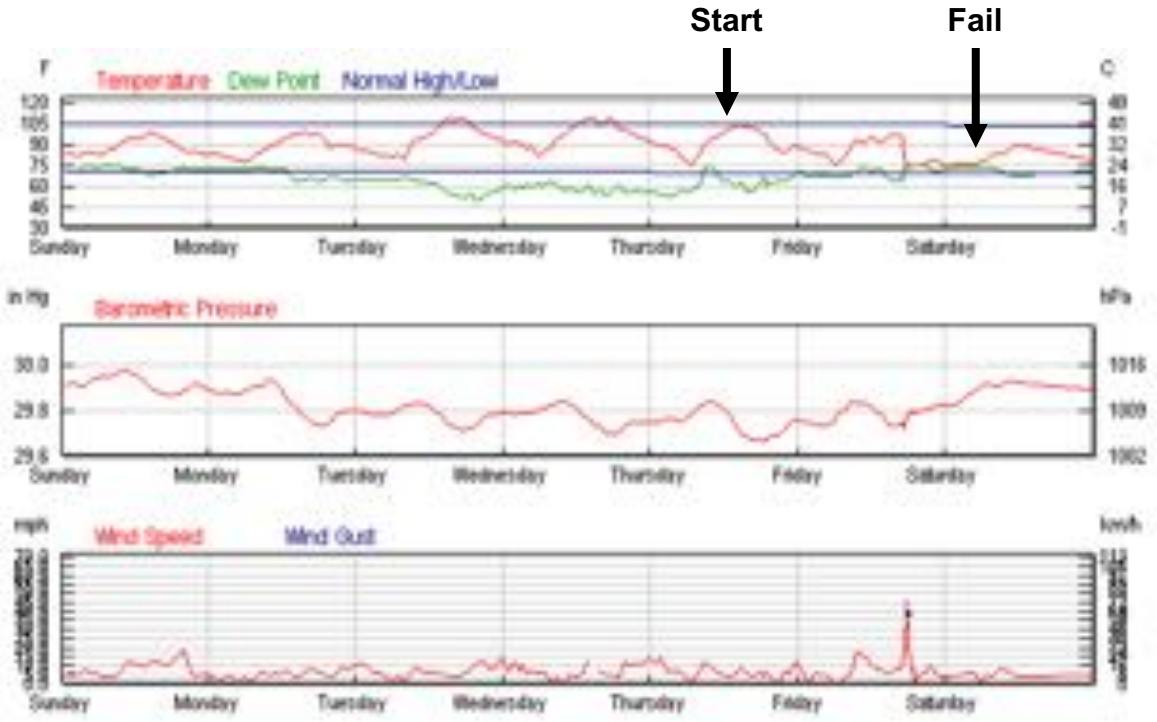
QUASR provides brighter endpoint signals with improved discrimination






9 units deployed in Pelican™ cases
suspended from stakes in soft ground
across 1– 2 sq. miles near Salton Sea for
2 weeks (1 month planned)

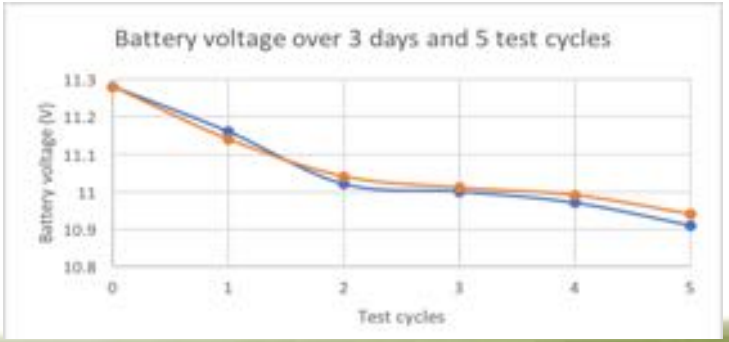
Harsh environmental testing revealed unexpected failure mode



- High temperatures (105ºF)
- Precipitation and high humidity (dew) from Friday evening until late Saturday
- Strong wind (and dust) Friday evening

Smart trap field trial yielded mixed results

- Units ran autonomously in the field, loading chips and moving through the whole process.
  - No positive detections, but conventional traps didn't get hits either
- Communication in a mesh network to coordinator Xbee and iPhone were effective.
- BUT, we lost power during a thunderstorm after Day 2 of the trial. No visible signs of leakage found, but all batteries drained. Failure analysis is ongoing.
- Lab testing predicts that batteries should last over 40 days on a single charge without solar recharging.



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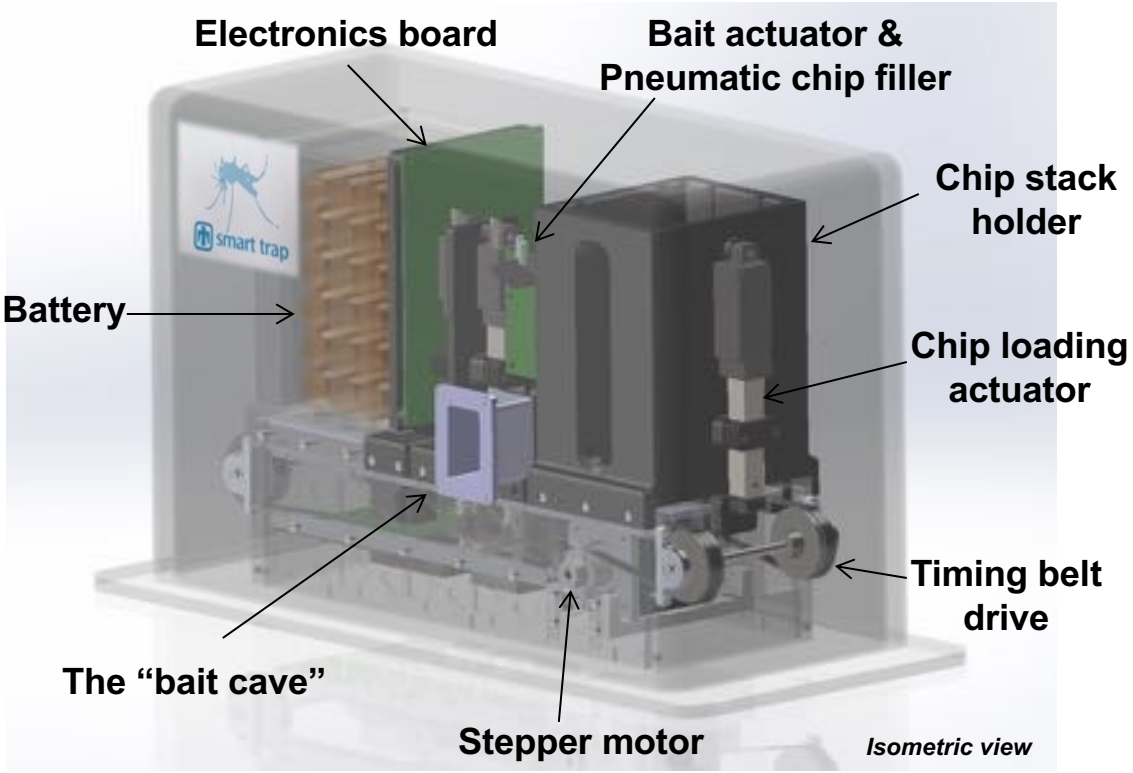
Automated sugar baiting is likely viable, but partial redesign and testing with infected mosquitoes is necessary

- QUASR LAMP is sensitive, robust, and broadly applicable to point of need testing
  - Beyond surveillance, direct testing of pathogens from whole blood (Ebola, plasmodium, hemorrhagic fever viruses)
- Chip redesign to seal enzymes against humidity or stabilize enzyme with humidity-agnostic stabilizers
- Chip prototyping & production by injection molding from the beginning
  - New advances in 3D printing of molds permits rapid prototyping
- Controlled lab studies (we had difficulty breeding infected mosquitoes)
- Potential customers (feedback from Air Force) care most about vector abundance, so addition of a counter or camera onto the device would improve commercialization potential

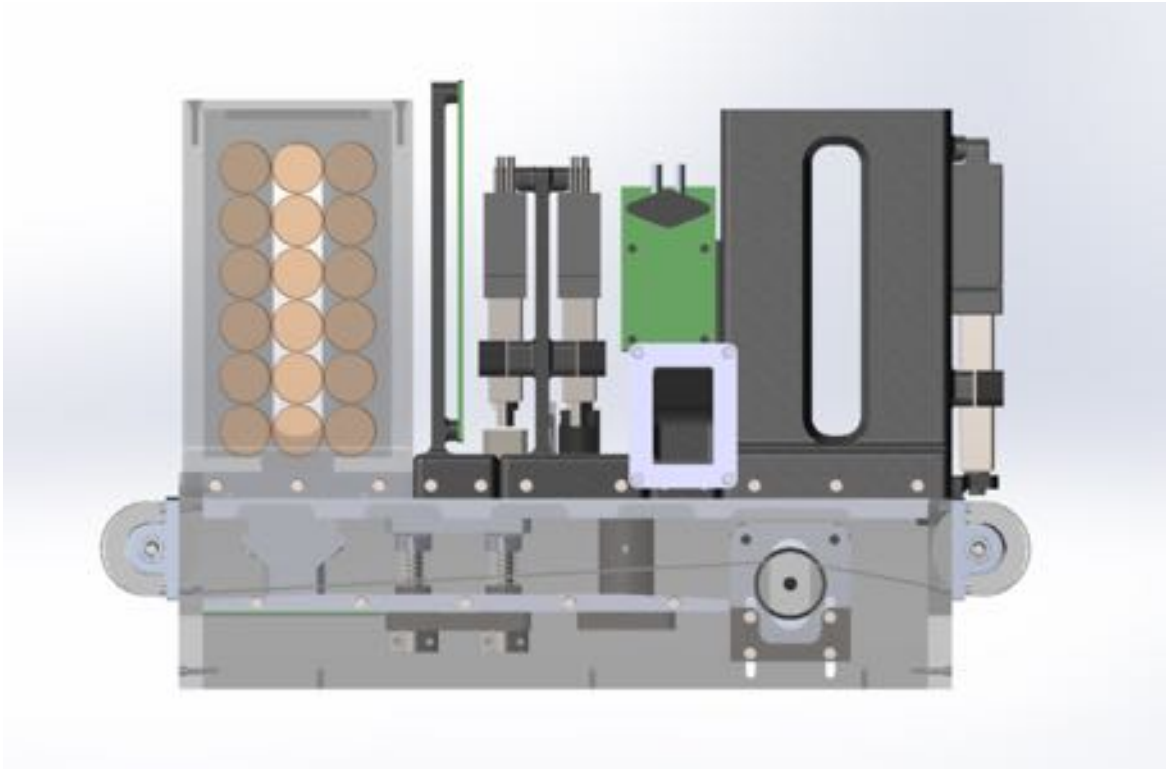
SUPPLEMENTARY SLIDES

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



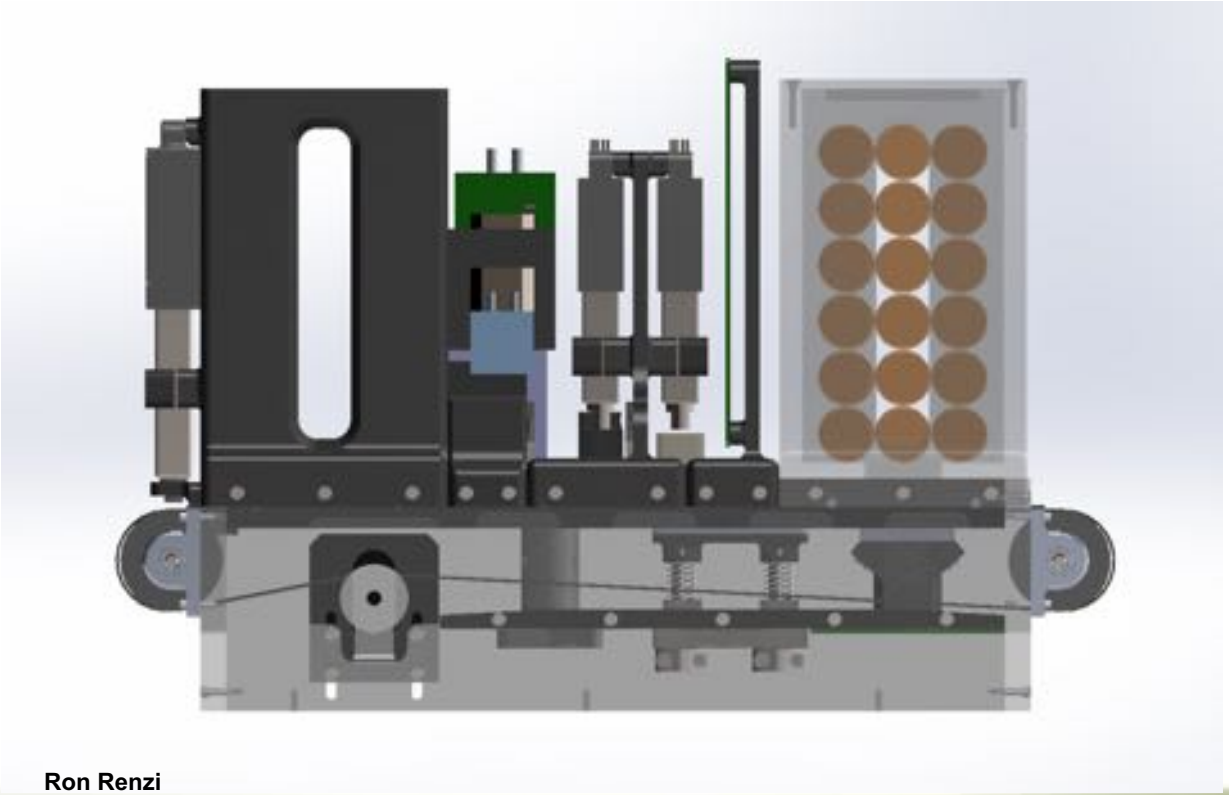
Ron Renzi

Front view



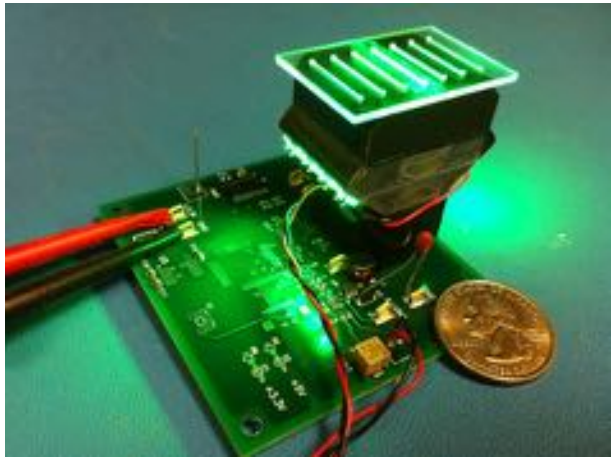
Ron Renzi

Rear view

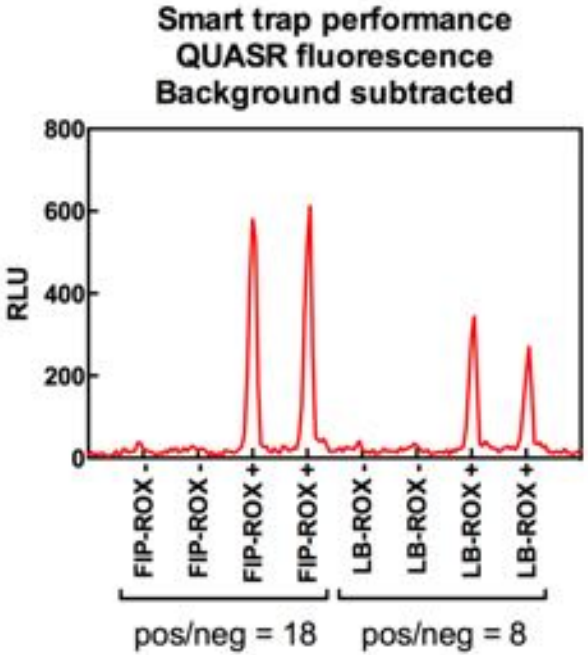


Ron Renzi

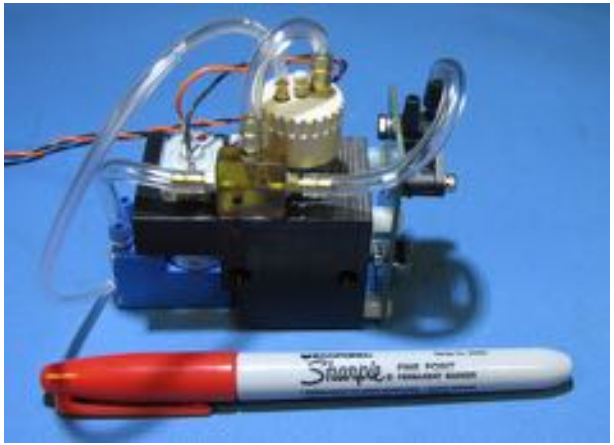
Detector module



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



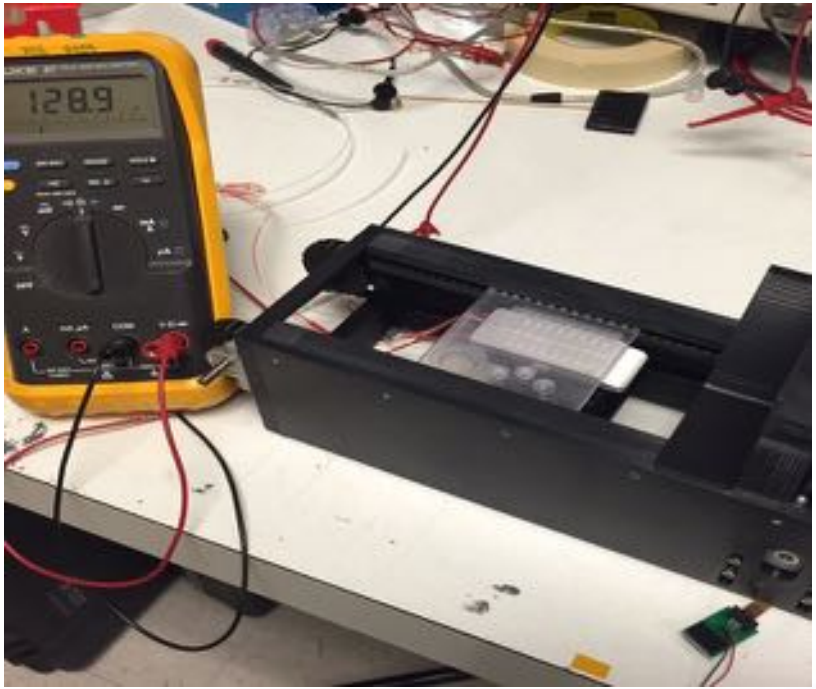
Pneumatic system fills chip



- 24 psig pump from Parker
- Coupled to small volume reservoir with Tygon tubing
- Integrated pressure sensor for system health monitoring

- Actuator-mounted pneumatic head
- Soft O-ring for low pressure seal

Spring-mounted heater controls reaction channel temperature



Networked electronics run trap and communicate with the cloud

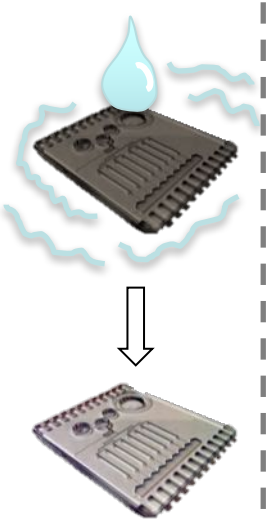
- Modular electronics design
- Onboard system state of health diagnostics
- XBee communication among smart trap neighbors
- Master trap enabled with 4G smartphone
- Data to Amazon web services and DTRA biosurveillance ecosystem

Limit of detection for West Nile was good

Target	LOD_{50}	LOD_{50} 95% confidence	LOD_{90}	LOD_{90} 95% confidence	N total[1]
WNV RNA	160	110 – 220	680	460 – 1300	163

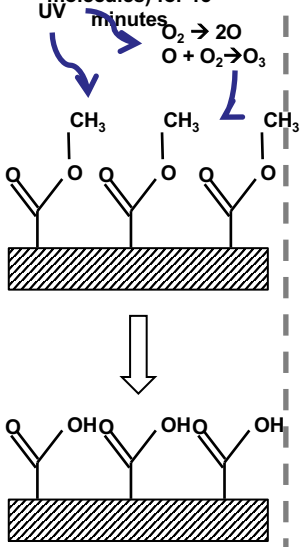
A 5-step thermal bonding process seals the PMMA chips

Chip components rinsed in water and isopropyl alcohol



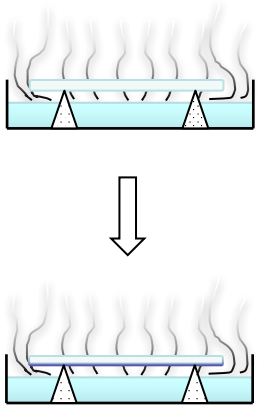
Rids components of gross contamination from lubricants, dirt, and oils

Chip components exposed to UV (185 nm—generates ozone; 254 nm—excites organic molecules) for 15 minutes



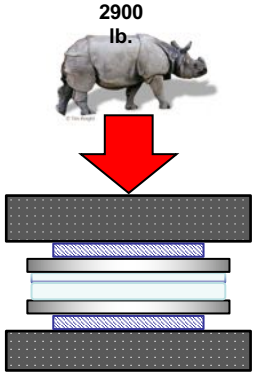
Creates a hydrophilic surface on the PMMA chip to promote bonding and wetting

Thin PMMA piece exposed to chloroform vapor at 20° C for 10 minutes



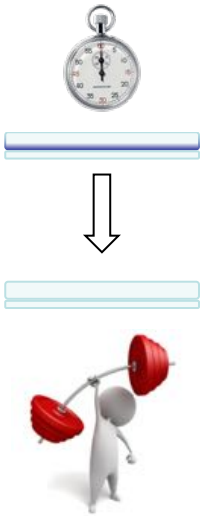
Swells the surface PMMA, which becomes tacky and improves bond strength

Chip components bonded at 70° C for 5 min 'kiss', 10 min @ 2900 lb., cooled under pressure to 30° C



Bonds the 0.2 mm PMMA backing to the machined PMMA chip with heat and pressure

Chips cured for 24h at 60° C



Strengthens the bond and removes chloroform