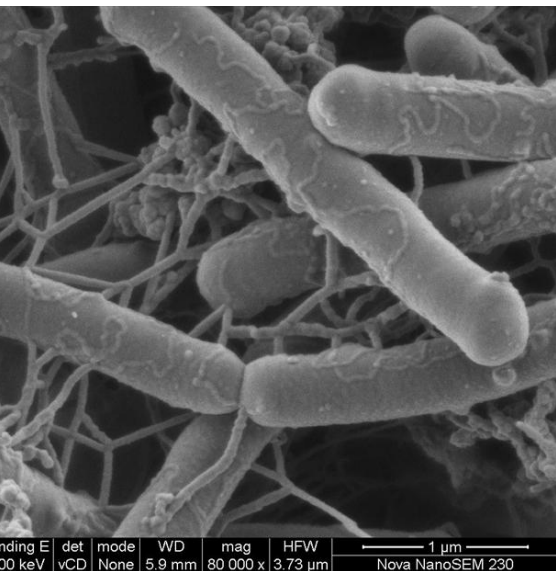


Innovation in rapid microbiology diagnosis. A step forward for food safety

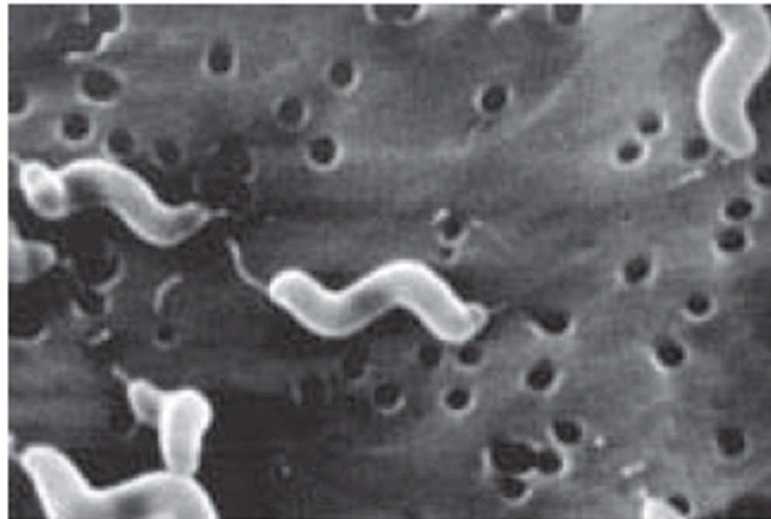
Javier Atencia, NIST Guest Researcher
Research Faculty at UMD, USA

May 9, 2019

Salmonella and Campylobacter: 410,000 antibiotic-resistant infections in USA/year



<https://www.fei.com/>



<https://www.sigmaaldrich.com/technical-documents/articles/analytix/on-the-trail-of-campylobacter.html>

Top 5 resistant infections from germs in food and animals

ANTIBIOTIC RESISTANCE
from the farm to the table

RESISTANCE Animals can carry harmful **bacteria** in their intestines

When **antibiotics** are given to animals...
Antibiotics kill most bacteria
But resistant bacteria can survive and multiply

SPREAD Resistant bacteria can spread to...

- contaminated animal products
- produce through contaminated water or soil
- prepared food through contaminated surfaces
- the environment when animals poop

EXPOSURE People can get sick with resistant infections from...

- contaminated food
- contaminated environment

Learn 4 steps to prevent food poisoning at www.foodsafety.gov

IMPACT Some resistant infections cause...

- illness
- severe illness and may lead to death

About **1 in 5** resistant infections are caused by germs from food and animals.
Source: *Antibiotic Resistant Threats in the United States, 2013*

Learn more about antibiotic resistance and food safety at www.cdc.gov/foodsafety/antibiotic-resistance.html

<https://www.cdc.gov/foodsafety/challenges/from-farm-to-table.html>

A nightmare in the food industry



Chipotle lost \$8 B in 6 mos.



*1,000 outbreaks/year in US,
generating \$55B losses*



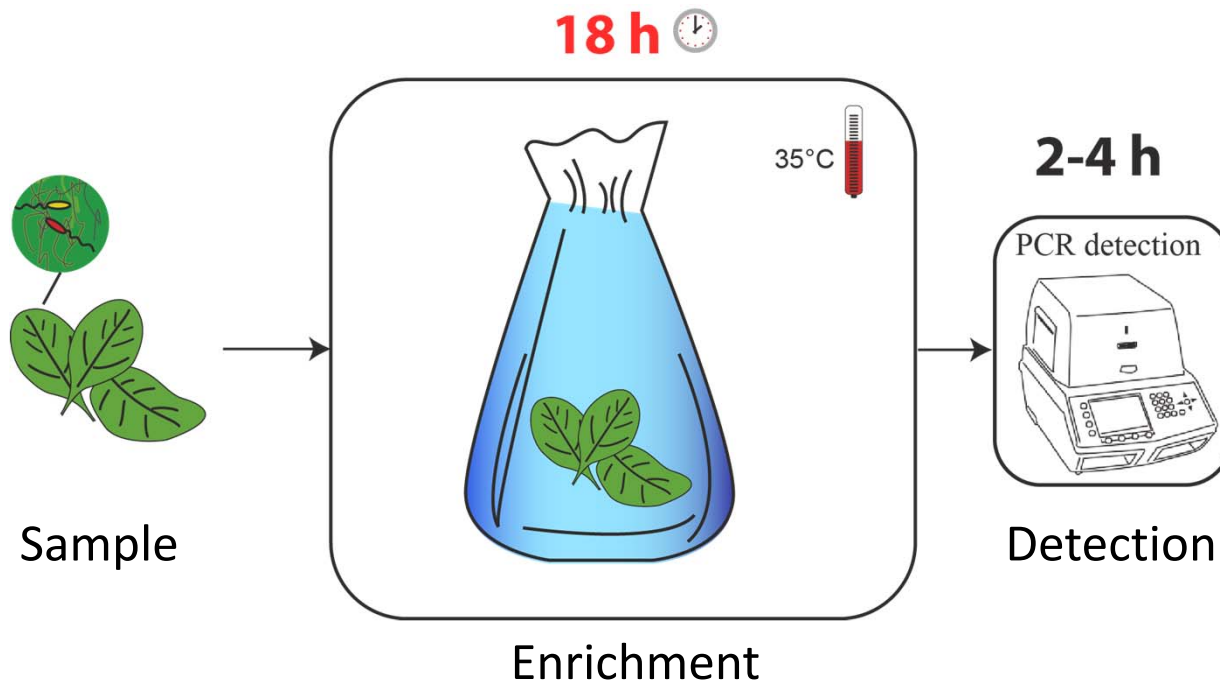
*Lettuces are tested
within 4 days before harvest*

Problem

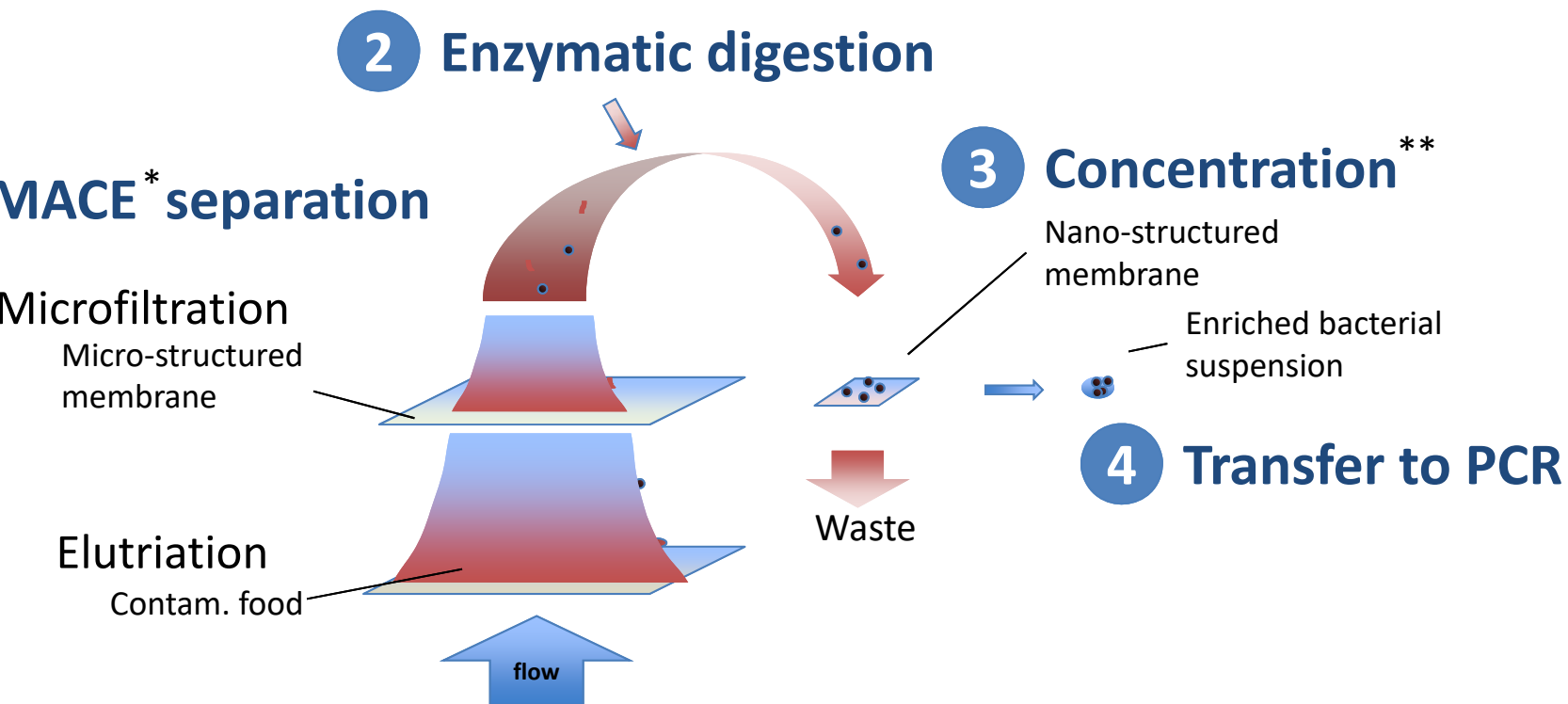
...the 'rapid' safety tests are too
slow: 1-3 days ...

...companies are compelled to
balance profit against food-safety

Bottle neck: Enrichment



Next Generation Enrichment



Our solution



Our solution



E. coli in 325g ground beef



inoculation:
E. coli O157H7 (20ul)
10⁸ CFU

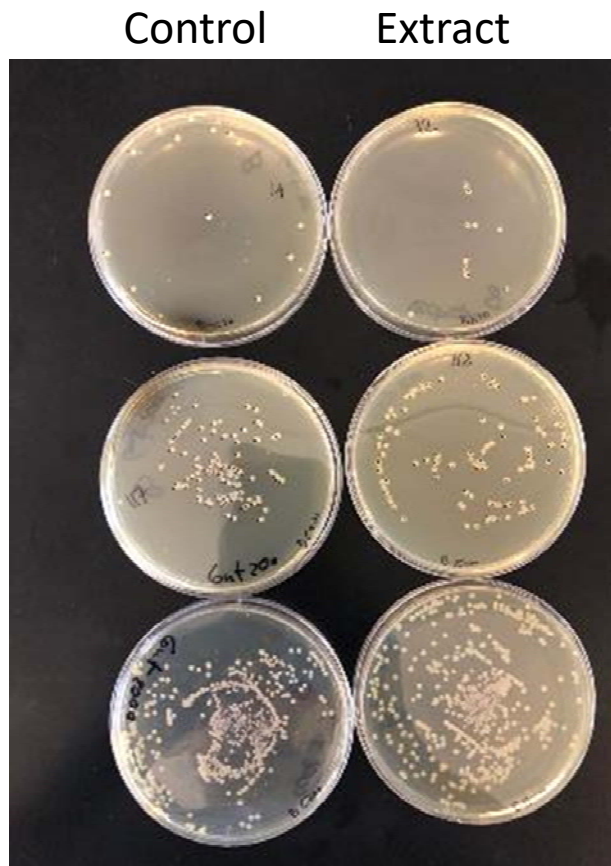


FIG. A

Efficiency 95.7%

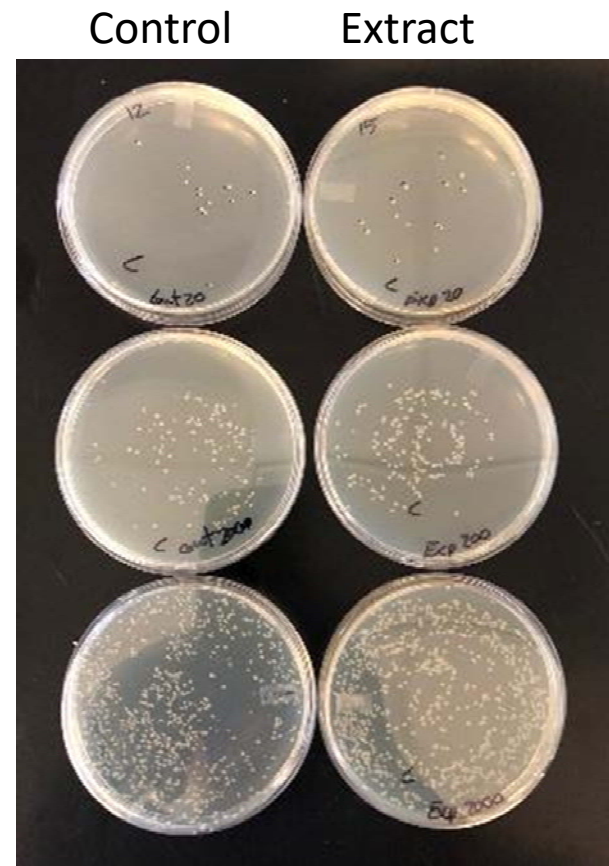


FIG. B

Efficiency 80%

Salmonella in 325g ground beef



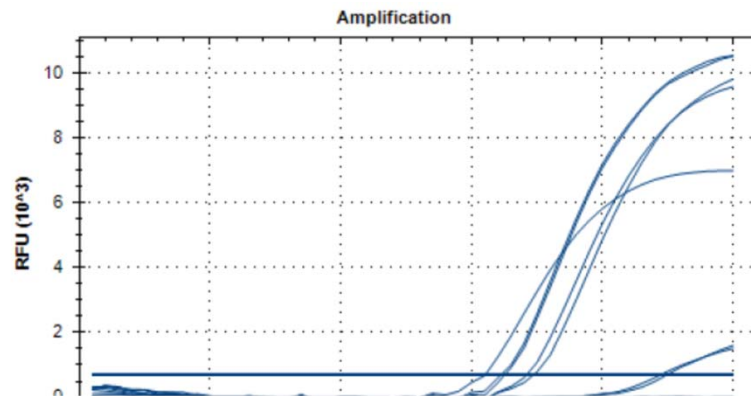
	1	2	3	4	5	6	7	8	9	10	11	12	
A													A
B		ext Positive	ext Positive	-3 5ul Positive	-3 Positive	-4 Positive	-4 Positive	Pos Ctrl Valid Ctrl	Neg Ctrl Valid Ctrl				B
C													C
D													D
E													E
F													F
G													G
H													H

-3: (control, pure culture) 5ul of 268+-31 CFU

-4: (control, pure culture) 5ul of 26.8+-3.1 CFU

ext: inoculation of 20 ul with 1075+-129 CFU in 325g. Extracted 10 ul, run 2 x 5ul PCR

Sample Id	Cq Target	Cq Internal Control	Result
ext	44.58	N/A	Positive
ext	45.04	N/A	Positive
-3 5ul	32.57	31.41	Positive
-3	32.29	31.31	Positive
-4	34.79	31.8	Positive
-4	34.24	31.69	Positive
Pos Ctrl	31.08	31.94	Valid Ctrl



Salmonella in 25g Lettuce



	1	2	3	4	5	6	7	8	9	10	11	12	
A													A
B													B
C		blank Negative	-5 Positive	-4 Positive	-3 Positive	Pos Ctrl Valid Ctrl	Neg Ctrl Valid Ctrl						C
D													D
E													E
F													F
G													G
H													H

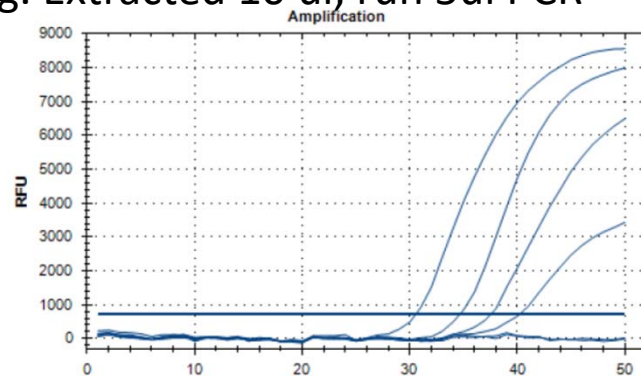
Blank: 25g lettuce without inoculation. Extracted 10 ul, run 5 ul PCR

Inoculation of 20 ul with 16.0+-1.8 CFU in 25g. Extracted 10 ul, run 5ul PCR

Inoculation of 20 ul with 160.0+-17.9 CFU in 25g. Extracted 10 ul, run 5ul PCR

Inoculation of 20 ul with 1600+-178.9 CFU in 25g. Extracted 10 ul, run 5ul PCR

Sample Id	Cq Target	Cq Internal Control	Result
Blank	N/A	32.45	Negative
5	37.48	32.87	Positive
4	40.16	32.92	Positive
3	34.61	32.43	Positive
Pos Ctrl	30.51	31.61	Valid Ctrl
Neg Ctrl	N/A	32.08	Valid Ctrl



E. coli in 25g Lettuce

	1	2	3	4	5	6	7	8	9	10	11	12	
A													A
B			blank Negative	pos cont Positive	neg cont Negative								B
C			-3 Positive	-4 Positive	-5 Positive	Pos Ctrl Valid Ctrl	Neg Ctrl Valid Ctrl						C
D													D
E													E
F													F
G													G
H													H

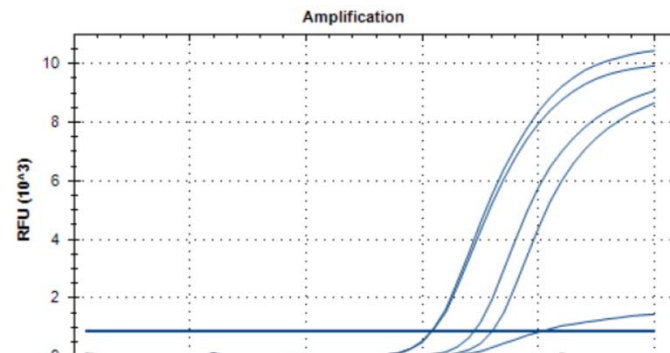
Blank: 25g lettuce without inoculation. Extracted 10 ul, run 5 ul PCR

-3: inoculation of 20 ul with 7.0+-1.7 CFU in 25g. Extracted 10 ul, run 5ul PCR

-4: inoculation of 20 ul with 70.0+-16.7 CFU in 25g. Extracted 10 ul, run 5ul PCR

-5: inoculation of 20 ul with 700+-167 CFU in 25g. Extracted 10 ul, run 5ul PCR

Well	Sample Id	Cq Target	Cq Internal Control	Result
3	blank	N/A	32.27	Negative
3	-3	34.38	34.6	Positive
4	pos cont	30.77	32.3	Positive
4	-4	40.1	35.27	Positive
5	neg cont	N/A	32.86	Negative
5	-5	35.95	34.58	Positive
6	Pos Ctrl	30.69	32.39	Valid Ctrl
7	Neg Ctrl	N/A	33.38	Valid Ctrl



Solution

The logo for pathtrak is displayed within a white circular frame. The word "path" is in a green, sans-serif font, and "trak" is in a black, sans-serif font. A stylized, orange-red line graphic starts above the "p", loops around the "h", and continues as a wavy line below the "k".

pathtrak

Next Generation Food Safety Tests: results in 4 h

Solution

patho  trak



PathoTrak's disposable NGE Kits reduce pathogen detection time in food from 1-3 days to 4 hours

Acknowledgements

NIST - Gregory Cooksey, Laurie Locascio, Jayne Morrow,
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USDA ARS – Jeffrie Karns, Dan Shelton

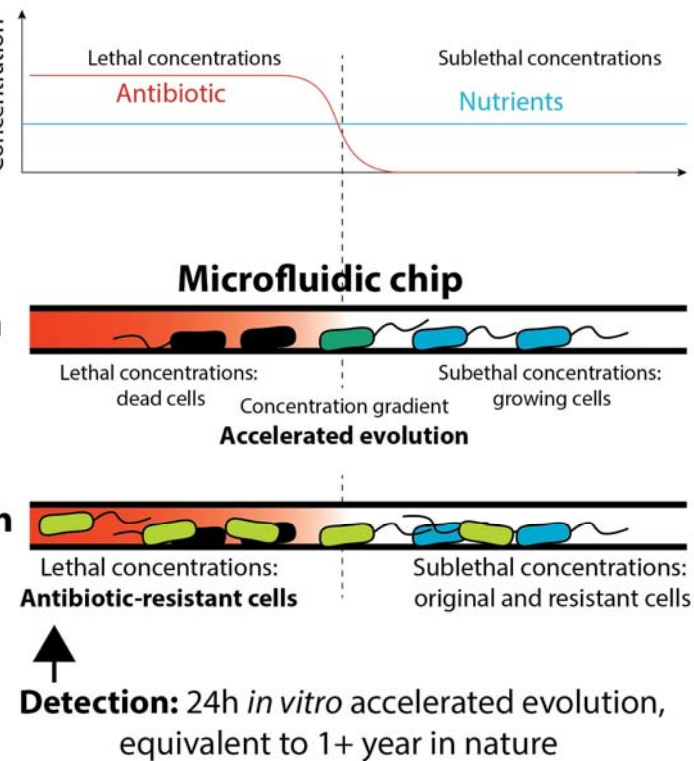
UMD – Robert Buchanan, Akbar Dawood

Funding

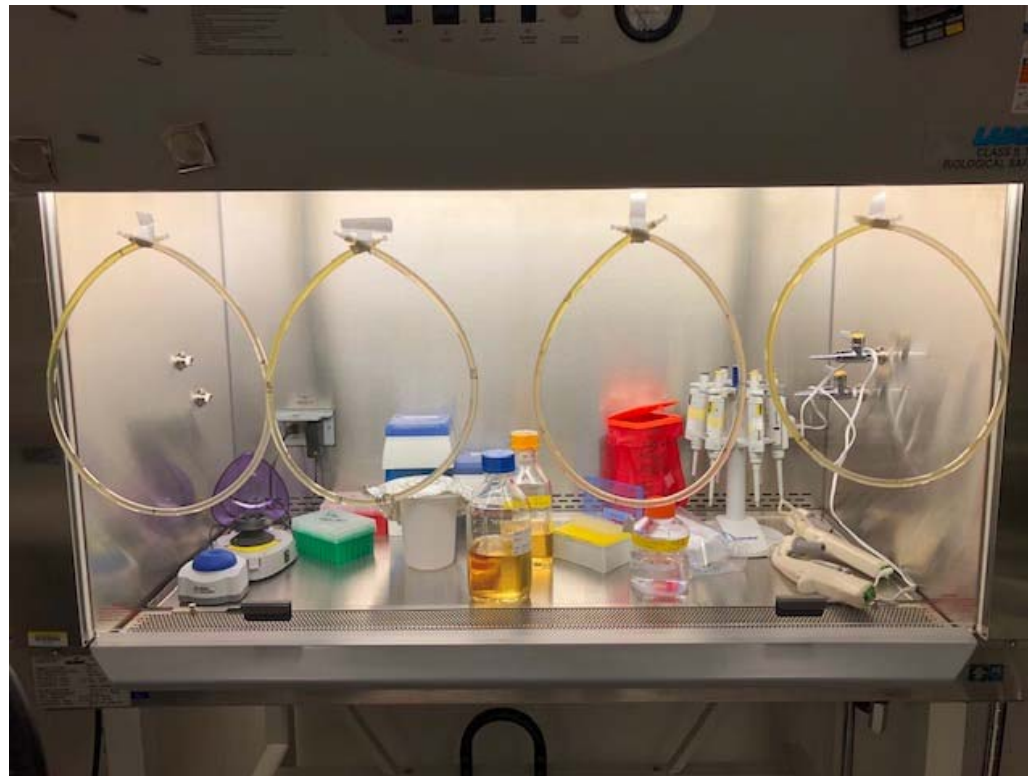
- MML-NIST
- Office of Special Programs-NIST
- TEDCO MD,
- DC I-corps - NSF

Javier Atencia, jatencia@nist.gov

In vitro tools to predict resistance



In vitro tools to predict resistance



In vitro tools to predict resistance



In vitro tools to predict resistance



In vitro tools to incentivize industry

al strains were fully
sensitive to cipro activity (black
lines)

Samples extracted from high cipro
environments (purple compared to
green) had higher MICs.

Mutagenic strain rapidly became
cipro resistant (right half compared
to left half)

E. coli strain MG1655

mutS
MG1655 Reference
E. coli Strain

MG1655 mutS
Knockout Strain

mutagenic strain. Deleted
mutS protein, results in
rapid increase in mutation

