

Why Does A Better Pathogen Test Matter?



*Your Food Safety Decisions are Only as Good as
the Data Behind Them*

Test Results Drive your Food Safety Decisions



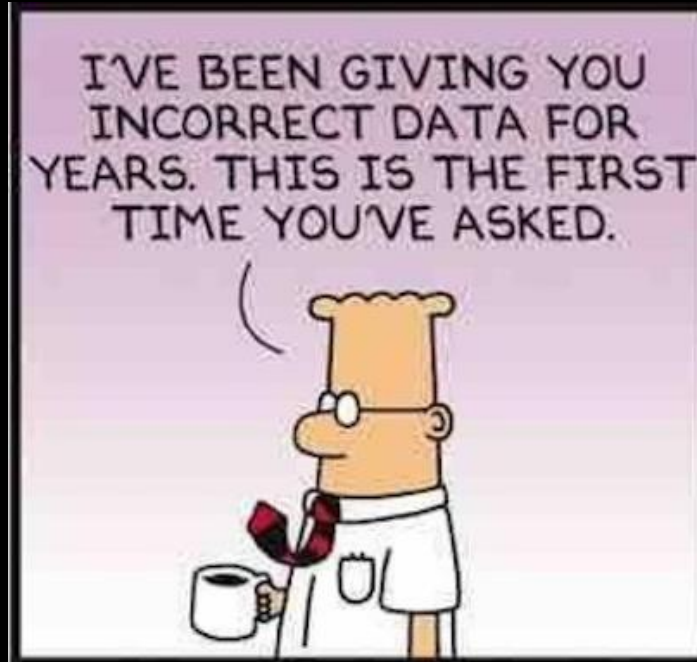
- ✓ Raw Material Acceptance
- ✓ Finished Product Release
- ✓ Process Control Monitoring
- ✓ Sanitation Decisions
- ✓ Intervention Validation / Verification

Inaccurate Test Results expose
your brand & consumers to
unnecessary risk



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How Often Do You Question Your Pathogen Test Results?



Accurate Testing is Needed to Protect your Brand



- **FALSE POSITIVES**
 - Excess cost and unnecessary disruption to operations
- **FALSE NEGATIVES**
 - Risk of recall
 - Lack of knowledge of effectiveness of food safety programs
- **INVALIDS**
 - Loss of shelf life due to retest time

The New Regulatory & Legal Landscape

FDA's role – from Inspector to Investigator

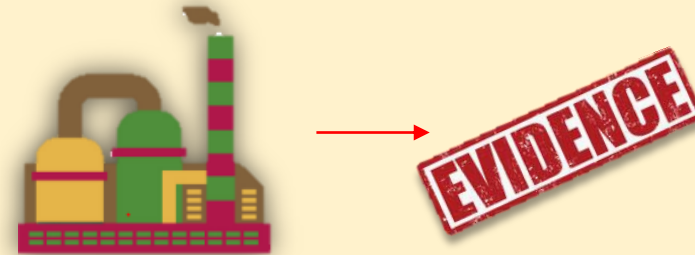
- “Swab-a-thon” sessions
- Product testing at retail level
- Stronger compliance & enforcement power
- Review of HARPC Plan & Test Results



Outbreak Surveillance

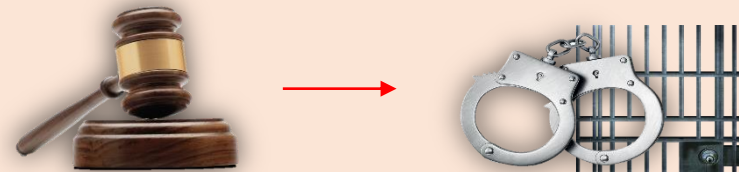
Genome Trakr Database - link outbreak to source

- Whole Genome Sequencing (WGS)
- CDC PulseNet
- All positives found by FDA now sequenced



Criminalization of Foodborne Illness

- Department of Justice Involvement
- Prosecution and conviction
- **Ignorance is not a viable defense**



Evolution of the Pathogen Test

Pathogen testing technology differences and improvements over time...



Cultural

Since 1880's

Target: **Colonies on plate**

Result: **5 days**

Result requires **interpretation**

Very labor intensive



Immunoassay

(ELFA/ELISA) 1980's

Target: **Proteins** on cell

Results: **2-3 days**

Detection level: **1,000,000 cells**

Cheap and simple

Poor accuracy (false positive/negative results)



PCR

1990 -2000's

Target: **DNA** (only 1 copy/cell)

Result: **1 - 2 days**

Detection level: **10,000 cells**

Regulatory method

Poor performance with many food types (no sample purification)

Complicated, error-prone



Roka – Atlas

Since 2009

Target: **RNA** (thousands of copies/cell)

Result: **12 – 27 Hours**

Detection Level: **100 cells**

Superior accuracy regardless of food type (onboard sample purification)

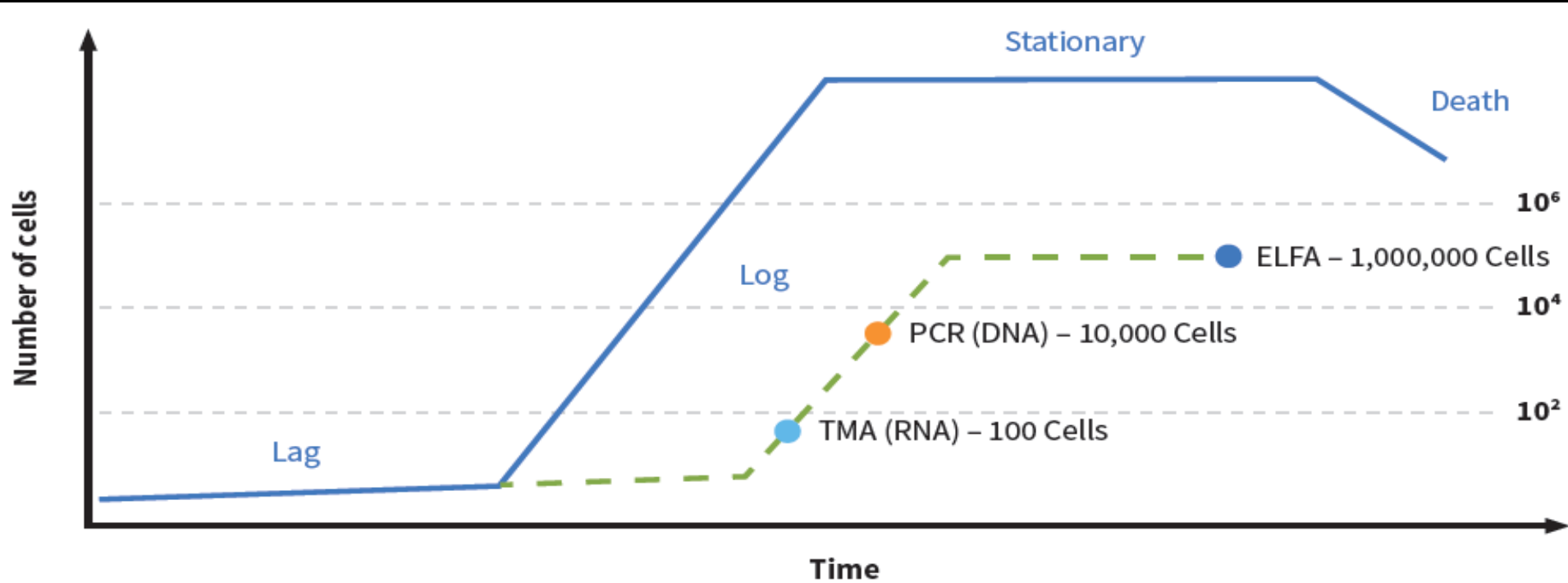
Full automation & traceability



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Pathogen Detection Methods are not Created Equal

If you truly want to find it: You need to be using a sensitive detection method



A detection method with a low Limit of Detection will overcome performance challenges created by routine intervention and the processing environment as well as deliver faster Time to Results.

Important Factors to Consider for Your Test Method

Validation

- Sample Size
- Enrichment Media
- Enrichment Time
- Sample to media ratio

Time to Result

- Meet operational needs
- Release product sooner
- Minimize food safety risk

Accuracy

- Detect true positives
- Minimize false positives
- Reduce invalids
- Minimize operator interaction

Case Study One: Environmental Testing

Atlas LSP vs. VIDAS LIS

| Roka Atlas LSP | | | | | ELFA LIS | | | | |
|----------------------|---------------|------------------|----------------|----------------|----------------------|---------------|------------------|----------------|----------------|
| Week # | Total Samples | Culture Positive | Atlas Positive | False Negative | Week # | Total Samples | Culture Positive | VIDAS Positive | False Negative |
| 1 | 35 | 10 | 11 | 0 | 1 | 35 | 10 | 7 | 4 |
| 2 | 35 | 11 | 11 | 0 | 2 | 35 | 10 | 7 | 3 |
| 3 | 35 | 10 | 9 | 1 | 3 | 35 | 10 | 3 | 7 |
| 4 | 35 | 15 | 13 | 2 | 4 | 35 | 15 | 6 | 9 |
| 5 | 35 | 7 | 7 | 0 | 5 | 35 | 6 | 2 | 4 |
| 6 | 35 | 15 | 15 | 0 | 6 | 35 | 15 | 8 | 7 |
| Total | 210 | 68 | 66 | 3 | Total | 210 | 66 | 33 | 34 |
| False Negative Rate* | | | | 4.4% | False Negative Rate* | | | | 51.5% |

Example of Sample Points: compactor drains, cooler drains, room drains, wet room drains, fork trucks, various containers

*Each method had one false positive during the study. Both in week 1.

*False Negative Rate = $\frac{\sum \text{False Negative}}{\sum \text{Culture Positive}}$

- Intentionally sampled challenging sites; Drains, Fork Lifts, etc.
- Samples taken from a higher positivity environment for 6 weeks
- **>50% False Negative Results on Vidas® System vs. 4.4% on Atlas**

- Preventative control plans need to be built on accurate data
- Negative results are almost never confirmed by plating
- Hidden Risk = Real Risk
- In today's regulatory landscape, a single missed positive could result in a significant financial and judicial impact

Listeria spp. Culture Confirmation Challenge Study

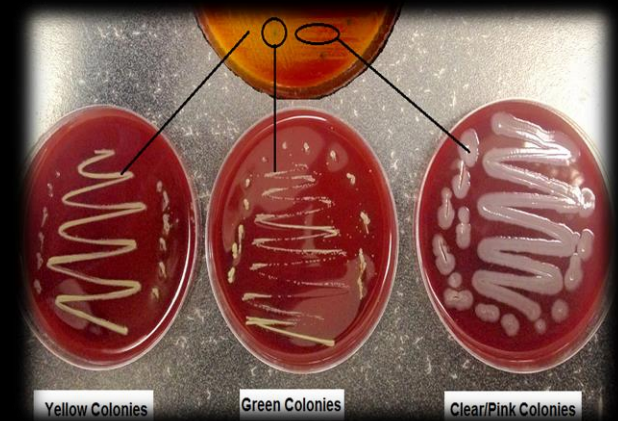
| | Total Samples Run | # Screen Positive by Atlas | Traditional Culture | | Extended Confirmation | |
|---------------|-------------------|----------------------------|---------------------|-------------------|-----------------------|-------------------|
| | | | # Confirmed | Confirmation Rate | # Confirmed | Confirmation Rate |
| Assay Round 1 | 1369 | 81 | 73 | 90.12% | 79 | 97.53% |
| Assay Round 2 | 1369 | 84 | 71 | 84.52% | 79 | 94.05% |

- Metrics To Date:

- 1,369 environmental swabs (2,738 Roka assays)
 - 14 facilities, representing 8 industry segments/production environments
 - HiCap Neutralizing Broth Sponge Swabs – WorldBioproducts
 - FoodChek Actero Listeria Enrichment Media
 - Atlas LE Assay with two analytical volumes

- Extended Confirmation Findings:

- Concentration improves recovery of low level Listeria
- Extended enrichment >48hrs improves recovery of Listeria
- Micro ID may fail to detect true Listeria spp. isolates including Lm
- Facility flora and sanitizers can influence sample flora in the bag and ability to culture
- Most labs are unlikely to run extended culture protocols



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Case Study Two: Salmonella in Processed Egg Products

Atlas vs. BAX

| Salmonella Recovery Testing | | Roka Atlas | | | PCR | | |
|-----------------------------|---------------|--|----------------|----------------|--|--------------|----------------|
| Matrix | Total Samples | Culture Positive | Atlas Positive | False Negative | Culture Positive | PCR Positive | False Negative |
| Dried Egg White | 20 | 10 | 10 | 0 | 10 | 1 | 9 |
| Dried Egg Yolk | 20 | 10 | 10 | 0 | 10 | 8 | 2 |
| Frozen Salted Egg Yolk | 20 | 10 | 10 | 0 | 10 | 10 | 0 |
| Frozen Liquid Sugar Yolk | 20 | 10 | 10 | 0 | 10 | 10 | 0 |
| Frozen Liquid Whole Eggs | 20 | 10 | 10 | 0 | 10 | 10 | 0 |
| Liquid Egg Whites | 20 | 8 | 8 | 0 | 8 | 3 | 5 |
| Total | 120 | 58 | 58 | 0 | 58 | 42 | 16 |
| | | False Negative Rate[†] | | | False Negative Rate[†] | | |
| | | 0% | | | 27.6% | | |

[†]False Negative Rate = $\frac{\sum \text{False Negative}}{\sum \text{Culture Positive}}$

- 10 non-inoculated and 10 inoculated samples run per matrix
- Inoculated samples spiked at a high level 20 CFU/sample
- **27.6% False Negative Results on BAX PCR**
- **Atlas had 100% agreement with culture**

- Product verification testing requires accuracy
- Challenging matrices can inhibit test methods without sample clean up
- False Negatives = Product Risk
- In today's regulatory landscape, a single missed positive could result in a significant financial and judicial impact

Case Study Three: Atlas vs. PCR in Diverse Produce Matrices

| Roka Atlas | | | | | |
|-----------------|------------------|------------------|----------------|----------------|---------------|
| Assay | Culture Positive | Culture Negative | False Positive | False Negative | Indeterminate |
| E. coli O157:H7 | 53 | 67 | 0 | 0 | 0 |
| Salmonella | 66 | 54 | 0 | 0 | 0 |
| STEC / EHEC | 78 | 66 | 0 | 0 | 0 |
| Listeria | 30 | 30 | 0 | 0 | 0 |
| Total | 227 | 217 | 0 | 0 | 0 |
| N=444 | | Rates* | 0% | 0% | 0% |

| Previous Molecular Method | | | | | |
|---------------------------|------------------|------------------|----------------|----------------|---------------|
| Assay | Culture Positive | Culture Negative | False Positive | False Negative | Indeterminate |
| E. coli O157:H7 | 55 | 65 | 0 | 3 | 0 |
| Salmonella | 43 | 29 | 0 | 2 | 0 |
| STEC / EHEC | 72 | 60 | 3 | 3 | 12 |
| Listeria | 30 | 27 | 2 | 1 | 3 |
| Total | 200 | 196 | 5 | 9 | 15 |
| N=411 | | Rates* | 2.8% | 4.5% | 3.8% |

E. coli O157:H7
 Broccoli, Cabbage, Celery, Cucumber, Flowering Kale, Strawberries IQF, Pasilla Peppers, Radicchio, Red Swiss Chard, Spinach

Salmonella spp.
 Broccoli, Cantaloupe, Cauliflower, Green Bell Peppers, Green Cabbage, Romaine Lettuce, Spinach, Spring Mix, Tomatoes, Strawberries IQF

STEC / EHEC
 Arugula & Baby Greens, Baby Carrots, Brussels Sprouts, Butternut Squash, Celery, Coleslaw 3 Color Deli, Garden Salad, Iceberg Lettuce, Spring Mix (Mesclun), Sugar Snap Peas, Water, Watermelon

Listeria spp.
 Red Chard, Red Leaf Lettuce, Romaine Lettuce, Spinach IQF, Strawberries IQF

- Product verification testing requires accuracy
- False Positives = Financial Loss
- False Negatives = Product Risk
- Indeterminate Results = Delayed Product Release

- 6 non-inoculated and 6 inoculated samples run per matrix
- Inoculated samples spiked at a high level 30 CFU/sample
- PCR resulted in false positive, false negative, and invalid results
- Atlas had 100% agreement with culture

Key Takeaways

- **The new regulatory environment and incorporation of WGS has enabled a more effective, efficient source trace back to outbreaks and recalls**
- **The consequences of recalls & outbreaks has been elevated**
- **New technology and detection method can provide higher resolution, lowering your risk, and enabling more effective preventative control programs.**
- **Your Detection Method Matters**

Be Positive

Be Certain

Be In Control



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Thank you

edreyling@rokabio.com

*Visit Roka at Booth 405 for
Further Discussion*



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