Rapid Microbiological Methods

August 31st, 2021

A presentation for Dairy Management Inc.

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Introduction

About Us

About you and your experiences

David Legan, PhD

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About Eurofins

Purpose

To provide our customers with innovative and high quality laboratory, research, and advisory services while creating opportunities for our employees and generating sustainable shareholder value

Values

- Customer focus
- Competence and team spirit
- Integrity
- Quality

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Eurofins Group Overview

Eurofins is the **global leader in biological testing** with an unrivaled reputation for unbiased analysis

200,000 reliable analytical methods for characterizing the safety, identity, purity, composition, authenticity, and origin of products

Our **diverse laboratories** navigate seamlessly through a dynamic and ever-changing global marketplace

55K EMPLOYEES

900 LABORATORIES

> 50 COUNTRIES

450M+ TESTS ANNUALLY

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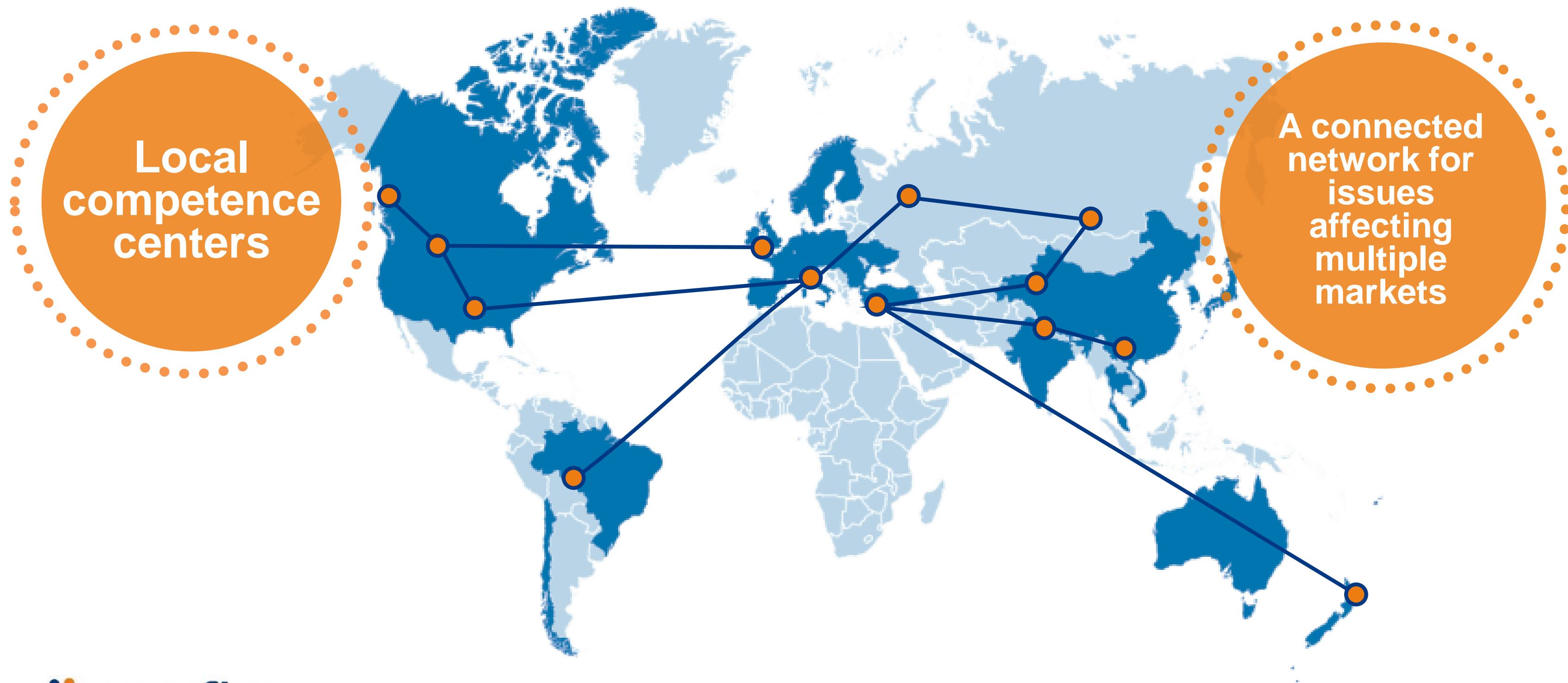
Local Service with a Global Network



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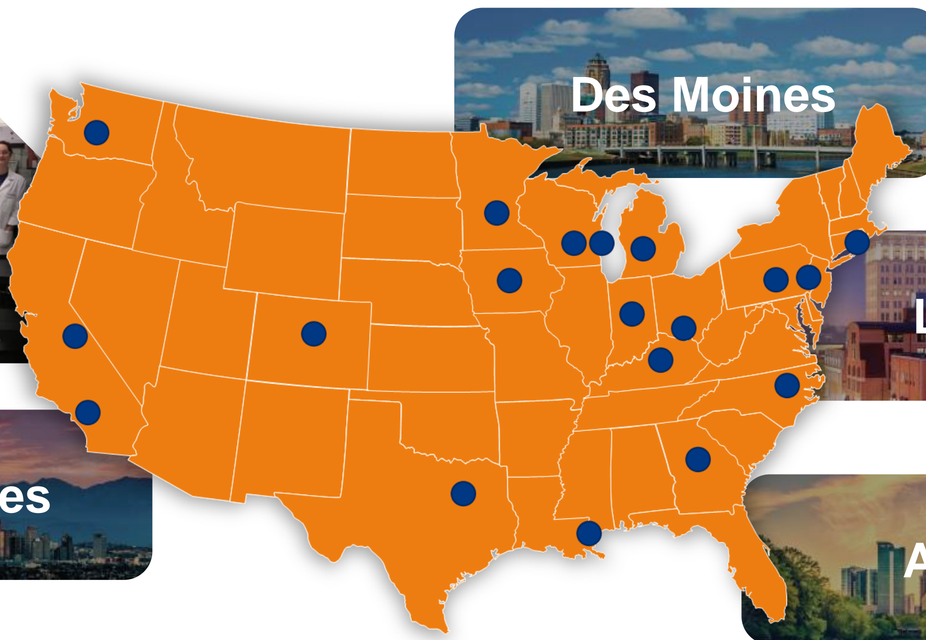
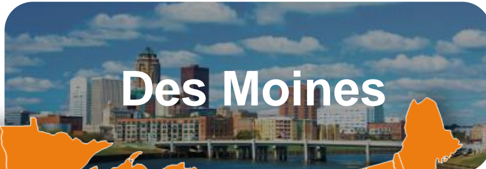
Eurofins Food Testing Laboratories

29 Laboratories in 19 Cities across the US

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Reliable Results

Quality Results, On Time and Accurate

❶ Accreditations & Approvals

ISO 17025 and other relevant standards

❷ Proficiency Testing

Industry approved programs for various methods

❸ Client Audits

Eurofins' open door policy invites you to audit us at any time

❹ Internal Standards

Internal controls to safeguard against false positives and negatives

❺ Technology Investment

Commitment to industry leading infrastructure and cutting edge technologies

❻ Industry Experts

Leaders and knowledgeable experts attuned to the latest trends

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2. Job function

Job role	% responses
Quality Assurance	55.6
Management	44.4
Production	22.2
Research and Development	22.2
Laboratory Technician	11.1
Total responses	**155.5**

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3. Is your company currently using rapid pathogen testing technologies?

Answer	% responses
Yes	55.6
No	44.4
Total respondents	100

Number of platforms	% of responses
0	44.4
1	22.2
2	22.2
3	10.1
4 or more	0

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4. If so, what pathogen platform(s) are you using?

Platform	% responses
VIDAS	100
BAX real time PCR	25
BioRad IQ check real time PCR	25
3M MDS	25
Neogen Reveal or other rapid immunoassay	25
Other: Neogen ANSR	25
Total Respondents	**225**

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5. What microorganisms are you currently testing for in your facility?

Organism	% responses
Coliforms/E. coli	77.8
Salmonella	66.7
Listeria spp.	66.7
Yeasts and Molds	66.7
Enterobacteriaceae	55.6
Listeria monocytogenes	33.3
Other: Cronobacter (2), total plate counts, gram negatives (general), Staph, Bacillus	
STEC (stx positive big six E. coli + O157:H7)	11.1
E. coli O157:H7	0
None of the above	22.2

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6. Where/how are you conducting your micro testing?

Testing location	% responses
Third party testing lab	75
In house at each individual production facility	62.5
In house at a central laboratory	25
Total Respondents	162.5

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7. How much do you trust the results of your pathogen tests?

Level of trust	% responses
1- Not at all	0
2- A little	0
3- Moderately	**12**
4- A lot	**62**
5- Completely	**25**

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8. How much do you trust the results of your quality / indicator tests?

Level of trust	% responses
1- Not at all	0
2- A little	0
3- Moderately	**12**
4- A lot	**62**
5- Completely	0

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11. What would you like that you do not currently have today?

Answer	% responses
Lower price	75
Faster turnaround time	62.5
Greater specificity (i.e. fewer non-confirming detections)	62.5
Higher throughput	50
Greater sensitivity (i.e. fewer false negatives)	12.5
Additional targets	0

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What did we hear?

- Mix of current users and non-users of rapid methods
- Mix of in-house and third-party labs
- Generally a high level of trust in both pathogen and quality tests
- You want methods that are: Cheaper > Faster = More Specific > Higher Throughput >> More Sensitive and No New Targets
- Almost all do some level of confirmation of pathogen results
  - in a 3rd party or corporate central lab
  - none at manufacturing locations.
- Various levels of ID following confirmed pathogen detection (including some users of WGS/NGS!)
- All have some remedial actions tied to target detection
  - May vary by source of detection
  - Always vector follow-up on environmental detection
- Need to consider method validations

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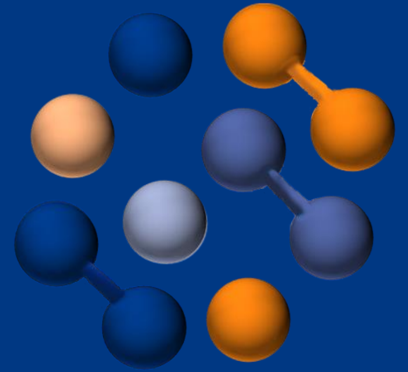
Rapid Methods in Food Pathogen Diagnostics

Challenges for Sample Preparation & Detection of Foodborne Pathogens



Daniel R. DeMarco, Ph.D.

Director of Science – Eurofins Microbiology Laboratories

08/31/21

Provide an overview of rapid methods in food pathogen testing including the biology and chemistry of detection in 10-15 minutes

…..and oh yeah if you can squeeze some terminology stuff in there, that would be nice too…

Detection Technologies in Food Pathogen Testing – A (Very) Brief Overview

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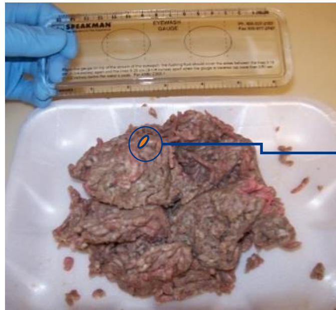
TABLE 1. Detection Technologies in Food Pathogen Testing

Type	Format/Specific Technology	High Level Description	Target Pathogen Capture Ligand(s)	Primary Detection modality	Pros	Cons	
Culture	Liquid/broth and plating/agar media	Growth of specific pathogens on differential/selective culture media with biochemical identification	na	Optical - visual interpretation	Only detects live cells, gold standard, easy, inexpensive, highly effective	very slow, labor intensive	BD, Thermo, etc.
Affinity Ligand	Lateral flow/dipstick	Specific capture of targeted pathogens on a lateral flow/dipstick device. Labeled secondary antibodies used to generate visible band	poly/monoclonal Abs, phage binding proteins	Optical - visual interpretation of presence/absence of bands	Inexpensive, easy to use, field deployable	Specificity gaps, sensitivity	SDI/Romer
	ELISA/ELFA	Specific capture of targeted pathogens using biological affinity ligands. Detection is accomplished through enzymatic routes to produce an optical signal	poly/monoclonal Abs, phage binding proteins	Optical - luminescence/fluorescence via enzymatic activity	Inexpensive, automatable	Specificity gaps, sensitivity, non automated methods very labor intensive	BM VIDAS, Eurofins BAC*Spec*
	Biosensors	Specific capture of targeted pathogens using biological affinity ligands. Detection is accomplished using a huge variety of physical/chemical methods with signal generation via signal transduction as a result of target binding	All types - phage proteins, aptamers, poly/monoclonal Abs, short chain Ab fragments, etc.	Many - SPR, EIS, surface acoustic wave, quartz crystal microbalance, evanescent wave, Raman, FRET, TIRF, QDs, etc.	sensitivity, speed	Expensive, specificity gaps, high variability in affinity/avidity, reproducibility of ligand production	None currently on market
Molecular (Amplification)	PCR	Use of polymerase chain reactin to amplify DNA and/or RNA sequences specific to target pathogens. Detection is accomplished following amplification (end point) or during amplification (real time) by optical detection of fluorescence or luminescence signals. PCR with thermal cycling is most common but isothermal PCR now widely available	na	Optical - Fluorescence detection post amplification (SYBR green melt curve)	highly specific, inexpensive, closed tube system, objective results calling by algorithm	slow, limited to single or biplex detection, detection of dead cells	Hygenia BAX
				Optical/Visual - visual identification of gel bands	very sensitive, highly specific, relatively fast, very inexpesive, low tech	labor intensive, subjective, PCR/amplicon contamination risk, detection of dead cells	IEH PCT
	real time PCR (rtPCR/qPCR)			Optical - fluorescence detection during amplification	fast, very sensitive, highly specific, many detection chemistries/formats widely available, multiplex up to 5 or 6 targets	expensive(ish), requires more highly trained staff, detection of dead cells	Everybody
	Isothermal (real time) PCR			Optical -luminescence detection during amplification	fast, very sensitive, highly specific	Currently limited to single plext target detection, dead cell detection	3M MDS
	Bacteriaphage based	Use of engineered bacteriophage that infect specific target pathogens. Phage carry genes for luminescence and signal is generated during phage replication (lytic cycle)Upon viral replication signal generation is accomplished through enzymatic or other biochemical means		Optical - luminescence detection	fast, cheap, potentially almost reagentless, only detects live cells	very new unproven approach, specificity gaps, inclusion of engineered phages causes issues in some European markets	Sample6/IEH
	Sequence based	Sequencing of either targeted segments or whole genome w/bioinformatics to identify		Optical - fluorescene labeling of bases for base calling	Potentially can identify anything	expensive, identifies anything, data security concerns (i.e. linkage to outbreaks), slow	Clear Labs
Hybrids and others	Immuno-PCR	Combination of both affinity ligand and molecular/amplification technologies or others	Antibodies, other	PCR amplification of specifc DNA targets, followed by detection of labeled specifc anti-DNA antibodies in lateral flow type format	Best of both worlds	Worst of both worlds	BM Invisible Sentinel

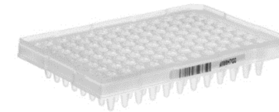


What we are trying to do is really, really, really hard!

~1mm = 1000X
actual size

Average weight of *E. coli* = 25kDa ~4 x 10^{-20}g
0.00000000000000000004G vs 375G

A single bacterium as a 6' 6" person would need to travel approximately 100,000 body lengths (~120 miles) to make it from one side of 375g ground beef sample to the other. (state of Delaware is 96 miles long)



Harder than finding a needle in a haystack!

- Total stack volume = 111 582 270.29 cm^3
- Needle volume = 0.04729861 cm^3
- Needle in HayStack = 4.24 x 10^{-10} = **42.4 ppb**

"Concentration" of needle in haystack is **170,000 time higher** than "concentration" of 1 E. coli in 375 g ground beef!

- Density of ground beef (90-94 % lean) = 0.92 g/cm³
- Ground beef = 375 g
- Ground beef volume = 4.076 x 10^{14} μm^3

- Volume of 1 cell E. coli ~1μm^3
- One E. coli in 375 g ground beef = 2.45 x 10^{-15} = **0.00245 ppt**

[haystack] / [Ground beef] = 172 781

*Thank you Dr. David Legan and Dr. Doug Marshall for the interesting factoids and corrections of my initial grossly wrong attempt to say something similar

- Over the past 30 years, detection has been the main focus of innovation
- In food pathogen dx innovation has mainly come from clinical and bio-threat applications
- Applications in food diagnostics have tended to follow 5-10yrs behind clinical

- Current technologies can provide accurate detection in seconds/minute
- Current technologies can detect one organism/molecule

- In essence the “detection problem” has been solved and has been for a long time

…provided the target gets into the detection system

The Current Food Testing Paradigm – Enrichments & Sampling

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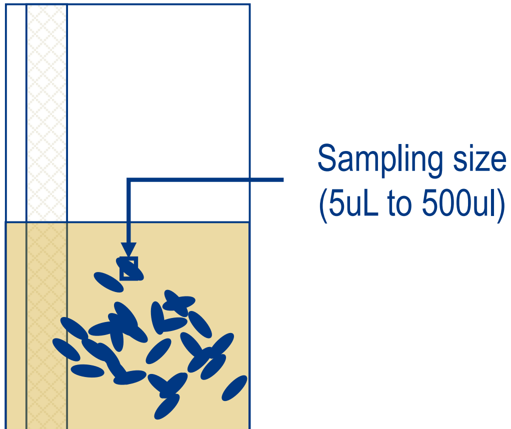
- Most food pathogen tests must be able to detect a single target pathogen in any given sample (25g-375g)
- With small sampling sizes (typical of almost all rapid methods) how do you ensure that the sample you test will contain the target of interest (if present)?

- Long enrichment + Small volume sampling



Advantages:

- Simple, effective
- Can add selectivity (i.e. suppress competing background flora) & dilutes inhibitors
- Works for all matrices

Disadvantage:

- Enrichments take time (16-48h or more) → slower TATs

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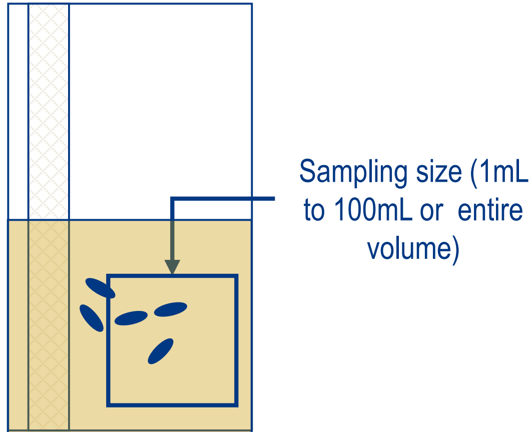
- Shorter enrichment + Larger volume sample



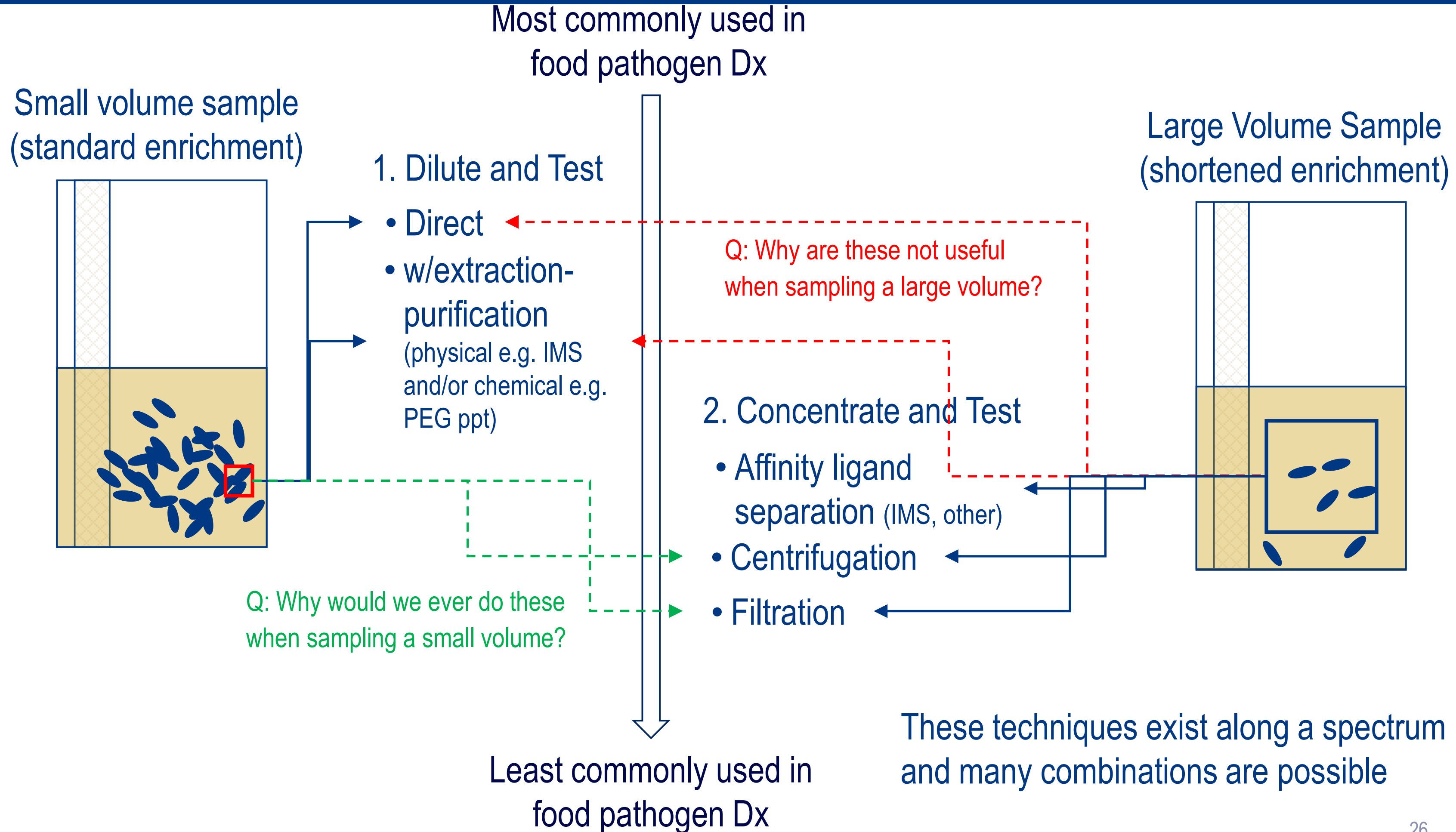
Advantage:

- Hypothetically improved sensitivity→shortened enrichment→ faster TAT

Disadvantages:

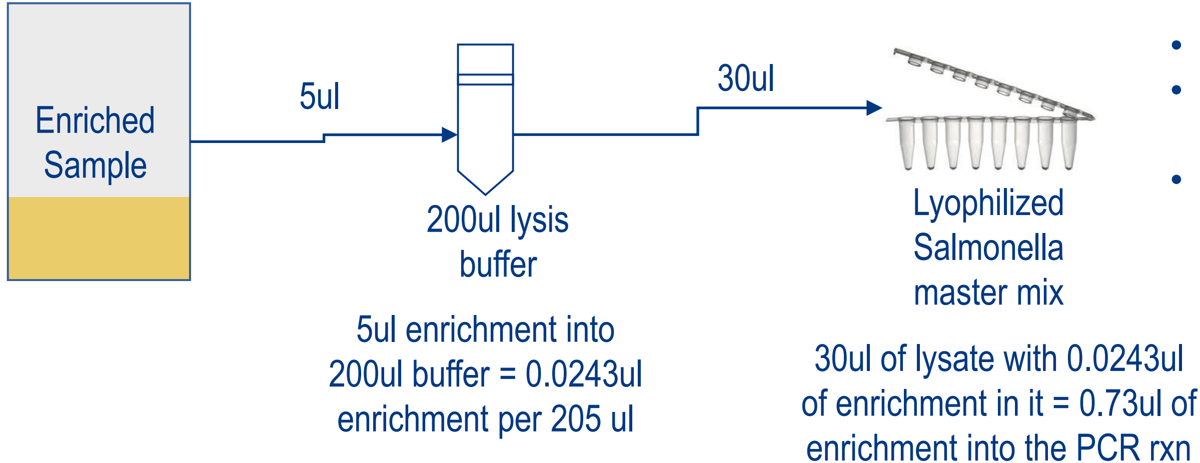
- Difficult as additional time/costs usually outweigh advantage of sensitivity improvements. Difficulties increase with ⬆ sample weight and ⬇ dilution ratio (aka sample weight:volume)
- Only possible for some matrices and each may requires unique method
- Detection method inhibition

Presenting the Target to the Detection System (aka Sample Prep)



Example Case – PCR Detection of *Salmonella*

- A commonly used real time PCR based method for *Salmonella* detection

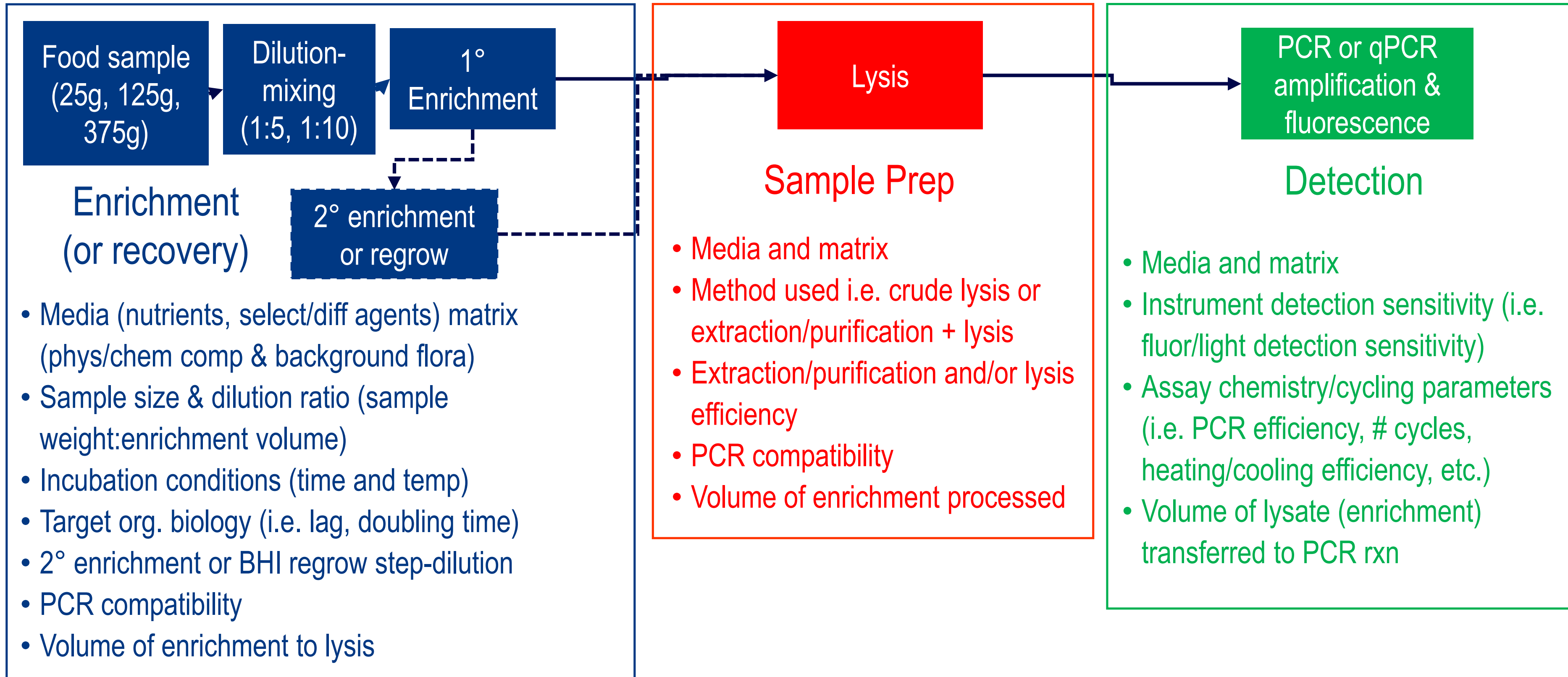


- **0.73ul** of original enrichment to PCR
- For a 25g sample (250ml enrichment) = **0.0003% of the entire volume.**
- For a 375g sample (3.75L enrichment) = **0.000015% of the entire volume**

If you ever wondered why we need to enrich for so long this is your answer

Factors that Influence Method Performance – PCR Method Workflow Example

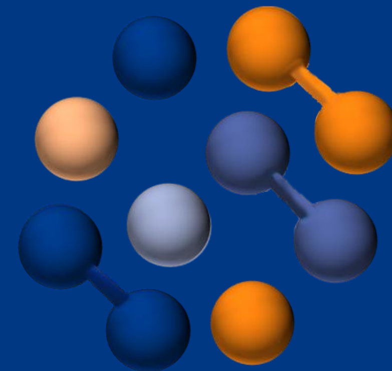
- Rapid food pathogen methods can be broken down into three core components.
- It is the combination of all three that determines overall method sensitivity and robustness
- Each component contributes to overall method sensitivity and robustness in different ways and many factors influence how



Shifting Gears

The Terminology of Detection

Presumptive (Positives) and the Great Non Confirming Presumptive (NCP) Debate



Non-Confirming Presumptives on the Rise?

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- True or False

- There has been a large increase in the number of presumptives that cannot be confirmed by culture in recent years

Why Is a Particular Result Classified as "Presumptive (Positive)"?

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- Performance
  - We question one or more performance characteristics of the method. i.e. sensitivity/specificity, robustness, etc. is believed to be non optimal or inferior (or sometimes maybe even superior) to the "gold standard" method
  - The targets of a multiplex PCR (both of which are required to indicate a positive result) may be found in different organisms in the same enrichment (STEC – stx/eae)

- "Defensibility" of results
  - We lack full confidence in the initial presumptive (positive) result because it is possible that it may be due to artifacts of the analysis
  - Improper sample preparation, laboratory contamination, misidentification during analysis, etc.
  - Further verification of a presumptive result = more defensible result, very important when result demands regulatory action

Why Is a Particular Result Classified as “Presumptive (Positive)”?

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- New/unknown science
  - The science behind the method and/or the target analyte is new and/or actively evolving
  - Not enough is known yet about the analyte and/or the method and how they behave in the “real world” to have great confidence in the results
- Misleading
  - Positive result would be misleading in terms of actual risk method is said to assess. e.g. detection of dead and/or non culturable organisms. The presence of dead cell DNA/RNA should present no risk from a food safety perspective therefore a secondary method should be used to “confirm” the presence of live cells.

False Positive (FP) vs. Non Confirming Presumptive (NCP)

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- NCP ≠ FP

TABLE 4. False Positive vs. Non Confirming Presumptive

	False Positive	Non-Confirming Presumptive
Instrument Result	+	+
Initial Reported Result	Presumptive	Presumptive
Confirmation/Final Reported Result	Not detected	Not detected
Target DNA/RNA present	No	Yes
Causes	X-reactivity w/non target org. (i.e. primer/probe or affinity ligand specificity problem), signal aberration resulting in algorithm miscall	Everything else, e.g. dead cell DNA/RNA, confirmation method failure, etc.
Other Terms	N/A	Non culturable presumptive, Detected not recoverable (DNR)

- Very few diagnostic test customers understand or appreciate this distinction. For the majority FP=NCP. In either case they must be acted on as if true positives

Discussion

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- The detection “problem” was solved decades ago. Many technologies exist that are capable of detecting a single molecule This includes PCR which is widely used in food pathogen testing today
- What remains is essentially a sample prep problem and it is very difficult because
    - Need to detect targets that are present at ultra low concentrations in large masses of material
    - These “masses of material” (aka foods or environmental samples) are extremely diverse and complex and span the gamut of forms of matter, components/ingredients, and sizes
    - Often contain substances which are inhibitory to rapid detection methods
    - Reluctance to pay more for testing
    - Difficulties/cost to make high throughput compatible

- There is ***always*** a tradeoff between speed and sensitivity…

- To date detection has seen much more research effort and dollars spent than sample preparation.
    - Detection is more “sexy”
    - Advances in detection have tended to trickle down to food from clinical dx but their lives are much easier on the sample prep front (very few matrices, target present at high concentration) and we should not expect sample prep innovation to come from clinical dx research
    - Biothreat agent dx r&d dollars have not helped and the economics (and applications) are very different

- Non confirming presumptives and false positives are not the same. Confusion between the two has resulted in no end of headaches and difficulties for diagnostic test kit developers, testing laboratories, and end users

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Considerations when choosing and using rapid microbiological methods.

David Legan, PhD

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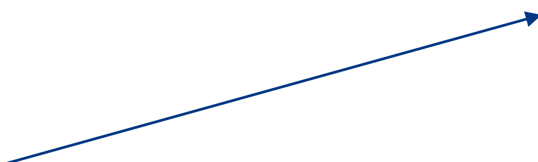
Considerations

Attributes

- Target
- Validation
- Speed
- Cost
- Safety
- Need for confirmation
- Customer and regulatory acceptability
- Operational efficiency



Validation

- Sensitivity
- Accuracy
- Inclusivity
- Exclusivity
- Enrichment / incubation time
- Reliability / robustness

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Detection methods: too much choice?

AOAC RI PTM certificates listed on 2021-08-16	
AOAC RI PTM certificates – type microbiological (including toxins)	284
Pathogens and potential pathogens (including *Listeria* spp.)	230
Of which, foodborne pathogens	**224**
Indicators	**24**
Spoilage / general	**22**
SARS-CoV-2	6
Aspergillus fumigatus and other spp. (Cannabis)	5
Misclassified allergen / foreign species	3

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Range of technologies

Affinity assays

- **Immunoassays** (VIDAS, Solus, BAC*Spec*)
- **Phage capture** (VIDAS-UP)
- **Lateral flow devices** (Invisible Sentinel/BioMerieux)

Culture-based "amplification"

- **Metabolite detection** (Soleris/Biolumix)

Molecular amplification assays

- **PCR/rtPCR** (BAX, BioRad iQ-check, BACGene, GENE-UP, Pall GeneDisc, PolySkope, PathogenDx)
- **LAMP** (3M MDS)
- **RNA detection** (Neogen ANSR, Roka/IEH)

Other

- **Phage-based detection** (Sample6/IEH)
- **Reactive swabs:**
  - **Chromogenic** (Paradigm PDX, Hygiena InSite)
  - **Bioluminescent** (ATP, Hygiena MicroSnap)
- **Sequence-based detection** and/or profiling (Clear Labs, Rheonix)

Assume all system names are trademarked. List is not comprehensive. **Inclusion does not imply endorsement. Exclusion does not imply disapproval.**

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Sensitivity

Limit of Detection (LOD) per mL of enrichment broth

Technology	CFU/mL (after enrichment)	Source
ELISA/ELFA	10^4 - 10^5	K.M. Lee et al. *Food Control* **47** (2015) 264 – 276
PCR	10^4	K.M. Lee et al. *Food Control* **47** (2015) 264 – 276
LAMP	10^3	Eurofins internal

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Validated LOD / test portion: *Salmonella*

Principle	Sample prep	Method	AOAC RI PTM	Test portion (g)[2]	LOD_{50} CFU /test portion[1]	Time to result
Culture	Enrichment & plating	FDA BAM	N/A	25[6]	1 (assumed)	84-126 h[4]
ELFA antibody	Enrichment	VIDAS SLM	20901	25	0.37-1.06	< 48 h[3]
ELFA phage tails	Enrichment	VIDAS UP	71101	25 - 375	0.41 - 0.90	22-32 h[4]
DNA rtPCR	Enrichment	BAC*Gene*	121501	25 - 375	0.75-1.06	16-30 h[4]
DNA LAMP	Enrichment	3M MDA2	91501	25 - 325	0.35-0.99	16-30 h[4]
RNA TMA	Enrichment	ATLAS	41303	25 - 375	0.17-1.50	16-30 h[4]
PCR Microarray	Extract & concentrate	PathogenDx	092001	4" x 4" stainless steel	47-73	8 h[4,5]

[1] Concentration where probability of detection is 50 %; [2] Portion size in data set submitted for certification; [3] Manufacture claim; [4] Depending on matrix; [5] Environmental only, [6] Generally, many options available

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Time to result

Broadly:

- Culture TTR (not rapid) > Affinity methods > Amplification methods > Concentration methods

For rapid screening methods time to result is primarily driven by enrichment time.

Why not just use “no-enrichment / concentration” options?

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No-enrichment methods

Some options (examples)

RNA detection, e.g.

- Neogen ANSR *Listeria* Right Now (for swabs only)

PCR with concentration step(s), e.g.

- PathogenDx
- GENE-UP *Salmonella* quantification

Integrated concentration and detection, e.g.

- SnapDNA

Pros

- Shorter time to result

Cons

- Currently limited applications
- Sensitivity
- Modified workflows
- Short experience

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Beware the “need for speed”

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- Constant desire for faster methods.
- Push to shorten enrichments for faster results.
- Means lower target concentration for detection.

POTENTIAL CONSEQUENCES

Public health risk.
Reputational and regulatory risk.
Loss of brand value.

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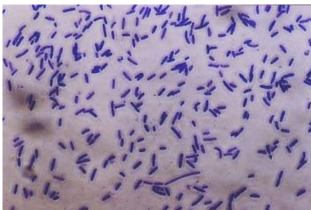
Interferences / failure to detect

Enrichment interference Detection interference



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High competitive background

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Antimicrobials – esp. environmentals, spices

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Deep colors (some detection technologies)

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Amplification inhibitors, e.g. spices, botanicals

True false negative rate is “impossible” to know.

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Interferences / failure to confirm

- "Non-confirming presumptive"
  - Method detected target but isolate cannot be cultured.
- Detection of target from "dead" cells.
  - Digestion of extraneous DNA, e.g. PR*Eraser*,
- Isolates that don't perform as expected on culture media
- True cross-reaction with non-target strain (rare)

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Validation and verification

Validation commonly through a recognized certification body.

- US market – usually AOAC
  - Performance tested methods (PTM) common for rapid methods. Validation study through reputable lab.
  - Official Methods (OMA) less common for newer methods. Requires multi-lab study and extensive experience of the method
- European markets, AFNOR, MicroVal, NordVAL
- So… Which dairy matrices are validated?

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PTM *Listeria* spp. validations – selected platforms

AOAC Dairy Categorization: Fermented and non-fermented products											
H_2O %	Fat %	Examples	1	2	3	4	5	6	7	8	9
< 20	<10	Powders: milkshake, buttermilk, NFDM, whey, casein					X				
	10-30	Dry whole milk, grated parmesan cheese									
	30-70	Powdered cream									
	>70	Butter, margarine									
20-50	<10	Canned condensed milk									
	10-30	Cheeses: American, Brie, Gouda, Monterey, Colby, goat			X				X		
	30-70	Margarine									
50-80	<10	Ice-cream, low-fat yogurt, Ricotta, milkshake, evap. milk	X				X	X	X		
	10-30	Sour cream, whipped cream, mozzarella	Q	S	Q	Q	Q	Q		Q	Q
	30-70	Heavy cream									
>80	<10	Fat-free half-&-half, whey, yogurt, cottage cheese, milk, buttermilk	X	X				X	X	X	X
	>10	Half-&-half regular									

Certificate states: Q, Queso Fresco; S, “soft white cheese”; X, maps to category example

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Operational integration

- Facilities / containment
  - on-site or offsite lab; containment of pathogens
- Method complexity / required skill level
  - Enrichment methods generally fit easily into lab workflows
  - Extraction / concentration methods generally harder, may need extra equipment
- Documentation, training and proficiency testing
  - Required for any new method
- Verification
  - Method works “in your hands”
- Fit for purpose
  - Method works for matrix.

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Summary

Many Rapid Methods on the market;

- Sensitivity/test portion
- Accuracy
- Precision
- Robustness

} Similar

- Speed
- Validated matrices

- Ensure that the method is validated for your matrix
  - Or generate your own validation
- Use an accredited lab
- If you have any concerns ask questions

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Beyond Technology, What Else Do You Need To Consider?

Douglas Marshall, PhD, CFS

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Practical Testing Considerations

- Ingredient vs. finished product vs. environmental samples?
- How often do you test?
- How many samples do you test?
- What sample size do you collect?
- Indicator vs. pathogen?
- What performance specifications do you use?
- In-house testing vs. out-source testing?
- Speed to test result, how fast is fast?
- How do you manage false positives and false negatives?
- How do you handle presumptives that don’t confirm?

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What to Test For – Pathogens, Spoilers, or Indicators?

Common Indicators

- Aerobic Plate Count
- Indicator organisms
- Coliforms
- Fecal coliforms/*E. coli*
- Enterobacteriaceae
- Enterococci
- Lactic acid bacteria
- Yeast and molds
- ATP

Specific Pathogens

- *Salmonella*
- *Cronobacter sakazakii*
- *Listeria* spp.
- *Listeria monocytogenes*
- *Staphylococcus aureus*
- *Yersinia enterocolitica*
- Pathogenic *Escherichia coli*

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Product Sample Considerations

- Direct testing liquid dairy samples allow for a lower LOD/LOQ
- Solid samples (cheese, ice cream, powders) require initial dilution, which increases LOD/LOQ
- Powders have potential to create dust, which is a cross contamination risk. Is the lab set up to handle this risk?
- Some ingredients can interfere with method performance, such as pigments, high lipid content, or some metallic cations like Zn

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How Many Product Samples?

Is one grab sample from an entire production lot adequate?

- For liquid samples that are easily homogenized – maybe?
- For solid samples that are often stratified due to settling – not a good choice?

How do you collect a representative sample?

- Products that are palletized?
- Products that are super sacked?
- Products that are in process?

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FDA-BAM Salmonella Sampling Guide

Food Category I. Greatest Risk, 60 25-g samples per lot

- Foods that would not normally be subjected to a process lethal to Salmonella between the time of sampling and consumption and are intended for consumption by the aged, the infirm, and infants

Food Category II. RTE Foods, 30 25-g samples per lot

- Foods that would not normally be subjected to a process lethal to Salmonella between the time of sampling and consumption

Food Category III. Lowest Risk, 15 25-g samples per lot

- Foods that would normally be subjected to a process lethal to Salmonella between the time of sampling and consumption

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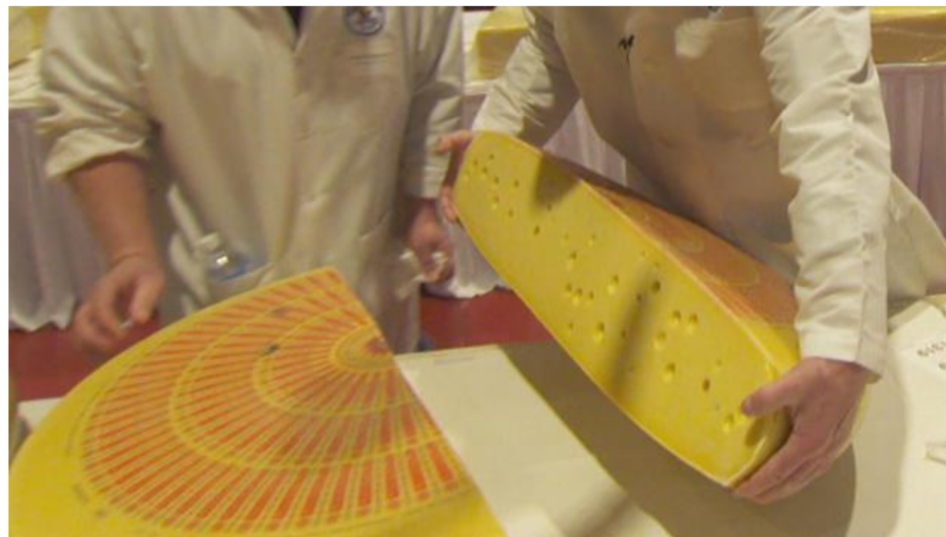
Sample Size?

Infectious dose for *Salmonella* in Cheddar cheese reported as low as 1 CFU/500 g (see Fontaine et al., 1980. Am. J. Epidemiol. 111:247–253)

What sample size is adequate?

- 25 g
- 125 g
- 375 g
- 750 g
- 1.5 kg



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Environmental Monitoring: What to Test For?

Wet Processing Environment

- *Listeria monocytogenes* – pathogen of concern
- *Listeria* species – best indicator

Dry Processing Environment

- *Salmonella* and *Cronobacter* – pathogens of concern
- Enterobacteriaceae count or coliform/*E. coli* count – which is the best indicator?

What about other pathogens?

- If you control *Listeria* and *Salmonella* the odds are good you're controlling other pathogens

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Transient vs. Persistent Strains

Transient strain – enters facility but does not permanently reside

- Control programs eliminate organism
- Unable to adapt and replicate
- Occasionally can be found during routine surveillance

Persistent strains – enters facility and establishes permanent residency

- Control programs fail to eliminate organism
- Establishes niche in harborage locations such as biofilms
- A frequent contaminant found during routine surveillance

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How Many EMP Samples?

- It varies depending on risk
- Size and complexity of facility
- Number of skews
- Number of sanitation runs
- Range from daily/multiple times a day to weekly
- Sites are typically pre-determined, but also may be randomly rotated
- At least weekly

- U.S. dairy Innovation Center recommendation
- A minimum of 30 swabs per 50,000 sq. ft. per week.
- > 55 swabs per 50,000 sq.ft. per week
- Significantly increase sample number when out-of-spec occurs

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Questions?

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