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Pathogen Screeni

Background, Methodology, and Application

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Food Industry – “Overheard”

- FSMA…
- FDA Draft Guidance - Zone 1 Environmental…
- Documentation…
- Whole Genome Sequencing…
- Recall, Risk…
- Validation and Verification…
- Environmental Monitoring…
- Audits…
- Methods…

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Pathogen Screening: An Industry Spaghetti Junction

- FSMA…
- FDA Draft Guidance - Zone 1…
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Unpacking Pathogen Screening

Unpacking Pathogen Screening

- Key Aspects of Pathogen Screening and Methods
  - Validation and Verification
    - Relation to Pathogens
    - Manufacturer, 3rd Party, End User
  - Pathogen Process and Methods
    - Enrichment, Analysis, Confirmation
    - Categories of Methods
  - Application Questions

Unpacking Pathogen Screening: Validate and Verify

- Validation (Pathogens)
  - “Demonstration that adequate confidence is provided when the results obtained by the alternative method are comparable to those obtained using the reference method using the statistical criteria contained in the approved validation protocol.” (FDA)

- Verification (Pathogens)
  - “The confirmation by examination and provision of the objective evidence that specified requirements of the performance of a method have been fulfilled by an individual laboratory. Also, the means used to demonstrate that the method functions in the user’s laboratory on matrices not included in the original method validation” (FDA)

Unpacking Pathogen Screening: Validate and Verify

- Manufacturers
  - Kit validation can include data and specifications to include, but not limited to: individual matrices, method instructions, sensitivity, specificity, reproducibility, recovery, and ruggedness as compared to Reference Method

Table 1. Inclusivity and Exclusivity results

Results	Analysis
100/100 *Salmonella* strains were detected (results were "positive")	100% Inclusivity
100/100 non-*Salmonella* strains were not detected (results were "negative")	100% Exclusivity

See Appendix, Tables 4a and 4b, for list of cultures tested

	Family / group	Genus / species / serotype	
Exclusives	*Enterobacteriaceae*	*Buttiauxella agrestis* *Buttiauxella noackiae* *Citrobacter farmeri* *Citrobacter hormaechei* *Citrobacter brakii* *Citrobacter diversus* *Citrobacter freundii, n=2* *Citrobacter koseri* *Cronobacter dublinensis* *Cronobacter malonaticus* *Cronobacter muytjensi* *Cronobacter turicensis* *Cronobacter sakazakii* *Edwardsiella tarda* *Enterobacter aerogenes* *Enterobacter gergoviae*	*Escherichia coli* O157:H43, O157:H7 (20), O18:K1:H7, O26:H11, O3:H2, O44:H18, O55:H6, O55:H7, O6:H10, O6:H6, O78:H11, O78:K80:H12, O96:H43 & O92:H33 *Hafnia alvei* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Kluyvera ascorbata* *Kluyvera spp.* *Leclercia adecarboxylata* *Morganella morganii* *Pantoea agglomerans* *Proteus mirabilis* *Proteus vulgaris*

Matrix	Reference method		Size	Enrichment volume, mL	Enrichment time, hours	3M versus Reference
Raw ground beef Raw ground chicken	USDA MLG		25 g	225	10-24	No statistical differences
Raw ground beef Raw ground chicken	USDA MLG		325 g	975	10-24 beef 14-24 chicken	No statistical differences
Chicken carcass rinse	USDA MLG		30 mL	30	18-24	No statistical differences
Chicken carcass sponge	USDA MLG		1 sponge	50	18-24	No statistical differences
Cooked breaded chicken	USDA MLG		325 g	2925	18-24	No statistical differences
Pasteurized liquid whole egg	USDA MLG		100 mL	900	18-24	No statistical differences
Raw whole shrimp	FDA BAM		25 g	225	18-24	No statistical differences
Dry dog food	FDA BAM		25 g	225	18-24	No statistical differences
Dry dog food	FDA BAM		375 g	1500	18-24	No statistical differences
Instant non-fat dry milk	FDA BAM		25 g	225	20-24	No statistical differences
Processed American cheese	FDA BAM		25 g	225	18-24	No statistical differences
Black pepper	FDA BAM		25 g	225	18-24	No statistical differences
Cocoa powder	FDA BAM		25 g	225	24-28	No statistical differences
Raw bagged spinach	FDA BAM		25 g	225	18-24	No statistical differences
Spent sprout irrigation water	FDA BAM		375 mL	3375	18-24	No statistical differences
Creamy peanut butter	FDA BAM		25 g	225	18-24	No statistical differences
Creamy peanut butter	FDA BAM		375 g	3375	18-24	No statistical differences
Sealed concrete	USDA MLG		1 sponge	225	18-24	No statistical differences
Stainless steel	USDA MLG		1 swab	10	18-24	No statistical differences
Sealed ceramic tile	USDA MLG		1 sponge	50	18-24	No statistical differences

Unpacking Pathogen Screening: Validate and Verify

- 3rd Party Accreditations
  - Organizations that review data submissions on method performance and may issue certifications if data backing performance claims meets that organizations standards
    - e.g. AOAC, ISO/AFNOR, individual country-based Organizations
    - AOAC-PTM/RI (Individual Lab) vs. AOAC-OMA (Collaborative)

Unpacking Pathogen Screening: Validate and Verify

- End Users and Verification
  - After a review of the matrices included in Manufacturer's Validation
  - After a review of the matrices included in 3rd Party Accreditation
  - Creating data using specific products (especially ones not included in Validation) to demonstrate the method functions, using method instructions, as intended for your needs
- Anti-Microbial and Anti-Assay factors
  - Can my target bacteria grow and be recovered? (e.g. Cinnamon, Cloves)
  - Does my product interfere with the detection mechanism?
    - False + or False -?

Unpacking Pathogen Screening: Process and Methods

- Enrichment
  - Taking a sample (environmental sample or product sample) and endeavoring to grow your target organism to your assay's Level of Detection
  - Multiple steps and different media types may be needed
- Analysis
  - Processing an aliquot of your enrichment and deriving a result through a reference method or commercialized kit
  - Many technologies commercially available
- Confirmation
  - Analysis of a presumptive sample to culturally recover the target pathogen

Unpacking Pathogen Screening: Process and Methods

- Cultural Methods
  - Cultural methods use classic agar schemes to recover the target pathogen
  - Examples: FDA-BAM, USDA-MLG, ISO
  - Reference Methods (e.g. FDA BAM – Chapter 11 – *Listeria monocytogenes*)
  - Regulatory Confirmatory Methods are foundationally Cultural
  - Typically take 4+ days to complete and often using multiple enrichments
  - Relatively complex and require a high level of expertise and numerous materials

Unpacking Pathogen Screening: Process and Methods

- Protein-based Methods
  - Use a variety of approaches to identify specific proteins of target pathogen
  - Examples: Lateral Flow, ELISA, ELFA
  - Typically a faster time to result (24-48 hours) compared to Cultural Methods
  - Vary between multiple and single enrichment methods
    - Relatively more sensitive and specific than agar (Assay)
  - Typically more objective than Cultural Methods
  - More efficient than Cultural Methods
  - Presumptive Screening methods

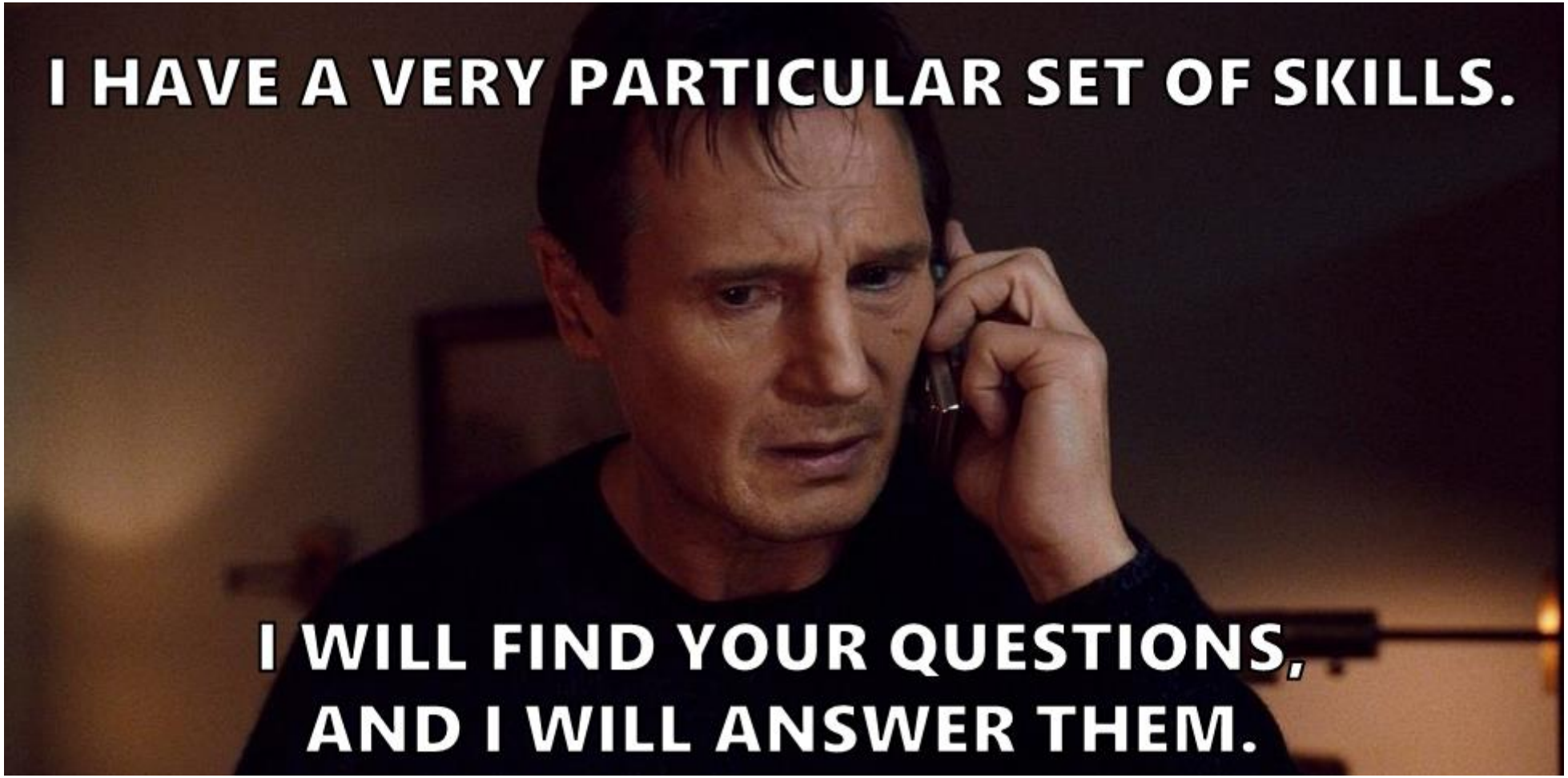
Unpacking Pathogen Screening: Process and Methods

- Molecular (DNA) Methods
  - Use a variety of approaches to identify specific DNA of target pathogens
  - Examples: PCR, LAMP, other Isothermal DNA Amplification variants
  - Typically a faster time to result (~24 hours or less) versus Protein Methods
  - Usually single enrichment, in some scenarios a second may be needed
    - Relatively more sensitive and specific versus Protein Methods (Assay)
  - Automated detection methods
  - Can be more efficient than Protein Methods
  - Presumptive Screening methods

Pathogen Screening: Application Questions

- What data points are included by Manufacturer for their kit?
- What was included in the 3rd party accreditation and what kind of accreditation?
- Do my products match the validations? Do my samples work? (Grow? Detect?)
- Is my enrichment process (e.g. compositing) validated by the manufacturer? Verified by end user?
- What is my risk of true false positives? False negatives?
- What is my risk of cross contamination or LE? How can I mitigate that risk?
- Confirmation: (how) is it to be done? Regulatory Confirmation Method? SOP?
- Does the level of sensitivity of my method impact other aspects of the process (e.g. Confirmation)
- Are the environmental samples my team collects conducive to getting valid results?
- Cultural versus Protein versus Molecular?

Questions?



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Thank You!

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