United States Department of Agriculture
Food Safety and Inspection Service

Design of E. coli O157:H7 sampling and testing programs by Industry

FSIS EIAO Correlation
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Background

- Establishments sample and test components and raw ground beef (RGB) products for *E. coli* O157:H7
  - Mitigate the probability of releasing adulterated product
  - assess the adequacy of process controls for *E. coli* O157:H7
  - Reduce amount of product subject to recall or diverted to cooking when a positive test result is obtained.
  - Required by customer

Background: Sample Definitions

- Lot: total material to be defined by establishment. Should be produced under uniform conditions
- Sample: material selected from a lot to be tested for the presence of *E. coli* O157:H7 cells
- Sampling plan: procedure for sampling a production lot or environment
- Lot prevalence: proportion of positive to total pieces
- Statistical sampling: Provides X level of confidence for detecting (rejecting) lots contaminated at Y lot prevalence (% pieces testing positive)

Background: Sampling Plan

- Define product to be tested
- Define product (i.e, the lot) represented by the sample
- Define target microorganism
- Describe how to sample the product
  - How many independent pieces (N)
- Describe how the sample is analyzed in the laboratory
  - How much of the sample is used
  - What analytical method(s) is used
- Describe how to evaluate the data
  - What is the acceptable level in each piece (m)
  - How many unacceptable pieces to reject the lot (c)

Background: ICMSF Sampling Plans

- International Commission for Microbiological Specification of Foods Microorganisms in Foods 7 (2002)
- Recommended statistical sampling procedures chosen on basis of hazard and degree of health concern after sampling
- Case 15 (severe hazard, increasing health concern)
  - N=60 pieces
  - m = 0 per 25 grams
  - c = 0
- If assumptions met, case 15 provides high confidence of rejecting lots contaminated at 5% (i.e., 3 of 60 pieces contain detectable *E. coli* O157:H7)

Background: ICMSF Sampling Plans 2

- Higher N detects lower lot prevalence

N	Probability of accepting lot with 5% contamination
5	77
15	46
30	21
60	5
100	1

Background: ICMSF Sampling Plans 3

- Statistical sampling plans assume
  - Uniform manufacturing conditions
  - Equal probability of contamination throughout lot ("homogeneous distribution")
  - Independent and random sampling (equal probability of sampling throughout lot)

Background: Testing Definitions

- Analytical Test: Procedure for detecting organism of interest (*E. coli* O157:H7, *Salmonella*) in sample
- (Selective) Enrichment: Procedure for increasing the (relative) number of organisms of interest in the sample by allowing it to grow in the laboratory; resuscitate injured organisms
- Portion: The amount of sample tested by the laboratory
- Composite: combining pieces of sample together for purposes of testing.
  - Dry composite: combine sample
  - Wet composite: combine enrichment cultures
- Sub: the total portion is divided into smaller portions (subs) to accommodate the needs of the analytical test

Background: Testing Definitions 2

- Sensitivity: probability that truly positive samples are detected as positive by analytical test
  - 1-false negative rate
- Specificity: probability that truly negative samples detected as negative by analytical test
  - 1 – false positive rate
- Level of detection (LOD): lowest level of contamination reliably detected by analytical test
  - LOD expressed as ratio of organisms to quantity tested material
  - e.g., CFU per gram, MPN per mL, CFU per square-ft

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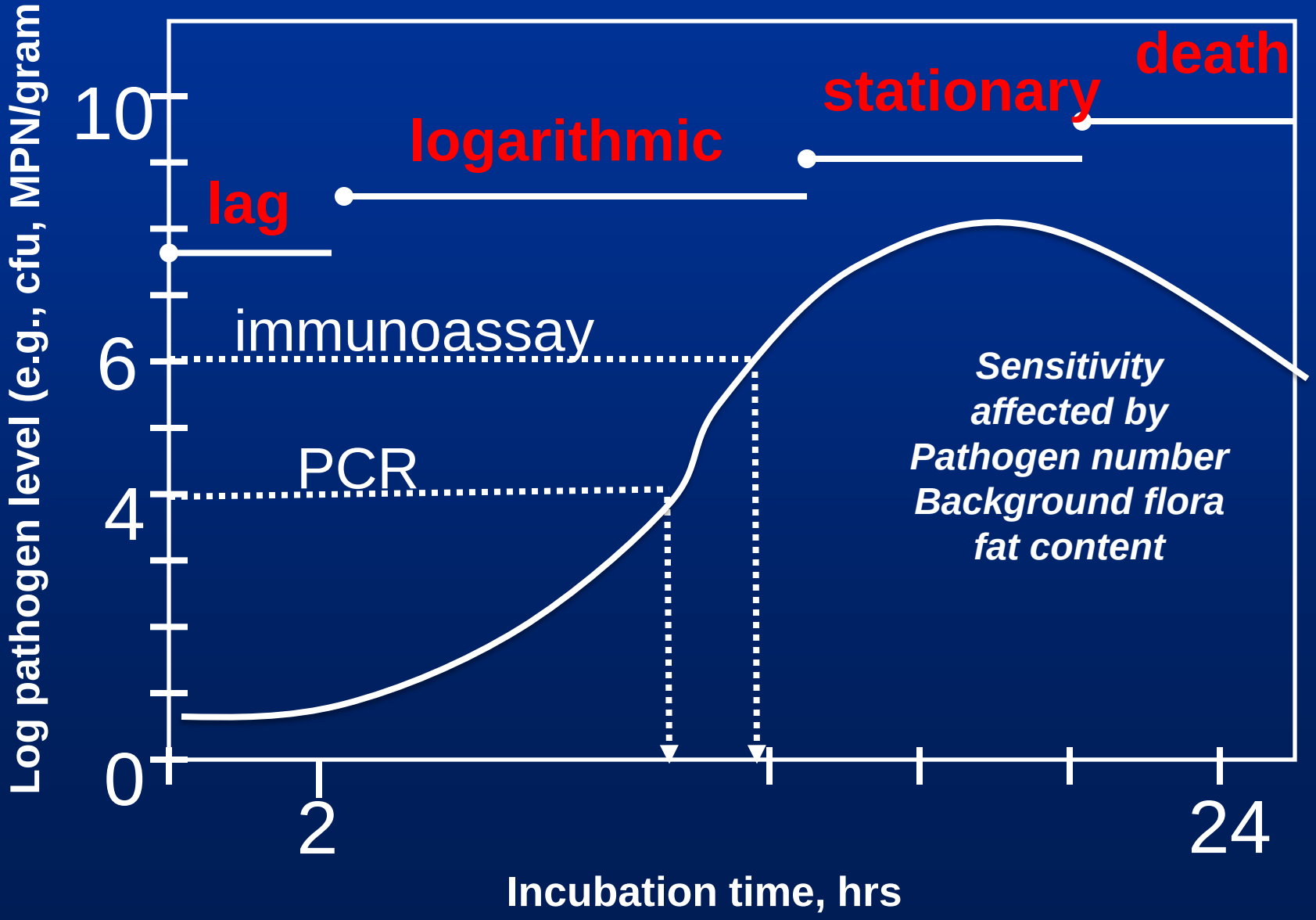
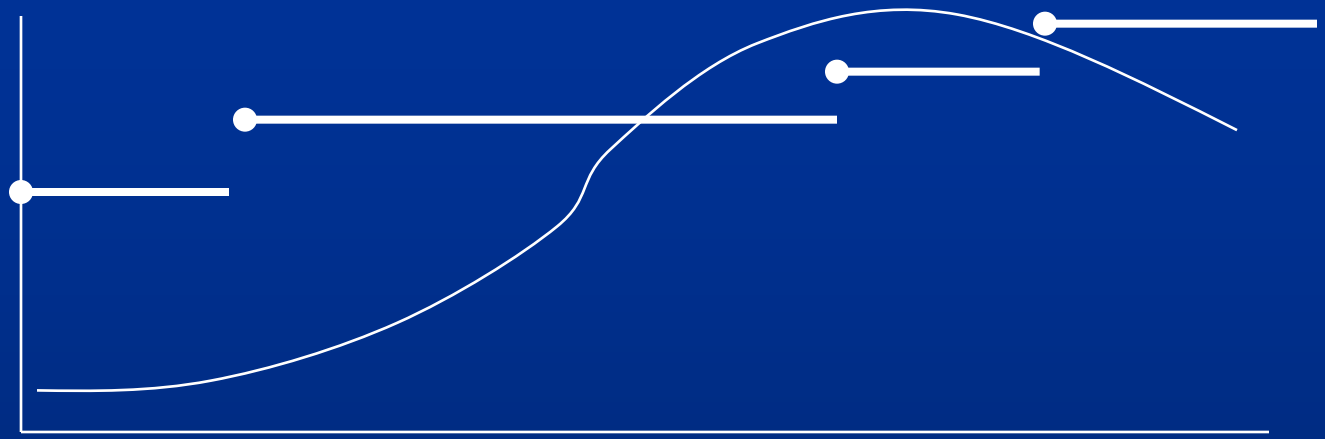
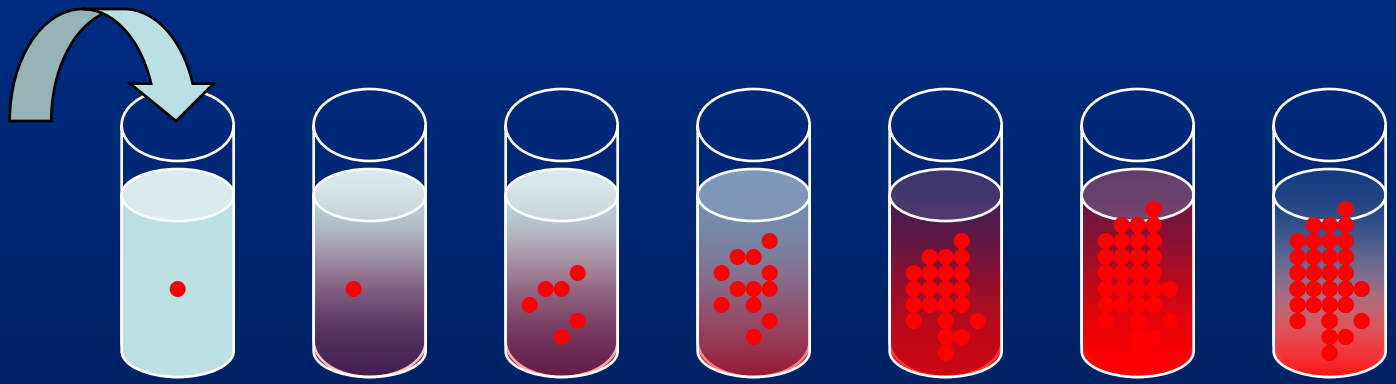
Pathogen Growth During Enrichment



Role of Enrichment





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E. coli O157:H7 Test Kits

- EIA (Enzyme Immunoassay)
  - Biomerieux VIDAS® UP E.coli O157 including H7 (VIDAS ECPT)
  - Biomerieux VIDAS® E.coli O157 (ECO)
  - 3M TECRA E. coli O157 Visual Immunoassay
- LFI (Lateral flow immunoassay)
  - Neogen Reveal for E. coli O157:H7 Test
  - Merck Singlepath® E. coli O157 Lateral Flow Assay
  - DuPont Lateral Flow System E. coli O157 Test Kit
  - FoodChek Systems FoodChek™ E. coli O157
  - SDI RapidChek® SELECT™ E. coli O157
  - EMD Chemicals Duopath® Verotoxin Lateral Flow Assay (Shiga toxin identification)

E. coli O157:H7 Test Kits

- PCR (Polymerase Chain Reaction)
  - Dupont Qualicon BAX® E. coli O157:H7 MP
  - Dupont Qualicon BAX® Real-Time PCR Assay E. coli O157:H7
  - Dupont Qualicon BAX® System Real-Time PCR Assay for E. coli O157:H7
  - Bio-Rad Laboratories iQ-Check E. coli O157:H7 Real-Time PCR Test Kit
  - Biocontrol Assurance GDS for Escherichia coli O157:H7 in Selected Foods
  - Biocontrol Assurance GDS for Shigatoxin Genes
  - Idaho Technology, Inc. E. coli O157:H7 R.A.P.I.D.® LT Test Kit

E. coli O157:H7 Test Kits

- PCR (Polymerase Chain Reaction)
  - BIOTECON Diagnostics foodproof® E. coli O157 Detection Kit
  - Pall Genesystems GeneDisc E.coli O157:H7
  - ADNucleis HQS E.coli O157:H7
  - Applied Biosystems MicroSEQ® E. coli O157:H7 Detection Kit
  - AES Chemunex ADIAFOOD Rapid Pathogen Detection System for Escherichia coli O157 and Escherichia coli O157:H7
  - IEH E. coli O157, Stx-producing E. coli (STEC) with Intimin

E. coli O157:H7 Test Kits

- Plating media
  - RAPID'E.coli O157:H7
  - BBL™ CHROMagar™ O157
- Immunoconcentration
  - Pathatrix ULTRA/Auto E. coli O157 Test System

E. coli O157:H7 Tests should be

- fit for intended purpose
  - For *E. coli* O157:H7 detection methods, this means able to detect low levels of potentially injured cells
- used by a regulatory body or validated by a recognized independent body (e.g., AOAC, AFNOR, ISO, Microval, Nordval) or validated using a robust experimental design with results subject to FSIS review
- used under the validated conditions
- used in a laboratory that ensures the quality of the results

FSIS Issuances

Design of *E. coli* O157:H7 sampling and testing programs by Industry

- Directive 10,010.1 section IV.B "How IPP Verify Establishment Testing for *E. coli* O157:H7"
- Compliance Guideline for Sampling Beef Trimmings for *Escherichia coli* O157:H7 (Draft for Stakeholder Comment, August 12, 2008)
- Guidance for Small and Very Small Establishments on Sampling Beef Products for *Escherichia coli* O157:H7 (Draft for Stakeholder Comment, August 12, 2008)
- Sampling and Testing Procedures for *Escherichia coli* O157:H7 in Beef Manufacturing Trimmings, Federal Register Vol. 73 No 198 (October 10, 2008)
- Compliance Guidelines For Establishments On The FSIS Microbiological Testing Program And Other Verification Activities For *Escherichia coli* O157:H7 (April 14, 2004)

Other References

- FSIS Microbiology Laboratory Guidebook (MLG) chapter 5.05
- BIFSCo Best Practices for Using Microbiological Sampling (March 2008)
- ICMSF, *E. coli* O157:H7 in Frozen Ground Beef Patties *in* Microorganisms in Foods 7 (2002)

AskFSIS Q&As

Design of *E. coli* O157:H7 sampling and testing programs by Industry

Title	Last updated
AOAC approval and FSIS acceptability of micro methods	05/19/2009 10:14 AM
Specificity and Sensitivity Criterion for testing methods	05/19/2009 10:08 AM
micro method "fit for use" determination	05/19/2009 10:22 AM
Evaluation criteria for *E. coli* O157:H7 testing methods	05/21/2009 04:52 PM
Using different sample sizes than original AOAC validation for micro methods	05/19/2009 10:18 AM

AskFSIS Q&As
Design of E. coli O157:H7 sampling and testing programs by Industry

Title	Last updated
E. coli O157:H7 Test Portion Size	08/09/2010 08:46 AM
E. coli O157:H7 Test Portions Smaller than 325 grams	08/03/2010 01:09 AM
Confirming *E. coli* O157:H7 Screen Positive Test Results	04/26/2010 12:22 PM

FSIS Sample Size: RGB components and products

- Manufactured and bench trimmings (large pieces)
  - Projects MT 50, 51, 52, 53, 55
  - Each piece is 1x3 in-squared (1/8 th inch deep)
  - excised from original external surface
  - Sampled equally from combo bins composing lot
  - Combined sample weight (goal): ~¾ lb (~340 grams)
  - Companion sample (1 ¼ lb) collected

FSIS Sample Size: RGB components and products

- Other RGB components and bench trimmings (small pieces)
  - Projects MT 54 and 55
  - Collect 1 – 2 lbs (LTRB, AMR, head, heart, cheek weasand meat) or 2 lbs (small pieces of bench trimmings)
- RGB products
  - Projects MT 5, 6, 8, 43, 44, 44T
  - Collect 1 lb sample

FSIS Testing: RGB components and products

- Test portion is 325 gram 10% product
- Test portion range: 292.5 – 357.5 grams
- FSIS labs will test as 5 individual subs (65 grams per sub)
- FSIS labs will move to new procedure—single composite

FSIS Evaluation of Industry Testing

- OFO compares industry sampling and testing programs to FSIS programs
- Some industry programs may sample and test more frequently

E. coli O157:H7 Test Portions Smaller than 325 grams

- AskFSIS Q&A (last updated 08/03/2010 01:09 AM)
- There are circumstances where test portions smaller than 325 grams of RGB products would be acceptable:
  - all source materials are tested at N=60
  - multiple samples taken on a single production line in one day or half shift that add up to over 325 grams
  - each smaller test portion represents sampling at multiple points within the defined ground beef lot
  - (e.g., small pieces collected at 15 min intervals combined to 65 gram composite)

E. coli O157:H7 Test Portions Smaller than 325 grams NOT appropriate

- AskFSIS Q&A (last updated 08/09/2010 08:46 AM)
- Circumstances where test portions of 325 – 375 grams of RGB products would be a more appropriate choice:
  - large N pieces in sampling plan
    - If 65 grams of an typical N60 sample is tested, only ~17% of sample is represented
    - Violation of the assumptions of the statistical sampling plan
  - investigative or follow-up testing to determine the extent of contamination and to prevent release of adulterated product following a contamination event

Advantages of larger test portions

- Accommodate all material represented by sample
  - Discussed in AskFSIS Q&A and Draft Guidelines
  - Avoid violating assumptions of statistical sampling plan
  - Greater quantity of material per “N”
    - N represented by 0.42 grams for 25 gram test portion, or 6.25 grams for 375 gram test portion.
  - Lower theoretical LOD:
    - Test with LOD 1 per 25 grams (0.04 CFU/g) could be capable of detecting 1 CFU in 325 grams (0.003 CFU/g)

Disadvantages of larger test portions

- More work for labs
  - Need to have equipment to deal with larger mass and volumes
- Most methods validated for 25 gram test portions
  - *E. coli* O157:H7 enrichment from larger test portions may be slowed (compared to 25 grams)
  - Expectation that methods can detect low levels of potentially injured organisms (not proportional to test portion)
  - Need to re-validate for larger test portions (65, 75, 325, 375 grams)

Take-homes

- Industry samples and tests RGB components or product for *E. coli* O157:H7 for several reasons
- Sampling and testing are different activities
- Assumptions of statistical sampling plans may be violated if the entire sample is not tested by the lab
- OFO uses FSIS sampling and testing procedures as a benchmark to evaluate industry procedures
- Some establishments sample more frequently than FSIS
- In some circumstances, samples less than 325 grams may be an appropriate choice by an establishment

  (see Ask FSIS Q&A)
- Analytical methods for larger test portions should be validated by the lab, or the test kit manufacturer