MICROBIOLOGICAL ANALYSIS OF SPICES

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Outline

- Sampling
- Methods of Analysis
  - Quantitative
  - Qualitative
    - Culture Based
    - Genetic Based
- Further Characterization
- Moisture Content

Sampling

SAMPLE CONDITION IS *EXTREMELY* IMPORTANT

- **Improperly collected samples = meaningless laboratory results**

- **Chapter 1: Food Sampling/Preparation of Sample Homogenate; FDA Bacteriological Analytical Manual**
- http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063335.htm

SAMPLE COLLECTION

- Whenever possible, collect samples in original containers
  - Retail Package
  - milk carton
  - can or bottle

SAMPLE COLLECTION

- When original containers cannot be collected (bulk items)
- whenever possible, obtain at least 100 g (4 ounces) for each sample unit

SAMPLE DELIVERY TO THE LABORATORY

- foods that are not perishable and are collected at ambient temperatures need not be refrigerated
- minimize transport time (<24 hours)

SAMPLING PLANS

- **Sampling plans:**
  - **A representative sample is essential**
  - **The number of units that comprise a representative sample from a designated lot of a food product must be statistically significant**

SAMPLING PLANS

- Sampling plan depends on:
- 1) the sensitivity of the consumer group
- 2) the possibility that the food may have undergone a step lethal to *Salmonella* during the manufacturing process or in the home
- 3) The history of the food (whether there was a past history of contamination)

SAMPLING PLANS - SALMONELLA

- **Food Categories**
- **Food Category I. - 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.**

SAMPLING PLANS - SALMONELLA

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

- FDA considers spices to be category 2

SAMPLING PLANS - SALMONELLA

- **Food Category III. - Foods that would normally be subjected to a process lethal to *Salmonella* between the time of sampling and consumption.**

SAMPLING PLANS - SALMONELLA

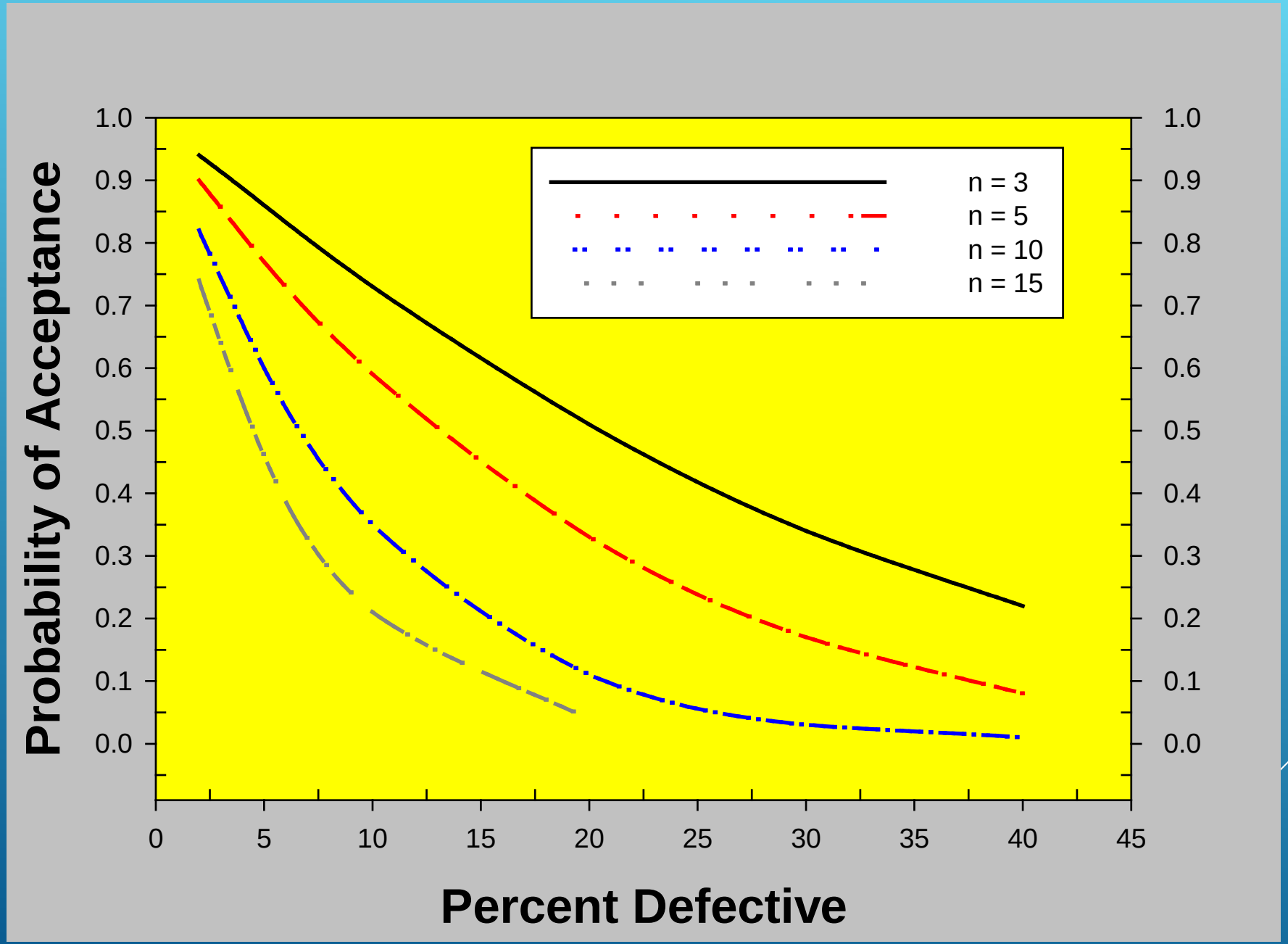
- Number of samples based on category
  - Food Category I - 60 sample units
  - ***Food Category II - 30 sample units***
  - Food Category III - 15 sample units
- In more recent FDA surveys, they have taken 2 groups of 30 samples each (60 samples total)

SAMPLING PLANS AND PROBABILITY

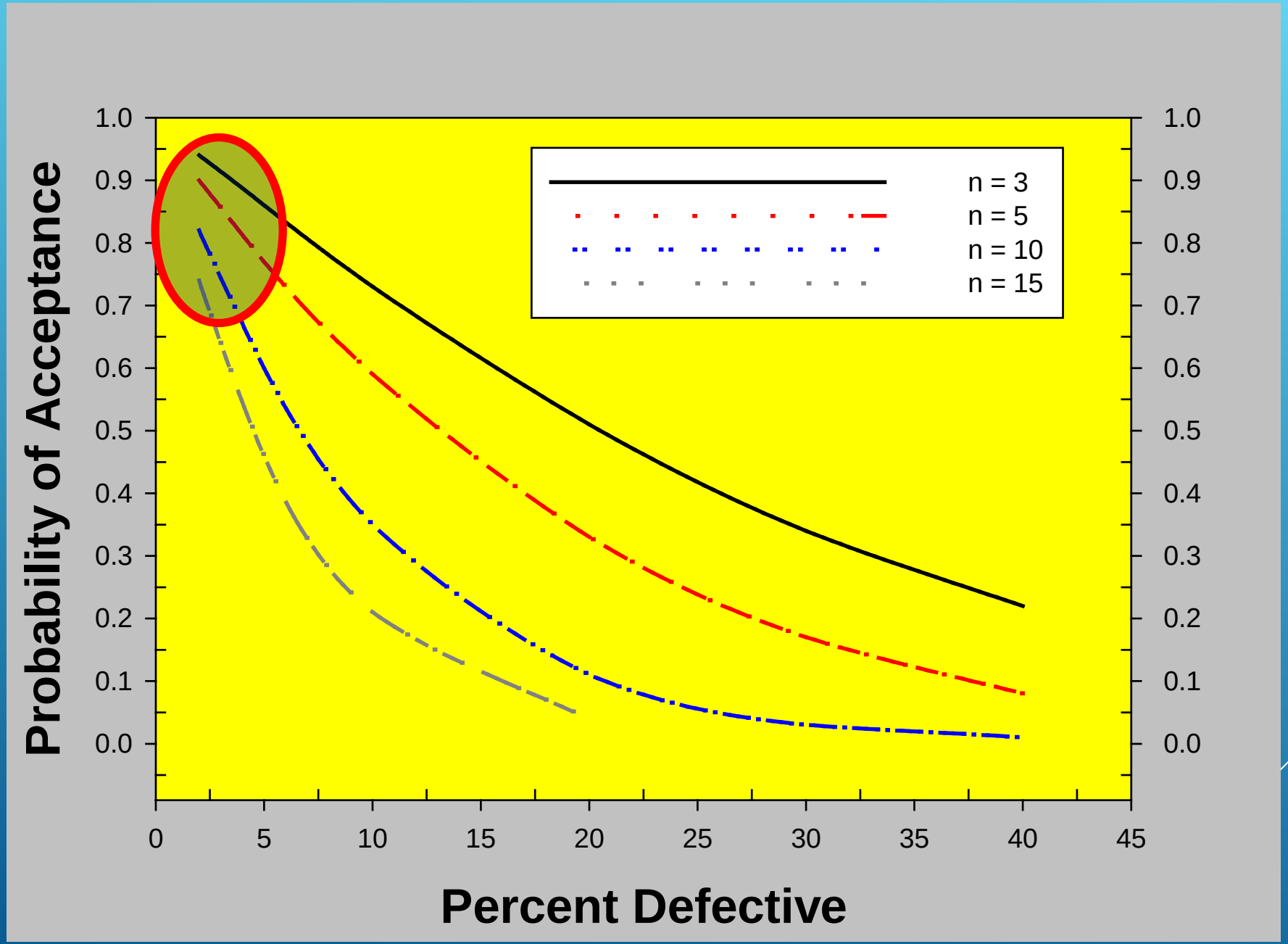
- Operating Characteristic Curve
- Probability of accepting a defective lot
  - Based on number of samples
  - Percent defective

- Military Standard 105 (Mil-Std 105)
- ANSI/ASQC Z1.4

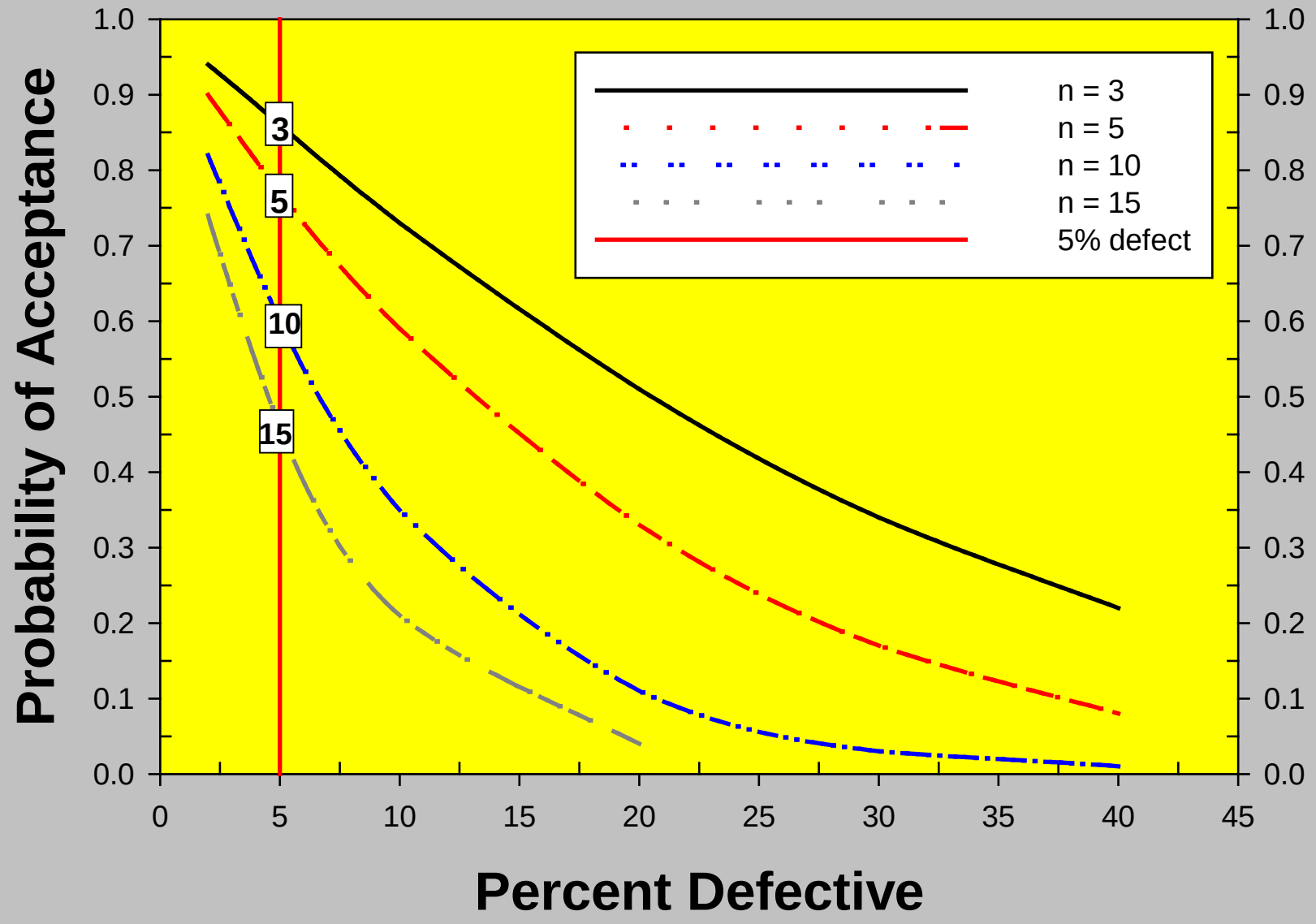
OPERATING CHARACTERISTIC CURVES



OPERATING CHARACTERISTIC CURVES



OPERATING CHARACTERISTIC CURVES



SAMPLING AND TESTING?

Row #	Expected Prevalence[1]	Number available samples from batch	Number samples taken from a batch[2]	Test Sensitivity	Pr. of NOT detecting
1	0.30%	8,000	1	90%	99.7%
2	0.30%	8,000	1	98%	99.7%
3	0.30%	8,000	1	99%	99.7%
4	0.30%	8,000	10	98%	97.1%
5	0.30%	8,000	100	98%	74.5%
6	0.30%	8,000	200	98%	55.5%

TWO AND THREE CLASS SAMPLING PLANS

- Two Class –
  - Presence or absence of a specific attribute
  - Typically used for Salmonella
- Three Class
  - Three categories: acceptable, marginal and unacceptable
  - Typically used for quality attributes

METHODS OF ANALYSIS

METHODS TO DETECT MICROORGANISMS IN FOODS

- **Quantitative - some regulatory standards are based on quantitative measures (e.g. population of bacteria allowed in raw milk; *E. coli* Biotype I on animal carcasses)**

- **Qualitative - some regulatory standards are based on qualitative measures (e.g. presence/absence of Salmonella in spices)**

QUANTITATIVE

- Often related to quality
- Total aerobic population
  - Pre-operational swabs
  - Shelf life determination
  - Overall Hygiene of food
- Coliforms or Enterobacteriaceae
  - Hygiene
  - Process Control Indicator

QUANTITATIVE

- Pathogens
  - Staphylococcus aureus
  - Clostridium perfringens
  - Bacillus cereus

- May be used for other pathogens during investigations (salmonella, E coli)

QUANTITATIVE

- Direct plating
- Direct microscopic count
- Most Probable Number

QUANTITATIVE

- Detection Limits
  - how many cells/ml or g can be detected
  - Function of sample size and dilution

- Sensitivity
  - proportion of population which are correctly identified
  - Selective media

DIRECT PLATING

- Principles of the technique

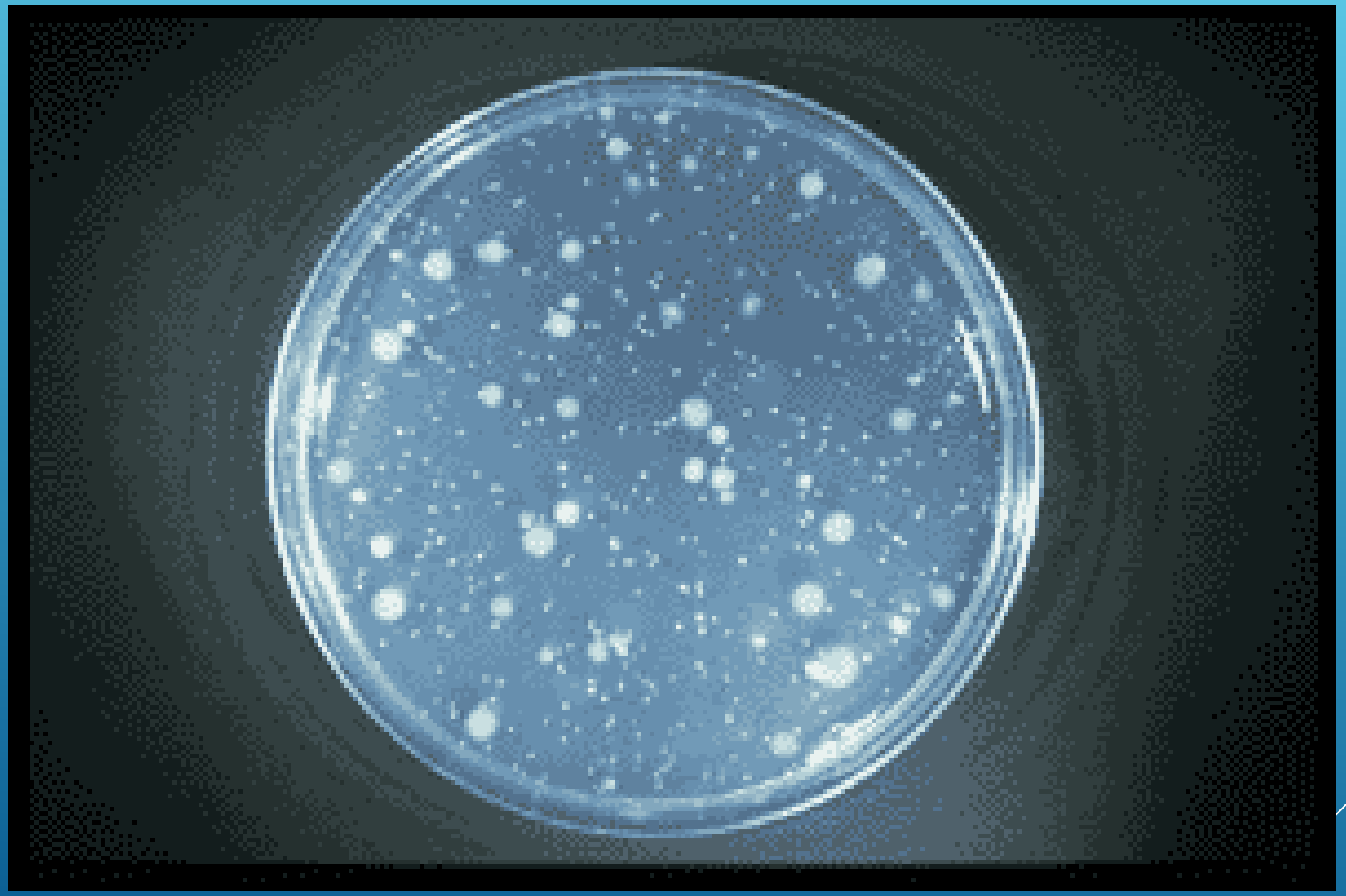
1. samples are homogenized and serially diluted
2. dilutions are plated on to a specified agar and incubated at a specific temperature and time
3. bacterial populations in the original sample are calculated by the population on the plates and the dilution factor

DIRECT PLATING

- Principles of the technique

4) The actual population enumerated depends on the media, the incubation temperature and the incubation time
5) Analysis can be used to enumerate specific groups of microorganisms
6) Time consuming; typical incubations 24 – 72 h

DIRECT PLATING



- http://www.biotec.com/images/r2amed.gif

THREE CLASS ATTRIBUTE

- Based on two criteria: a marginal (m) and a Defect (M) level
  - n = Number of samples
  - c = number of samples allowed to fail marginal criteria (m)
  - M = any sample exceeding M indicates a defective lot

- Example: Irradiate Spice – aerobic bacteria
  - n = 5, c = 1, m = 1,000 M = 10,000

Qualitative Methods of Analysis

- Culture Based
- Genetic Based

QUALITATIVE ANALYSIS

- Usually pathogen
  - Salmonella
  - Pathogenic E coli
  - Listeria
- Food Samples
- Environmental Samples

QUALITATIVE ANALYSIS

- Culture Based – Conventional Bacteriology
- ELISA – enzyme linked immuno- sorbent assay
- PCR

QUALITATIVE ANALYSIS

- **Sensitivity - proportion of actual positives which are correctly identified**
- **Specificity - the proportion of negatives which are correctly identified**
- **Detection Limits - how many cells/ml or g can be detected**

QUALITATIVE ANALYSIS

- **False Positives – Analysis gives a positive result when the sample is truly negative**

- **False Negative – Analysis gives a negative result when the sample is truly positive**

QUALITATIVE ANALYSIS

False Positive

	“True” Positive	“True” Negative
Test Positive	19	6
Test Negative	2	23

False Negative

QUALITATIVE ANALYSIS

- **Food borne pathogens, when present, are in very low populations**
- **Typically have high background (non-target) microflora**
- **Current standard for *Salmonella* in spices: < 1 cell (“negative”) in 325 grams**

QUALITATIVE ANALYSIS

- Most food borne pathogen analyses include:

a) Non-selective enrichment (18 – 24 h)

b) Selective enrichment (18-24 h)

c) Detection (ELISA, PCR)

d) Confirmation (cultural)

ELISA

- Principles of the test

1. Samples are enriched
2. Sample is placed on a filter
3. Antibodies specific for the pathogen react with the bacteria and produce a color change

ELISA

http://www.biocompare.com/images/bc/006/ArticleImages/BioAss_Works_pro_9.jpg

PCR

- Principles of the test

1. Isolate DNA from the sample
2. Samples usually require enrichment
3. Analyze sample DNA with specific primers

4. Fluorescent dye generates a signal when sufficient number of DNA copies are present

PCR

1. requires an enriched sample
2. detection limit ~ 10^3 – 10^4 cfu/ml of target in enrichment broth

PCR

TWO CLASS ATTRIBUTE SAMPLING PLAN

- Based on an essential criterion, such as presence or absence of a pathogen (m)
  - n = Number of samples
  - c = number of samples allowed to fail criteria

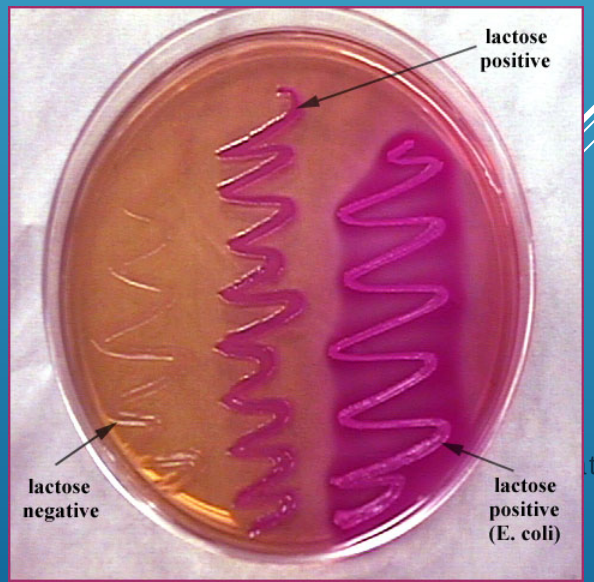
- Example: Spices
- n = 10, c = 0, m = positive in 325 g

FURTHER CHARACTERIZATION

ISOLATION AND IDENTIFICATION

- Conventional Bacteriology
  - Selective media
  - Biochemical reactions

www.mc.maricopa.edu/~johnson/labtools/Dbiochem/3mac.jpg



www.rapidmicrobiology.com/news/1054h29p.JPG

www.bacto.com.au/images/crystal_c.jpg

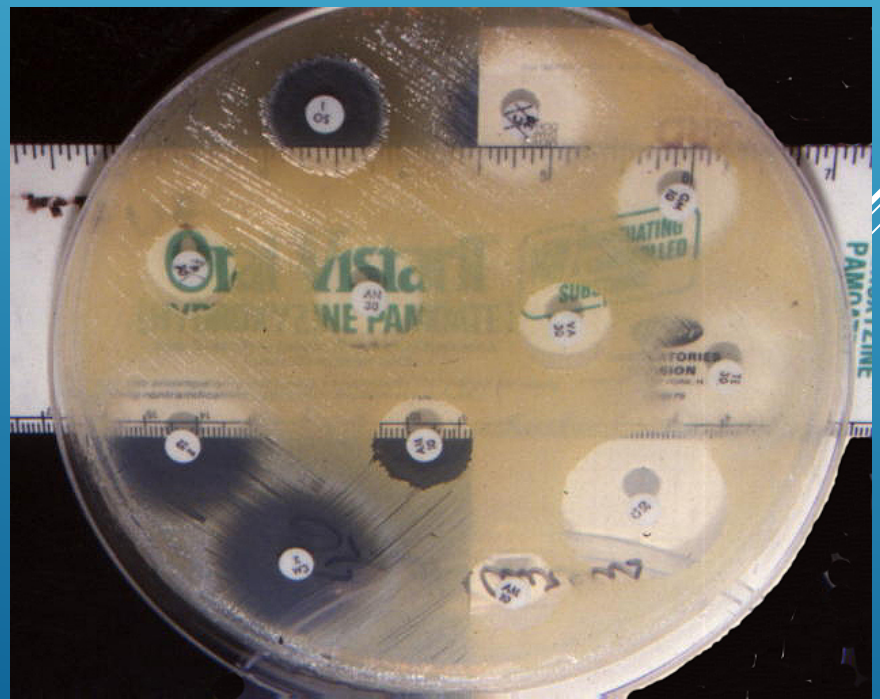
FURTHER CHARACTERIZATION

- Serotyping

- Antibiotic resistance profiles

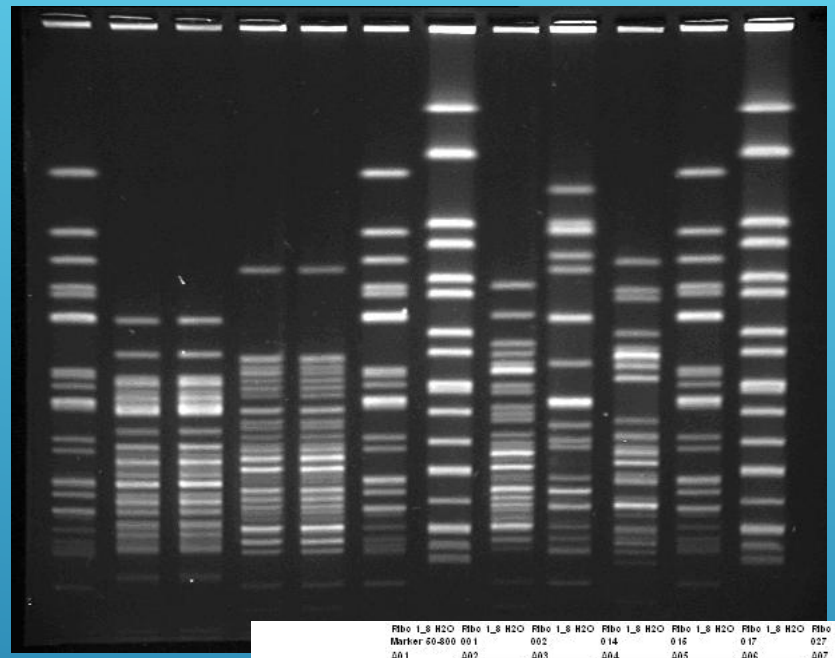
www.essum.se/images/gbs3.png

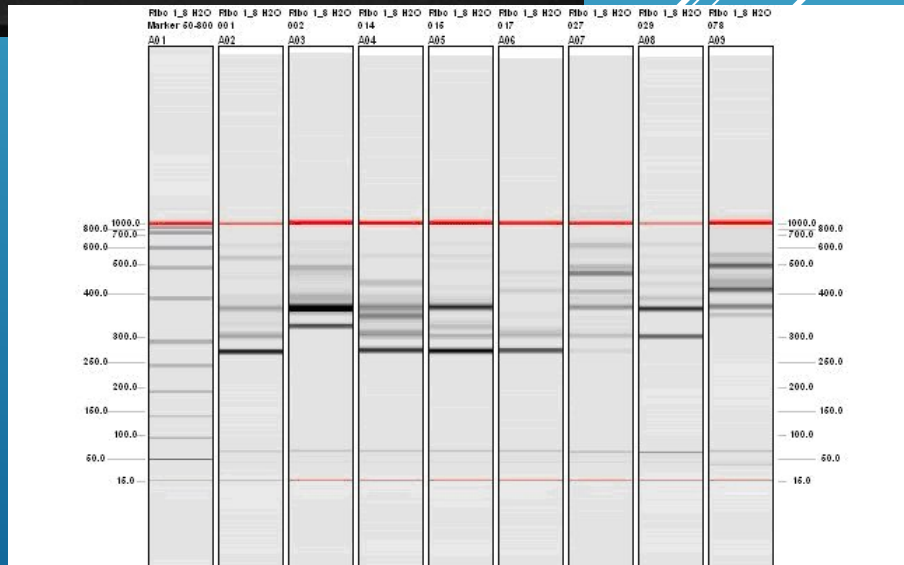
www.life.umd.edu/classroom/bsci424/Images/PathogenImages/AntibioticZonesofInhibition.jpg



GENETIC CHARACTERIZATION

- PFGE
- Multiple-Locus Variable Number Tandem Repeat Analysis (MLVN)
- Whole genome sequencing





OFFICIAL METHODS

- U.S. Food and Drug Administration Bacteriological Analytical Manual
  www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm

- USDA Food Safety and Inspection Service
  Microbiological Laboratory Guidebook
  www.fsis.usda.gov/science/microbiological_lab_guidebook/index.asp

OFFICIAL METHODS

- Association of Analytical Chemists (AOAC) International
  http://www.aoac.org/
- Official methods
- Performance Tested Methods

FOOD LABS REPORT FALSE NEGATIVES OF CAMPYLOBACTER 9 PERCENT OF THE TIME, STUDY FINDS

- 5/21/2013
- Food microbiology laboratories continue to submit false negative results and false positive results on a routine basis, according to a study of nearly 40,000 proficiency test results over the past 14.
- "***There is concern when laboratories report that pathogens are not found in a food sample, when in fact they are there***,". "This is known as a 'false negative'. Similar concerns arise when a laboratory reports a 'false positive' suggesting that pathogens are in the food sample, when indeed they are not."
- The study found that, on average, food laboratories ***report false negatives of 9.1 percent for Campylobacter and 4.9 percent for Salmonella. The false positive rate, on average, is 3.9 percent for Salmonella and 2.5 percent for both E. coli and Listeria.***

LABORATORY GUIDANCE

Establishment Guidance for the Selection of a Commercial or Private Microbiological Testing Laboratory

- http://www.fsis.usda.gov/PDF/Guidance_Selecting_Micro_Testing_Lab.pdf

USDA United States Department of Agriculture
Food Safety and Inspection Service

MOISTURE CONTENT

MOISTURE CONTENT

- Four Common Methods of Analysis

1) Karl Fischer method (titration)

   reagent is added; color change indicates moisture content

2) Organic solvent distillation

   condense the water and measure volume

MOISTURE CONTENT

- Four Common Methods of Analysis

3) **Physical Drying**

   Place sample in oven (may be under vacuum) and dry the sample. [May also lose volatile components which skews the results]

4) **Capacitance**

   Instrument measure; passes an electrical current through a sample. [particle size may cause erroneous results]

MOISTURE CONTENT

- Have to balance method of analysis with benefits and liabilities
- Primarily speed vs. accuracy

MICROBIOLOGY OF SPICES

- Summary

1) Microbiology of Spices
    - Basic Microbiology
    - Microbiology of Spices
2) Mitigation Processes
3) Microbiological Analysis

QUESTIONS?