Microbiological Testing of Foods

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Importance of detecting microorganisms in food

- Investigating outbreaks of foodborne disease
- Assessing the safety of the product to consumers.
- Assessing the stability or shelf life of the product under normal storage conditions.
- Determining the level of sanitation during product preparation.
- Regulatory compliance
- Incidence surveys for pathogens

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Bacterial pathogens

- *E. coli* O157:H7
- *Salmonella*
- *L. monocytogenes*
- *S. aureus*

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Indicator or spoilage microorganisms

- Aerobic/anaerobic plate counts
- Coliforms
- *E. coli*, yeast & mold counts
- Psychrotrophs

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Toxins and microbial metabolites

- *Bacillus cereus* enterotoxin
- *Clostridium perfringens* toxin
- *E. coli* O157:H7 enterotoxin
- Staphylococcal enterotoxin
- Aflatoxins and Fumonisin

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Bacteriological detection methods

Direct enumeration(Microscopic count)
(Colony Forming Unit (CFU) count)

- Non-selective media
- Non-selective differential media
- Selective media
- Selective differential media

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Bacteriological detection methods

Indirect Determination

- Most Probable Number Method (MPN)
- Enumeration of Injured Cells by Selective Media Overlay Method
- Thin Agar Layer Method

Bacteriological detection methods

Pathogen Isolation

- Sample does or does not contain microorganism of interest
- Pre-enrichment step
- Selective enrichment step
- Testing on medium containing selective and/or differential agents

Testing for bacterial toxins

- Agglutination
- Radioimmunoassay (RIA)
- Enzyme Linked Immunosorbent assay (ELISA)
- Enzyme Linked Fluorescent Inmmunoassay (ELFA)

Regulatory compliance testing

- *USDA-FSIS "Mega-Reg" Testing*
- Meat and poultry slaughter plant and raw ground products processing facilities are required to test for generic *E. coli* and *Salmonella* under the provisions of the HACCP program or Pathogen Reduction Final Rule.
- Quantitative testing for generic *E. coli*
- Qualitative testing for *Salmonella*

Regulatory compliance testing

- FDA: Seafood or other food products
- Examples include microbial analysis for spoilage microorganisms or pathogens in seafood or cheese.
- State Dairy Testing
- Pasteurized Milk Ordinance (PMO)
- These tests relate to the quality of various dairy products.
- Microbial testing and analysis include coliform counts, standard plate counts (SPC).

Testing considerations

- Selection of sampling techniques
- Selection of sampling kits
- Use of AOAC-approved methods

Testing methods

- Standard Methods for the Examination of Dairy Products
- Standard Methods for the Examination of Water and Wastewater
- Standard Methods for the Examination of Seawater and Shellfish
- Compendium of Methods for the Microbiological Examination of Food
- Bacteriological Analytical Manual of Food and Drug Administration

ISO 17025

- General Requirements for the Competence of Testing and Calibration Laboratories
- For international benchmark for approving the competence of the testing and calibration
- ISO 17025 allows laboratories to carry out procedures in their own ways, but an auditor may require the laboratory to justify using a particular method
- ISO/IEC 17025 is divided into two principal parts:
- Management requirements
- Technical requirements

ISO 17025 Management requirements include paragraphs on

- **Organization and management**
- **Quality system**
- **Document control**
- **Review of request**
- **Subcontracting of tests and calibrations**
- **Purchasing services and supplies**
- **Service to the client**
- **Complaints**
- **Control of non-conformity testing**
- **Corrective action**
- **Preventive action**
- **Records**
- **Internal audits**
- **Management reviews**
- **Technical Requirements**

ISO 17025 Technical requirements include paragraphs with much detail on

- **General**
- **Personnel**
- **Accommodation and environmental conditions**
- **Test and calibration methods including sampling**
  This includes requirements for method validation (laboratory developed, non-standardized, standardized but used outside of their intended range) and measurement uncertainty
- **Equipment**
- **Measurement trace ability**
- **Sampling**
- **Handling and transportation of test and calibration items**
- **Assuring the quality of test and calibration results**
- **Assuring the quality of test and calibration results**

Microbiological uncertainty

- It means a method used to estimate the uncertainty associated with model inputs, assumptions and structure/form
- Many microbiological laboratories have had procedures available for monitoring variability in duplicate results generated by laboratory analysts for some time
- Studies and more complex statistical calculations

Viruses and parasites — how are they "different"?

- Cannot multiply other than in specific, living host cells (rare exception with *Giardia*)
- Cannot multiply in food (no toxins or other metabolites) — either remains infectious or not
- Cannot be enriched for testing
- Usually, qualitative testing at the limit of sensitivity
- Subjectivity problems

Sensitivity = concentration method + detection method

- Concentration: start with serving-size sample of food or water?
- Drinking water samples often 10–100 liters
- Solid food samples can't be concentrated — separate agent from food solids into liquid phase
- Virus (~30 nm) concentration: adsorption-elution, precipitation, or brute force
- Concentrating protozoan cysts-oocysts (4–20 μm [larger than bacteria]): filtration, centrifugation (to bottom of tube or onto "cushion")
- Immunomagnetic capture

Detection

- Viruses: susceptible hosts unavailable — "molecular" methods used
- Most viruses RNA only — reverse transcription (RT) required for PCR
- Both RT and PCR are very susceptible to interference by substances in environmental samples; real-time PCR and nucleic acid sequence-based amplification (NASBA)
- PCR product analysis: gel electrophoresis; biosensors; verification; sequencing
- Protozoa: larger than bacteria, so microscopy is an option
- Staining, fluorescent or otherwise
- Immunofluorescent techniques
- PCR (multiple chromosomes)

Specificity absence of false positives

- Detecting only the target organism
- What if a "broad-spectrum" test is wanted?
- Detection of noninfectious (inactivated) agent = false positive?
- False positives from noninfectious viruses — look for alternations in the virus that
- Accompany inactivation (RNase sensitivity)
- False positives from noninfectious protozoa — excystation PCR for *Cryptosporidium*; in vitro culture of *Giardia*

Overview

- Methods for microbiological testing of foods are limited by sampling — spoilage
- Organisms and some indicators may be fairly homogeneously distributed, but pathogens are typically "spotty" in distribution and present at relatively low levels
- Because of distribution and sampling problems, sensitivity (false negatives) and specificity (false positives) present continuing challenges

Overview

- The key to detection of bacterial pathogens is usually enrichment (which is not an option with viruses and protozoa), detection and enumeration media may be selective, differential, both, or neither.
- Bacterial toxins are usually detected by some adaptation of serology
- With viruses and protozoa, sample processing and concentration, as well as a sensitive final detection method, are necessary to a satisfactory outcome, and problems of false positives with noninfectious contaminants remain.

Thank you !