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

Bacterial pathogens

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

Indicator or spoilage microorganisms

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

Toxins and microbial metabolites

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

Bacteriological detection methods

Direct enumeration (Microscopic count)
- (Colony Forming Unit (CFU) count)
- Non-selective media
- Non-selective differential media
- Selective media
- Selective differential media
Bacteriological detection methods

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

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 Immunoassay (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

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
- Records

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 traceability
- 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
- Microscopy, 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

Thank you!