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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

Sampling and testing of foods

- **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
- **Culture identification**
  Gram positive vs. Gram negative
  Rod, cocci, or spiral shaped
  Yeast or mold
  E. coli serotype (O and H)
- **Toxins and microbial metabolites**
  Bacillus cereus enterotoxin
  Clostridium perfringens toxin
  E. coli O157:H7 enterotoxin
  Staphylococcal enterotoxin
  Aflatoxins and Fumonisin

Bacteriological detection methods

- **Quantitative**: Enumerate or estimate directly or indirectly the bacterial load in the product.
  - *Direct enumeration*
  ☺ Microscopic count
  ☺ Colony Forming Unit (CFU) count
    ✳ Non-selective media
    ✳ Non-selective differential media
    ✳ Selective media
    ✳ Selective differential media
  - *Indirect Determination*
  ☺ Most Probable Number Method (MPN)
  ☺ Enumeration of Injured Cells by Selective Media
    ✳ Overlay Method

✳ Thin Agar Layer Method

- **Qualitative**: Determine the possible presence of certain microorganisms (mostly bacteria) or foodborne pathogens in the food.
  - *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*
- *FDA Import / Detention Testing*
  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

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 remain infectious or not
- Cannot be enriched for testing
- Usually, qualitative testing at the limit of sensitivity
- Subjectivity problems

Sample availability from outbreaks — incubation period of illness; shelf life of food vehicle; durability of agent

Sensitivity

- Absence of false negatives
- Sensitivity goals related to peroral infectious (pathogenic) dose?

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.
- 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.

Selected references

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