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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

ISO 17025

ISO/IEC has just published a standard, General Requirements for the Competence of Testing and Calibration Laboratories**.** The standard was first published in 1999, and a new version was

published in 2005, as an international benchmark for approving the competence of the testing and calibration laboratories that play a vital role in trade, in product development and manufacturing, and in protection of the consumer. 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. The modifications relate mainly to the management requirements in order to harmonize with the quality management standard ISO 9001:2000 in laboratories.ISO/IEC 17025 is divided into two principal parts:

- Management requirements
- Technical requirements

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

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

This refers to a method used to estimate the uncertainty associated with model inputs, assumptions and structure/form. Each qualified laboratory wishing to comply with the

requirements of ISO/IEC 17025:1999 needs to estimate uncertainty of measurement for their quantitative methods. Many microbiological laboratories have had procedures available for monitoring variability in duplicate results generated by laboratory analysts for some time. These procedures, however, do not necessarily include all possible contributions to uncertainty in the calculations. Procedures for estimating microbiological method uncertainty, based on the Poisson distribution, have been published; but, at times, the procedures can either underestimate uncertainty or require laboratories to undertake considerable experimental 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

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.

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