Challenges in the Food Industry

Nuno Reis

Workshop on Microbial Safety in Food Industry Using Rapid Methods
Presentation Overview

- Traditional Microbiology – Rapid Microbiology?

- Quality Indicators – Pathogen Detection

- Standard Methods & Criteria

- What is an Alternative Method
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Traditional Microbiology

- Introduced by Robert Koch (1843-1910) & Julius Richard Petri (1852-1921)

- 2-14 days of incubation

- Growth phase: 2 - 14 days

- Single cell

- Colony
Traditional Microbiology

- Most commonly used worldwide

- Traditional microbiology results are based on plate count methods

- It takes several days until results are available
  - Slow
  - Delays released finished products & ingredients; Delays response to env monitoring program data
  - Results vary with microbial population, media & conditions – can yield false negatives or positives with a large measurement of uncertainty
  - Labor intensive

- Products need to be kept in quarantine until micro results are available

- Raw materials cannot be used for production before Micro Lab delivers results
Food Industry – Real Situation

- Both internal & external pressure to:
  - Detect and react quickly to out-of-spec results
  - Fulfill customer orders at periods of high demand
  - Reduce inventory and improve cash flow
  - Confidently release manufacturing process

- The use of rapid microbiological methods
  - To improve quality and help prevent process “derailments” by providing early information of problems

- An excellent concept…but may be difficult to choose.
Rapid Microbiology

- Food Industry indeed needs Rapid Methods

- Rapid Microbiology
  - Which method?
  - On which criteria do I release products?
    - Quality Indicators (TPC, Y&M) or Pathogens (Listeria, Salmonella…)?

- REGULATION
- RISK ASSESSMENT OF MICROBES
- INADEQUATE TRAINED STAFF
- PROBLEMS IN THE FOOD INDUSTRY

- Different sort of food products
- Different microorganisms targeted
  - Often present in low numbers
  - Background flora; stressed or injured cells
- Different approaches
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Desserts, yogurts, soups, fruit juices, beverages…

Microbiological risks:
- Products spoiled by TPC, Yeasts or Fungi

Consequences:
- Blowing packs, off odors & flavors, visual deterioration

Release on:
- TPC or Y&M

Processed meats, fish, delicatessen, minced meat, cheese, elaborated meats…

Microbiological risks:
- Pathogen contamination (Listeria monocytogenes, Salmonella, E.coli O157:H7

Consequences:
- Public Health Risks…

Release on:
- Pathogens
Microbiology in the Food Industry

Focusing on Microbiology

- Pathogens
- Quality Indicators/Enumeration
- Bacterial Identification
- Microbial Genotyping/Strain-typing
- Environmental Control
- Microbial detection/Sterility testing

Available Methods: Rapid or Classical methods in compliance with ISO, FDA BAM, AOAC and other official compendia

- Classical methods = media (reference methods)
Microbiology in the Food Industry

Pathogens
- Presence/absence (positive/negative)
  - *Salmonella*
  - *Listeria spp & Listeria monocytogenes*
  - *E.coli O157:H7*
  - *Campylobacter*
  - Staphylococcal enterotoxins
  - *Bacillus cereus*
  - *Staphylococcus aureus*
  - *Pseudomonas aeruginosa*
  - *Shigella*
  - *Vibrio*
  - *Yersinia*
  - *Cronobacter sakazakii*
    - PIF: Powder Infant Formula
Microbiology in the Food Industry

Quality Indicators/Enumeration
  - Spoilage organisms (counts)
  - Microbial limits (cfu/g)

- Total aerobic plate count
- Enterobacteriaceae
- Coliforms
- E.coli
- Lactic acid bacteria
- Staphylococcus
- Yeast & Mold
- Campylobacter
- Clostridium perfringens
- Pseudomonas aeruginosa
- Listeria monocytogenes

Reference Methods: plating & MPN (Most Probably Number)
Microbiology in the Food Industry

**Bacterial Identification**
- All positive results (pathogen testing: presence/absence) must be confirmed according to ISO and FDA BAM reference methods.
  - Manual
    - Biochemical tests
    - API strips – “Gold Standard”
  - Automated
    - Gram-negative
    - Gram-positive
    - Yeast
    - Bacillus
    - Anaerobic
    - Corynebacterium

**Microbial Genotyping/ Strain-typing**
- PFGE (Pulse Field Gel Electrophoresis)
  - Reference Method / “Gold Standard”
- Contamination source tracking
- Typing cultures used in fermentation (breweries)
- Strain comparisons
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Pathogen Detection

**Reference Method / Microbiological criteria**

**Standardization:** methods, guides or rules elaborated by independent organisations (e.g., in food microbiology)

- Of Methods = Norms = Text of reference = Reference Methods = Standard Methods
- By standardization bodies (ISO, FDA BAM, AOAC, AFNOR Normalisation,...)
Standardization bodies and related Organizations

- International Organization for Standardization (ISO)
  - ISO TC34/SC9: structure in charge of Food microbiology

- European Committee for Standardization (CEN)
  - EN TC275/WG6: structure in charge of Food microbiology

- National Committees in European states (AFNOR Normalisation, BSI, DIN, UNI, AENOR, IBN, NEN, SNV…)
  - Endorse most ISO Standards as National Standards
  - Mandatorily endorse CEN Standards as National Standards

- 15 countries (European + Australia, Egypt, Thailand…) participate actively to ISO TC34/SC9

- Related organizations:
  - US FDA BAM, USDA MLG
  - Standard Method for Examination of dairy products (US/APHA, IDF,…)

Examples:

- **In food microbiology: Method of Analysis (Salmonella)**
  - ISO 6579:2002 “Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Salmonella* spp.”

- **In food microbiology: Method of Analysis (Listeria)**
  - ISO 11290 “Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of *Listeria monocytogenes*”
ISO Standards in food microbiology

*Salmonella*: Regulatory aspects/criteria

- **AFNOR area: European criteria**
  - Cf Dir 2073/2005
  - Absence in 25g or 10g sample
  - Detection with ISO 6579 or validated method
  - Preparation of samples: ISO 6887 standards

- **Food products**:
  - Meat: ground beef, poultry, …
  - Meat products ready-to-eat,
  - Cheese, butter,
  - Dairy products prepared with raw milk or pasteurized milk, …
  - Milk powder and whey powder,
  - Ice cream,
  - Egg products,
  - Cooked seafood,
  - Sprouts, Fruits, vegetables, Milk powder for babies…
Salmonella: ISO 6579 : 2002

X g into 10X mL BPW

16-20 hrs @ 37±1°C

0.1 mL into 10 mL Rappaport Vassiliadis Soya broth
21-27hrs @ 41,5 ±1 °C

1 mL into 10 mL MKTTn broth
21-27 hrs @ 37±1 °C

Other selective media
Other selective media

Manufacturer's recommendations
Manufacturer's recommendations

Pick up 1 to 5 colonies

Nutrient agar
21-27 hrs @ 37±1 °C

Biochemical confirmation Serological confirmation
ISO Standards in food microbiology

*Listeria monocytogenes*: Regulatory aspects

- **ISO area**: European criteria
  - Absence in 25g sample
  - Mandatory notification
  - Tolerance for some kinds of samples at the end of shelf-life (criteria = 100 cfu/g)
  - Detection with ISO 11290-1 or validated methods and enumeration with ISO 11290-2
# Microbiological criteria in EU

## Criteria for *Listeria monocytogenes*

<table>
<thead>
<tr>
<th>Foodstuffs</th>
<th>Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ready to eat food intended for infants and ready to eat foods for special medical purposes.</td>
<td>Absence in 25 g</td>
</tr>
<tr>
<td>Ready to eat food able to support the growth of <em>L. monocytogenes</em>, other than those intended for infants and for special medical purposes</td>
<td>Absence in 25 g&lt;br&gt;And (&lt;100) ufc/g during shelf life</td>
</tr>
<tr>
<td>Ready to eat foods unable to support the growth of <em>L. monocytogenes</em>, other than those intended for infants and for special medical purposes (*)</td>
<td>(&lt;100) ufc/g during shelf life</td>
</tr>
</tbody>
</table>

(*) : products unable to support the growth of L mono : acid products (ph \(<4.4\)), dehydrated products (aw \(<0.92\)) or products with shelf life \(<5\) days.
L. monocytogenes detection
ISO 11290-1

25 gr into 225mL
Half Fraser broth

22-26 hrs @ 30±2°C

0.1 mL into 10 mL
Fraser broth

46-50 hrs @ 35 or 37°C

Ottaviani Agosti Agar

24 hrs @ 37°C

1st reading

Additional
24 hrs @ 37°C

2nd reading
(if necessary)

Other selective media

Manufacturer’s recommendations

Confirmation
Microbiological criteria in EU

Criteria for pathogens

**Cronobacter sakazakii:**
- Absence in 30 x 10 g for « dried infant formula and dried dietary foods for special medical purposes intended for infant below 6 months of age ».

**Enterotoxins of *Staphylococci***:
- Absence in 25 g for cheeses, powder milk, whey powder when the result in coagulase positive *Staphylococci* is $> 10^5$ cfu/g.
Relation of ISO Standard in EU & Codex microbiological criteria

**Codex Alimentarius**

- The *international food standards setting body recognized by World Trade Agreements as being the reference point for food standards applied in international trade.*

- **Main objective:** to protect the health of consumers and ensure fair practices in the *international food trade.*

**Codex establishes contact with ISO to obtain information on the current status of food safety-related work within ISO to present findings to the Executive Committee, relevant to Codex work.**
Standardization: Regulatory (US)

- **USDA/FSIS Microbiology Laboratory Guidebook (MLG)**
  - United States Department of Agriculture / Food Safety and Inspection Service
  - Methods for Meat, Poultry and egg products

- **FDA/CFSAN Bacteriological Analytical Method (BAM)**
  - US Department of Health and Human service Food and Drug Administration / Center for Food Safety and Applied Nutrition
  - Methods for other food products
Example of Microbiological criteria in the US

Information are spread in different regulatory organization:

- **USDA / MLG for poultry, eggs and meat food**
  e.g.: limit of detection for the method MLG 4.04 Salmonella: less than 1 CFU/g in a 25 g sample

- **FDA / BAM for all for others products**
  e.g.: Zero tolerance policy of no detectable L mono in 2 x 25g analytical portions of food or beverage

- **Compendium**
  e.g.: Compendium of fish and Fishery Product Process, Hazard control
  e.g.: Aerobic Plate Count (ACT) Dried and frozen whole egg maximum of 50000 ACP/g

- **Codex alimentarius committee**
  e.g.: determined criteria for pathogenic microorganisms or powdered Infant Formulae, formula for special medical purposes and human milk fortifiers, E sakasaki 0/10 g and Salmonella 0/25g
  e.g.: determined criteria for process hygiene:
  - Mesophilic aerobic bacteria: m= 500/g and M=5000/g
  - Enterobacteriaceae 0 /10g
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- What is an Alternative Method
2 kinds of Methods are recognized:

1. Reference method
2. Alternative validated methods

Europe:
- Reference method = ISO
- Alternative methods must be validated according to ISO 16140 and certified

USA:
- Reference method = FDA BAM and USDA MLG
- Alternative methods must be validated by AOAC
What is an alternative method?

For a given scope, it shall estimate the same analyte than one measured by the corresponding reference method but which can also present some additional characteristics such as:

- Easier to perform / more automated than the reference method
- Shorter “time-to-result”
- Good or better performances (i.e. sensitivity, etc…)

Commercial method adapted to the requirements of an industrial production
Alternative Methods in EU/USA/some Asian countries

- AFNOR (Association Francaise de Normalisation)
- AOAC (Association of Official Analytical Chemists) International
- EMMAS Assessment (UK)
- Health Protection Branch of Canada
- DIN Committee (Germany)
- Chinese Government (SN & GB)
- …
Territorial Acceptability of Alternative Methods

3 different approaches depending on countries:

- No specific requirement
- Specific registration (i.e. India, Russia, Brazil, Mexico, China…)
- Third party validation and bodies in charge:
  - USA & others (AOAC International / AOAC RI)
  - Canada (MFHPBP Sante Canada)
  - Europe: AFNOR, Microval, NordVAL (ISO 16140)
Validation of Alternative Methods

**Objective:** To demonstrate the equivalence between the alternative method and the reference method

**According to International rules (ISO 16140):**
- AFNOR Certification (Validation)
- Microval
- NordVal

**According to other rules:**
- AOAC
- Health Canada
ISO 16140 & Validated Alternative Methods

ISO 16140:2003 (Microbiology of food and animal feeding stuffs – Protocol for the Validation of alternative methods)

Objective: To demonstrate the equivalence between the alternative method and the reference method

- Perform the validation as described (i.e. in EN ISO 16140)
- Manufacturer shall apply a quality system covering the production line of the product for which the certification is sought (i.e. ISO 9001, ISO 13485)
- Regular verification of the quality of certified method (regular audits, updated documentation…)

Salmonella: ISO vs. Alternative method (VIDAS)

X g into 10X mL BPW

0.1 mL into 10 mL Rappaport Vassiliadis Soya broth
21-27 hrs @ 41.5 ± 1°C

Other selective media + XLD
16-20 hrs @ 37 ± 1°C

1 mL into 10 mL MKTTn broth
21-27 hrs @ 37 ± 1°C

Other selective media + XLD

Food & envt.
225 ml of BPW + supplement (green)
41.5 +/- 1°C
18-24 h

Heat & Go 5 min*
*Except for poultry & egg products

Manufacturer's recommendations

21-27 hrs @ 37 ± 1°C reading
Pick up 1 to 5 colonies

Nutrient agar
21-27 hrs @ 37 ± 1°C

Biochemical confirmation
Serological confirmation

VIDAS UP Salmonella
48 min
**Listeria monocytogenes: ISO vs. Alt Method (VIDAS)**

1. **25 g into 225 mL Half Fraser broth**
   - 22-26 hrs @ 30 ± 2°C

2. **0.1 mL into 10 mL Fraser broth**
   - 46-60 hrs @ 35 or 37°C

3. **Ottaviani Agosti Agar**
   - 1st reading
   - 2nd reading (if necessary)

4. **Other selective media**
   - 24 hrs @ 37°C
   - Additional 24 hrs @ 37°C

5. **Confirmation**
   - Manufacturer's recommendations

6. **25 g samples**
   - + 225 ml prewarmed LMX broth
   - + 0.5 ml LMX supplement

7. **26-30 hours @ 37 ± 1°C**

8. **250 µl / strip**
   - 5 min heating

9. **VIDAS LMX**
In Conclusion…

**Reference Methods: Standardization**
- ISO, US FDA BAM

**Microbiological criteria**
- Pathogens: zero tolerance
- Enumeration: Specification limits

**Alternative Validated Methods:**
- ISO 16140
- AOAC

**Reason:** simpler, faster, LOD, specificity, sensitivity & more economical
Industrial Applications

- **Importance of short time-to-result for product release**
  - Raw material release

- **Conversion from manual to automated methods**
  - Limit possible human errors

- **Cost savings**

- **International customers / harmonization of quality practices**
Rapid Methods: Cost Savings

- Media preparation
- Time allotted to dispensing enrichment in tubes
- Selective enrichment cost
- # of selective enrichments
- Number of broth tubes & plates
- Total transfer steps
  - Time per transfer
  - Total transfers/day
- Primary tube labeling time
- Cost of test tube

- Total media related prep & QC time invested
- Total transfer time
- Total dispensing time
- Total labeling time
- Total value of labor
- Faster time to result with rapid alternative methods
Automated alternative methods make it possible to improve food safety testing and enables significant gains in productivity.

Automation = Standardization
Automation = Speed
Automation = Controlling laboratory costs
Automation = Added value