# Microbiological Testing: Vegetables/Produce

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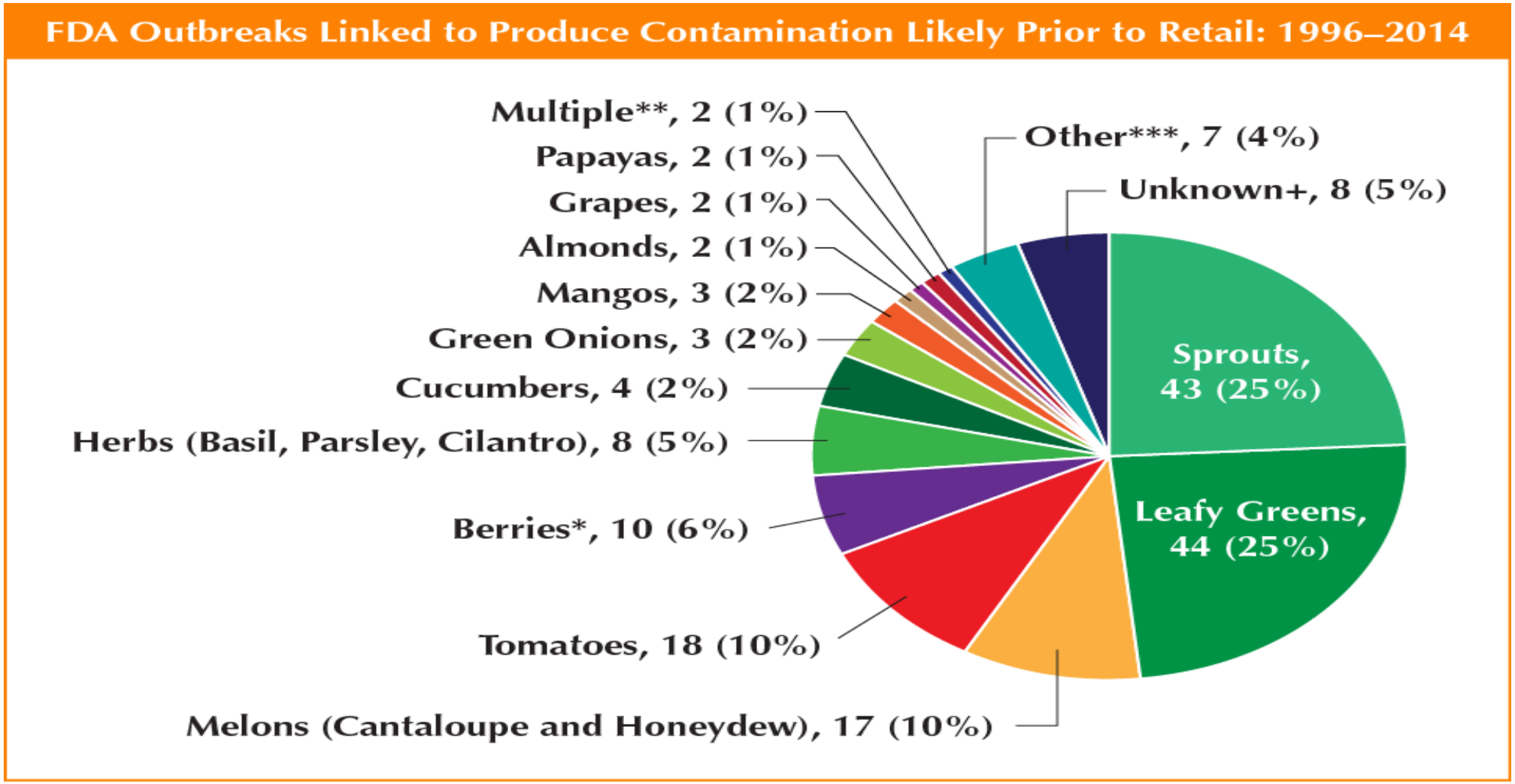
# What is Produce?

- Foods, other than grains, nuts, and some legumes, derived from plants
  - Vegetables
  - Fruits
  - Certain fungi
  - Sprouted seeds



- Important part of human diet as source of nutrients, fibre and vitamins
- Consumption of ***produce and their products*** increased significantly in many countries in recent years

# Outbreaks Associated with Produce - US



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# Surveillance of Foodborne Disease – US 2009-15

| Food Category | Outbreaks | Illnesses |
|---|---|---|
| **Aquatic Animal** | **344** | **2,288** |
| **Land Animal** | **565** | **13,709** |
| Dairy | 136 | 1,639 |
| Eggs | 36 | 2,470 |
| Beef | 106 | 1,934 |
| Chicken | 136 | 3,114 |
| **Plant** | **334** | **9,746** |
| Sprouts | 21 | 766 |
| Root and underground vegetables | 20 | 383 |
| Seeded Vegetables | 44 | 2,572 |
| Herbs | 7 | 476 |
| Vegetable row crops | 81 | 2,420 |
| Fruits | 78 | 2,420 |

Dewy-Mattia, D. et al., 2018
Surveill Summ 2018; 67:1-11

# Top 5 pathogen-food category pairs resulting in outbreak-associated illnesses – US 2009-15

| Etiology | Food Category | No. Outbreaks | No. Illnesses | No. Hospitalizations | No. Deaths |
|---|---|---|---|---|---|
| *Salmonella* | Eggs | 31 | 2,422 | 41 | 1 |
| ***Salmonella*** | **Seeded Vegetables** | **25** | **2,203** | **419** | **7** |
| *Salmonella* | Chicken | 49 | 1,941 | 372 | 0 |
| *Salmonella* | Pork | 43 | 1,539 | 206 | 3 |
| *Campylobacter* | Dairy | 60 | 917 | 51 | 1 |

Dewy-Mattia, D. et al., 2018 Surveill Summ 2018; 67:1-11

# Top 5 pathogen-food category pairs resulting in outbreak-associated hospitalizations – US 2009-15

| Etiology | Food Category | No. Outbreaks | No. Illnesses | No. Hospitalizations | No. Deaths |
|---|---|---|---|---|---|
| ***Salmonella*** | **Seeded vegetables** | **25** | **2,203** | **419** | **7** |
| *Salmonella* | Chicken | 49 | 1,941 | 372 | 0 |
| ***Salmonella*** | **Fruits** | **24** | **838** | **227** | **6** |
| *Salmonella* | Pork | 43 | 1,539 | 206 | 3 |
| ***L. monocytogenes*** | **Fruits** | **3** | **184** | **179** | **41** |

Dewy-Mattia, D. et al., 2018 Surveill Summ 2018; 67:1-11

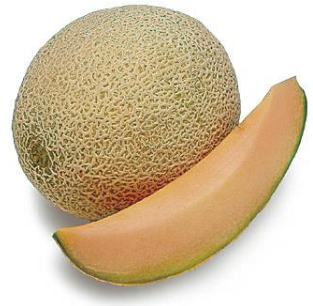
# Top 5 pathogen-food category pairs resulting in outbreak-associated deaths – US 2009-15

| Etiology | Food Category | No. Outbreaks | No. Illnesses | No. Hospitalizations | No. Deaths |
|---|---|---|---|---|---|
| ***L. monocytogenes*** | **Fruits** | **3** | **184** | **179** | **41** |
| *L. monocytogenes* | Dairy | 14 | 106 | 70 | 14 |
| ***Salmonella*** | **Seeded vegetables** | **25** | **2,2-3** | **419** | **7** |
| ***Salmonella*** | **Fruits** | **24** | **828** | **227** | **6** |
| ***L. monocytogenes*** | **Vegetable row crops** | **2** | **29** | **29** | **6** |

Dewy-Mattia, D. et al., 2018 Surveill Summ 2018; 67:1-11

# Notable Produce Outbreaks

- USA – August – October 2011
- Whole Cantaloupe
- *Listeria monocytogenes*
  - States – 28
  - Case Count – 147
  - Hospitalizations – 143
  - Deaths – 33
- At the time, most deadly outbreak in US history
- First Lm outbreak associated with whole produce
- Outbreaks strains found in packinghouse, not field



CDC Final Update, 2012

*Pictures from Online - USA Today

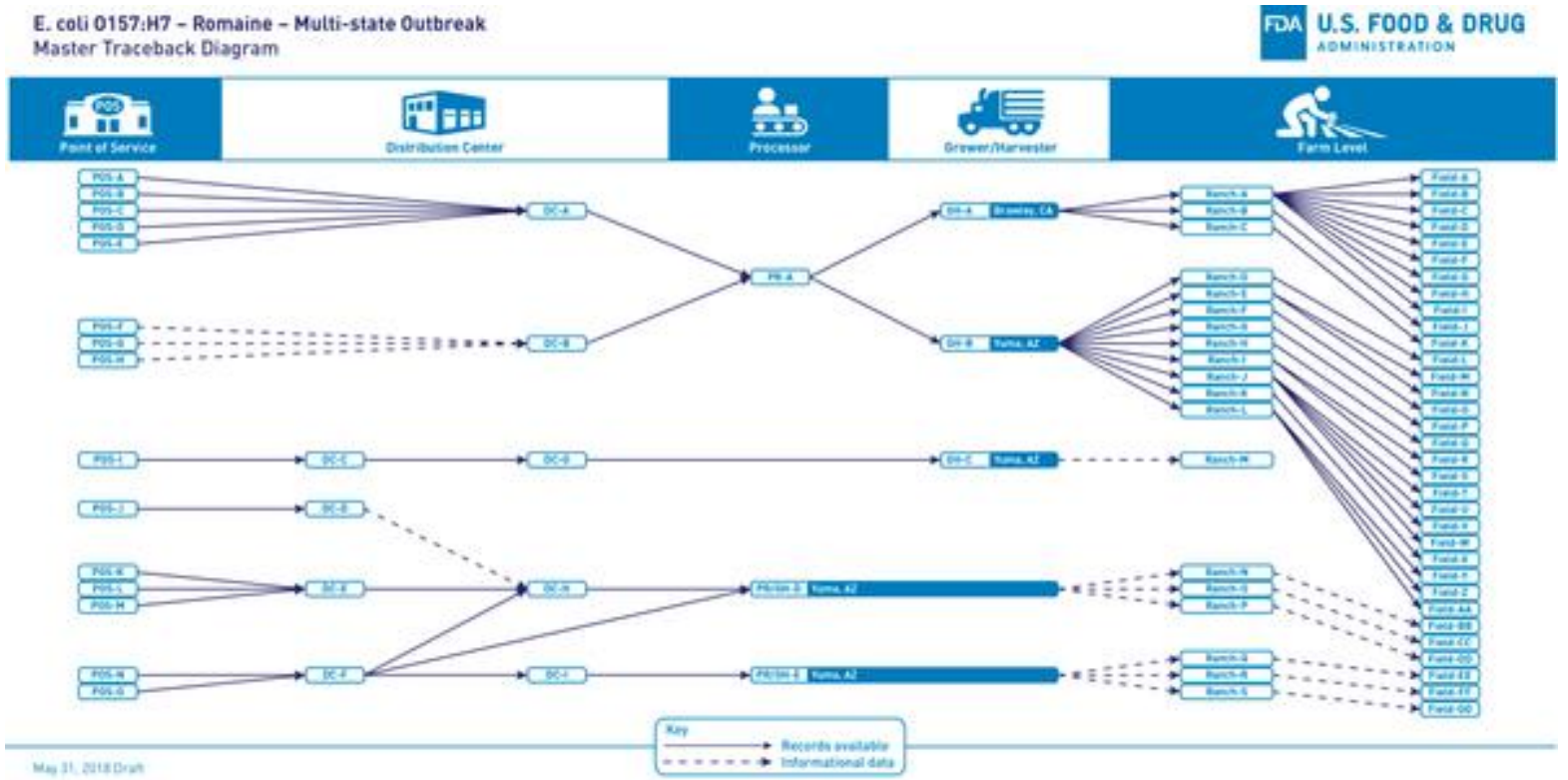
# Notable Produce Outbreaks

- EU – 2013
- Mixed berry products
- Hepatitis A
  - 12 European countries
  - At least 1,444 cases
  - No Deaths
- Two suspected origins
  - Single point source
  - High risk practice during freezing

EFSA Journal 2014; 12 (9):3821

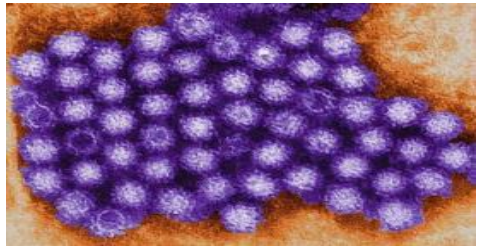
# Notable Produce Outbreaks

- US – March-June, 2018
- Romaine Lettuce
- *E. coli* O157:H7
  - States – 36 (and Canada)
  - Case Count – 210
  - Hospitalizations – 96
  - HUS - 27
  - Deaths – 5
- Traced back to a growing region, not an individual farm
- Strain found in canal waters and a nearby feedlot



# Microorganisms of Concern

- Bacteria
  - *Salmonella,* toxigenic *E. coli* (EHEC)*, Shigella, Listeria monocytogenes, Yersinia pseudotuberculosis, Clostridium botulinum* (low acid juices)
- Viruses
  - Norovirus, Hepatitis A
- Protozoa/Parasites
  - *Giardia lamblia*, *Cryptosporidium parvum, Cyclospora cayetanensis, Toxoplasma gondii, Fasciola hepatica*



# International Documents

CODEX ALIMENTARIUS

INTERNATIONAL FOOD STANDARDS

Food and Agriculture Organization of the United Nations

World Health Organization

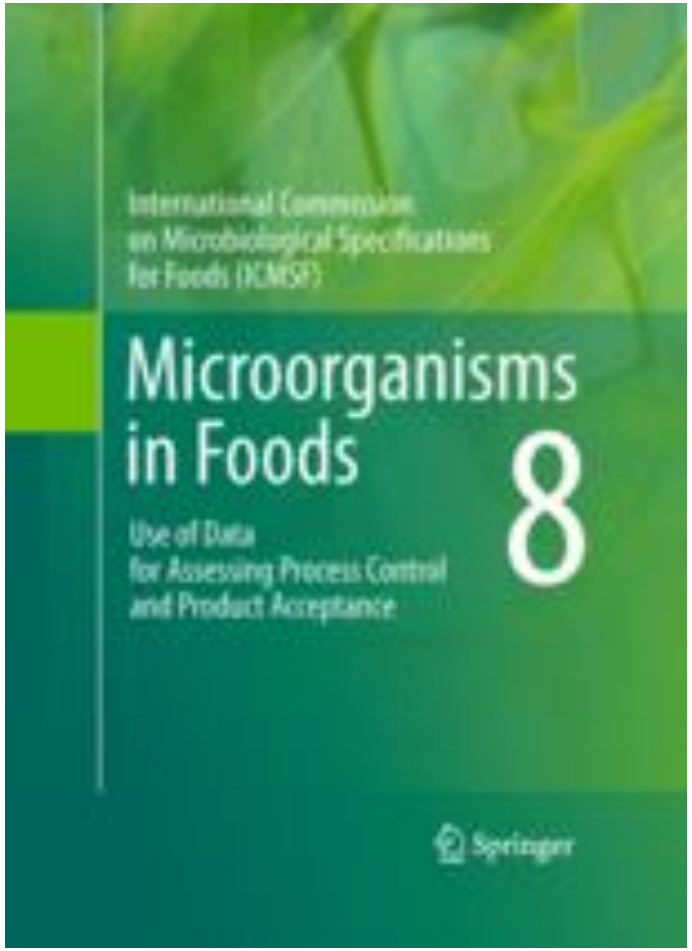
E-mail: codex@fao.org - www.codexalimentarius.org

**CODE OF HYGIENIC PRACTICE FOR FRESH FRUITS AND VEGETABLES**

**CXC 53-2003**

**Adopted in 2003. Revised in: 2010 (new Annex III for fresh leafy vegetables), 2012 (new Annex IV for Melons), 2013 (new Annex V for Berries), 2017.**

- Annex I – RTE fresh pre-cut fruits and vegetables
- Annex II – Sprout production
- Annex III – Fresh Leafy Vegetables
- Annex IV – Melons
- Annex V – Berries




# Useful Testing for Safety Management

All Values Are Scientific Advice Developed By The ICMSF And Have No “Official” Status

- Chapter 12 - Vegetables and Vegetable Products pg 147
- Chapter 13 – Fruits and Fruit Products pg 177

# Types of Microbiological Testing

- Routine
  - lot-by-lot, assess safety of lots, end-product or in process
- Verification
  - occasional, measure continuing effectiveness of controls
- Environmental
  - assess effectiveness of GAP and GHP program and potential for cross contamination
- Investigational
  - in response to failure or deviation, identify root cause
- Shelf-life
  - Validation of shelf-life and impact of factors affecting it; profile microbiological changes occurring in product during shelf-life of individual lots

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# Products Covered

## Vegetables and Vegetable Products

- **Fresh and Fresh-cut**
- Cooked
- Frozen
- Canned
- Dried
- Fermented and Acidified
- Sprouted Seeds
- Mushrooms

## Fruits and Fruit Products

- **Fresh Whole**
- **Fresh-Cut**
- Frozen
- Canned
- Dried
- Tomatoes and tomato products
- Fruit Preserves

*** Primary Production covered in both**

# Product Diversity

- “Vegetables”, “Fruits”, and “Produce” cover a huge range of foods and food products that vary from region to region

- All recommendations provided must be adapted to the specific fruits and vegetables being considered to account for differences in cultivation techniques, distribution and processing, end use, etc.

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# Primary Production

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# Contamination Sources

Humans

Soil

Produce

Animals

Buildings
Equipment Tools

Water

# Types of Microbiological Testing

- Extent of contamination can be strongly influenced by primary production practices and conditions
  - Good Agricultural Practices
- Verification testing may be beneficial for higher-risk fresh produce (e.g., leafy greens, sprouts)
  - Pre-harvest testing
- At primary production a focus is verification of **water sources** and **soil amendments**, as well as investigational sampling

# Evaluating Risk Related to Production Water

Three main impact points for produce safety risks related to production water are:

1. Production water source and quality
   - Public water supply, ground water, surface water
   - Testing frequency and sampling location
2. Application method
   - Water that does not contact the harvestable portion
   - Water that contacts the harvestable portion of the crop
3. Timing of application
   - At planting or close to harvest

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# Probability of Contamination

Rain

**Lower Risk** → **Higher Risk**

Improperly treated waste

| **Public Water Supply** | **Ground Water** | **Surface Water** |
|---|---|---|
|  |  |  |
| Treated | Deep Well | Shallow Well | Open to Environment |

# Method of Irrigation

- Overhead (sprinkler)
  - Higher risk:  A direct water application method resulting in contact with produce
- Flood (surface, furrow)
  - May avoid direct contact with produce
  - Consider risk of contact with contaminated soil during harvest or from splash
- Drip (trickle, subsurface, micro, under canopy)
  - Lower risk: Produce generally not in direct contact (except root crops), reduces foliar diseases, improves water use efficiency

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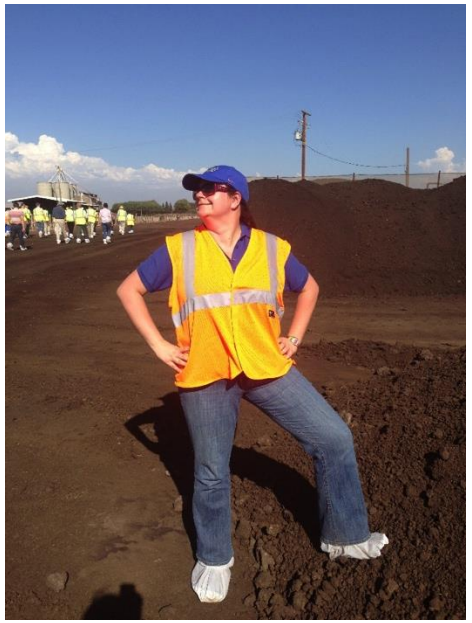
# Agricultural Water

| Use | Impor-tance | Hazard or Indicator | Testing method / Analytical Unit | n | c | m | M |
|---|---|---|---|---|---|---|---|
| Irrigation, RTE | High | *Escherichia coli* | ISO 9308-1 100 ml | 3 | 1 | 10 | $10^2$ |
| Irrigation, non-RTE | Mode-rate | *E. coli* | ISO 9308-1 100 ml | 3 | 1 | $10^2$ | $10^3$ |
| Pesticides, cleaning, etc. | High | *E. coli* | ISO 9308-1 100 ml | 5 | 0 | Absence in 100 ml | NA |

# Soil Amendments & Food Safety Risks

- Biological soil amendments, especially those that include untreated (raw) manure, pose significant microbial risks
  - This is also true of untreated human waste and improperly treated biosolids
- Synthetic (chemical) soil amendments can also impact food safety, if not prepared and applied properly
- Risks can be reduced by:
  - Selection of crop
  - Treatment
  - Application Timing
  - Application Method
  - Handling



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# Composted Organic Soil Amendments (1)

| Intended Use | Relative Importance | Hazard or Indicator | Testing Method/ Analytical Unit | n | c | m | M |
|---|---|---|---|---|---|---|---|
| Composted manures / Vegetables likely to be eaten raw | High | *Escherichia coli* | ISO 16649-2 | 5 | 2 | $10^2$ per g | $10^4$ per g |
| | | EHEC | ISO 16654 10g | 5 | 0 | Absence in 10g | NA |
| | | *Salmonella* | ISO 6579 10g | 5 | 0 | Absence in 10g | NA |
| Pasteurized manures / Vegetables likely to be eaten raw | Moderate | *E. coli* | ISO 16649-2 | 5 | 2 | $10^2$ per g | $10^4$ per g |
| | | EHEC | ISO 16654 10g | 5 | 0 | Absence in 10 g | NA |
| | | *Salmonella* | ISO 6579 10g | 5 | 0 | Absence in 10 g | NA |

# Composted Organic Soil Amendments (2)

| Intended Use | Relative Importance | Hazard or Indicator | Testing Method/ Analytical Unit | n | c | m | M |
|---|---|---|---|---|---|---|---|
| Composted manures / Vegetables not likely to be eaten raw | Low | *E. coli* | ISO 16649-2 | 5 | 2 | $10^3$ per g | $10^5$ per g |
| | | EHEC | ISO 16654 10g | 5 | 0 | Absence in 10 g | NA |
| | | *Salmonella* | ISO 6579 10g | 5 | 0 | Absence in 10 g | NA |
| Pasteurized manures / Vegetables not likely to be eaten raw | Routine microbiological testing not recommended . Periodic testing to verify effectiveness of process may be beneficial. | | | | | | |

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# Fresh and Fresh-cut

# Fresh and Fresh-cut

- Generally capable of supporting growth of bacteria and fungi
  - pH=4.5 to 7.0, aw > 0.98
- Allow survival of viruses and protozoa
- Minimal processing
- Cut surfaces and other routes of entry
- Often no cooking
- Temperatures and time for quality may be in range for microbial growth

- Enterobacteriaceae, coliforms, and fecal coliforms are part of the normal flora found on fresh produce, and these groups do not reflect the sanitary status of raw produce.

# Fresh and Fresh-cut

| | Relative Importance | Useful Testing |
|---|---|---|
| Critical Ingredients | Low | Rely on verification that GAPS were followed and verification testing at primary production and harvest |
| In process | High | Non-microbial testing of antimicrobial in wash water, flume water, etc for control of cross-contamination |
| Processing Environment | Medium | Periodic testing of food contact surfaces and processing environments to verify GMP and sanitization protocols |
| Shelf Life | Low | Validated through microbiological testing before initiation of a new product line and revalidated after any major change in process technologies |

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# Fresh and Fresh-cut

| Relative Importance | | Useful Testing | | | | | | |
|---|---|---|---|---|---|---|---|---|
| End Product (Fresh-cut Veg) | | Routine microbiological testing is not recommended. Periodic testing for specific indicators may be useful for verifying process control and conducting trend analysis. Test for specific pathogens only when other data indicate high potential for contamination or process failure. | | | | | | |
| | | Microorganism | Testing Method / Analytical unit | Case | Sampling Plan and Limits | | | |
| | | | | | n | c | m | M |
| | Medium | *E. coli* | ISO 7251 | 6 | 5 | 1 | 10/g | 100/g |
| | Low | *Salmonella* | ISO 6579 25 g | 12 | 20 | 0 | Absence in 25 g | NA |
| | Low | EHEC | ISO 16654 25 g | 15 | 60 | 0 | Absence in 25 g | NA |
| | Low | *Listeria monocytogenes* | ISO 11290-1 25 g | NA (Codex) | 5 | 0 | Absence in 25 g | NA |

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# Fresh and Fresh-cut

| Relative Importance | | Useful Testing | | | | | | |
|---|---|---|---|---|---|---|---|---|
| End Product (Fresh-cut RTE fruit supporting growth | | Routine microbiological testing is not recommended. Testing may be warranted when information indicates a potential for contamination. | | | | | | |
| | | Microorganism | Testing Method / Analytical unit | Case | Sampling Plan and Limits | | | |
| | | | | | n | c | m | M |
| | Low | *Salmonella* | ISO 6579 | 12 | 20 | 0 | 0 | NA |
| | Low | *L. monocytogenes* | ISO 11290-1 | - | 5 | 0 | 0 | NA |
| (Fresh-cut RTE fruit no growth) | Low | *L. monocytogenes* | ISO 11290-2 | - | 5 | 0 | 0 | NA |

# Concluding thoughts

- Microbiological testing is integral part of produce safety programs; but must be used appropriately and pragmatically
- No food safety program can rely solely on microbiological testing
  - Prevention through production, preventing cross-contamination during packing and processing
- Most current criteria developed by expert elicitation
  - Vary depending on production practice, commodity, end use
- Enterobacteriaceae, coliforms, and fecal coliforms are part of the normal flora found on fresh produce, and these groups do not reflect the sanitary status of fresh produce.