Food Safety and Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

Microbiology for EIAOs

Udit Minocha
Microbiologist

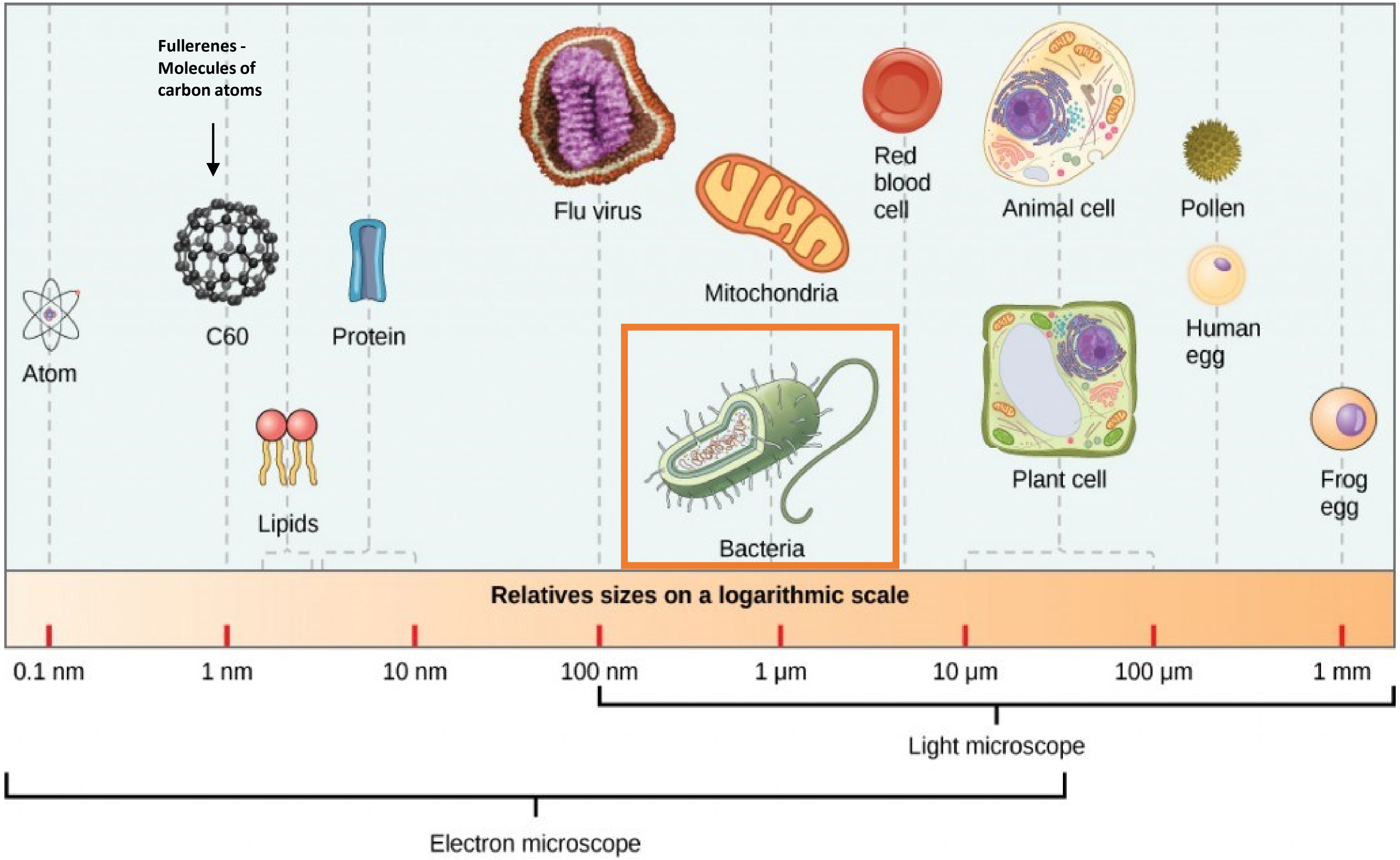
Overview

- A review of basic microbiological concepts.
- Review relationship between bacterial adulterants and meat and poultry products during processing.
- General hurdle technologies used and some general good food/meat manufacturing safety practices.
- Basics of reporting and use of whole genome sequencing by FSIS applicable to role of the EIAO.

What Are Bacteria?

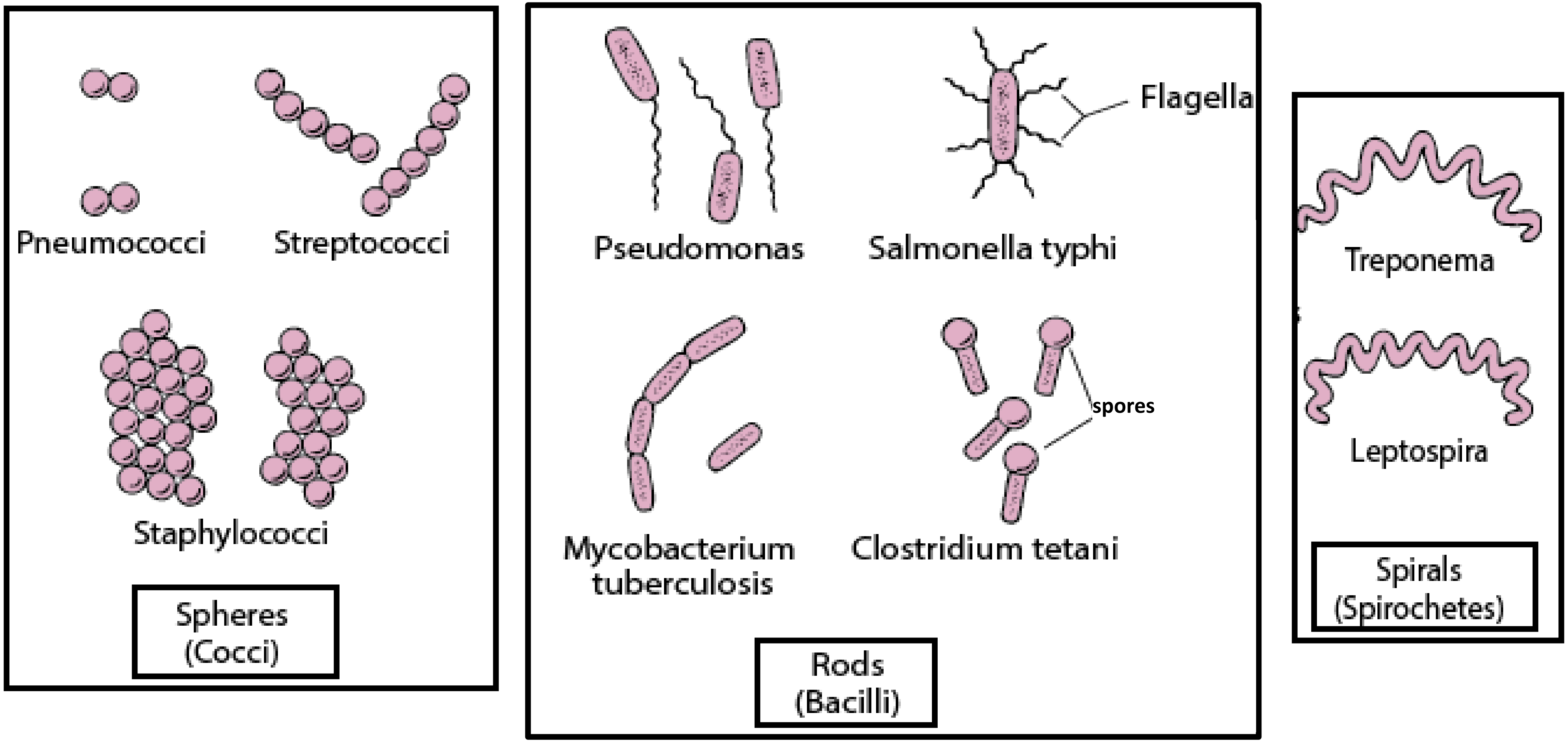
- Single-celled or unicellular microorganisms.
- Extremely small - you need a microscope to see them.
- Living organisms - carry out metabolism through respiration or fermentation.
- Prokaryotic cells - do not have a nucleus.
- Live in every environment on the earth (e.g., volcanos, at the bottom of the ocean, and in extreme cold temperatures -- Arctic).
- Enclosed by a cell membrane.
- Some bacteria have a thick cell wall others have a thin cell wall

The Size of Bacteria Relative to Other Types of Cells

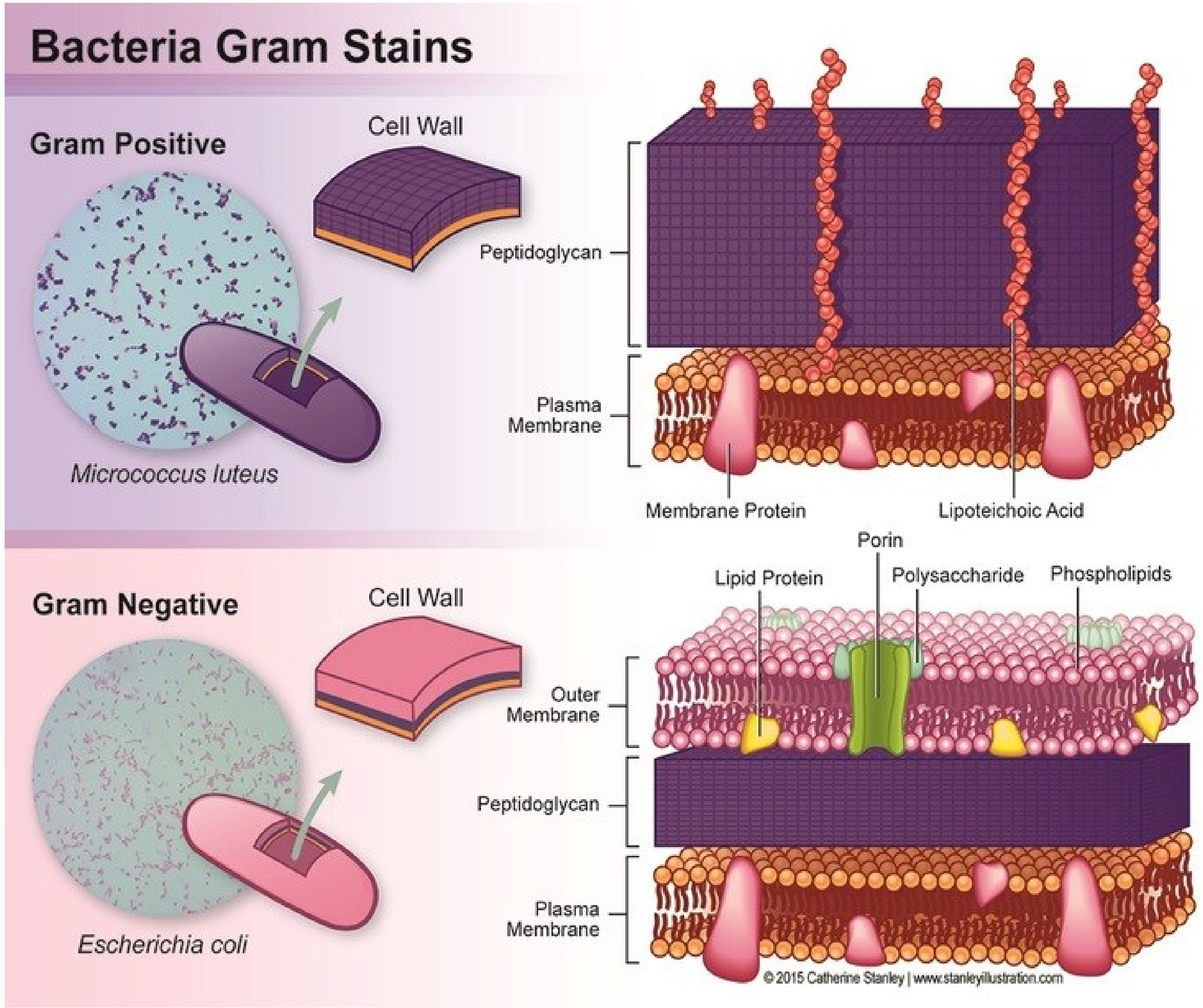


https://www.slideteam.net/business_powerpoint_diagrams/14063485-style-medical-3-microbiology-1-piece-powerpoint-presentation-diagram-infographic-slide.html

Basic Shapes of Bacteria



Color of Bacteria After Gram Staining Depends on Thickness of the Bacterial Cell Wall



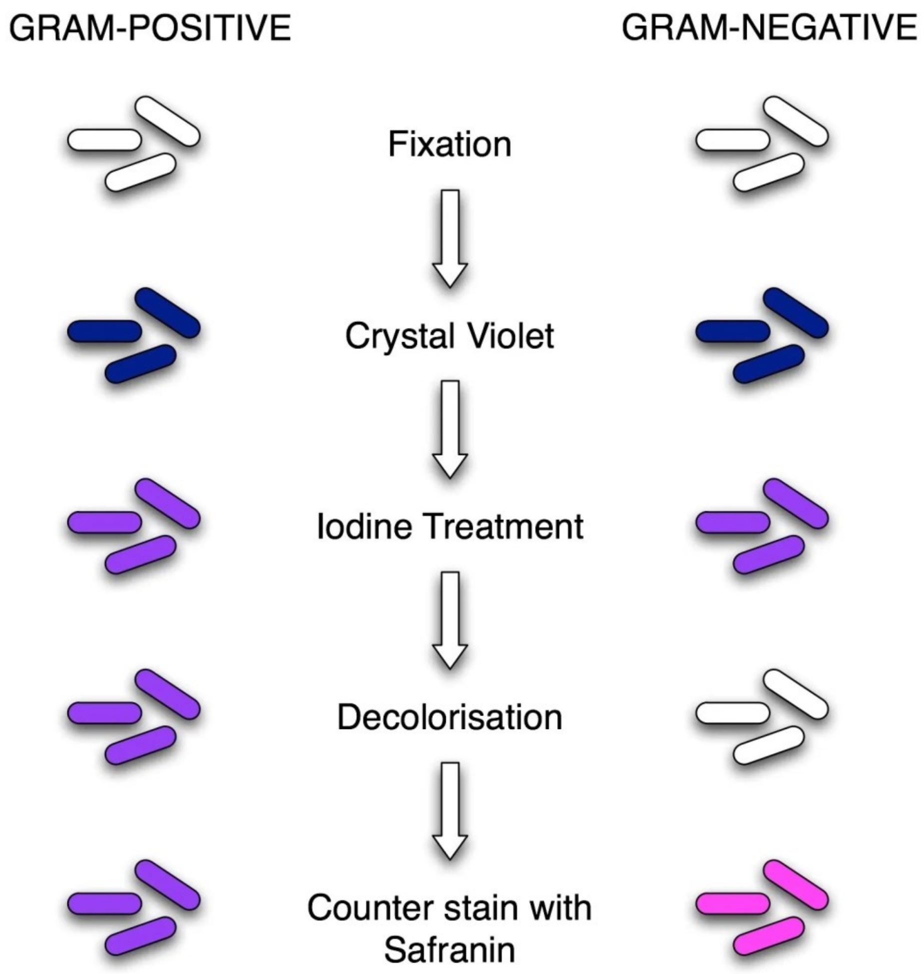
Thick cell walls of Gram-positive bacteria retain stain and will not decolorize, resulting in a purple-blue bacteria seen under light microscope.

Thin cell walls of Gram-negative bacteria allow stain to be decolorized and counterstained with safranin, resulting in a pinkish bacteria seen under light microscope.

The Gram Staining Method Used to Divide Bacteria Into Gram-Positive or Gram-Negative Bacteria

Examples of Gram-Positive Bacteria

Bacillus
Clostridium
Listeria
Staphylococcus



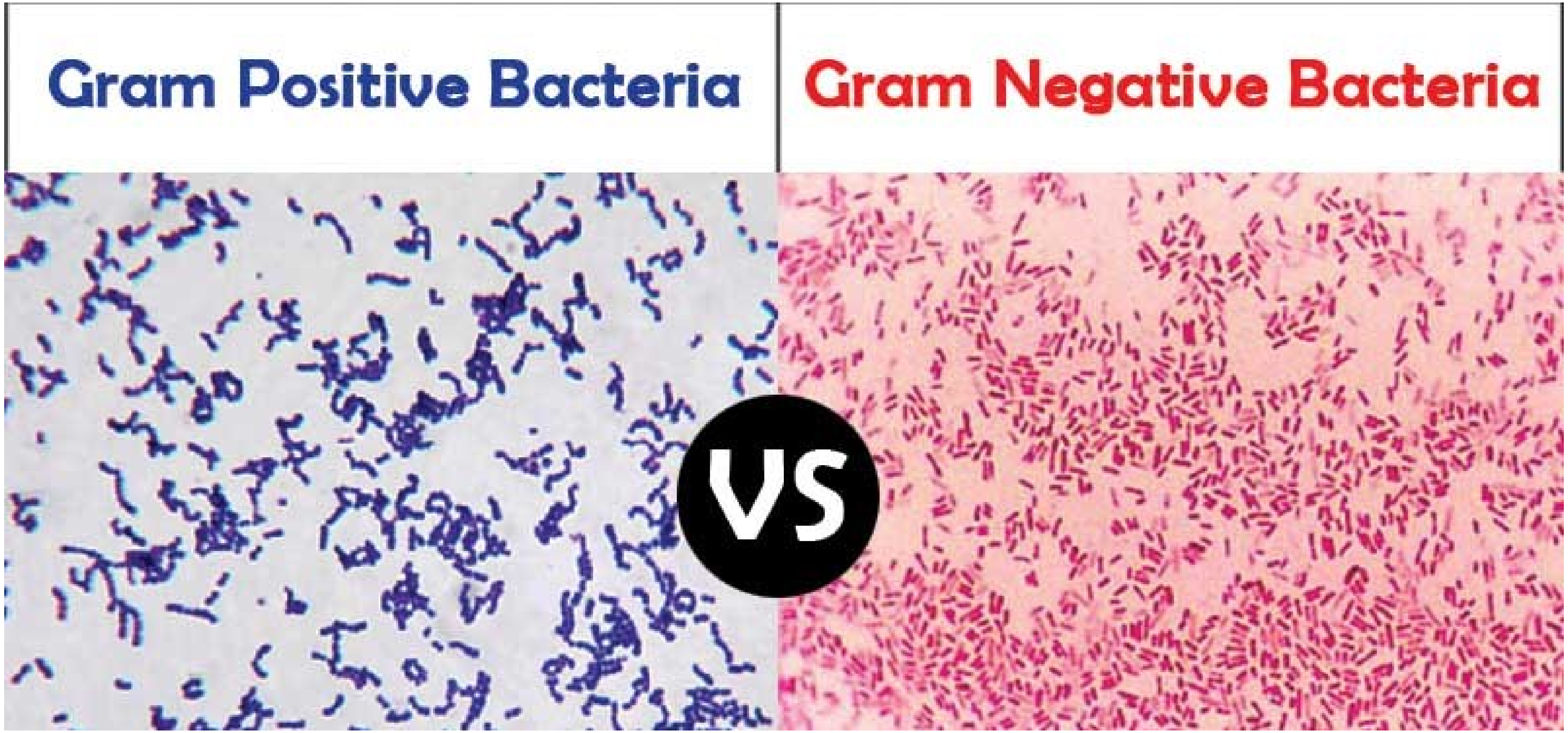
Examples of Gram-Negative Bacteria

Campylobacter
E. coli / STEC
Pseudomonas
Salmonella

Based on staining characteristics of the bacterial cell wall, bacteria are classified as Gram-positive or Gram-negative

https://microbeonline.com/key-facts-about-gram-staining-techniques-that-you-might-not-know/

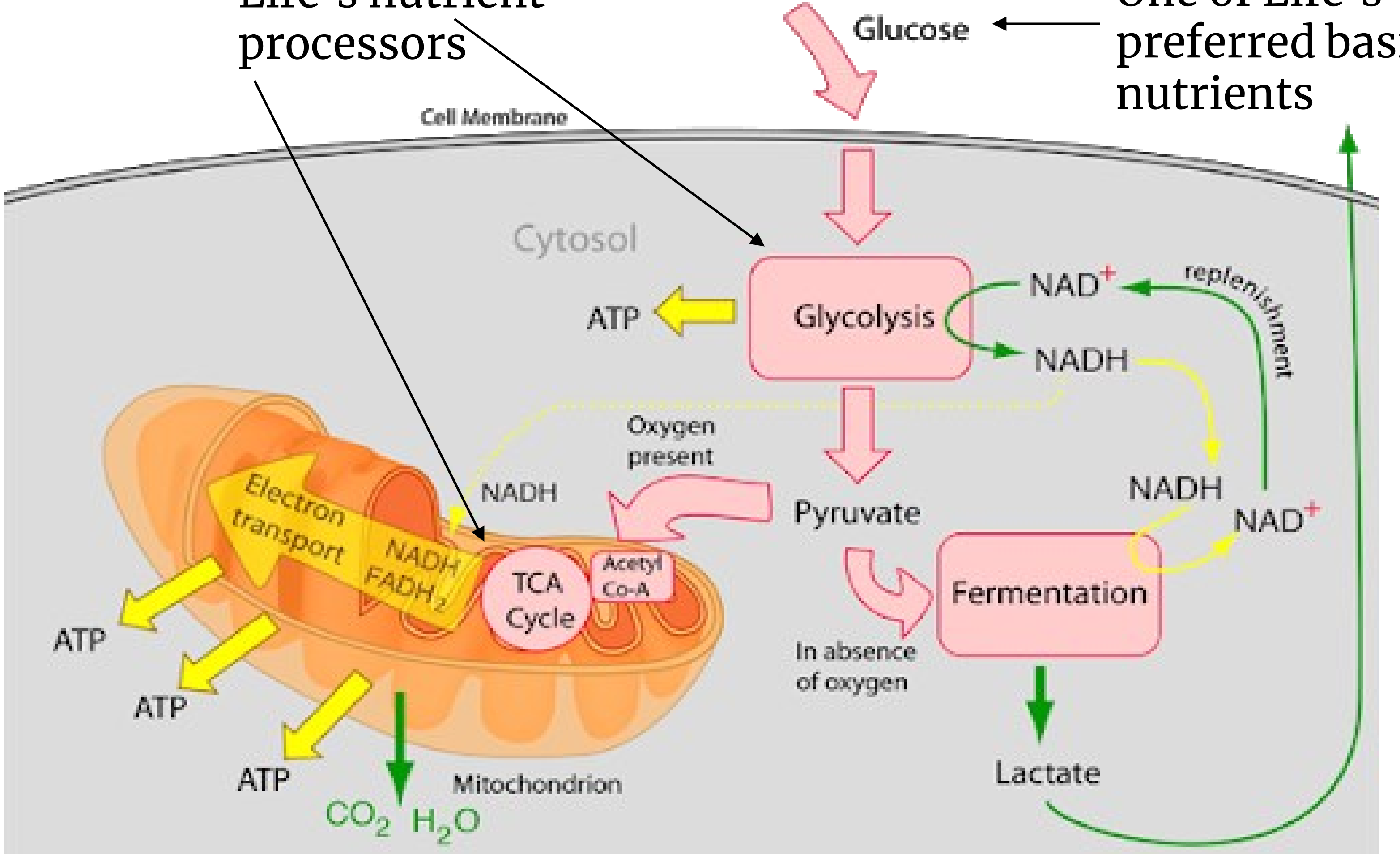
Micrographs of Gram-Stained Bacteria



Process of Cellular Respiration in Bacteria – Needed to Breakdown Nutrients, to Gain Energy to Grow

Life's nutrient processors

One of Life's preferred basic nutrients



Bacteria use the same respiratory mechanisms shown here in mitochondrion.

Respiration occurring aerobically uses oxygen. In contrast, during anerobic respiration oxygen is absent.

Bacteria Use Four Major Mechanisms for Respiration

1. Obligate aerobes – Requires oxygen to grow (e.g., *Mycobacterium*, fungi)

2. Obligate anaerobes – Cannot grow in the presence of oxygen. Oxygen is toxic to these bacteria (e.g., *Clostridium* spp.)

3. Facultative anaerobes – These bacteria can go under low and high oxygen conditions (e.g., *E. coli*, STEC, *Salmonella*, other enterics within the Enterobacteriaceae, and some yeasts)

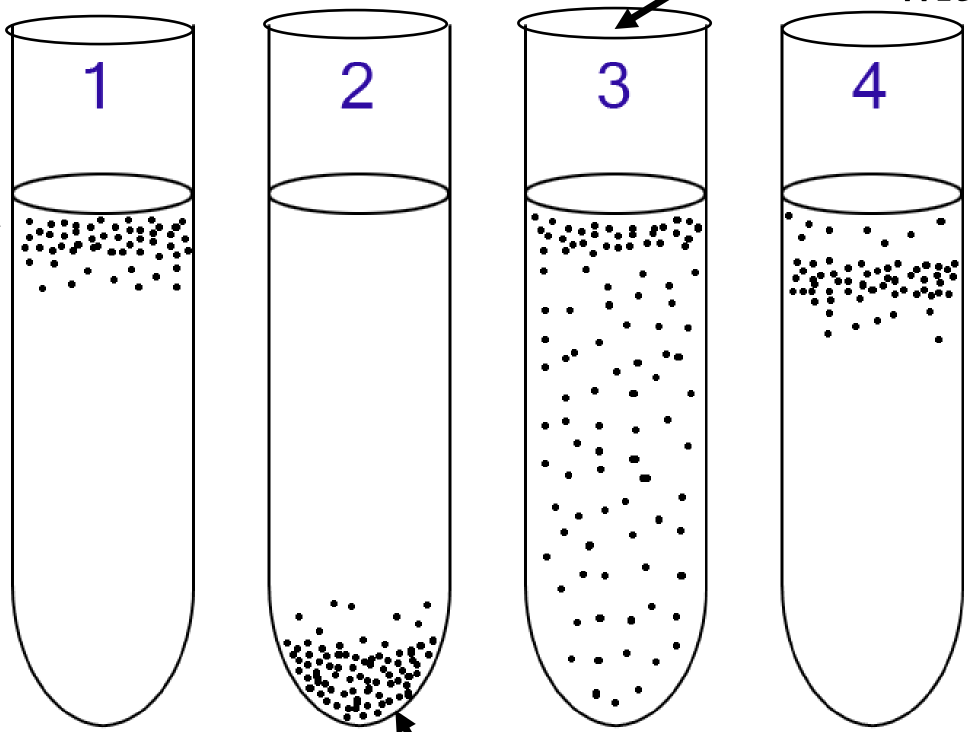
4. Microaerophilic (2-6% oxygen) – Grows best in low oxygen situations (e.g., *Campylobacter* spp.)

https://en.wikipedia.org/wiki/File:Anaerobic.png

Bacteria Use Four Major Mechanisms for Respiration

Facultative - bacterial growth throughout the broth, with O_2 and without O_2.

Aerobic - bacterial growth with O_2. Most of the growth is where most of the O_2, is located.



Microaerophile most of the bacterial growth is in areas of the broth with reduced O_2 .

Obligate anerobic - bacterial growth, without O_2, capped tubes.

https://en.wikipedia.org/wiki/File:Anaerobic.png

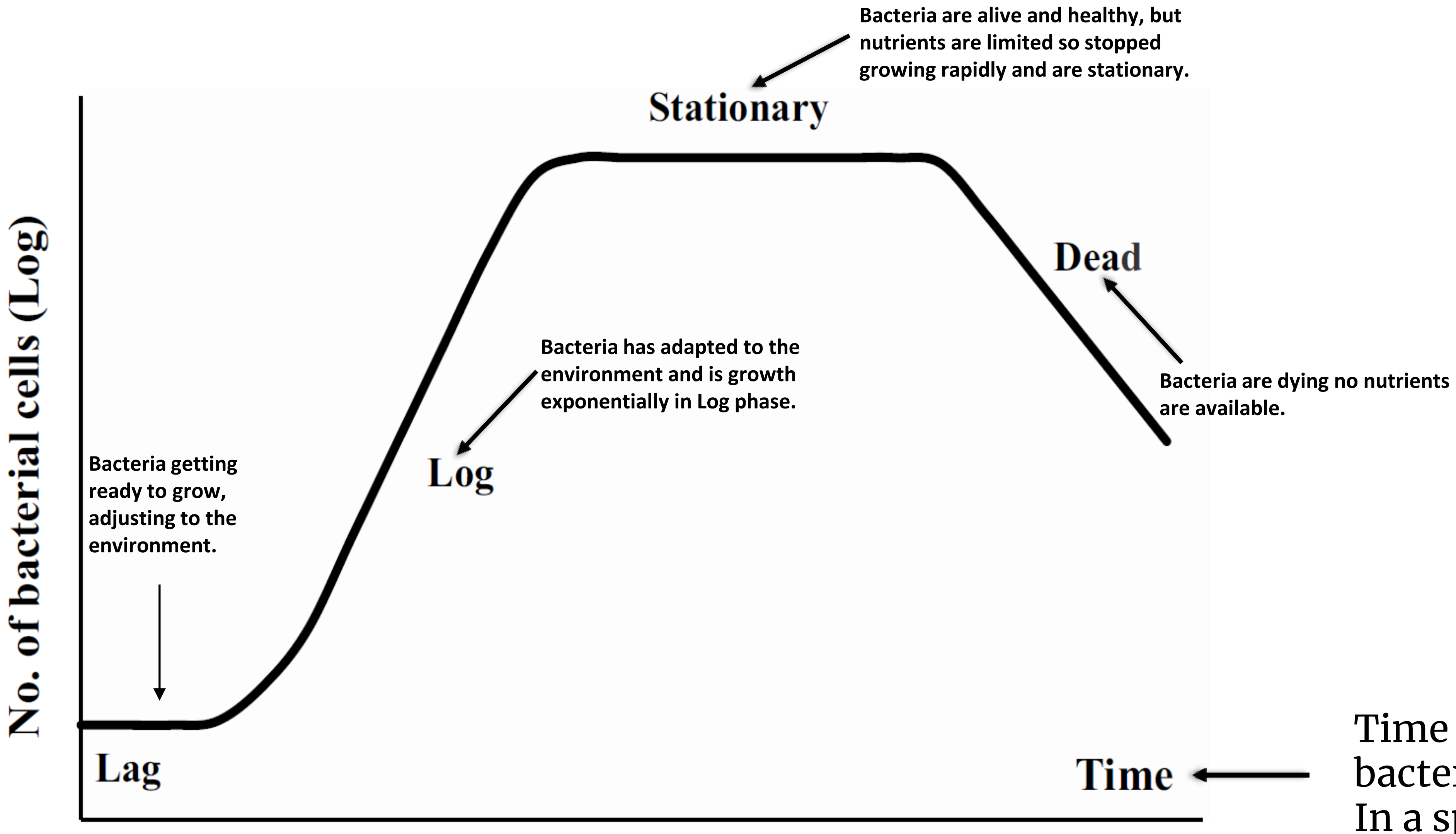
Temperatures at Which Specific Types of Bacteria Can Grow

Growth temperature classification	Growth Temp min / optimum range / max (°F)	Examples of representative bacteria
Psychrophiles (cold-loving)	23 / 32-59 / 68	*Achromobacter*, *Alcaligenes*, *Planococcus*, *Colwellia*
Psychrotrophs (tolerate cold)	32 / 68-77 / ~100	*Brochothrix*, *Listeria*, *Pseudomonas*
Mesophiles (love moderate temp)	50 / 86-113 / 122	*Campylobacter*, *Clostridium*, *Enterobacter*, *Lactobacillus*, *Listeria*, *Pseudomonas*, *Salmonella*
Thermophiles (heat-loving)	~110 / 113-122 / >125	*Deinococcus*, *Geobacillus stearothermophilus*, *Thermus* aquaticus

Note: Some species of bacteria can survive across a wide range of temperatures and fall within two categories, e.g. *Listeria and Pseudomonas*.

Growth of Bacteria and Bacterial Growth Requirements

A Typical Bacterial Growth Curve



Time to complete the bacterial growth cycle In a specific environment.

Describing Food-related Factors Affecting Bacterial Growth

Intrinsic factors – related to the food itself; characteristics “inside” of the food

Extrinsic factors – related to the environment where the food is or what is added to it; characteristics “outside” of the food

Intrinsic Factors That Affect Bacterial Growth

Intrinsic factors – inherent characteristics of food

- **pH** - >6.5-7.2; depends on amount of lactic acid produced in muscle after slaughter of animal.
- **Water activity** – raw meat ~0.98.
- **Nutrient content** – sugars, proteins, amino acids, organic acids.
- **Physical structure** – whole muscle, comminuted, chunked/formed.
- **Naturally occurring antimicrobial components** present in animal prior to slaughter.
- **Oxidation-Reduction Potential** (Redox potential) - measure of how easy it is for a molecule to accept or lose electrons; more oxygen = higher potential; inner side of meat anaerobic surface is aerobic; even after death respiration continues with formation of lactic acid.

Extrinsic Factors That Affect Bacterial Growth

Extrinsic factors – characteristics that modify the properties of a food

- **Storage temperature** – frozen, refrigerated, room temperature.

- **Relative humidity –** affects speed of drying and if the meat dries properly.

- **Presence of other bacteria** – affects growth of pathogens; naturally occurring spoilage bacteria or the addition of lactic acid bacteria can prevent *Staphylococcus* growth.

- **Gases in the environment** – modified air packaging; meat that is vacuum-packed or flushed with other gases (carbon dioxide, carbon monoxide, nitrogen, etc.).

Conditions for Bacterial Growth

Food **A**cidic environment **T**emperature **T**ime **O**xygen **M**oisture **(FATTOM)**

- **F**ood – meat and poultry are good sources of nutrients for bacteria

- **A**cidic environment – most all foods have a pH<7
  low acid foods have a pH>4.6 (a_w>0.85)
  high acid foods have a pH ≤ 4.6

- **T**emperature – 40 to 140°F = the Danger Zone; allows bacterial growth

- **T**ime – food in the Danger Zone for more than ~4-6 hours

  example – 1 bacteria; doubling every 20 min for 6 hours in the danger zone = 18 divisions = 5.4 $\log_{10}$ bacteria

Conditions for Bacterial Growth

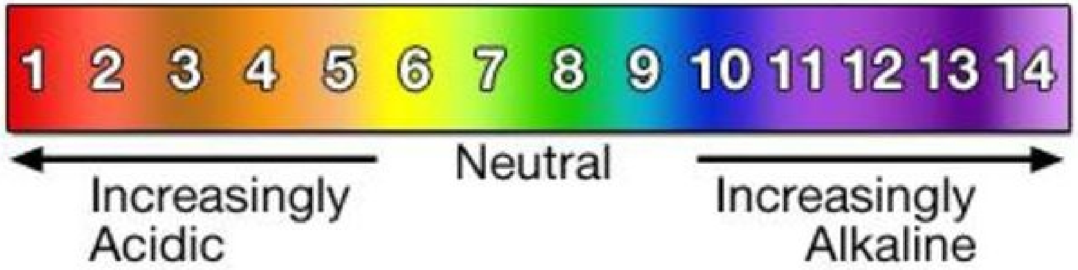
Food Acidic environment Temperature Time Oxygen Moisture (FATTOM)

- **Oxygen**
  - Obligate aerobes, facultatively anaerobes, and microaerophilic organisms require oxygen to grow well.
  - Obligate anaerobes will not grow in the presence of oxygen.

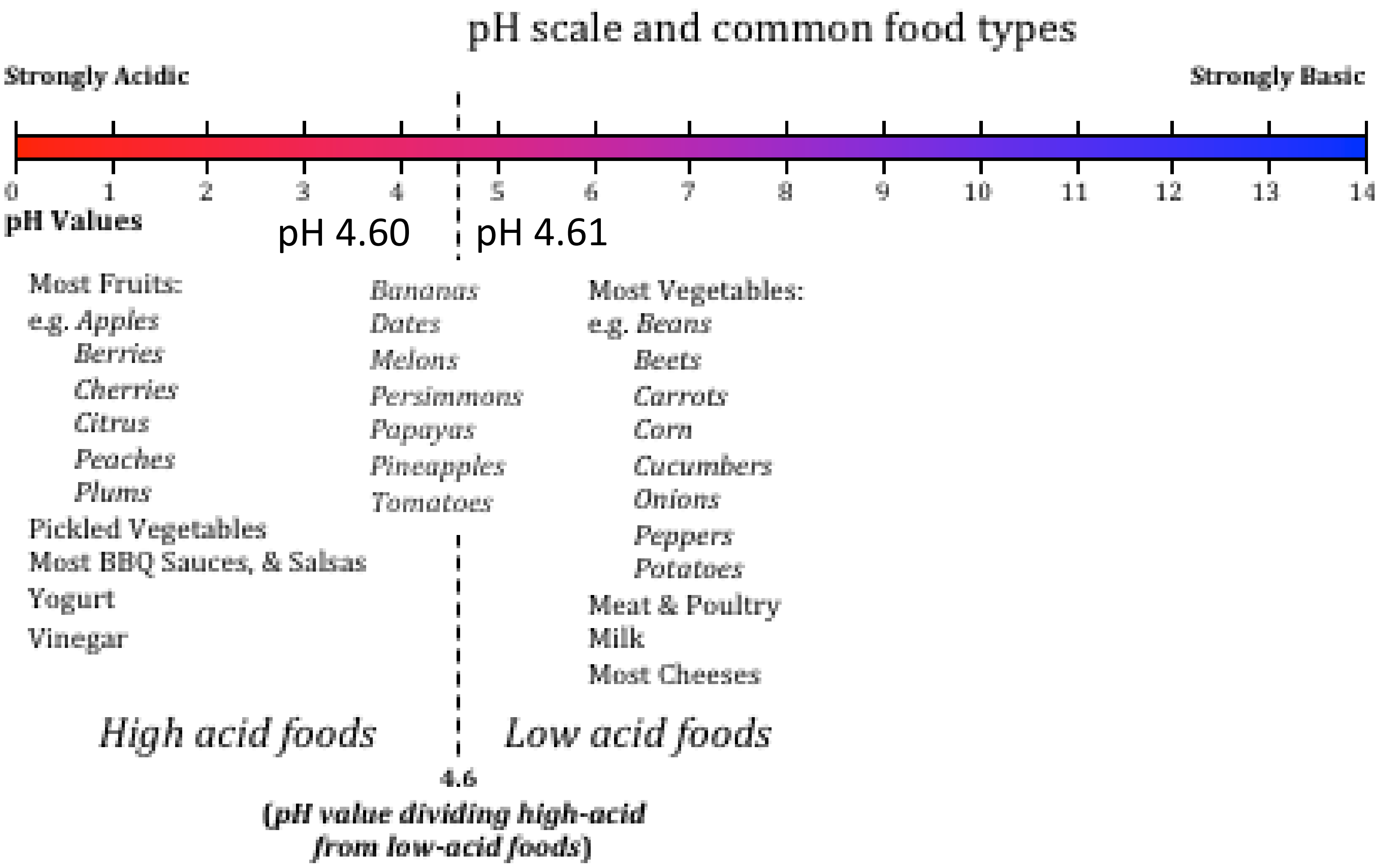
- **Moisture**
  - Moisture content is a measurement for food quality.
  - Water activity (a_w) is a measurement for food safety; a_w >0.85

What is pH?

- pH is the measurement of the concentration of positively-charged hydrogen ions [H+].
- Definition - the negative logarithm of the concentration of hydrogen ions [H+].
- Logarithmic scale - each change in a whole number represents a tenfold change in [H+] concentration.
  - A sample at a pH of 4.0 has 10x the concentration of [H+] ions as a sample at pH of 5.0, and 100x the concentration of [H+] than a sample with a pH of 6.0.
  - Raw meat and poultry has an approximate pH between 5.4 – 6.8.



What is pH?



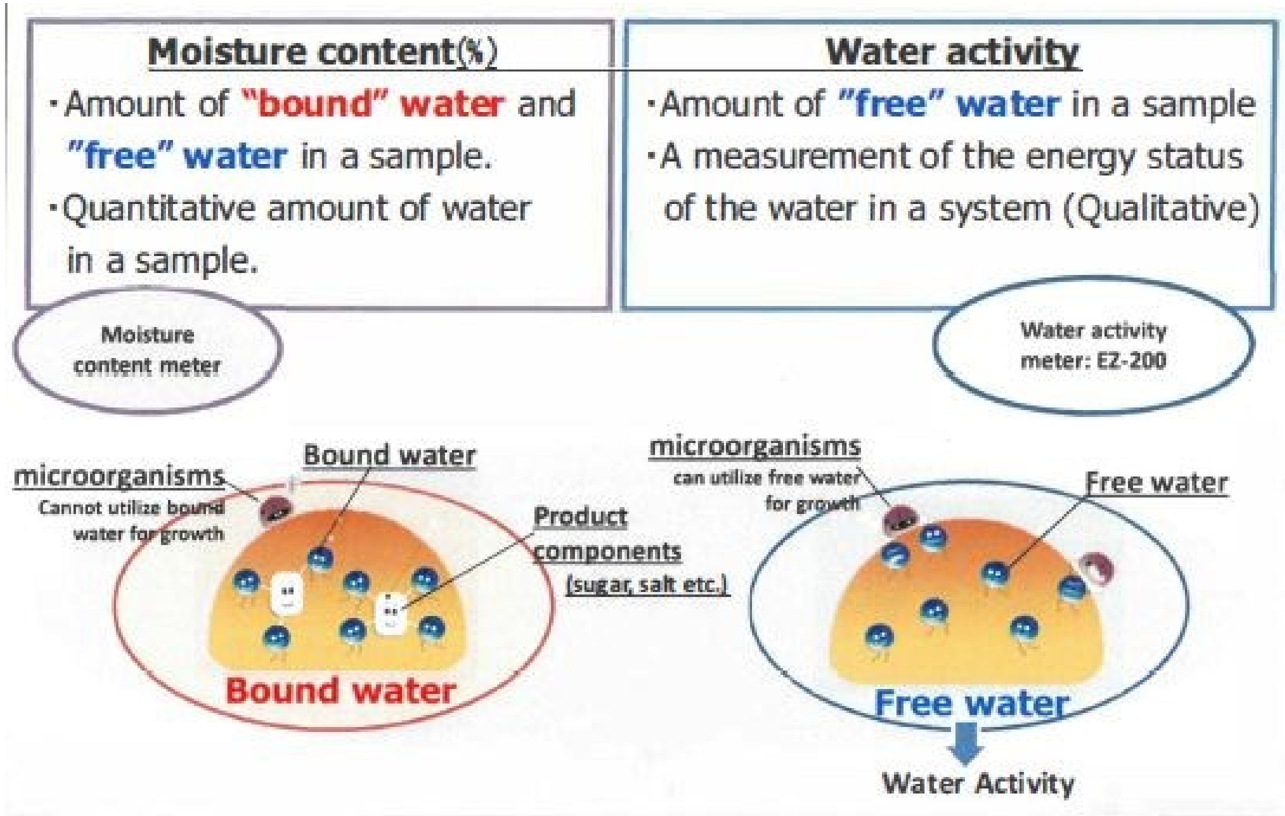
https://extension.okstate.edu/fact-sheets/the-importance-of-food-ph-in-commercial-canning-operations.html

pH of Some Common FSIS-regulated Foods

Food	pH
egg solids, whole	7.1 – 7.9
egg whites	7.0 – 9.0
beef (unaged)	7.0
egg yolk	6.4
egg solids, whites	6.5 – 7.5
chicken	6.5 – 6.7
ham	5.9 – 6.1
turkey (roasted)	5.7 – 6.8
fish (most fresh)	6.6 – 6.8
veal	6.0
beef (aged)	5.8
lamb	5.4 – 6.7
beef (ground)	5.1 – 6.2
sausage, dry	5.3 – 5.5
sausage, semidry	5.3 – 5.0

Definition of Water Activity (aw)

a_w is the ratio between the vapor pressure of the food and the vapor pressure of distilled water under identical conditions



Water Activity (a_w)

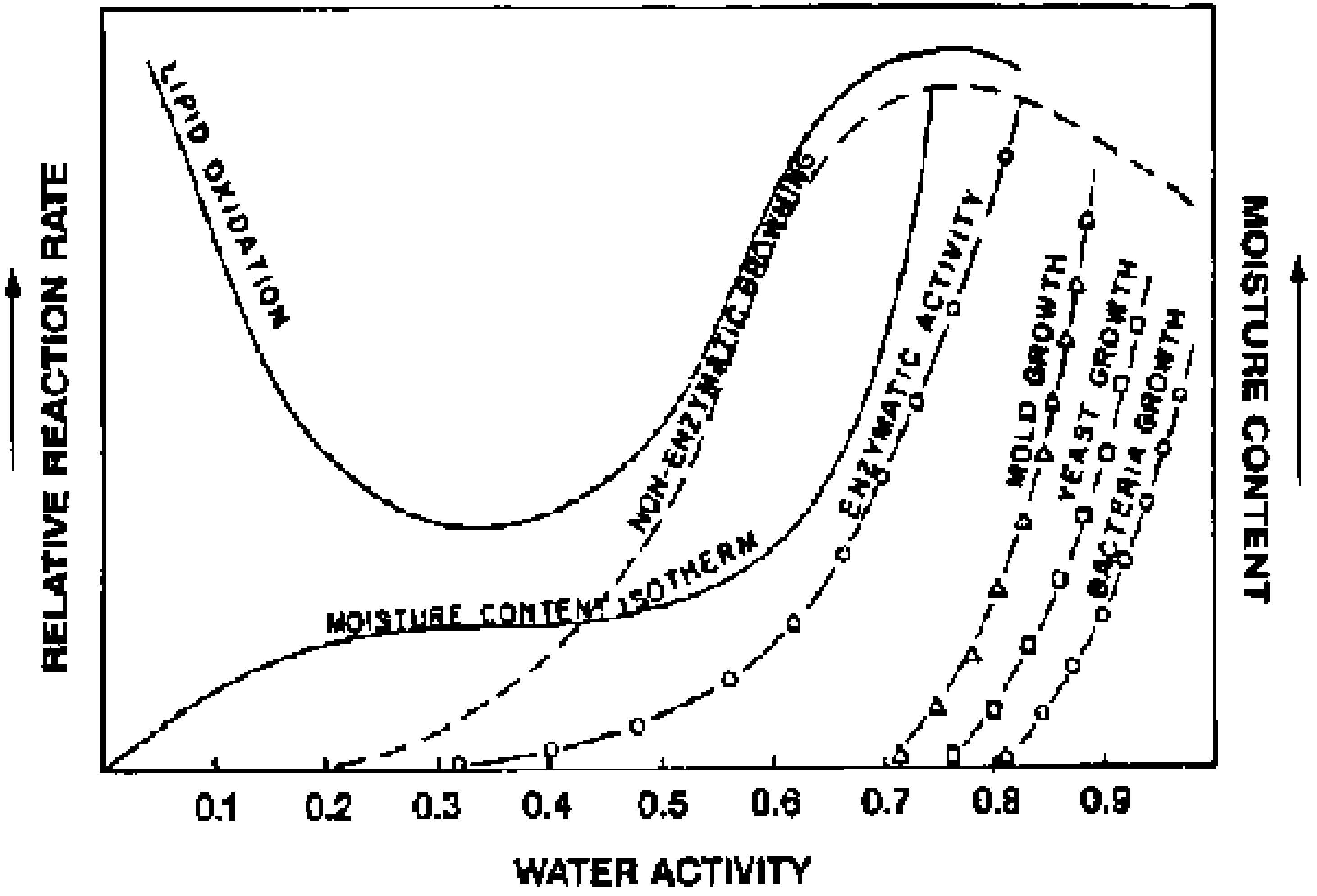
- Water that is strongly bound to ingredients cannot be used for bacterial growth.
  - Ingredients used in food process that bind water: NaCl, phosphates, gums, humectants, surface effect of the substrate, sugar (requires much higher concentration than salt).

- Water and solutes (NaCl, acetic acid, phosphates) move along a gradient to reach an equilibrium.
  - Low water activity outside a bacteria causes osmotic stress – cannot take up water and becomes dormant (not killed).

a_w range	Microorganisms generally INHIBITED by lowest a_w in this range	Foods generally in this range
0.98 – 0.95	*Pseudomonas*, *Escherichia*, *Proteus*, *Shigella*, *Klebsiella*, *Bacillus*, some yeasts, *C. perfringens*	raw meat, raw poultry, fish, milk, canned fruits, vegetables
0.95 – 0.91	Some molds & yeasts, *Salmonella*, *Pediococcus*, *Lactobacillus*, V. *parahaemolyticus*, *C. botulinum*	cured meat (ham, cold cuts), some firm cheeses, bread
0.91 – 0.87	*Many yeasts (Candida, Torulopsis, Hansenula), Micrococcus*	fermented sausage (salami), sponge cakes, dry cheeses, margarine
0.87 – 0.80	*Most molds (mycotoxigenic penicillia), S. aureus, most Saccharomyces (bailii), Debaryomyces*	beef jerky, most fruit juice concentrates, syrups, jams/jellies, soft pet food
0.80 – 0.75	*Most halophilic bacteria, mycotoxigenic aspergilli*	beef jerky, marmalade, marzipan, glacé fruits
0.75 – 0.65	*Xerophilic molds (Aspergillus chevalieri, A. candidus, Wallemia), Saccharomyces bisporus*	molasses, raw cane sugar, some dried fruits, nuts, snack bars, snack cakes
0.65 – 0.60	*Osmophilic yeasts and some molds*	dried fruits containing 15-20% moisture; some toffees and caramels; honey, candies
0.60 – 0.50 0.30 – 0.20	No microbial proliferation	dry pasta, spices, rice, confections, wheat whole egg powder, chewing, gum, flour cookies, crackers, breakfast cereals, dry pet food, peanut butter whole milk powder

Adapted from L.R. Beuchat, Cereal Foods World, 26:345 (1981)

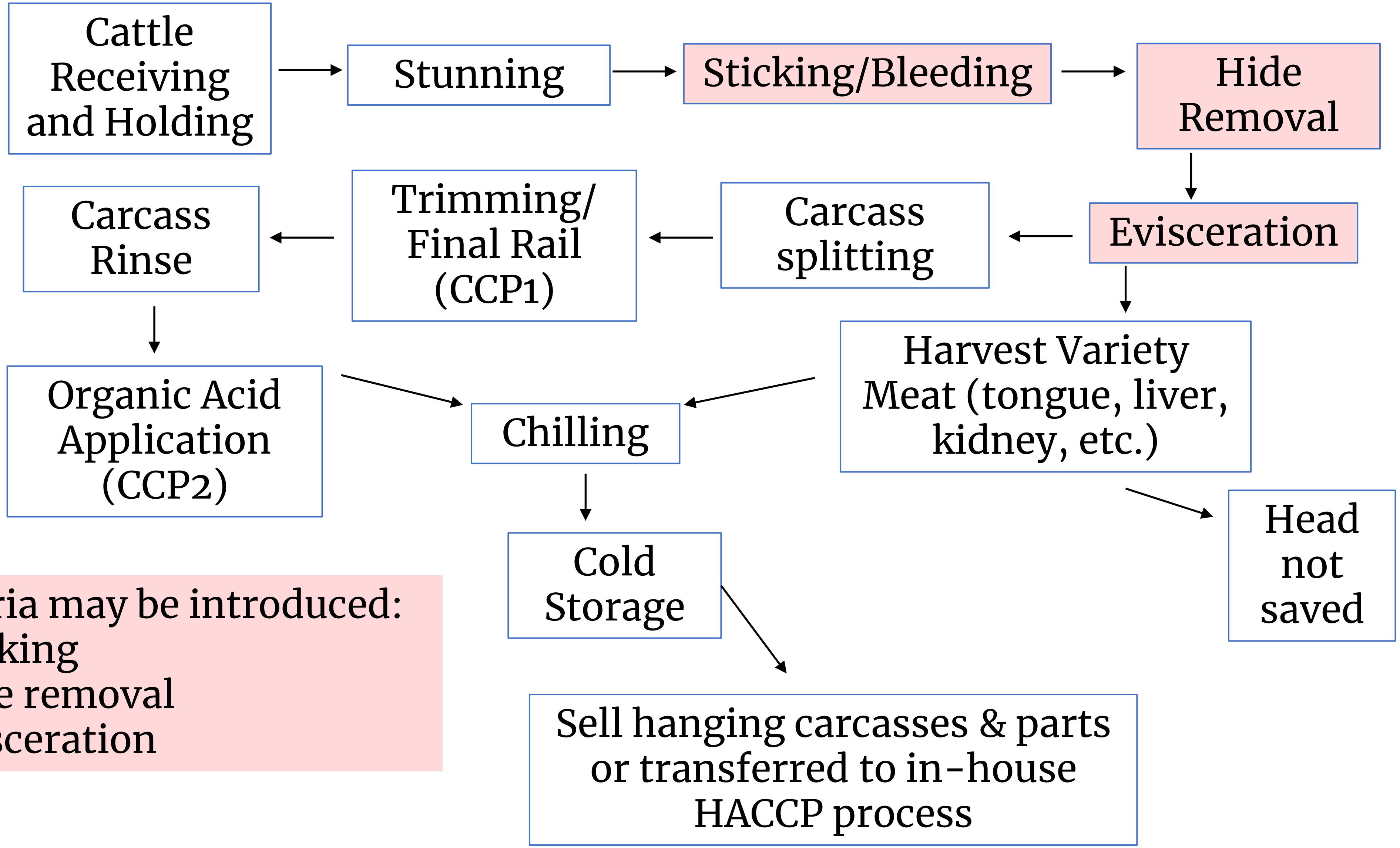
How a_w Affects Biological Systems



http://www.fao.org/3/Y4358E/y4358e06.htm

How Bacterial Foodborne-Pathogen Cross-contamination of Food Products Occur

Beef Processing Flow Diagram



Bacteria may be introduced:
- Sticking
- Hide removal
- Evisceration

How Contamination of Meat Occurs During Processing

- Carcasses are contaminated during hide removal and evisceration.

- Bacterial adulterants and fecal contaminates such as coliforms, aerobic bacteria, and generic *E. coli* and enteric pathogens such as *Salmonella* and Shiga Toxin-Producing *E. coli* (STEC) can be deposited on carcass during evisceration.

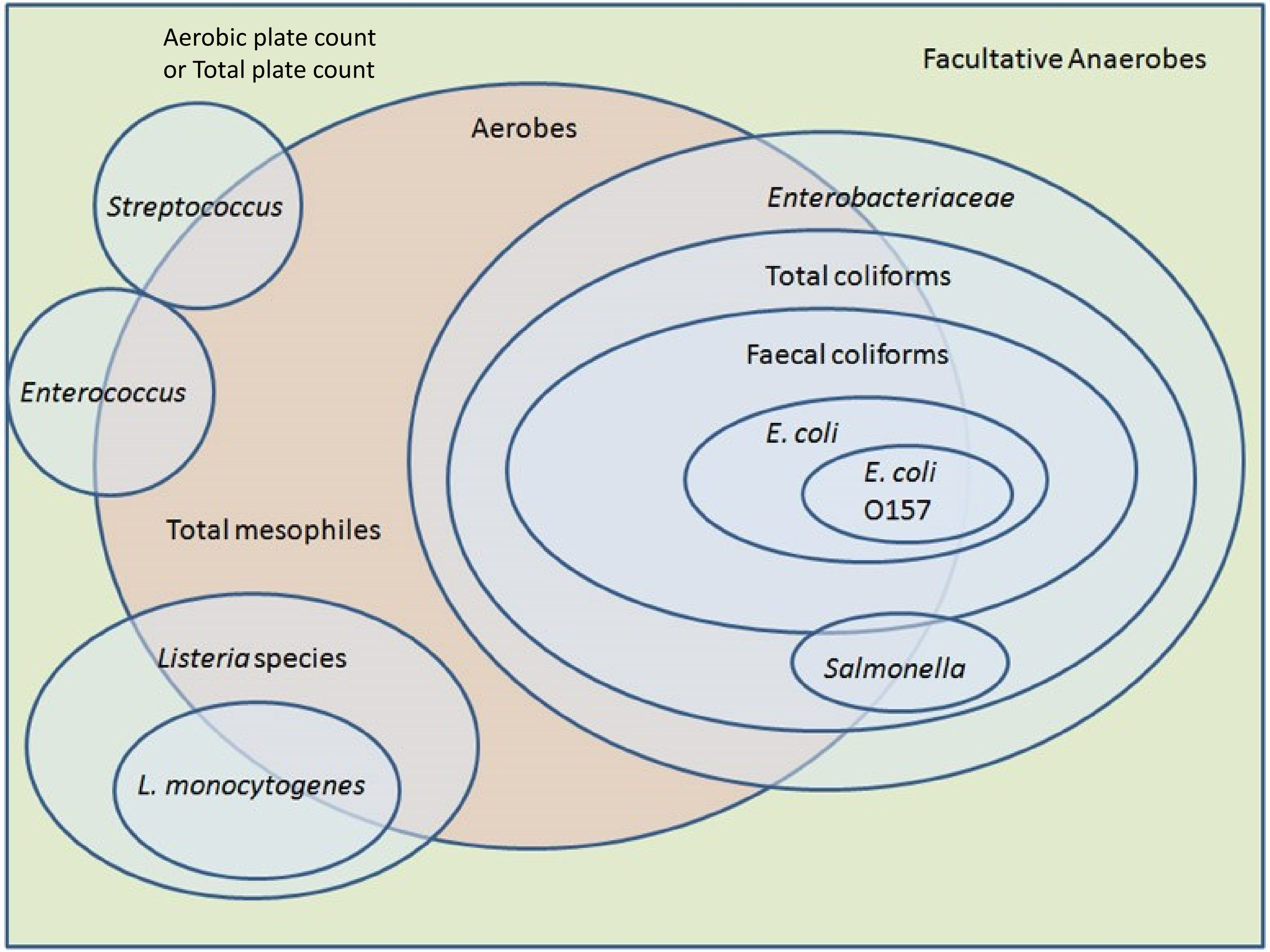
- Bacteria are main cause for concern because they can multiply and cause illness if under-cooked products are consumed.

- Cross contamination can occur via:
    - Knives and tools used during killing/processing of carcasses.
    - Employees' smocks, hands.
    - Food contact surfaces and the environment.

Antibacterial Intervention Controls Commonly Used in the USA

- Hot water/steam
- Organic acids – lactic, lactic/citric mixtures, peroxyacetic, lactate/diacetate, nitrite
- Inorganic compounds - acidified sodium chlorite, trisodium phosphate, chlorine
- Microbiological – bacteriophage (viruses that infect bacteria) bacteriocins (proteins/peptides produced by bacteria to inhibit or kill bacteria, lactic acid bacteria, food spoilage bacteria
- Chilling, freezing, cooking

Growth of Aerobic and Facultative Anaerobes & Their Overlap



Meat-associated Foodborne Bacterial Pathogens

Two Major Methods Used by Foodborne Pathogens to Cause Illness

- **Infection** – ingestion of pathogen and growth of pathogen results in physiological damage without enterotoxins. Bacteria grow within the intestinal tract and cause pathological damage resulting in illness.

  (e.g., *Campylobacter*, *Listeria*, *Salmonella)*

- **Intoxication**
  - Ingestion of food containing a pre-formed toxin.

  (e.g., *Bacillus cereus*, *Clostridium botulinum, Staphylococcus aureus*)

  - Bacterial growth and toxin production in the intestinal tract. The toxin made during infection is the major virulence factor responsible for the symptoms and pathological effect of illness.

  (e.g., *Clostridium perfringens,* STEC)

Meat-associated Foodborne Pathogens – *Bacillus cereus*

- Widespread in nature – found in soil, hide/skin/feathers, cereals, herbs, spices, and unpasteurized milk.

- Food-associated sources – can be found on meat; also found on starchy foods such as cooked rice, potatoes, puddings.

- Characteristics:
  - Gram-positive rods, spore forming facultative anaerobe
  - Temperature growth range min/max - 41°F / 131°F
  - Optimum temp range - 82 °F to 120°F
  - lower pH limit - pH 4.9
  - lower a_w limit - 0.93
  - Survival in salt - 7.5%

Meat-associated Foodborne Pathogens – *Bacillus cereus* (continued)

- Foodborne illness - *B. cereus food* poisoning
  - Infective dose 10^5 CFU/g

- Causative agents
  - Intoxication; Diarrheal toxin (heat-labile)
    - ✓ Diarrheal toxin – diarrhea and abdominal pain

  - Emetic toxin (cereulide; heat-stable); both toxins mimic *S. aureus* enterotoxin
    - ✓ Emetic toxin – nausea and vomiting

Meat-associated Foodborne Pathogens – *Campylobacter* spp. (*C. jejuni*, *C. lari*, *C. coli*)

- Widespread in nature – intestinal tract of animals, birds (e.g., cattle and poultry).

- Food sources – poultry and meat

- Characteristics:
  - Gram-negative spiral-shaped and microaerophilic (5%)
  - Temperature min/max - 86 °F / 122°F
  - Optimum temp range - 98 °F to 108°F
  - lower pH limit - pH 5.0
  - lower a_w limit - 0.98
  - Survival in salt - No

Meat-associated Foodborne Pathogens – ***Campylobacter* spp. (*C. jejuni*, *C. lari*, *C. coli*; continued)**

- Foodborne illness - Campylobacteriosis
  - Infective dose 500 CFU/g

- Illness
  - Gastroenteritis - host cell invasion, toxin production, inflammation, and epithelial cell destruction. mimics *S. aureus* enterotoxin leading to nausea and vomiting.

- Sensitive to freezing, drying, acidic conditions (pH < 5.0), and salt.

Meat-associated Foodborne Pathogens – *Clostridium botulinum*

- Widespread in nature – soil, intestinal tract, and hides of animals.
- Food sources – cooked meat and poultry products (e.g., roast beef).
- Characteristics:
  - Gram-positive rods obligate spore forming anaerobes
  - Temperature min/max - 50 / 122°F
  - Optimum temp range - 95 – 104 °F
  - lower pH limit - pH 4.7
  - lower a_w limit - 0.93
  - Survival in salt - 10%
  - Nitrite – 100 ppm inhibits growth and toxin production; use with cure accelerator (sodium erythorbate or ascorbate) or a high salt concentration
  - Phosphate and lactate/diacetate – can have an inhibitory effect

Meat-associated Foodborne Pathogens – *Clostridium botulinum* (continued)

- Foodborne illness – botulism
  - Infective dose 10^3 CFU/g

- Illness
  - Intoxication caused by a neurotoxin ingested with food.

- Controlling growth of *C. perfringens* will control *C. botulinum* and *B. cereus*

- FSIS indicates is there must be no multiplication of *C. botulinum*

Meat-associated Foodborne Pathogens – *Clostridium perfringens*

- Widespread in nature – soil, intestinal tract and hides of animals.

- Food sources – cooked meat and poultry meat from beef or pork and poultry cooked with sauce highest risk. (e.g., roast beef).

- Characteristics:
    - Gram-positive rods spore-forming obligate anaerobe
    - Temperature min/max - 43 / 126°F
    - Optimum temp range - 109.4 – 117 °F
    - lower pH limit - pH 5.0
    - lower a_w limit - 0.93
    - Survival in salt - 7%
    - Nitrite - 100 ppm inhibits growth; use with cure accelerator (sodium erythorbate or ascorbate) or a high salt concentration
    - Phosphate and lactate/diacetate – can have an inhibitory effect

Meat-associated Foodborne Pathogens – ***Clostridium perfringens*** **(continued)**

- Foodborne illness - gastroenteritis
  - Infective dose 10^5 CFU/g
- Illness
  - Intoxication –Toxin produced in the gut after ingestion of vegetative cells.
- Controlling *C. perfringens* growth will control *C. botulinum* and *B. cereus* due to the fast growth rate of *C. perfringens*, as fast as 15 minutes.
- No more than 1-$\log_{10}$ multiplication of *C. perfringens* to comply with 9 CFR 318.17(a)(2).

Meat-associated Foodborne Pathogens – *Listeria monocytogenes*

- Widespread in nature – environment, intestinal tract and hides of animals, soil

- Food sources:
  - undercooked or under-processed foods (fermented, dried, and ready-to-eat foods).
  - Post-lethality contaminated meat such as cooked deli meats, hotdogs, fermented sausages/meats.

- Characteristics:
  - Gram-positive rods facultative anaerobes
  - Temperature min/max - 32 / 113°F
  - Optimum temp range - 86 - 99 °F; grows at refrigerator temperatures
  - lower pH limit - pH 4.4 (survives in low acid)
  - lower a_w limit - 0.92
  - Survival in high salt - 10%
  - lactate/diacetate – can have an inhibitory effect

Meat-associated Foodborne Pathogens – *Listeria monocytogenes* (continued)

- Foodborne illness - listeriosis
  - Infective dose ~<100 CFU in a 25g sample
  - Mortality rate as high as 30% in immunocompromised persons, neonates, pregnant women, elderly and other people with medical issues.

- Illness
  - Invades and grows inside intestinal epithelial cells (enterocytes) and can move inside cells from cell-to-cell.
  - Can cross the gut epithelial barrier into blood stream. Can lead to inflammation of the membranes and fluid surrounding the brain (meningitis).

- Can persist in establishments (harborage) due to formation of biofilm and spreads by cross contamination events by water (splashes/aerosols).

- Very difficult to eradicate after it establishes itself in a biofilm.

Meat-associated Foodborne Pathogens – *Salmonella*

- *Salmonella* spp. – Gram-negative; rod; facultatively anaerobic; two species:
  - *Salmonella enterica* – human pathogen; >2500 serovars; six subspecies.
  - Six subspecies (ssp.) of *Salmonella enterica*: enterica, indica, salamae, houtenae, diarizonae, and arizonae

Proper nomenclature:

- *Salmonella enterica* subspecies enterica serovar Enteriditis
  - Generally written as *Salmonella* Enteritidis

- *Salmonella enterica* subspecies enterica serovar Typhimurim
  - Generally written as *Salmonella* Typhimurium

Meat-associated Foodborne Pathogens – *Salmonella* (continued)

- Widespread in nature – environment, beef, pork, and poultry.

- Food sources
  - Raw meat and poultry
  - undercooked and under-processed RTE products (fermented or dried meat products) made with beef, pork, and poultry to achieve a ~6-$\log_{10}$ reduction.

- Characteristics:
  - Gram-negative rod-shaped facultative anaerobes
  - Temperature min/max - 36 °F/129°F (can become heat resistant)
  - Optimum temp range - 95°F - 99°F
  - lower pH limit - pH 4.1 (can become acid resistant)
  - lower a_w limit - 0.93

Meat-associated Foodborne Pathogens – *Salmonella* (continued)

- Foodborne illness - salmonellosis
  - Infective dose 10^5–10^6 CFU/g

- Illness
  - Gastroenteritis - diarrhea, fever, abdominal cramps, and vomiting.
  - Secondary illness - reactive arthritis can last months or years and can lead to chronic arthritis.

- Can persist for several weeks on foods with low a_w (jerky a_w=0.80; egg powder a_w=0.5) and can survive at a_w<0.5.

Meat-associated Foodborne Pathogens – *Salmonella* (continued)

Salmonella serovars that cause foodborne illness:

- Enteriditis
- Newport
- Typhimurium
- Javiana
- I:4, [5],12:i:-

Meat-associated Foodborne Pathogens – **Shiga Toxin-Producing *E. coli* (STEC)**

- Widespread in nature – intestinal tract, fecal contamination of animal hides of bovine.
- Food sources – pork, leafy greens, vegetables, and acidic fruits (e.g., apples/apple cider).
- Characteristics:
  - Characteristics:
    - Gram-negative rods facultative anaerobes and psychroterant and grow at low temperatures (1 to 45 °C = 33°F to 113°F)
    - Temperature min/max - 40 °F / 113 °F
    - Optimum temp range – 98.6 °F
    - lower pH limit - pH 3.3
    - lower a_w limit - 0.90
    - Survival in salt – 3.5%
    - lactate/diacetate – can have an inhibitory effect

Meat-associated Foodborne Pathogens – **Shiga Toxin-Producing *E. coli* (STEC; continued)**

- Foodborne illness – gastroenteritis
    - Infective dose 1-100 CFU

- Illness
    - Gastroenteritis, hemorrhagic colitis (bloody diarrhea), severe cramping (abdominal pain), vomiting, low-grade or no fever.

- Survives fermentation, drying, storage, and 69 ppm nitrite.

- Can become heat and acid resistant

- Designated by O antigen (somatic) and H antigen (flagella), (i.e., *E. coli* O157:H7).

- Seven serovars that FSIS consider adulterants:
  O26, O45, O103, O111, O121, O145, O157:H7

Meat-associated Foodborne Pathogens – *Staphylococcus aureus*

- Narrow source in nature – skin and nasal passages, not all strains are pathogenic.
- Food sources – improperly handled foods due to poor worker hygiene or lack of process control; meat carcasses.
- Characteristics:
  - Gram-positive cocci facultative anaerobes
  - Temperature min/max - 45 °F / 118°F
  - Optimum temp range - 95 °F - 104 °F
  - pH limit - pH 4.2
  - aw limit - 0.83 (tolerates low water activity)
  - High salt tolerance (15%); nitrite has little inhibitory effect
  - Does not grow well in the presence of competing bacteria

Meat-associated Foodborne Pathogens – *Staphylococcus aureus* (continued)

- Foodborne illness – gastroenteritis due to *Staphylococcus* Enterotoxins (SET)
  - Infective dose 10^6 CFU/g

- Illness - nausea, vomiting, abdominal pain, diarrhea.

- Can survive improper fermentation (Summer sausage).

- *S. aureus* - not affected by high salt concentration (~15%) or nitrite, can grow below aw 0.91. Does not grow well when other bacteria are present.

- SET not affected by cooking or proteases. SEA 80%, SEB 10%, SED 37% (SEC and SEE also implicated).

- If *S. aureus* growth is suspected, you must look for SET (not for *S. aureus*) because *S. aureus* could be dead (or non-culturable) but the SET will be present (and make people ill).

Comparison of Foodborne Pathogen Characteristics

Bacteria	Growth temp min / optimum range / max (°F)	lower pH limit	lower a_w	Infectious dose
Bacillus cereus (salt 7.5%; IT and TI)	41 / 82-104 / 131	4.9	0.93	10^5 CFU/g
Campylobacter spp. (5% O_2; IN)	86 / 98-108 / 122	5.0	0.98	500 CFU/g
Clostridium botulinum (salt 10%; NS; IT)	50 / 95-104 / 122	4.7	0.93	toxin: 1 ng/kg body weight
Clostridium perfringens (salt 7%; NS; TI)	43 / 109.4-117 / 126	5.0	0.93	10^6 CFU/g
Shiga Toxin-producing *E. coli* (salt 3.5%; TI)	39 / 95-99 / 113	3.6	0.90	1-100 CFU/g
Listeria monocytogenes (salt 13-25%; IN)	32 / 86-99 / 113	4.4	0.92	1000 CFU
Salmonella spp. (IN)	36 / 95-99 / 129	4.1	0.93	10^5 – 10^6 CFU/g
Staphylococcus aureus (salt 15%; NIS; IT)	45 / 95-104 / 118	4.2	0.83	10^5 CFU/g toxin: 1 µg/kg

IN = Infection; IT = Intoxication preformed toxin; TI = Intoxication toxin made during infection in situ; NS = nitrite-sensitive; NIS = nitrite insensitive

General Measures to Prevent Bacterial Outgrowth

- Preventing foodborne illness
    - Cooking or Lethality step – refer to Appendix A
    - Ensure food is maintained either at a temperature above 140°F or refrigerated below 40°F (outside the danger zone)
    - Proper stabilization – refer to Appendix B; cool cooked foods that will not be immediately consumed to below 40°F.

Hurdle Technology in Meat and Poultry Processing

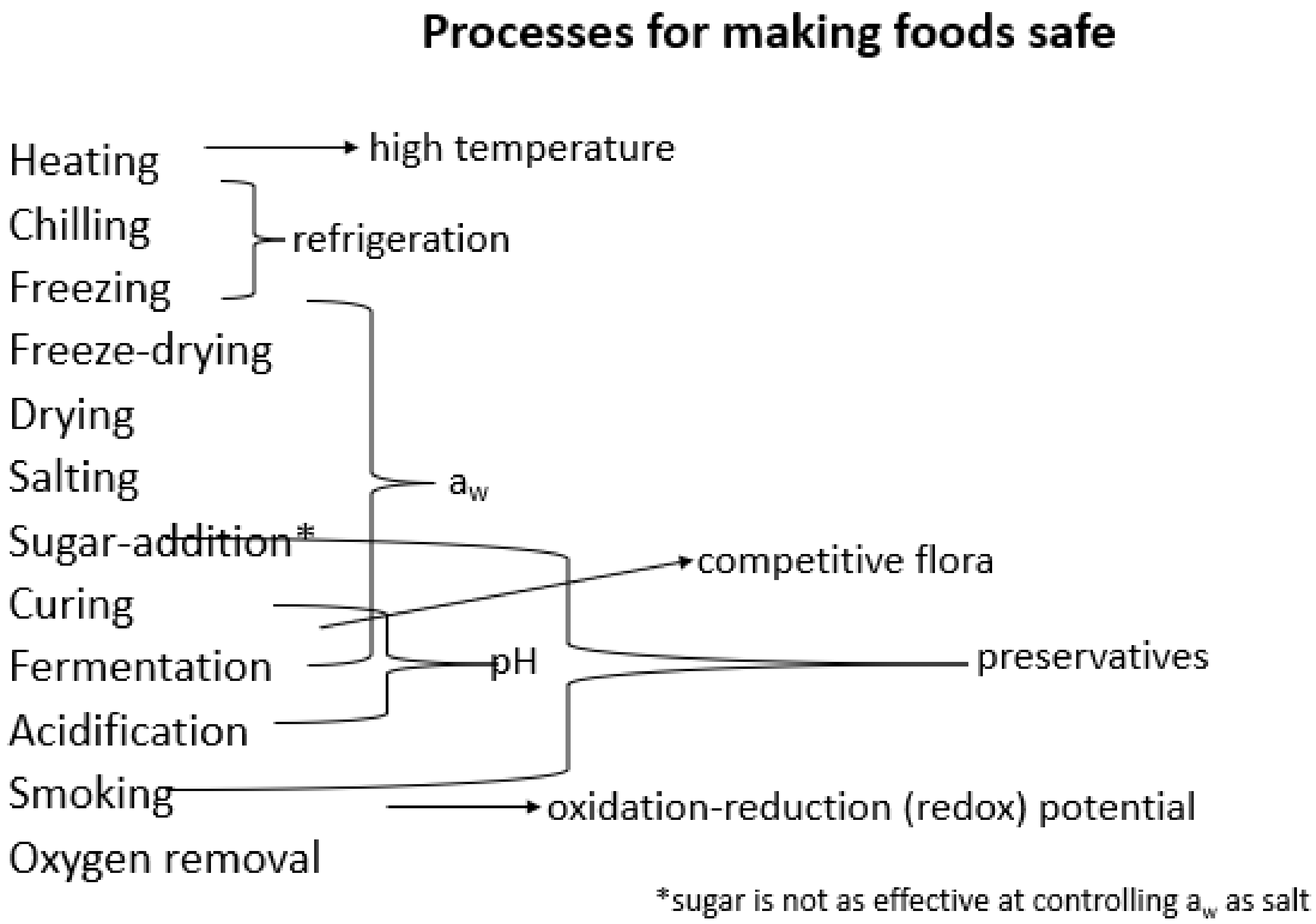
- Hurdle technology involves several inhibitory factors used together to control or eliminate pathogen growth, which would be ineffective if used alone (Leistner, 1976, 1992).

- Deliberately combines existing and new preservation techniques (salting, curing, drying, etc.) to establish a series of preservative factors that microorganisms are unable to overcome.

- Hurdle technology combines attempts to disrupt one or more homeostatic mechanism of bacteria to cause the microbes to become inactive or die.

- The best plans use multiple small hurdles to disturb several bacterial homeostatic mechanisms.

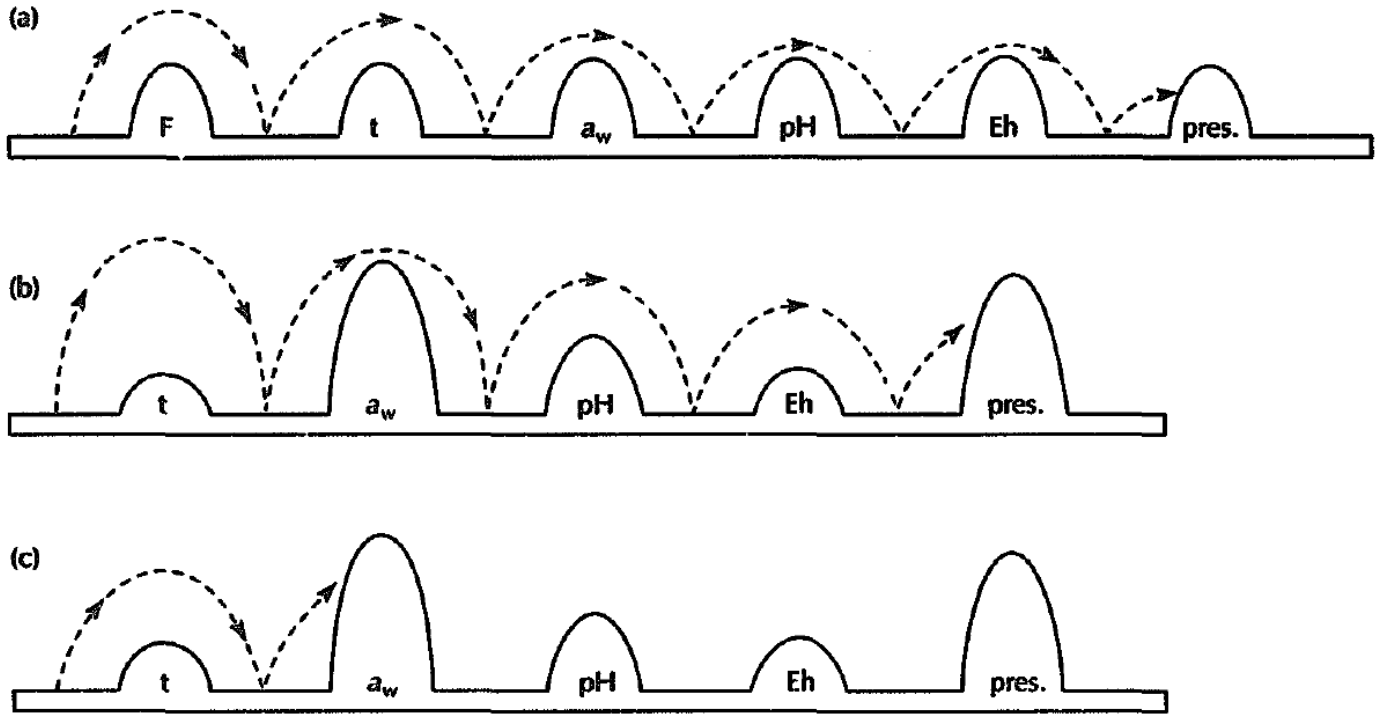
Hurdle Technology: Use of Multiple Food Safety Interventions



Graphic Depiction of Hurdle Technology

F = heating
t = refrigeration
a_w = water activity
pH = acidification
Eh = Redox potential
pres. = preservatives

Leistner (1995)



A single intervention may not be enough to kill or prevent bacteria from growing.

Combining multiple interventions, where each alone may have with a minor contribution, but together have an additive or synergistic effect by disrupting one or more physiological pathways.

Example: Food Code – Interaction of Hurdles: pH and a_w

Interaction of pH and a_w for control of spores in FOOD heat-treated to destroy vegetative cells and subsequently PACKAGED

Aw Values	pH: 4.8 or less	pH: > 4.6-5.6	pH: > 5.6
≤ 0.92	Non-TCS Food*	Non-TCS Food	Non-TCS Food
> 0.92 -0.95	Non-TCS Food	Non-TCS Food	PA**
> 0.95	Non-TCS Food	PA	PA

***TSC FOOD means TIME/TEMPERAURE CONTROL FOR Safety Food**
**** means Product Assessment required**

Interaction of pH and a_w for control of vegetative cells and spores in FOOD not heat-treated or heat-treated but not packaged

Aw Values	pH: < 4.2	pH: 4.2-4.6	pH: > 4.6-5.0	pH: > 5.0
< 0.88	Non-TCS Food*	Non-TCS Food	Non-TCS Food	Non-TCS Food
0.88 - 0.90	Non-TCS Food	Non-TCS Food	Non-TCS Food	PA**
> 0.90 – 0.92	Non-TCS Food	Non-TCS Food	PA	PA
> 0.93	Non-TCS Food	PA	PA	PA

FDA Food Code 2017

Cold Storage Deviation – An Example

- Heat Treated, Not Fully Cooked, Not Shelf Stable sausage (vacuum-packed) and Heat Treated, Fully Cooked, Not Shelf Stable (vacuum-packed)
  - Bacon
  - Head cheese
  - Pork sausage, low sodium sausage, sausage with jalapeno and cheese, andouille, hot pork

- During an FSA, the cooler temperature at the coldest part was 48°F.

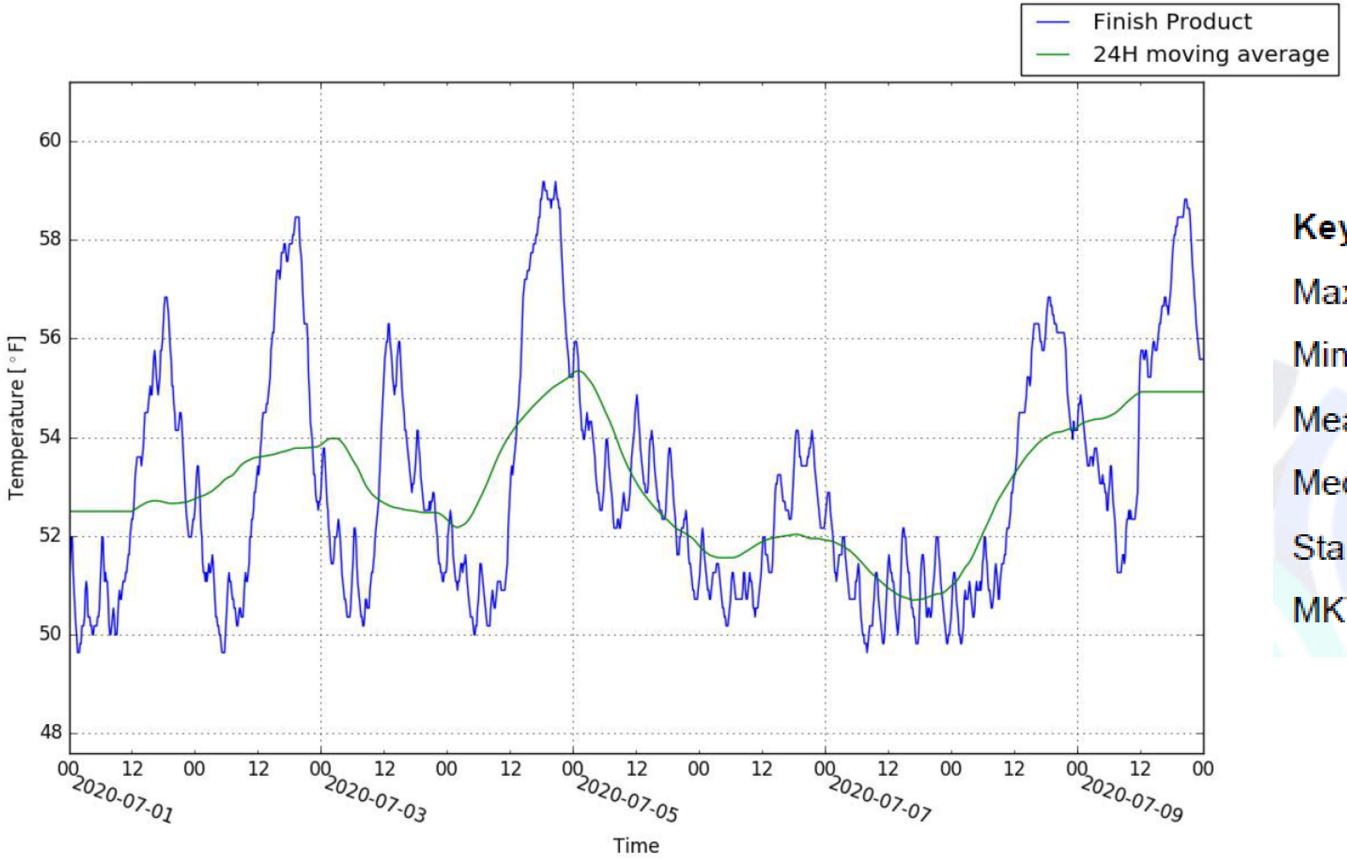
- Internal product temperatures varied between 48 and 53°F.

- Previous month's temperature log showed temperatures fluctuated between 45.1 and 59.2°F.

Note: Every situation is different and must be handled individually.

Cold Storage Deviation – An Example

Details		**Interval**	
Sensor ID:	1300067C:DE:0	Time Zone:	Central Standard Time
Sensor Name:	Finish Product Cooler	Interval:	One Time Report
Location:		From:	2020-07-01 00:00:00
Description:	Finish Product Cooler	To:	2020-07-10 00:00:00

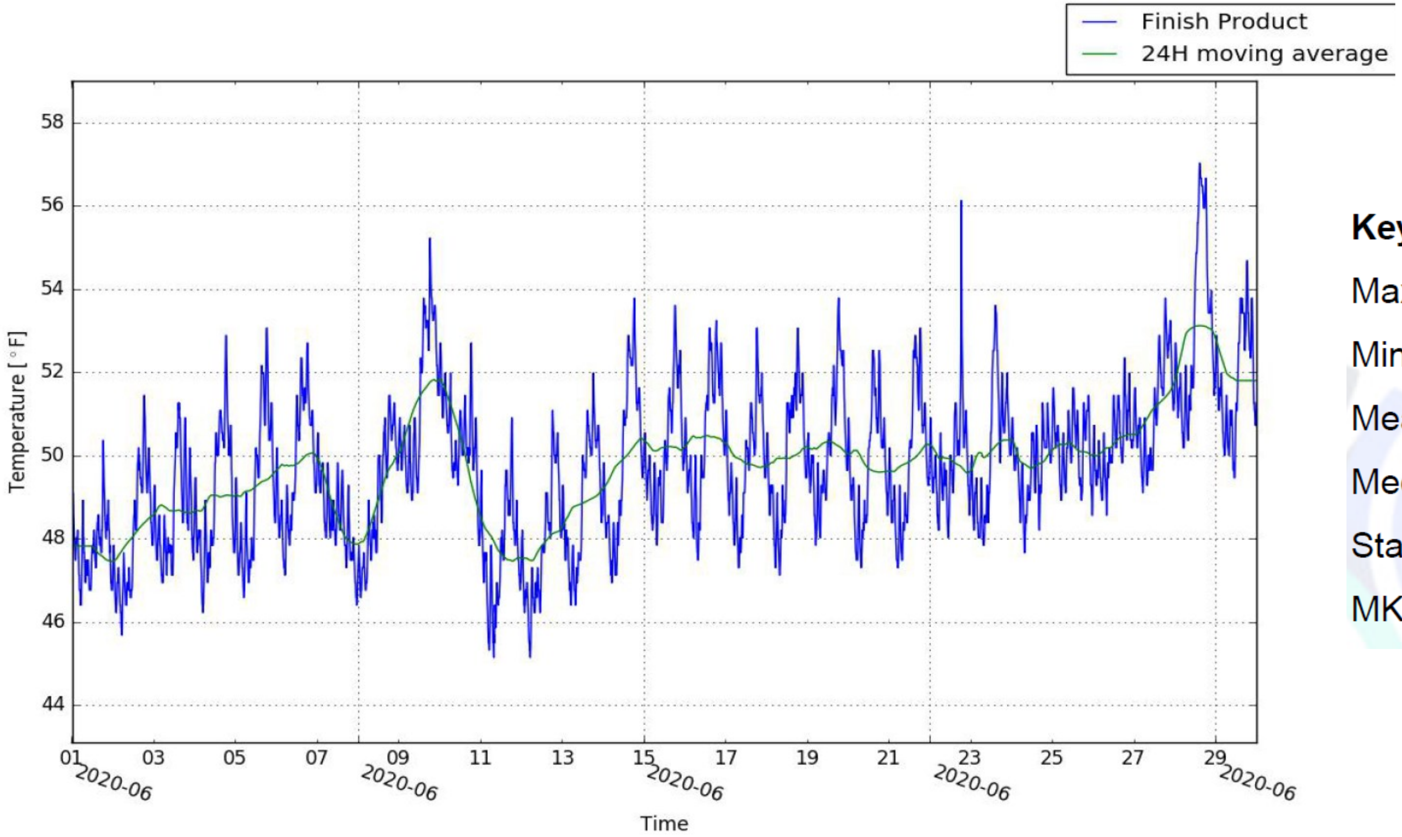


Key Values

Max Value:	59.2
Min Value:	49.6
Mean Value:	53
Median Value:	52.5
Standard Deviation:	1.3
MKT:	53.2

Cold Storage Deviation – An Example

Details		**Interval**	
Sensor ID:	1300067C:DE:0	Time Zone:	Central Standard Time
Sensor Name:	Finish Product Cooler	Interval:	One Time Report
Location:		From:	2020-06-01 00:00:00
Description:	Finish Product Cooler	To:	2020-06-30 00:00:00



Key Values

Max Value:	57
Min Value:	45.1
Mean Value:	49.7
Median Value:	49.6
Standard Deviation:	1.0
MKT:	49.9

Cold Storage Deviation – An Example

Concerns with –

Heat-treated, Not Fully Cooked, Not Shelf Stable (vacuum-packed)

- *Clostridium perfringens* (*C. botulinum*)
- *Listeria monocytogenes*
- *Salmonella*
- *Staphylococcus aureus* enterotoxins

Concerns with –

Heat-treated, Fully Cooked, Not Shelf Stable (vacuum-packed)

- *Clostridium perfringens* (*C. botulinum*)
- *Listeria monocytogenes*
- *Staphylococcus aureus* enterotoxins

- Multiple discussions between OFO, OPPD and OPHS.
- Decided on multiple tests to be performed.

Note: Every situation is different and must be handled individually.

Whole Genome Sequencing (WGS)

Whole Genome Sequencing – A Collaborative Approach

- FSIS worked with the Food and Drug Administration, the Centers for Disease Control and Prevention (CDC), with PulseNet partners on:
  - How to perform WGS – methodology (aligned methods)
  - Analyze WGS data
  - Interpret WGS data

- FSIS began performing WGS for *Listeria monocytogenes* (Lm) in FY13 (along side PFGE) and for all pathogens starting in early FY16.

- FSIS suspended PFGE analysis for Lm and started using WGS data Jan 15, 2018.

Whole Genome Sequencing – Benefits

- WGS benefits FSIS and its mission to protect public health:
  - Detects harborage and cross-contamination of pathogens in FSIS-regulated facilities,
  - Traceback from human illness outbreak data to regulated food products, and
  - Identification of unique genes related to virulence, pathogenicity, survival, adaptation, and resistance to biocides (sanitizers, metal, etc.) and antimicrobials.

Whole Genome Sequencing – Analysis

- FSIS uses different tools to analyze WGS information including:
  - Public Sequence Typing
  - Multi-locus Sequence Typing (MLST)
  - Core genome analysis (~1800 genes for Lm)
  - Phylogenetic analysis
  - High-quality Single Nucleotide Polymorphisms (hqSNP)

Whole Genome Sequencing – Single Nucleotide Polymorphism (SNP)

Single Nucleotide Polymorphism (SNP)

ATGTT**C**CTC isolate A

ATGTT**G**CTC isolate B

Whole Genome Sequencing – Sequence Typing

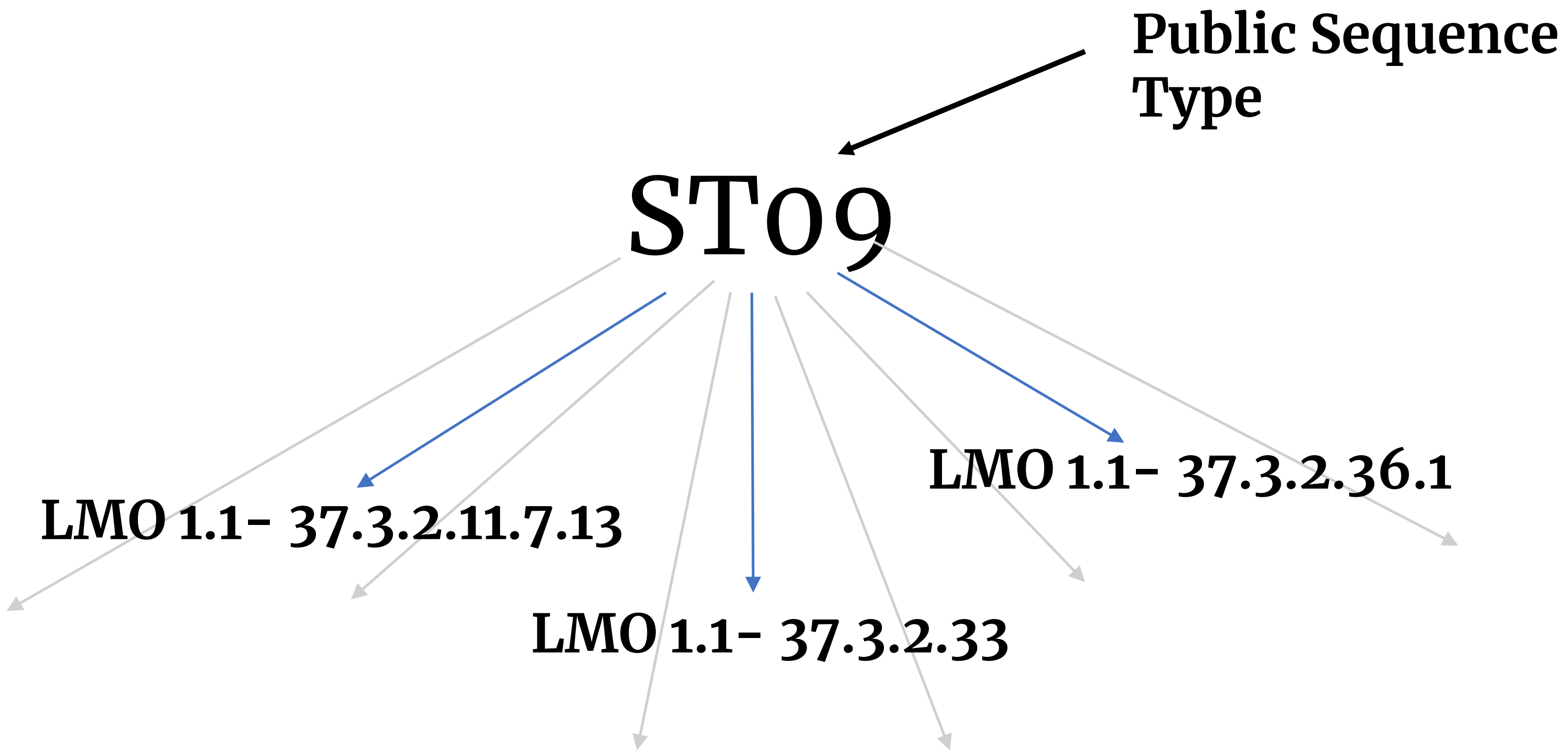
Multi-locus Sequence Typing (MLST)

- MLST can generate a **pattern name or designation** based on differences in a pre-defined set of genes.

- MLST Results will be Provided by FSIS as Follows:
  - **Public Sequence Type** (“MLST ST”, “ST”, or “pubST”)
    - small number of genes (i.e., 6-12)
    - named using the publicly available database developed by Jolley & Maiden (2010) (e.g., publicST09)

- **Allele Code**
  - compares ~1,800 genes for Lm
  - named by using CDC PulseNet numerical code (e.g., LMO1.1-5.1.1.2.5.1)

Whole Genome Sequencing – Single Nucleotide Polymorphism (SNP)



Allele Code is more specific than Public Sequence Type; one Public Sequence Type can be inclusive of many Allele Codes.

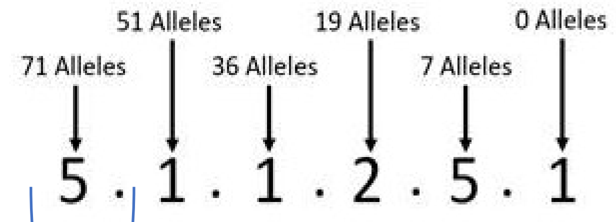
Whole Genome Sequencing – Single Nucleotide Polymorphism (SNP)

Example: LMO1.1 - 5.1.1.2.5.1

LMO – *L. monocytogenes*
Version 1.1

Allele codes are a nomenclature scheme created by CDC

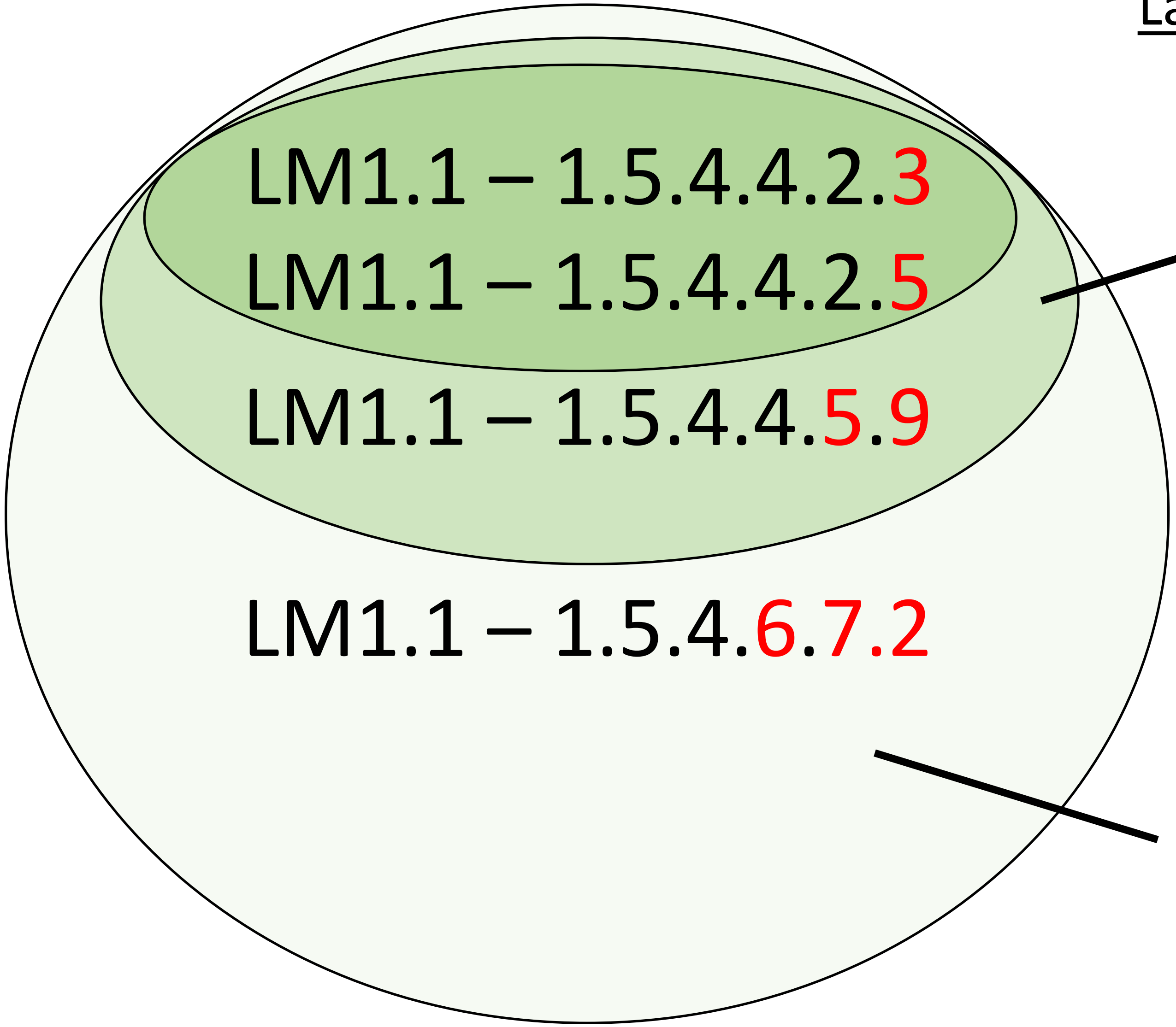
Like PFGE patterns, allele codes simplify how we communicate about pathogen strains



a "field"

If the first four fields between two isolates match, the isolates may be closely related

Background: What does allele code tell you?



<u>Last digit same or different</u>
differ by 0 – 19 alleles
"closely related"

<u>3rd to last digit different</u>
differ by > 19 alleles
"not closely related"

Establishment-specific Datasets

Allele codes for Lm have been reported since 2019

Fields were created for *Salmonella* and STEC allele codes (*Campylobacter* in development)

Date Stamp format (allele code (space) date mm/dd/yyyy

LM1.0-23.5.6.0 04/05/2022 → Retrieval of the allele code from PulseNet

Allele codes may change over time, a date-stamp supports use of the data in static reports

Whole Genome Sequencing – Allele Codes

- Allele codes are a nomenclature scheme created by CDC.
- Like PFGE patterns, allele codes simplify how we communicate about pathogen strains.
- Allele codes can be used for trend analysis and to interpret relatedness.

Whole Genome Sequencing – Analysis - Microbial Characterization Branch (MCB) - Eastern Lab, Athens, GA

Establishment	Field	590668019	590668018	201074252	201047328
M54-P54 (LocID: 9542)	FormID	102595413	102595402	11629154	11610429
	Collect Date	2020-11-09	2020-11-09	2012-03-20	2011-10-11
	Allele Code	LMO1.1 - 5.1.2.5.4.1	LMO1.1 - 5.1.2.5.4.1	LMO1.1 - 5.1.2.5.2	LMO1.1 - 5.1.2.5.2
	MLST ST	ST204	ST204	publicST204	publicST204
	Project	INTENV_LM_M	INTCONT_LM_M	INTENV	RTE001
	FSIS Identifier	FSIS22029688	FSIS22029687	FSIS11816785	FSIS11816784
	NCBI Accession Number	SAMN16839333	SAMN16839186	SAMN10645629	SAMN10645628
	NCBI SNP Cluster (Retrieve Date)	PDS000024493.9 2020-11-23	PDS000024493.9 2020-11-23	PDS000024493.9 2020-11-23	PDS000024493.9 2020-11-23
	Min Food Env (SNP)*	0	0	8	5
	Indicative of Potential Harborage**	Yes	Yes		
	Indicative of cross-contamination***	Yes	Yes		
	Min Clinical (SNP)*	None	None	None	None
	Potentially related to a clinical isolate****	No	No		

LIMS ID

Form ID

Allele Code

FSIS Identifier

*A value of "None" indicates greater than ~50 SNPs for this isolate source

**Harborage, or repeated introduction is suggested if WGS analysis indicates closely related Lm isolates are found in product, food contact, or non-food contact environmental samples collected over multiple days, weeks, months, or years. FSIS Notice 48-18

***Cross-contamination is suggested when closely related Lm isolates are found in product, food contact, and environmental (nonfood contact) samples collected during the same sampling event. FSIS Notice 48-18

**** Clinical isolates collected and uploaded within two years of the new isolate based on available NCBI metadata.

Harborage and Cross-contamination

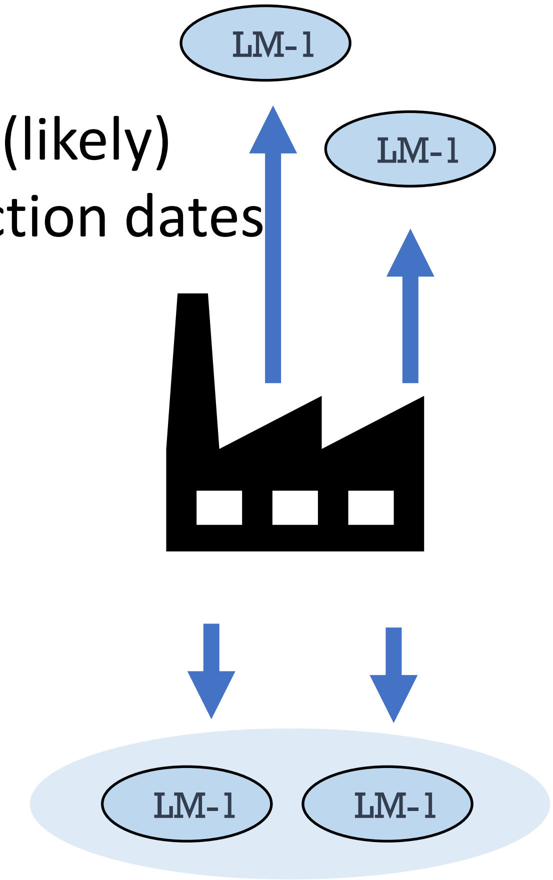
- **Harborage** or **persistent** contamination of the post-lethality environment, is suggested if WGS analysis indicates closely related *Lm* isolates are found in product, food contact, or non-food contact environmental samples that were collected over multiple days, weeks, months, or years.

- **Cross-contamination** is suggested when closely related *Lm* isolates are found in product, food contact, and environmental (non-food contact) samples collected during the same sampling event.

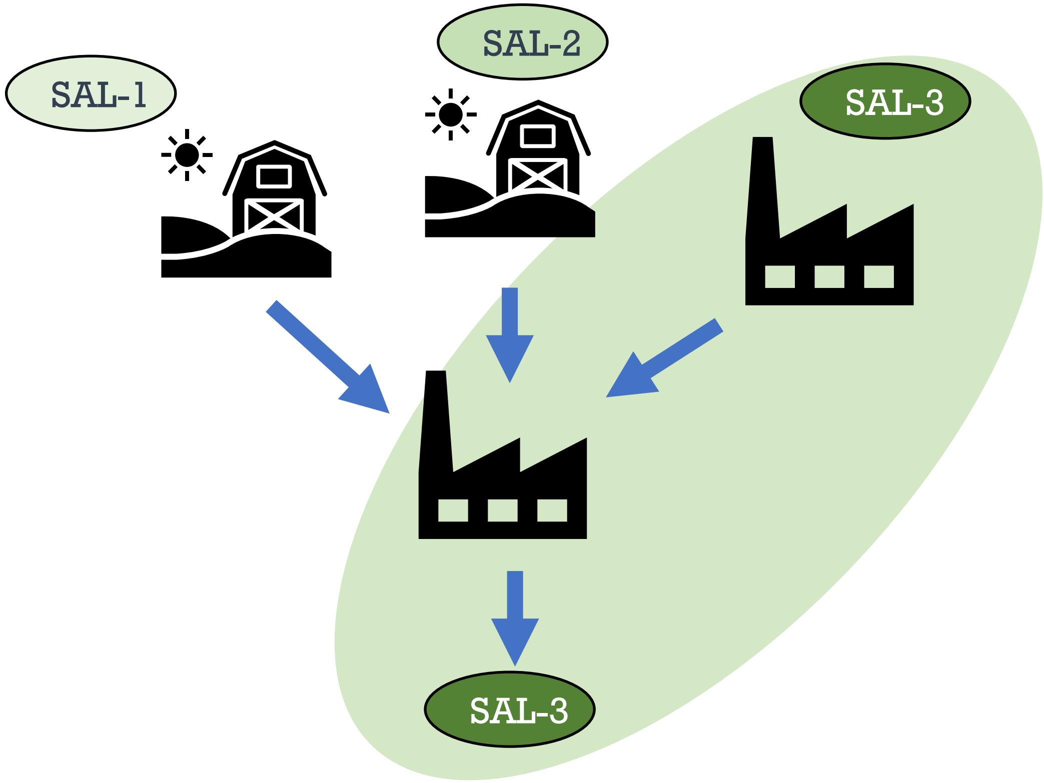
  If *Lm* is isolated from a post-lethality exposed product sample and from a food contact surface sample, the food contact surface is more likely to be the source, unless under-processing of RTE product is suspected.

Harborage and Cross-contamination

Harborage (likely)
different collection dates



Cross-contamination (possible)
same collection date



Source

Recommend corrections to food safety controls

Asking for More Information

- When performing a PHRE in establishments with more than one positive RTE sample, EIAOs are to:
    - **Use the Form ID to Request WGS analysis** of previous matches from the OPHS - Microbial Characterization Branch (OPHS-MCB) from Outbreaks_WGS@fsis.usda.gov
    - The WGS analysis will indicate if there is a history of harborage or cross-contamination in the establishment.

- After an IVT/R*Lm* positive, EIAOs are to make a request through the Outbreaks_WGS@fsis.usda.gov Outlook mailbox for WGS analyses.

Summary - I

- Bacteria are either cocci, bacilli (rods) or spirochetes (cork-screw) shaped; bacilli (rods) are about 1 x 0.5 µm and are broadly characterized by their reaction to a Gram stain.

- Many foodborne pathogens are Gram(-), mesophilic, facultatively anaerobic, enterics (*Campylobacter*, *E. coli*/STEC, *Salmonella*).

- Some foodborne Gram(+) bacteria include *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Listeria monocytogenes*, *Staphylococcus aureus*.

- Intrinsic factors are those characteristics that are inherent to the food, i.e., pH, a_w, nutrient content, physical structure.

Summary - II

- Extrinsic factors are those characteristics that are related to the external environment of the food, i.e., temperature, relative humidity, gases in the environment (packaging), presence of other bacteria.

- The conditions for bacterial growth:
  Food, Acidic environment, Temperature, Time, Oxygen, Moisture (FATTOM).

- The Danger Zone is the temperature range where mesophilic bacteria are most easily able to grow and is between 40 to 140°F.

- Water activity describes the physio-chemical characteristic of water bound by food compounds making it unavailable to bacteria.

- pH describes the acidity of a solution and is a logarithmic scale; most meats are generally between pH 5.4-6.8.

Summary - III

- *Bacillus cereus*, *Clostridium botulinum*, and *Clostridium perfringens* are Gram(+) spore formers.

- *Clostridium* is nitrite-sensitive; *C. perfringens* grows quickly and controlling it generally means *C. botulinum* and *Bacillus cereus* will also be controlled.

- *Listeria monocytogenes*; G(+); can grow at refrigerated temperatures, in high salt, and low pH. Lactate/diacetate hinder growth. Spreads due to cross contamination by moisture. Forms a biofilm that is hard to detect and eradicate.

- *Salmonella*; G(-); is hardy; can survive in low water activity foods for weeks; can become heat- and acid-resistant.

- STEC; G(-); is hardy, can survive in low pH, can become heat- and acid-resistant; FSIS considers some foods containing any of the seven serovars as adulterated.

Summary - IV

- *Staphylococcus aureus*; Gram(+); human-associated and transferred by poor worker hygiene; tolerates high salt, low aw; does not grow well in the presence of other bacteria; nitrite has little effect.

- Hurdle technology is the additive/synergistic effect of combining multiple interventions that each have a minor contribution in killing pathogens.

- Foodborne illness is generally caused by one of three mechanisms: ingesting pathogenic bacteria, consuming food containing pre-formed toxin, or when pathogenic bacteria make toxin in the intestine.

- FSIS reports Listeria monocytogenes by an allele code; if the first four fields between two isolates match, the two isolates may be closely related
  (≤19 alleles apart).

Food Safety and Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

fsis.usda.gov