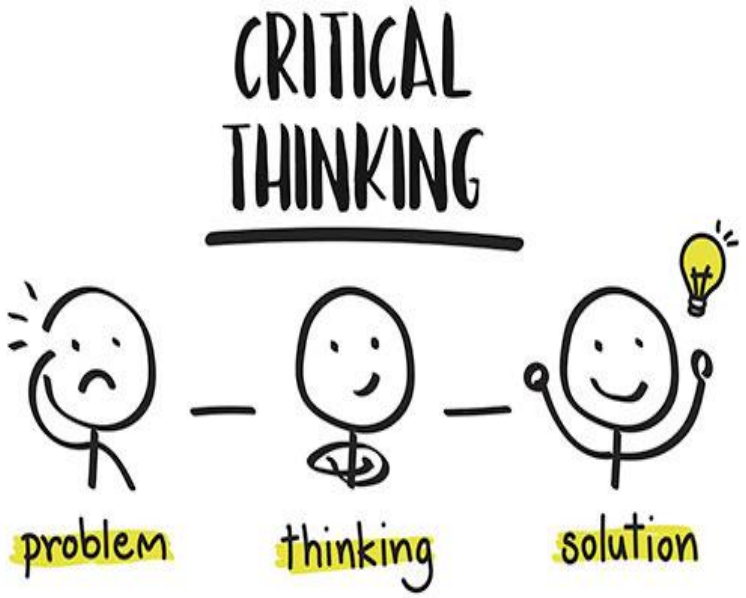
Guidance on Pre-Harvest Testing

Overview

- Micro Basics as they pertain to pre-harvest testing on produce
- What do I want to test for and why?
- Sampling
- Lotting
- Choosing a lab
- Questions to explore when choosing a lab
- Choosing an assay or method
- Questions to explore when choosing an assay/method
- Resources

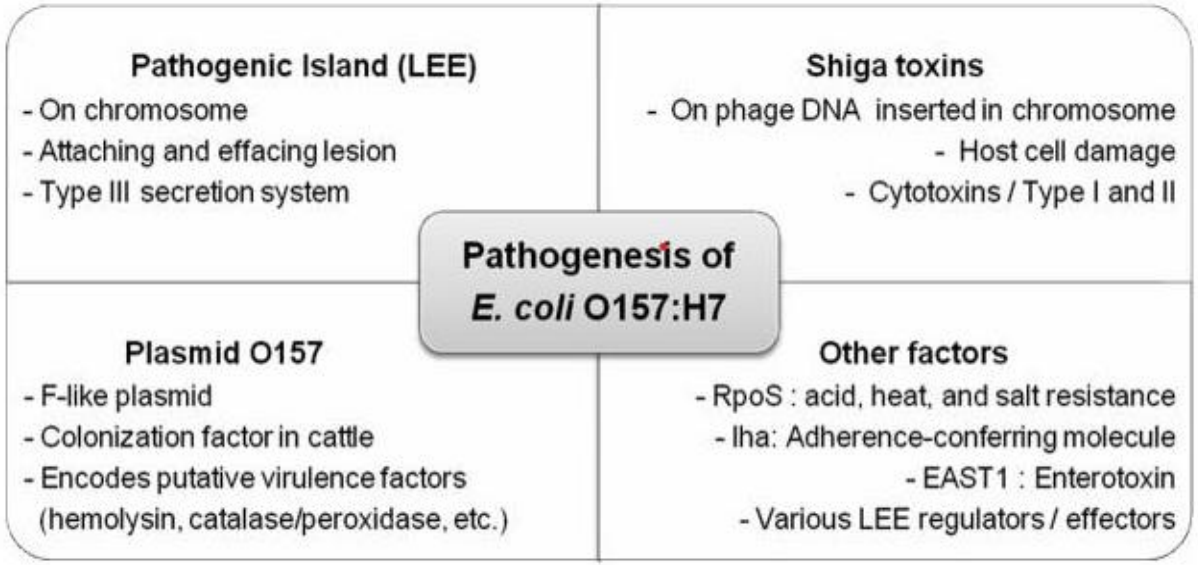


Quick Micro Overview

Why do we have to test particular organisms?

E. Coli O157:H7

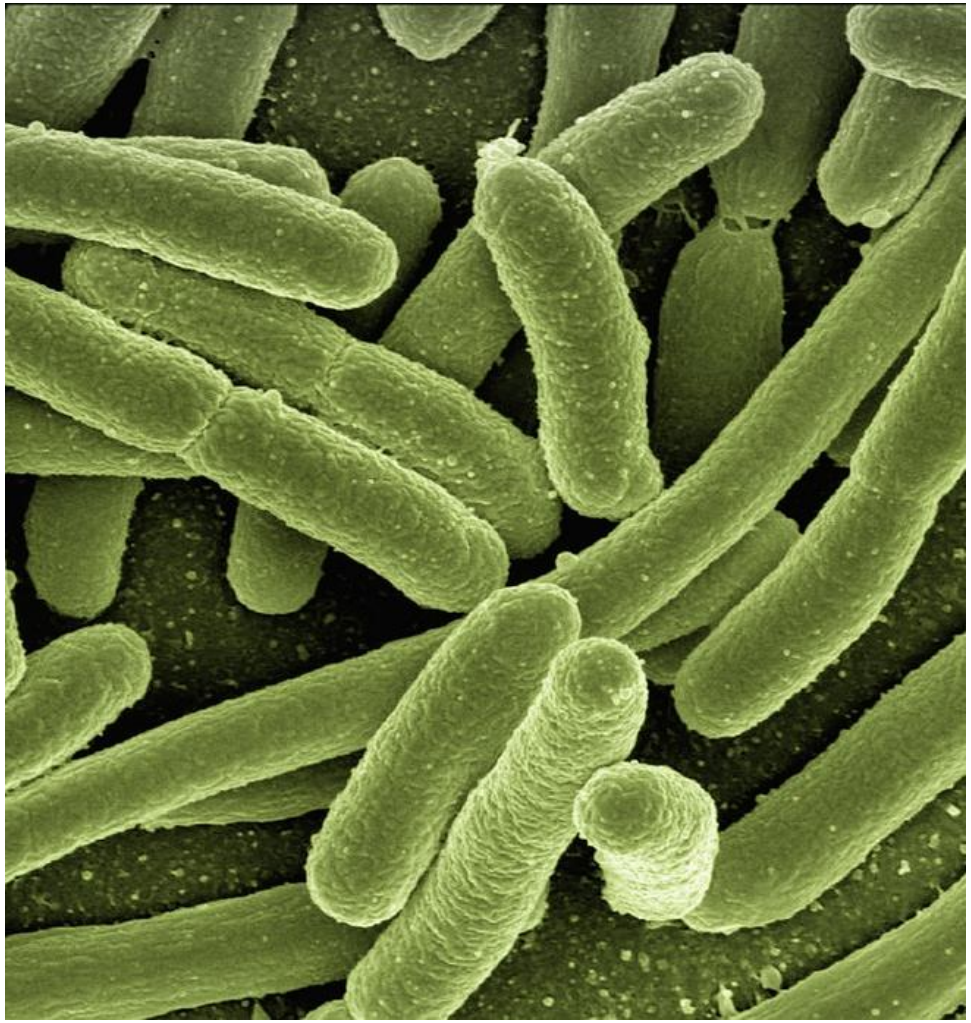
- Most *E. coli* strains harmlessly colonize the gut of humans and animals as normal flora
- Most common in foodborne illness
- This particular strain of *E. coli* expresses somatic (O) antigen 157 and flagella (H) antigen 7
- Gram –negative rod shaped, facultative anaerobic bacterium
- Found in the gut and feces of many animals (cattle major reservoir)
- Involved in foodborne illness
- Pathogenic and produces Shiga Toxins
  - Stx 1
  - Stx 2 (thought to be more toxic)
- Has the EAE gene



What are the "Big Six"?

- Six serotypes or strains of E. coli are known to cause illness:
  - *E.coli* 026 (2nd most common)
  - *E. coli* O45
  - *E. coli* O103
  - *E. coli* O111
  - *E. coli* 0121
  - *E.coli* O145
- Why are they called STECs?
  - Shiga Toxin Producing *E. coli*
- These strains are the most commonly identified non-O157 found in foods and cause over 200,000 foodborne illnesses in the US each year

- **Note: These will become important later when we discuss testing**



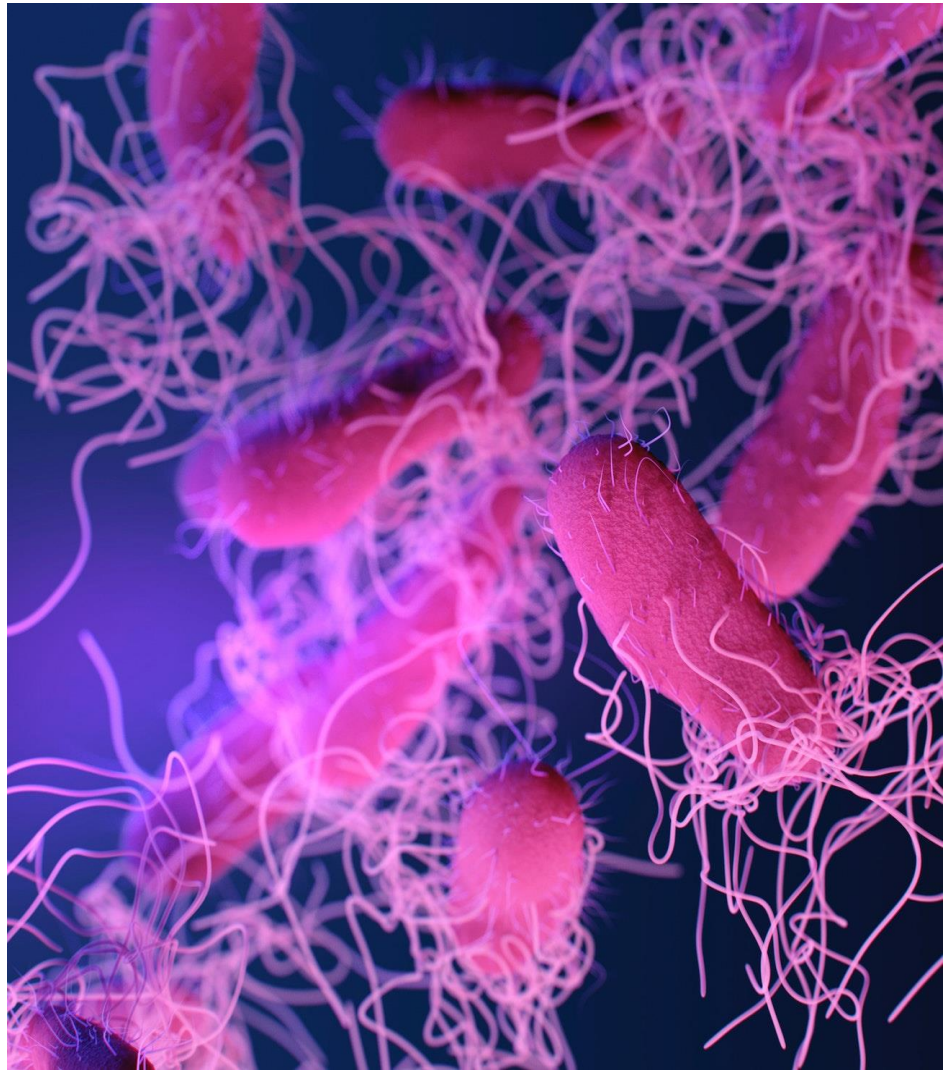
Are there other *E. coli* serotypes that are pathogenic?

- **Yes!**
- Pathogenic *E. coli* strains are categorized into "pathotypes":
  - Enterotoxigenic *E. coli* (ETEC)
  - Enteropathogenic *E. coli* (EPEC)
  - Enteroaggregative *E. coli* (EAEC)
  - Enteroinvasive *E. coli* (EIEC)
  - Diffusely adherent *E. coli* (DAEC)
- **Why then do we focus on STECs?**
  - Most associated with foodborne illnesses, often very serious illnesses
  - Also referred to as EHEC-enterohemorrhagic *E. coli* (some labs report this way)

Salmonella spp.

- Salmonella is a gram-negative rod belonging to the Enterobacteriaceae
- All strains of Salmonella are pathogenic and have ability to invade, replicate, and survive in human cells
- Over 2,500 different serotypes/serovars identified to date
- What are the most common strains involved in foodborne illness?
  - Salmonella Enteritidis
  - Salmonella Typhimurium
  - Salmonella Newport
  - Salmonella Javiana
  - Salmonella Heidelberg
- Estimated 1.35 M Salmonella cases occur annually in US

Note: Important to serotype your positive salmonella strains to help work through root cause



What should we test for?

Think of pathogens that pertain to produce…

How to choose pathogens to test for?

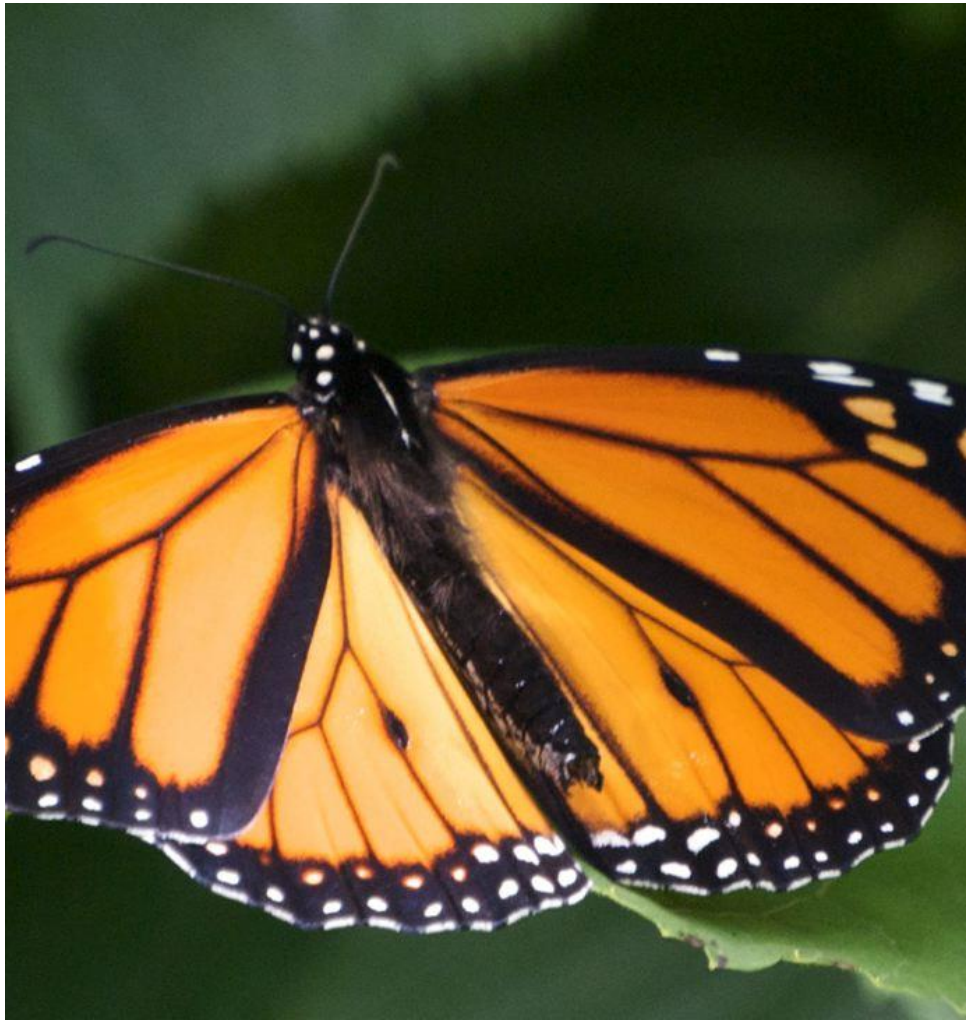
- Use your resources to identify which pathogens have been involved in Produce outbreaks or could be risk factors associated with Produce
- E. coli O157:H7- the most common of *E. colis'* involved in foodborne illness in Produce. This would be a good one to test
- Salmonella- Salmonella has so many serotypes that are all pathogenic and come from so many places. This would be a good one to add.
- The "Big Six" –these have been identified as STECs and have been associated with some illness. You may want to consider adding these.
- Remember to assess risk
- Extended testing- (discussion point)

Can I Change my Program?

Change is good…

Assess program by using data

- Start with a program by discussing with business and identifying what is it you want to accomplish
- Have monthly business updates and go through data
- If you get a positive conduct root cause assessment
  - Try to identify anything that could have been a contributing factor
  - Take additional samples (tissue, water, environmental)
- After having enough data (you decide what enough is) start to mold your program
- Things can change for example “season after season I get a positive in this field around this lot”
  - Have a discussion with Ag Ops and Grower
  - Try and identify any hazards, risks, etc
- The program should be a living document and you learn as you go
- Maybe you test at different times of the year in different ways
- Maybe you have an escalation protocol



Sampling

Who is going to sample this product? How? When?

Identifying the right sampling program for you…

- Samplers can be internal
  - Participate in the training
  - Spend time watching the process
  - Conduct unannounced visits to ensure SOPs are being followed
- Samplers can be third party
  - Recommended doing on cost analysis based on volume
  - Get multiple quotes
  - Review how they are trained
  - Conduct unannounced visits to ensure SOPs are being followed
- Samplers need training on how to sample, aseptic technique, environmental assessments, and the "whys" behind sampling
  - Third party resources conduct this training and can certify your samplers
  - Look at acreage you want sampled and determine how many samplers are needed
  - Understand where samples get maxed out (i.e. 96 samples)
  - Determine regions and commodities to be sampled and how to this will be coordinated
  - Determine tools needed for sampling, add to budget
  - Partner with Ag Ops teams to help get this information

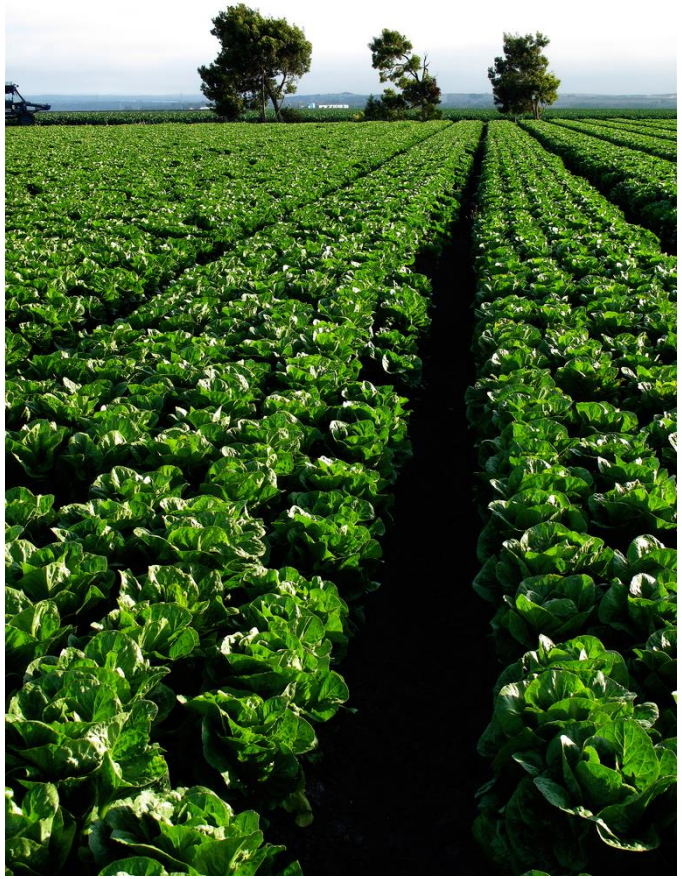
Lotting

How should we set up sampling lots?

What is a sampling lot? How do we decide?

Always partner on these discussions

- Sampling lot can be of your choosing
- Recommend holding a strategy meeting with key partners in your business to help figure out what works best
- Use your resources, such as the work LGMA and Western Growers have done on confidence levels to help with the conversation
- Understand how product will be tested and understand the consequences and required actions if you get a positive result
  - For example, 1,500 grams sampled across 10 acres-all 10 acres my have to be destroyed if you get a positive
  - For example, 1,500 grams sampled across 1 acre-could potentially release by the acre and save some of the crop
  - For example, 1,500 grams sampled across 2 acres in a 10-acre field-could release in 2-acre increments should you get a positive
  - Have multiple meetings on this and come together on making the decision. Have scenarios identified and any papers, resources, or consultants you may want to bring into the conversation
  - If you are doing spot purchases, develop criteria for those as well
  - Always discuss pros, cons and consequences



Choosing a lab

Ask lots of questions…

How should I choose a lab?

- Research labs in the area or in close proximity
  - Ask about courier services
  - Set up realistic pick-up times
- Ask if they have ever tested produce before
- Ask about current customer load and if they can support your volume
  - You should have acreage in mind to give them along with sampling plan
- Meet the team-lab mangers, technicians, subject matter experts
- Ask about customer service and communication
  - Once lab is chosen, set up calls with some sort of frequency
  - Set up transition calls so labs can be prepared
- Take a field trip and visit the lab-have them walk you through the entire process from start to finish so you have a visual understanding of what occurs when your sample arrives at the lab
- Ask about technician training and proficiency testing
- Ask about protocols in place to ensure lab is not the cause of or contributing to sample contamination
- Set up customer profiles, requirements, or work instructions and ask that they be readily available for all techs

Choosing an assay or method

Good idea to have opens…

How to pick a method/assay? Some questions to ask

- Microbiological assays can be extremely technical, so it is important to research and spend time asking questions
- Examples of questions could be:
  1. Is the method DNA or RNA based? What is the difference?
  2. What genes make something positive?
     i. For example, Stx1, Stx2 and EAE are genes to look for in pathogenic E. coli assays in food
  3. How does the assay work overall?
  4. How many preparation steps are there and is there room for human error?
  5. What type of training is involved at the lab level for lab technicians?
  6. Are there ever false positives or negatives?
  7. Ask for the validations and ask if someone from the developing company can walk you through them?
  8. Incubation time and what does that mean?
  9. Turn around time?
  10. With the microflora present on produce does it cause any interference with the assay? If so, how?
  11. If you get a positive how accurate it is? What type of confirmation is being used?
  12. What is the percent confirmation you get on you assay?
  13. How many primers are being used?

Diversity in Methods? Example of two methods

	3M Isothermal DNA Amplification	PCR (Polymerase Chain Reaction)
Enzyme	*Bst* DNA Polymerase	*Taq* DNA Polymerase
Number of Primers	6 (+)	2
DNA Denaturation	Strand Displacement	Heat
Reaction Temperature	Isothermal (60°C)	Thermal cycling consisting of cycles of repeated heating and cooling of the reaction for DNA denaturation (94°C) and enzymatic DNA replication (55°C then 72°C)
Amplification	Continuous	Cycling
Detection	Bioluminescent Light	Fluorescent Light

Resources

Places you can go for help

Helpful Articles and Studies

- Center for Produce Safety Webinar, Optimizing Rapid Test Methods for Shiga toxin-producing E. coli (STEC) on Fresh Produce and in Ag Environments, August 2014
- LGMA Appendix C – Product testing protocol, Version 8-27-21.1 • IFPA - Field Sampling, Truth and Consequences
- United Fresh. 2010. Microbiological testing of fresh produce. A white paper on considerations in developing and using microbiological sampling and testing procedures if used as part of a food safety program for fresh fruit and vegetable products.
- United Fresh. 2021. Would my sampling plan have detected contamination levels that resulted in an outbreak? Reverse-engineered preharvest sampling plan thought experiment, Jan 2021.
- United Fresh Produce Association, Key Questions Around Sampling and Testing Fresh Produce, August 2019 • Western Growers Lunch & Learn Webinar, Produce Testing: Current state, benefits and challenges, February 2017
- LGMA press release, https://lgma.ca.gov/news/whats-behind-lgmas-preharvest-testing-requirement
- Wilhelmsen, E. 2015, Lot Acceptance Testing for RTE Salads, Food Safety Magazine 2015