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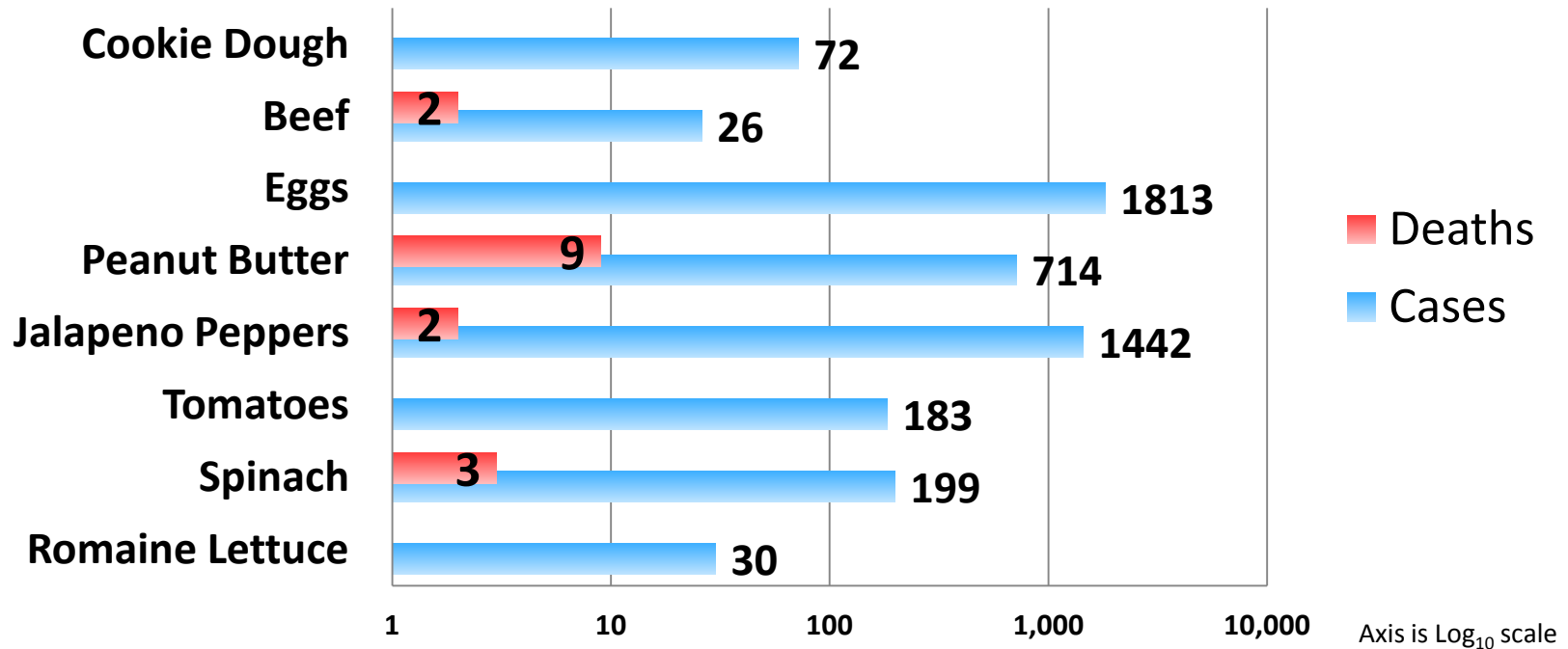
HUMAN PATHOGENS IN THE GREENHOUSE?!



Food Safety

Data Source: CDC.gov

Foodborne Pathogen Outbreaks 2006-2010

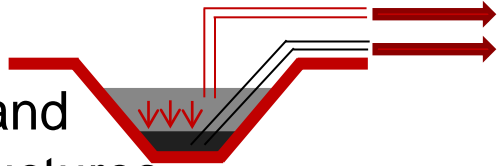


Wastewater & Irrigation

Cows



Storage and treatment structures



Agricultural land



Agricultural runoff



Food crops

Wastewater treatment plants

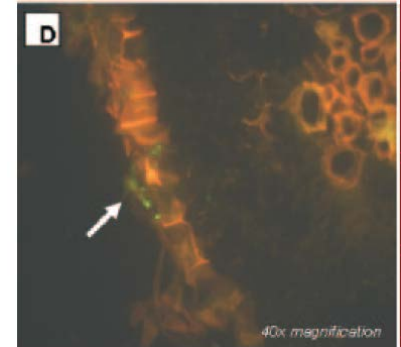
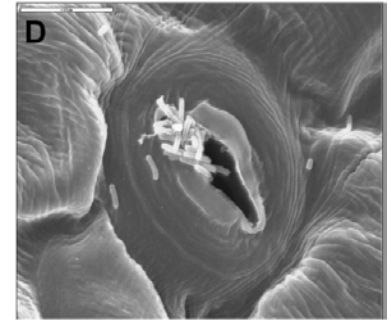


Humans

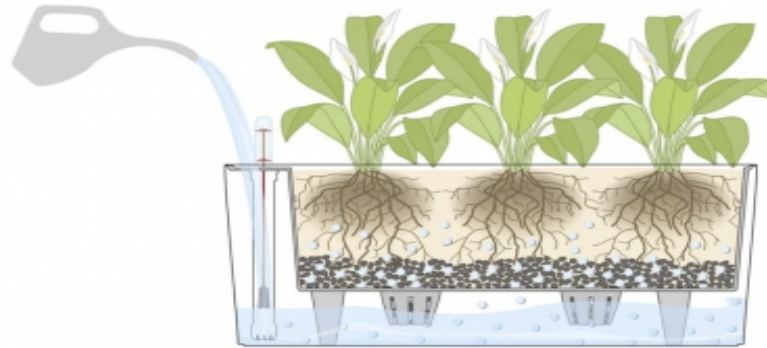


Precedence

- Microbes:
 - Through leaf surface: internalization involves chemotaxis and penetration through open stomata (Kroupitski et al 2009).
 - Through root surface: Salmonella spp. enters lettuce and accumulates at the root-shoot transition region (Klerks et al. 2007).



Experimental Design



Factors	Levels
Concentration in water	10^5 vs. 10^7 CFU/mL
Lettuce cultivar	Green star vs. Green salad bowl
Lettuce age	3 vs. 4½ vs. 6 weeks



Risk Assessment

Hazards/Concerns

- Agent
- Contaminated waste water
- Waste disposal
- Aerosolization?
- Researcher Experience

Procedures

- Preparing pathogen cultures
- Inoculating Plants
- Harvesting infected plant tissue



Facility Components/Features

BSL-2 compliant (for pathogens)

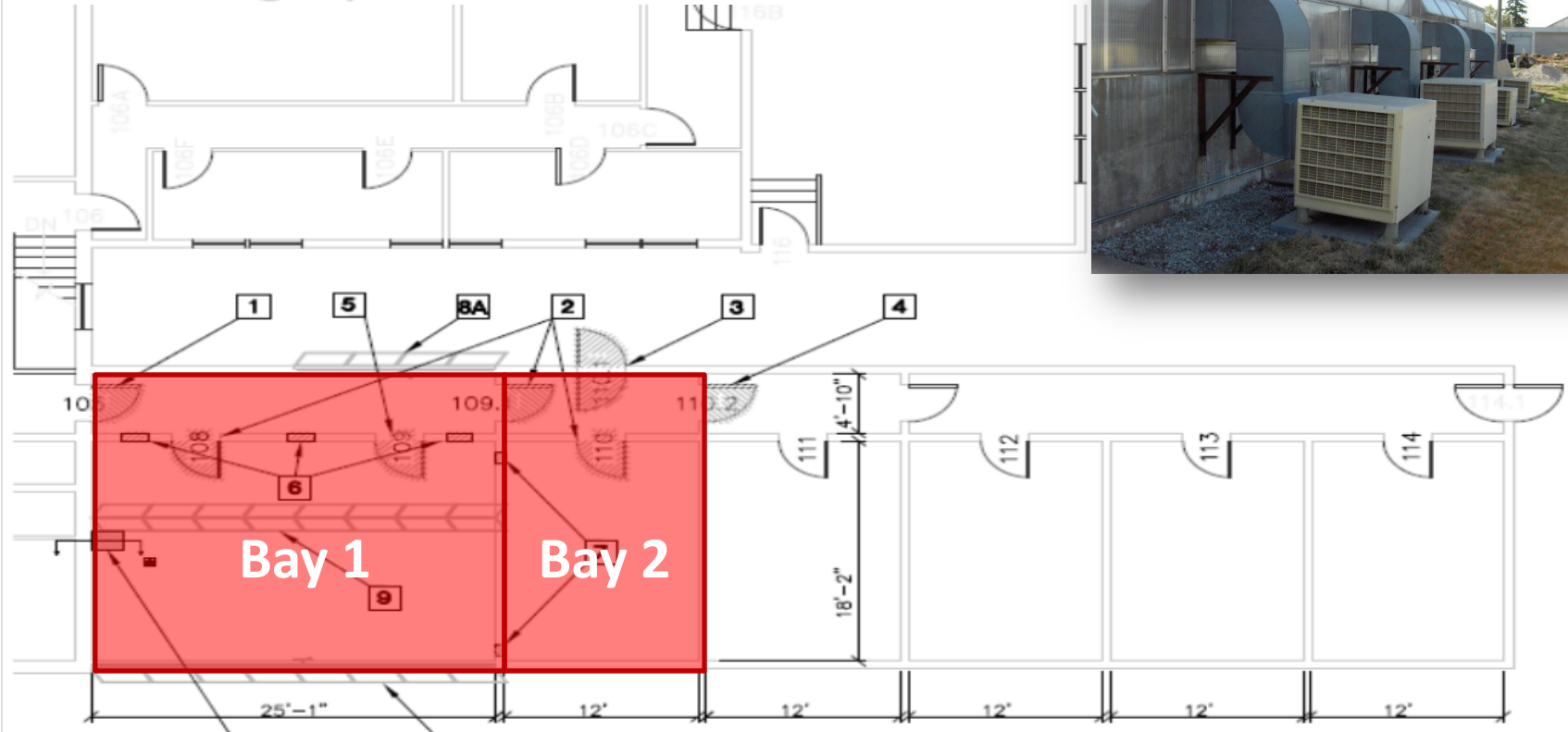
- Negative airflow
- Disinfectable surfaces
- Insect and pest prevention program
- PPE
- Effluent treatment?
- Security
- Autoclave or other waste treatment

Greenhouse (for plants)

- Glass roof
- Growth lights
- Temperature Control
- Cement floor
- Tables for plant trays or pots
- Soil
- Water
- Humidity Control

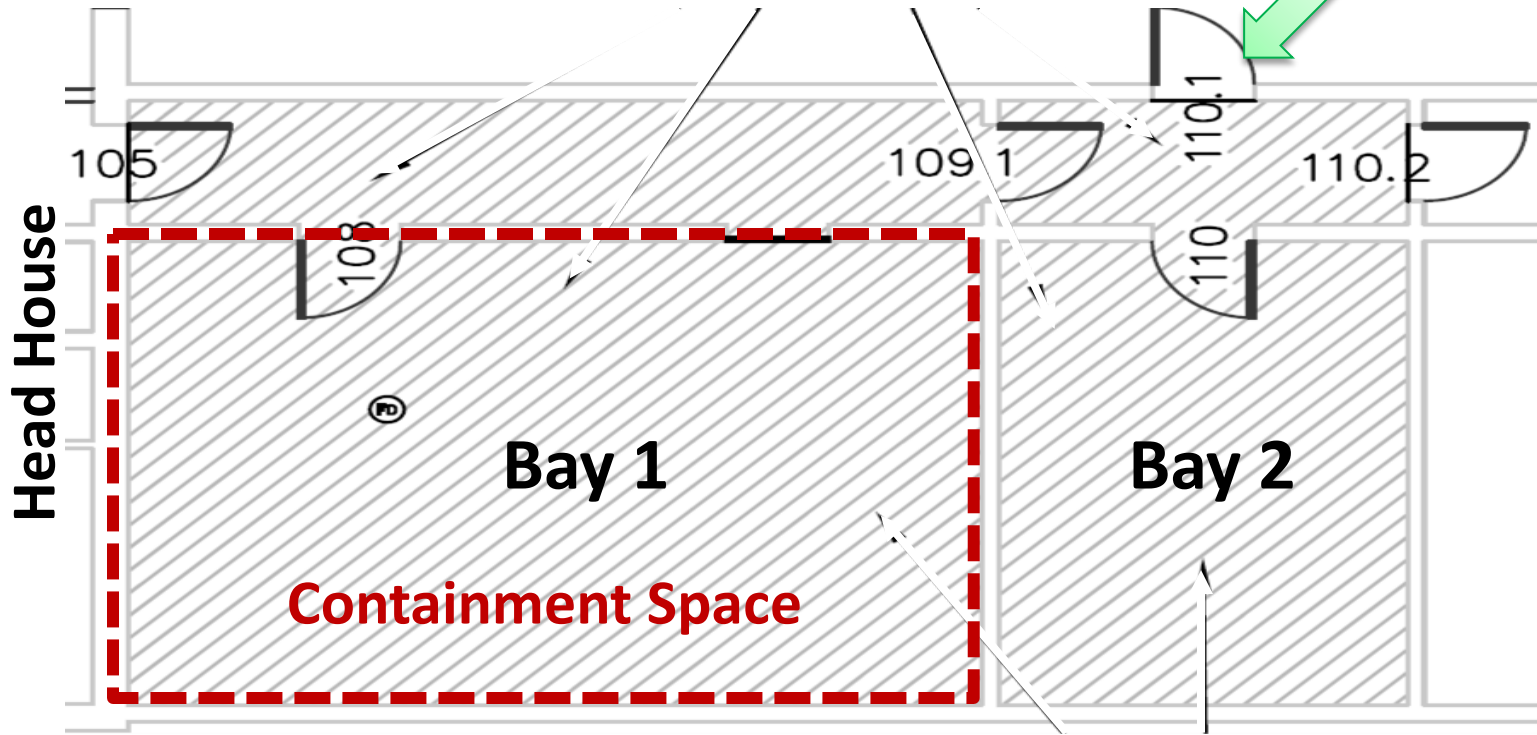


Existing Space

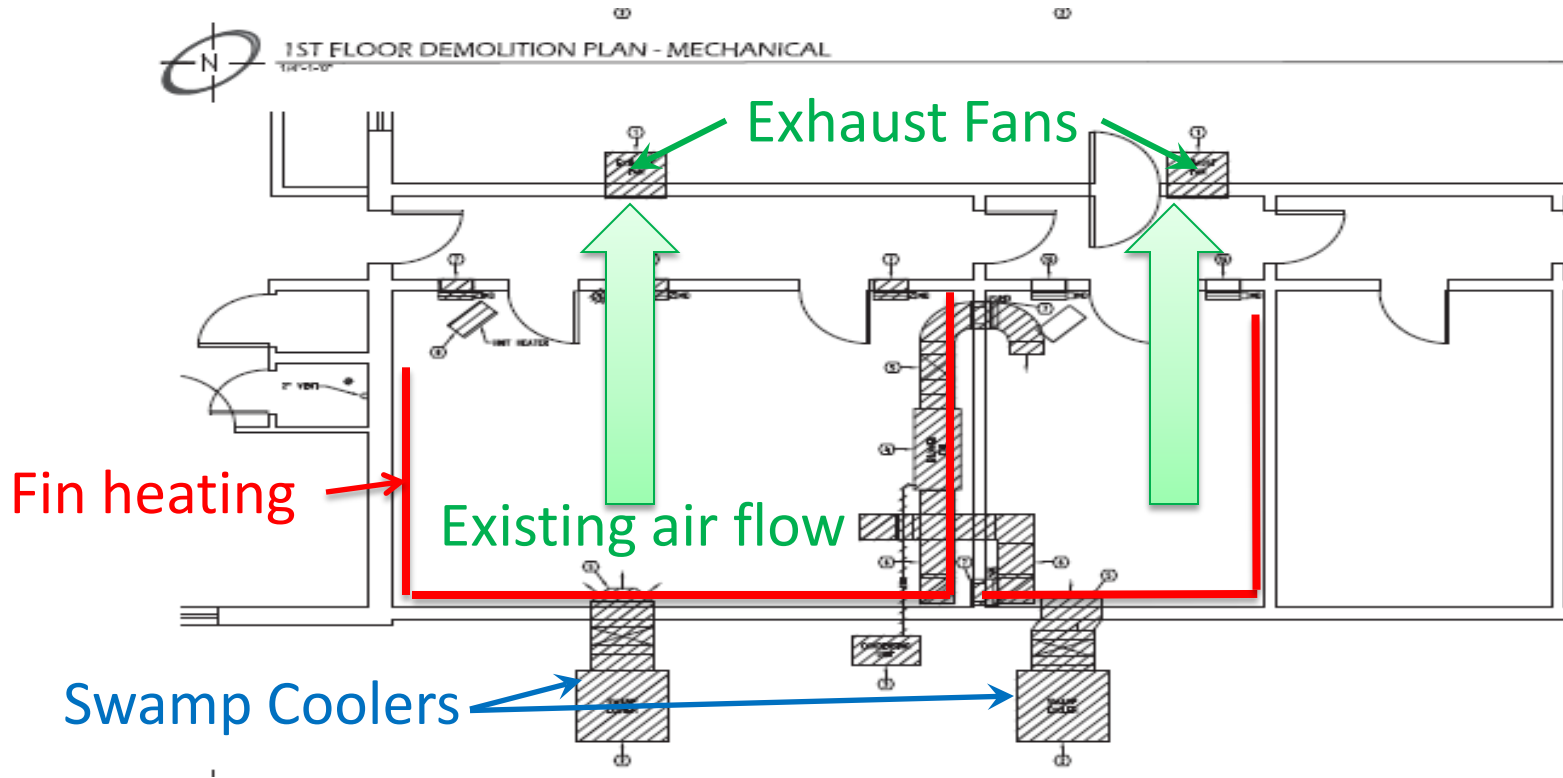


New Floor Plan

Outside Entry Door

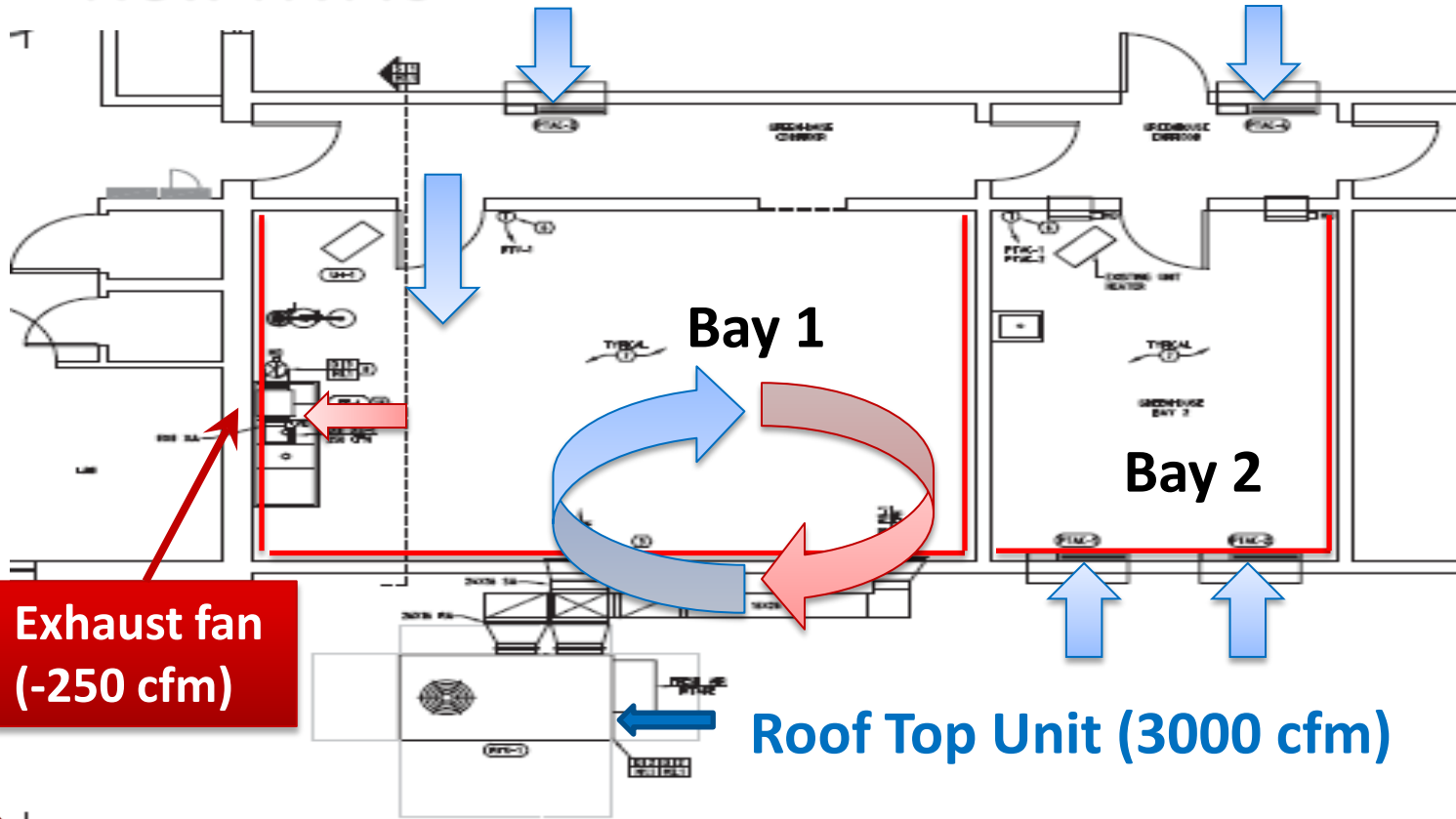


Existing Heating and Cooling



New HVAC

← Supply ← Exhaust



**Exhaust fan
(-250 cfm)**

Roof Top Unit (3000 cfm)



Lever lock access control



Security lighting



Insect netting on
roof drain tubes



Eyewash & Shower



Stainless steel tables



Drain plug in floor drain



Additional Improvements

- Sealed noticeable penetrations in walls and ceiling.
- Left original screens in place to help reduce bugs
- Screwed louvers on walls and in roof peak in place to prevent operation and sealed with caulk from outside
- Door sweeps installed to prevent pests
- Hand washing sinks in both bays. Bay 1 has a deep basin sink.





Exhaust fan



Exhaust fan duct coming out of greenhouse roof



Roof Top Cooling Unit



Window cooling units in Bay 2

PPE Use in Containment Bay

- Tyvek suits are used in the bay because the room is the primary containment barrier.
- Goggles
- Gloves
- Respiratory protection is voluntary
 - ❖ Aerosol-generating activities are prohibited by policy
- Booties



Lab in Operation



Accumulation of Salmonella in Lettuce

Growth stage	<i>Salmonella</i> level (CFU/mL)	Fresh weight ^a (g)	<i>Salmonella</i> internalization ^b
3-week	10 ⁵	0.9±0.3	1/10 (9.8×10 ²)
	10 ⁷	0.5±0.2	0/10
4.5-week	10 ⁵	10.9±2.9	1/10 (9.8×10 ²)
	10 ⁷	6.9±3.1	2/10 (8.0×10 ⁴)
6-week	10 ⁵	29.9±11.3	0/10
	10 ⁷	33.6±13.9	1/10 (2.5×10 ²)

^a mean and standard deviation (n=10). ^b detection frequency (concentration, CFU/g fw).

Summary/Lessons Learned

- Risk assessment is a team effort
- Don't underestimate the power of **perceived risk**
- Consider and educate other users in a facility if pathogen use is new
- Human pathogens can be studied in a greenhouse setting with specific design characteristics and proper procedures
- Hybrid facilities will be valuable for future study of food safety problems



Acknowledgements

- Research group

- Xu Li, PhD (PI)
 - Yuping Zhang
 - Brett Sallach
- Laurie Hodges, PhD (Co-PI)
- Shannon Bartelt-Hunt, PhD (Co-PI)
- Daniel Snow, PhD (Co-PI)

- IBC Sub-Committee

- Rodney Moxley
- Thomas Clemente
- Amy Hilske
- Peter Angeletti

M. Malendia Maccree
(UC Davis)

Dann Adair (Convion)

