PRODUCE TESTING:

YOUR SAMPLE IS PRESUMPTIVE, NOW WHAT?

Joelle Mosso

Assoc. CSO, Produce

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THE CHALLENGE

You walk the field, you pick some product, and you drop them off at the lab for testing. Not long after, you hear back your sample is presumptive for a pathogen.

Now what?

TESTING IN FRESH PRODUCE

What is testing really doing?

What are **you** trying to do with your testing?

HOW DID WE GET HERE?

A test result is the visible outcome of many decisions made before…

- Sampling design
- Sample definition
- Test selection

TEST PLAN DESIGN

- Surveillance
- Lot acceptance
- Investigatory

SURVEILLANCE TESTING

- Determines normal occurrence of target (prevalence)
- Can help identify when risk increases
- Can be used to dictate appropriate lot acceptance sampling plan

LOT ACCEPTANCE TESTING

- Detection is used to accept or reject a lot
- Assumes sample is representative/robust enough
- Prevalence is high enough to be detected
- Cumulative Data can shows trends/prevalence

INVESTIGATORY TESTING

- Root cause testing
- Testing conducted to identify a better “sample”
- Vectoring or exploration

WHAT IS A SAMPLE?

sample noun

Save Word

sam·ple | \ ˈsam-pəl \

Definition of *sample* (Entry 1 of 3)

1 : a representative part or a single item from a larger whole or group especially when presented for inspection or shown as evidence of quality : SPECIMEN

2 : a finite part of a statistical population whose properties are studied to gain information about the whole

https://www.merriam-webster.com/dictionary/sample

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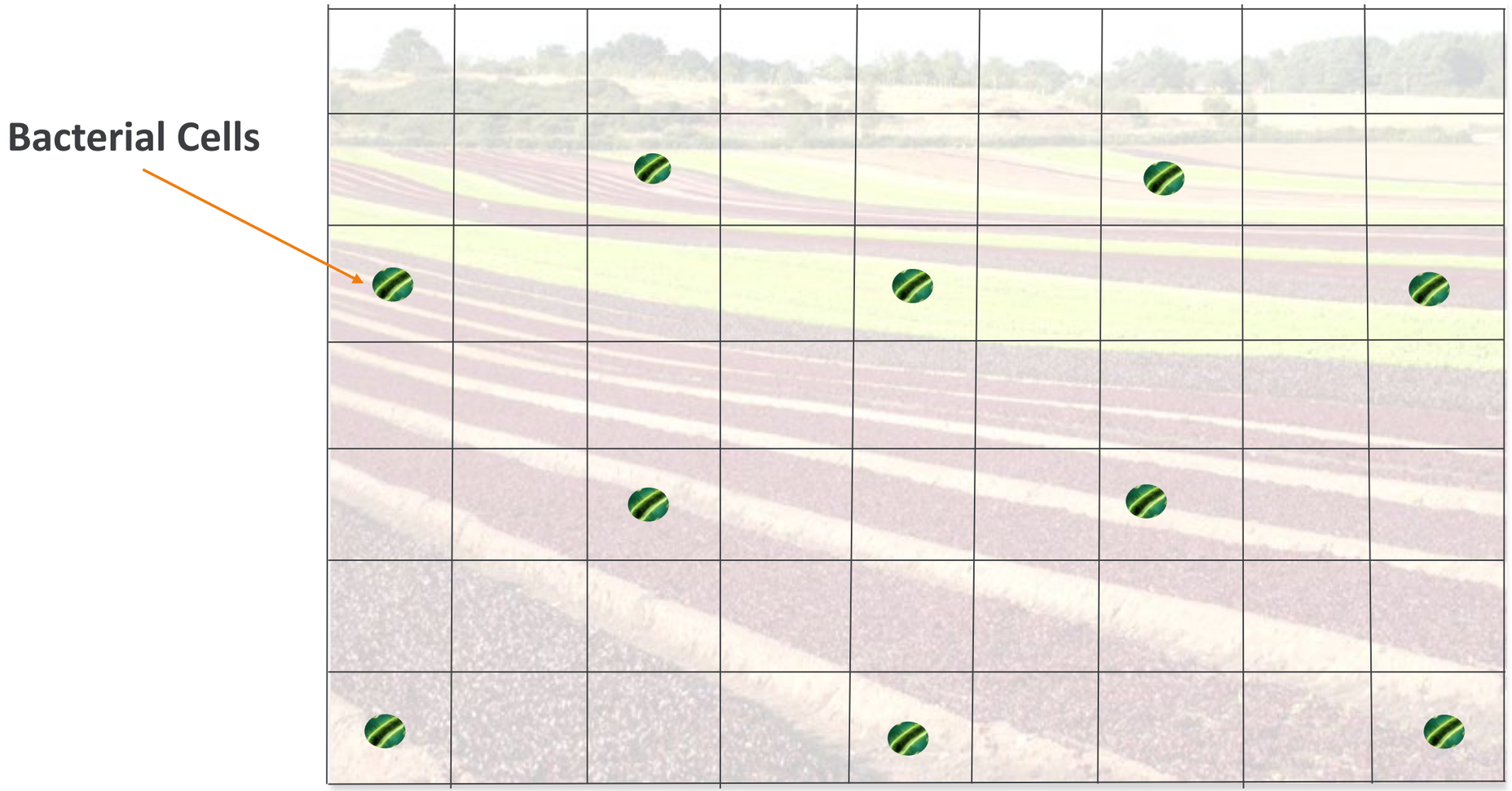
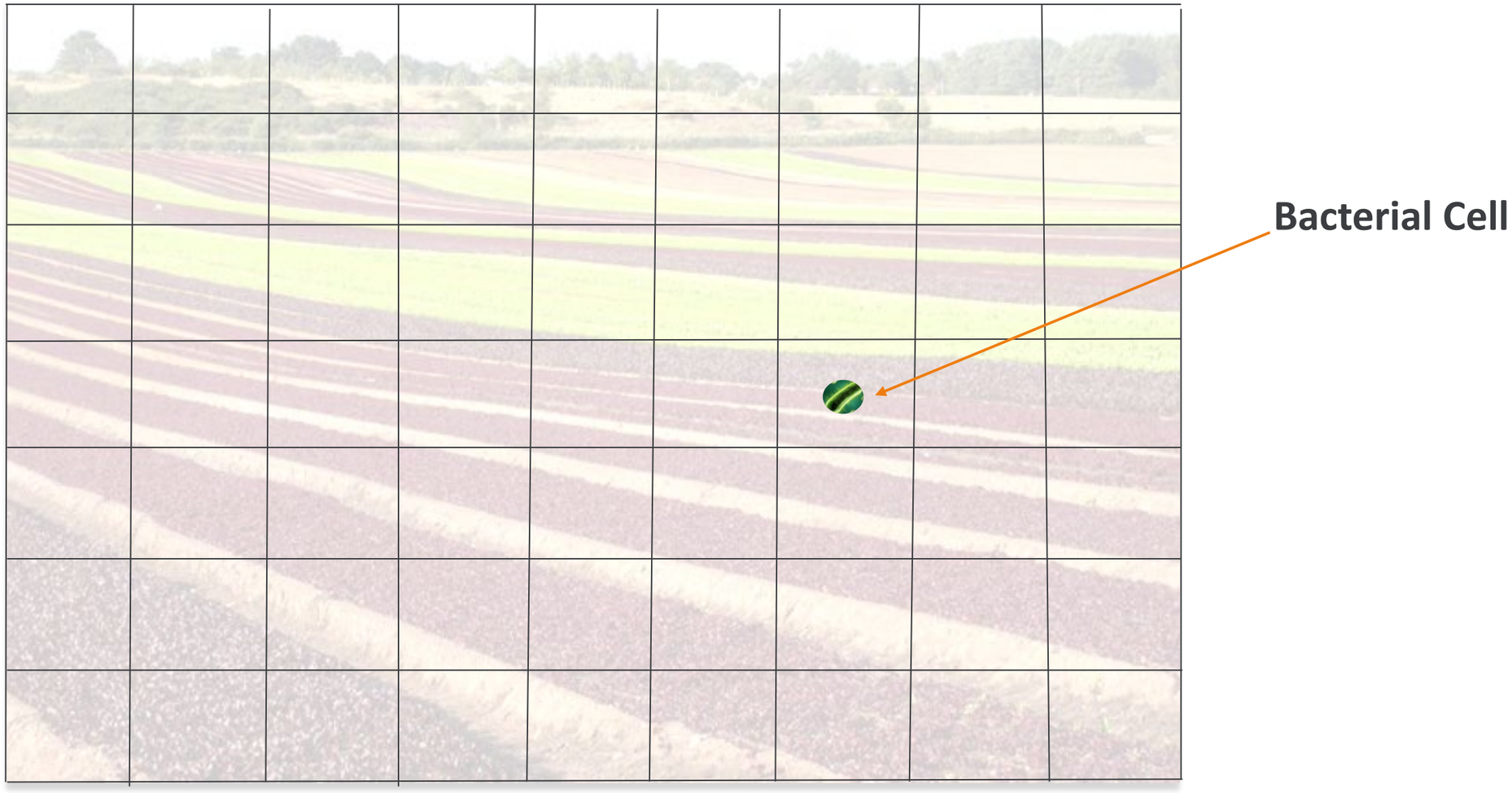
METHODS ASSUME UNIFORM PREVALENCE



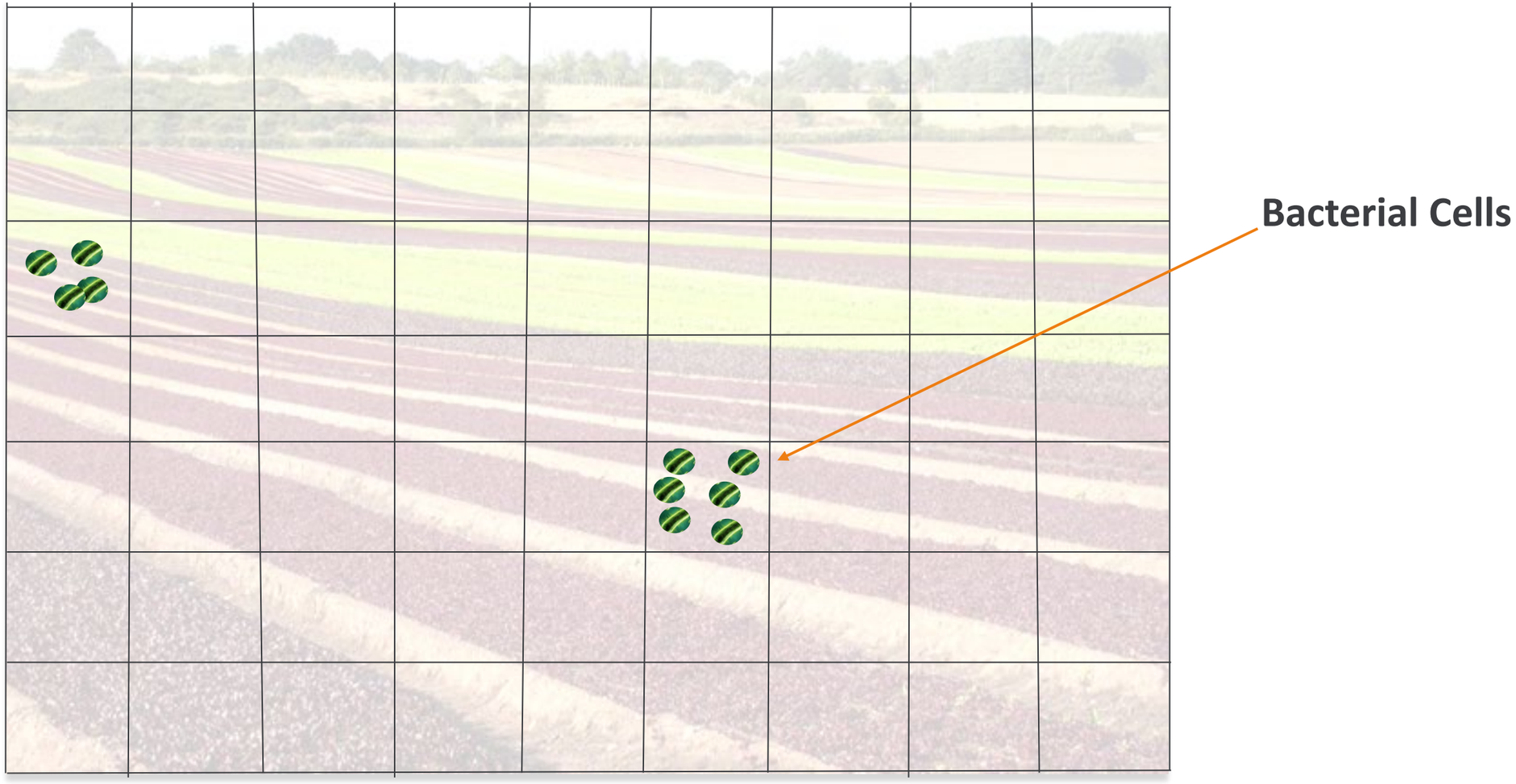
Each square can be a sampling unit from a production lot or an environmental sampling site

POOR ODDS AT LOW PREVALENCE



Each square can be a sampling unit from a production lot or an environmental sampling site

POOR ODDS DUE TO NON UNIFORMITY



Each square can be a sampling unit from a production lot or an environmental sampling site

HOW MANY SAMPLES?

Two-Class Plans: Probabilities of Acceptance c=0

Composition of Lot		Number of Sample units tested				
% Acceptable	% Defective	5	10	20	60	100
98	2	0.9	0.82	0.67	0.3	0.13
95	5	0.77	0.6	0.36	0.05	0.01
90	10	0.59	0.35	0.12	<	<
80	20	0.17	0.11	0.01		
70	30	0.03	0.03	<		
50	50	0.03	<			
40	60	<				
30	70					

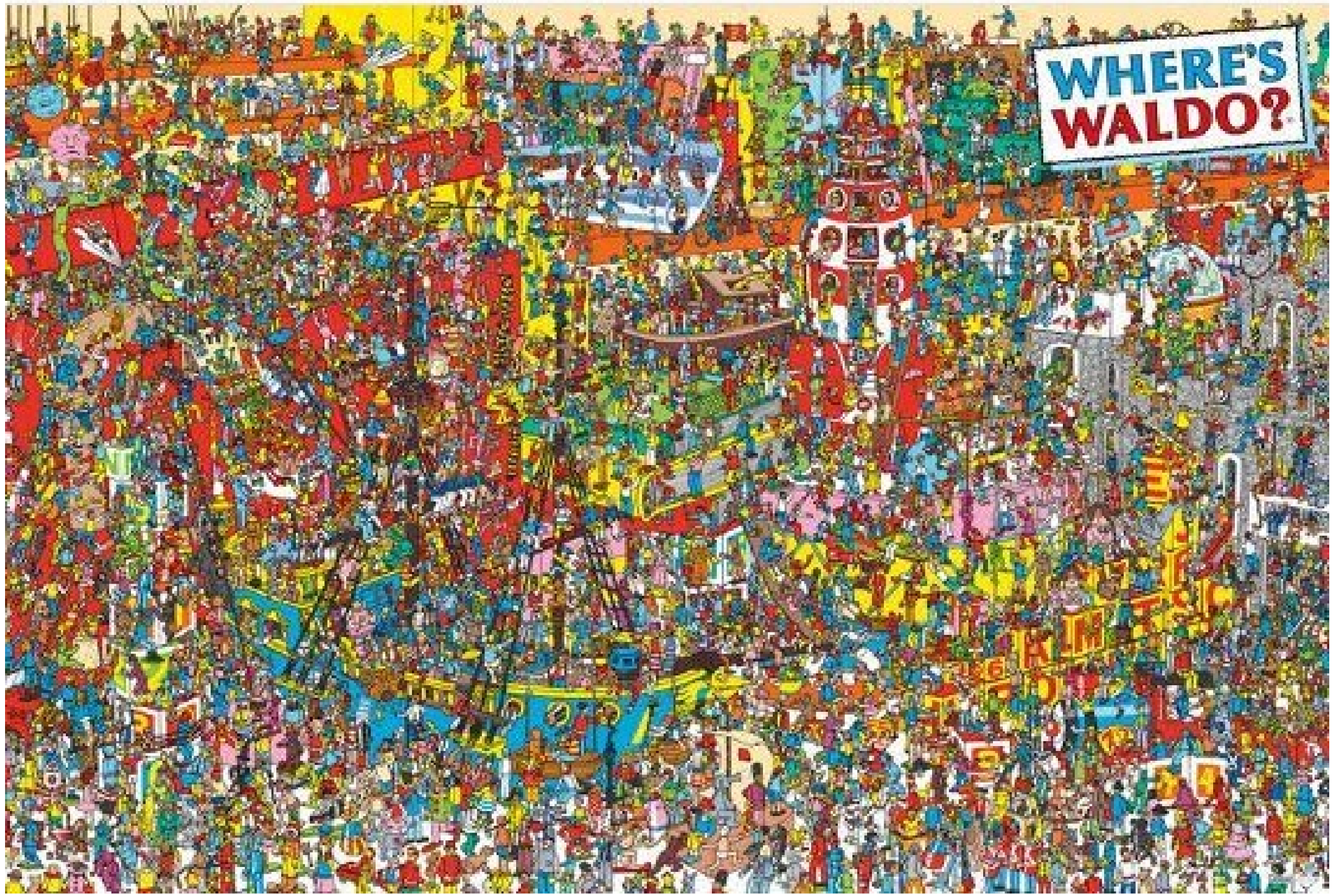
ICMSF, 1986. Microorganisms in Foods, Sampling for microbiological analysis: Principles and applications, University of Toronto Press, Toronto.

Testing first helps you define your appropriate sampling program

DEFINE YOUR GOAL

Testing then becomes the tool to monitor changes in risk

SAMPLING EXAMPLE



https://www.allposters.com/-sp/Where-s-Waldo-Toys-Posters_i15527017_.htm

SAMPLING DESIGN

- What is your purpose?
  - Surveillance
  - Lot acceptance
  - Investigatory
- What is your sample?
  - Make sure it is reflective of your product (*representative* sample)
  - A perfect sampling plan will not detect if sample is not representative
- How do you test?
  - Every test has trade-offs
  - How do you choose?

A CONCEPT OF “TOOLS”

- A scale will tell weight
- A scale will not make you gain or lose weight
- Some scales are more accurate than other scales
- Weight changes during the day. When to use the scale?

LIMITS TO RAPID TESTING

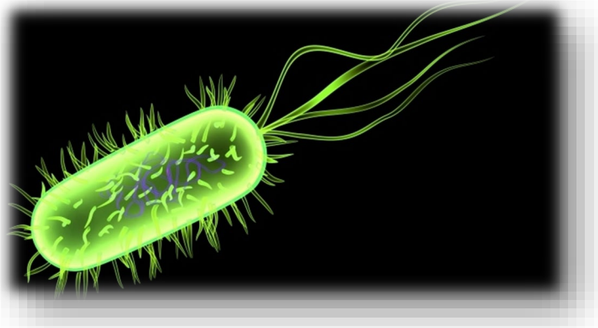
We are looking for “low levels” of contamination. 1 CFU in 25–375g.

Current methods require a “growth step” to increase numbers of cells to 10,000 CFU.

- What impacts growth?
  - Injury
  - Competition
  - Limits of biology
  - Stress
  - TIME!!!

SCIENCE MUST DELIVER…

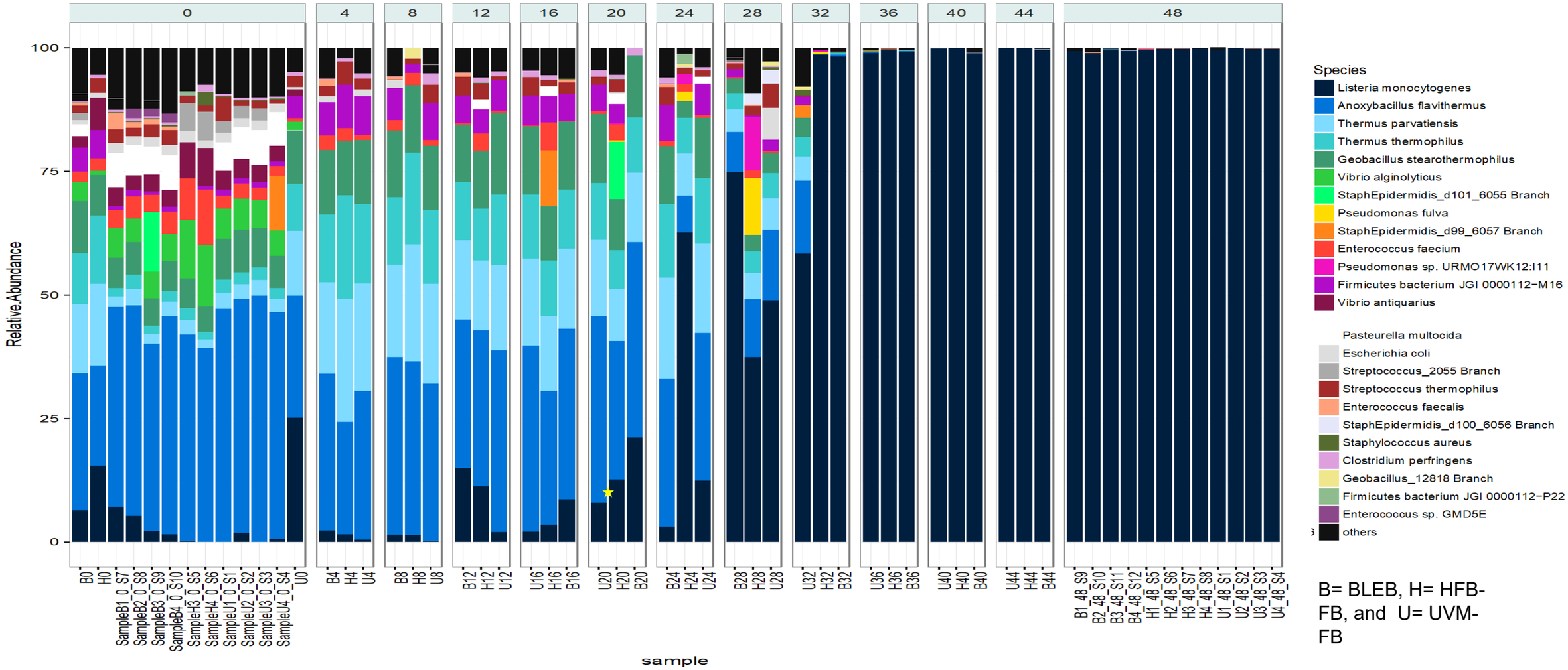
Find 1 *Listeria* cell in a sample size:



- Trend is for testing larger sample sizes
- Avg. weight of a Listeria cell is ~ 25 kDa = ~4 x 10-20 g
- Looking for **0.00000000000000000004 g** in a 375 g sample size
- If a single cell were a **6’ 6” person** it would need to travel approximately 100,000 body lengths or **~120 miles to make it from one side of 375 g sample** to the other side. (For reference, state of Delaware is 96 miles long)*

*Dr. Doug Marshall, Dr. David Legan & Dr. Dan DeMarco

ENRICHMENT MICROBIOME PROFILES (ICE CREAM)

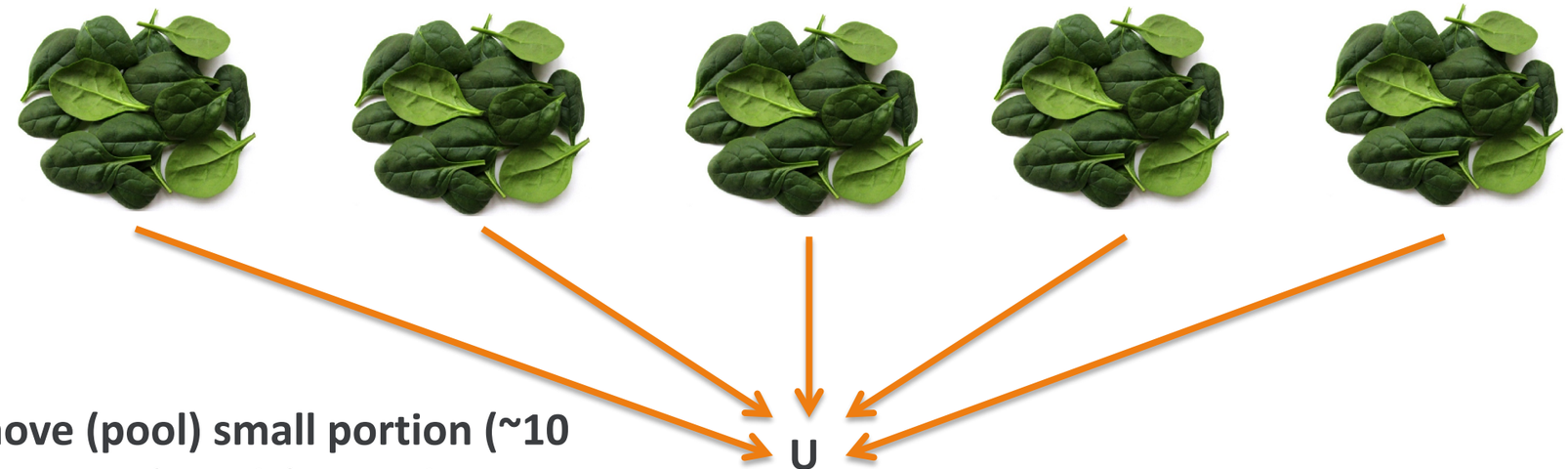


B= BLEB, H= HFB-FB, and U= UVM-FB

Ottensen et al., BMC Micro, 2016; figure courtesy of N. Hassan, CosmosID

WET POOLING CONCEPT

5 sample subs, each enriched separately



Remove (pool) small portion (~10 µl) from each enrichment into a small tube

U

Run one reaction from pooled tube

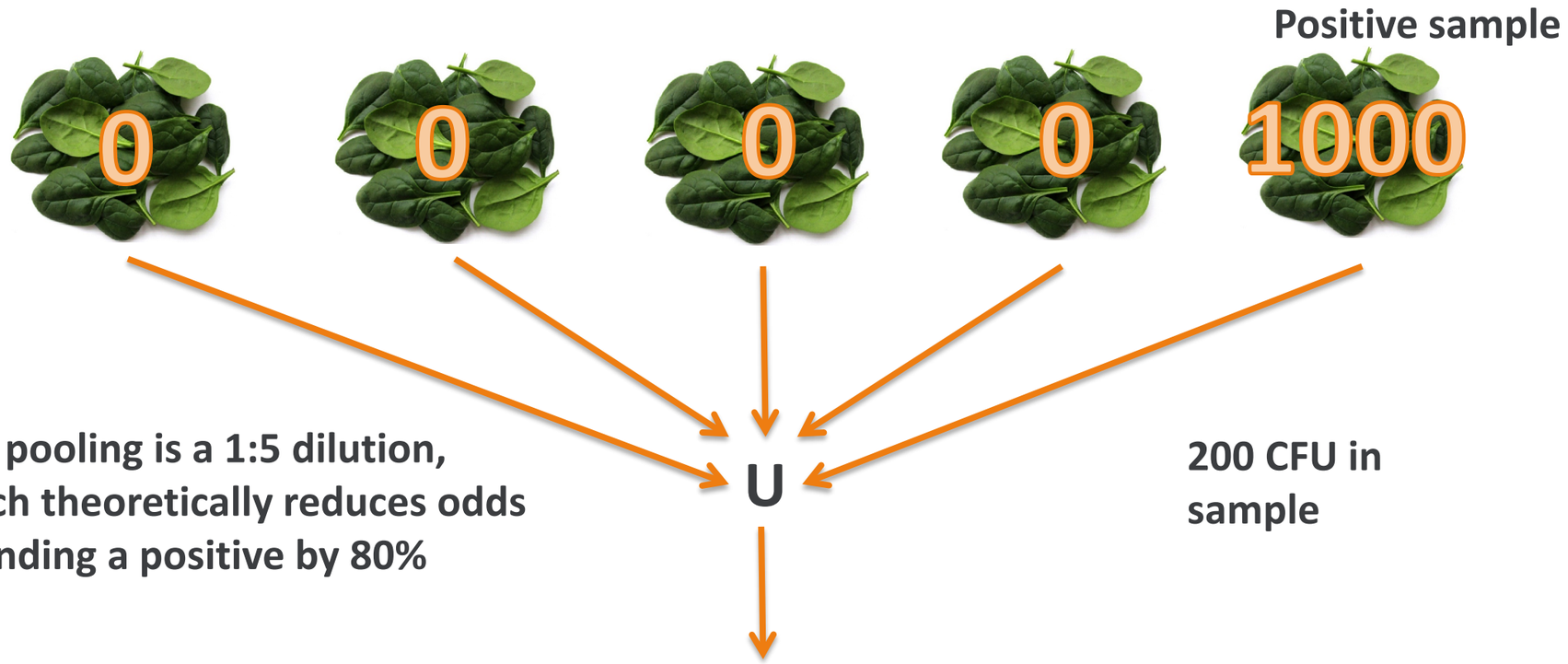
PCR, ELISA

Not Detected → ship lot

Detected → run detections on each individual enrichment

WET POOLING MATH

Best PCR & ELISA assays have Limits of Detection of 1,000 to 10,000 CFU



This pooling is a 1:5 dilution, which theoretically reduces odds of finding a positive by 80%

200 CFU in sample

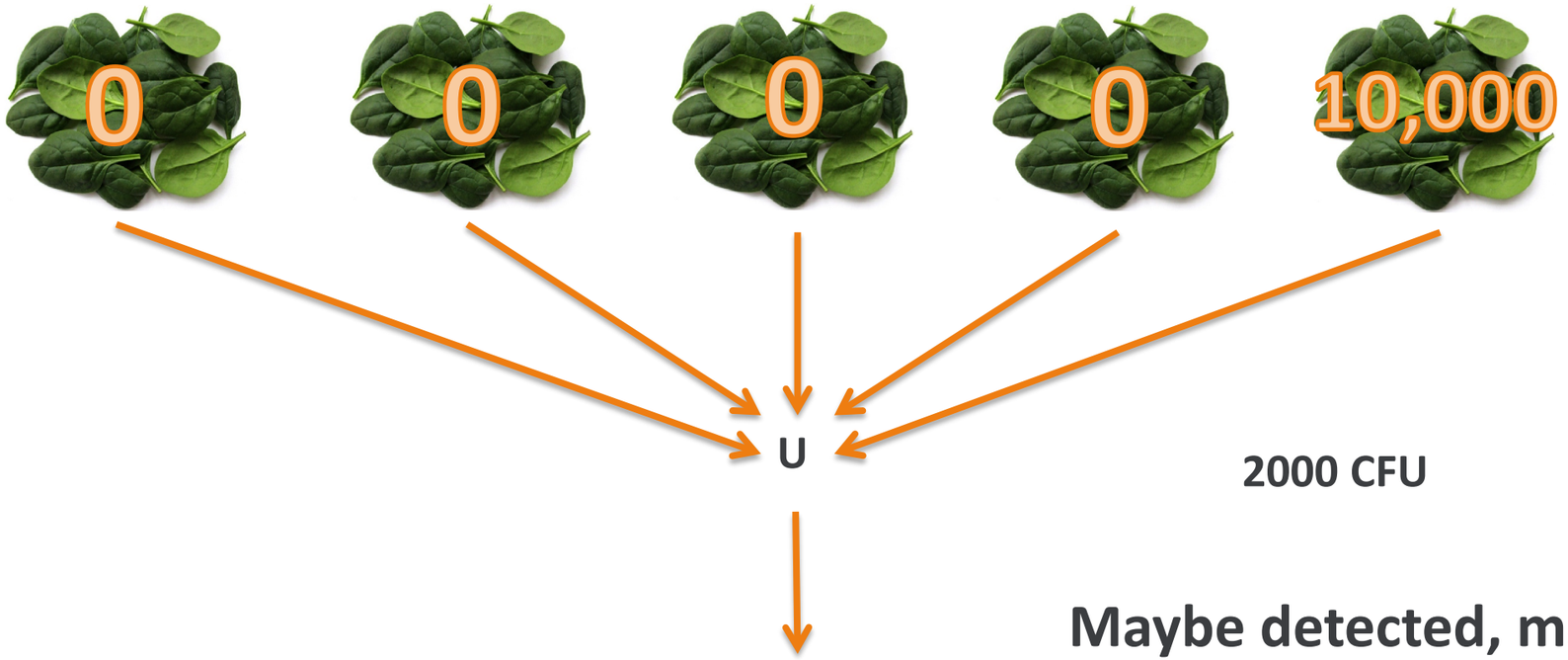
Limit of Detection ~1000 CFU

PCR, ELISA

Not Detected → ship contaminated lot!

WHAT IF ENRICHMENT IS MORE SUCCESSFUL?

Best PCR & ELISA assays have Limits of Detection of 1,000 to 10,000 CFU



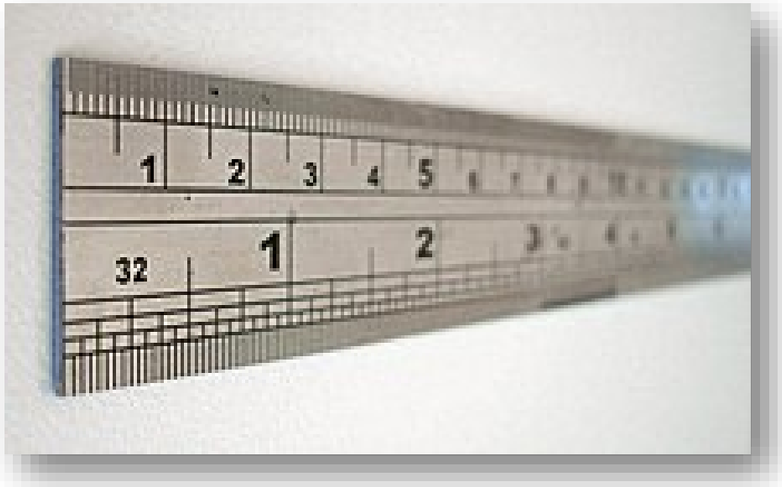
For pooling to work, enrichments must have target populations greater than 100 times the LOD

REMEMBER: A TEST IS “JUST A TOOL”

- Tests are critical to our discovery & decision making process

- Tests are JUST tools, they rarely are perfect
  - Know the upper and lower limits
  - Recognize the trade-offs

- Every test has its limits & assumptions



WHAT TO MONITOR?

- Pathogen
  - *Listeria monocytogenes*
  - *Salmonella*
  - Pathogenic *E.coli*
    - *Big 7, or more* STEC
- Indicators
  - *Listeria species*
  - Generic *E.coli*
  - Coliform
  - *Enterobacteriacae*
  - Pathogenic risk assays

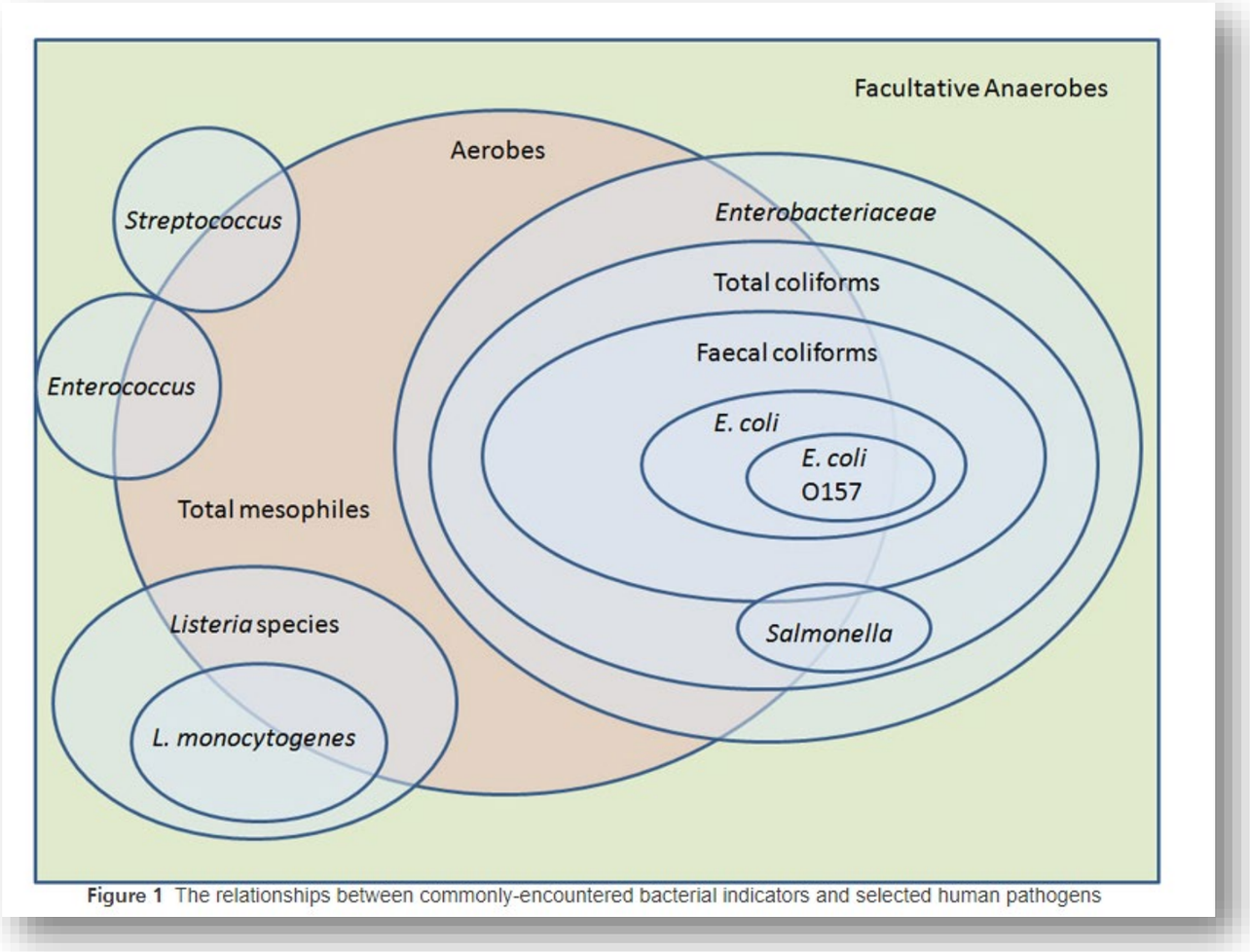


Figure 1 The relationships between commonly-encountered bacterial indicators and selected human pathogens

IMAGE citation: Food Standards of Scotland http://freshproducetool.foodstandards.gov.scot/node/21023

YOUR PRESUMPTIVE POSITIVE

What does it mean?

- DNA is present
- What was your sample plan designed to find?
  - Surveillance, lot acceptance, investigatory, undefined?
- What limitations to your tool?
  - Trust your positive, trust your negative
- Use you data as a “data set”, not a data point

STEP BACK FROM YOUR PLAN

- Define success
  - Design how to measure it
  - Select a tool that measure what matters
  - Develop a plan when a positive is found
  - Understand limits to your plan

- Activity vs. value
  - More isn’t always more
  - Focus on value, not activity
  - Don’t be afraid to change approaches

DESIGNING PURPOSE TO YOUR TESTING

You walk the field, you pick some product, and you drop them off at the lab for testing. Not long after, you hear back your sample is presumptive for a pathogen.

We're here to help. #eurofins

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THANK YOU