

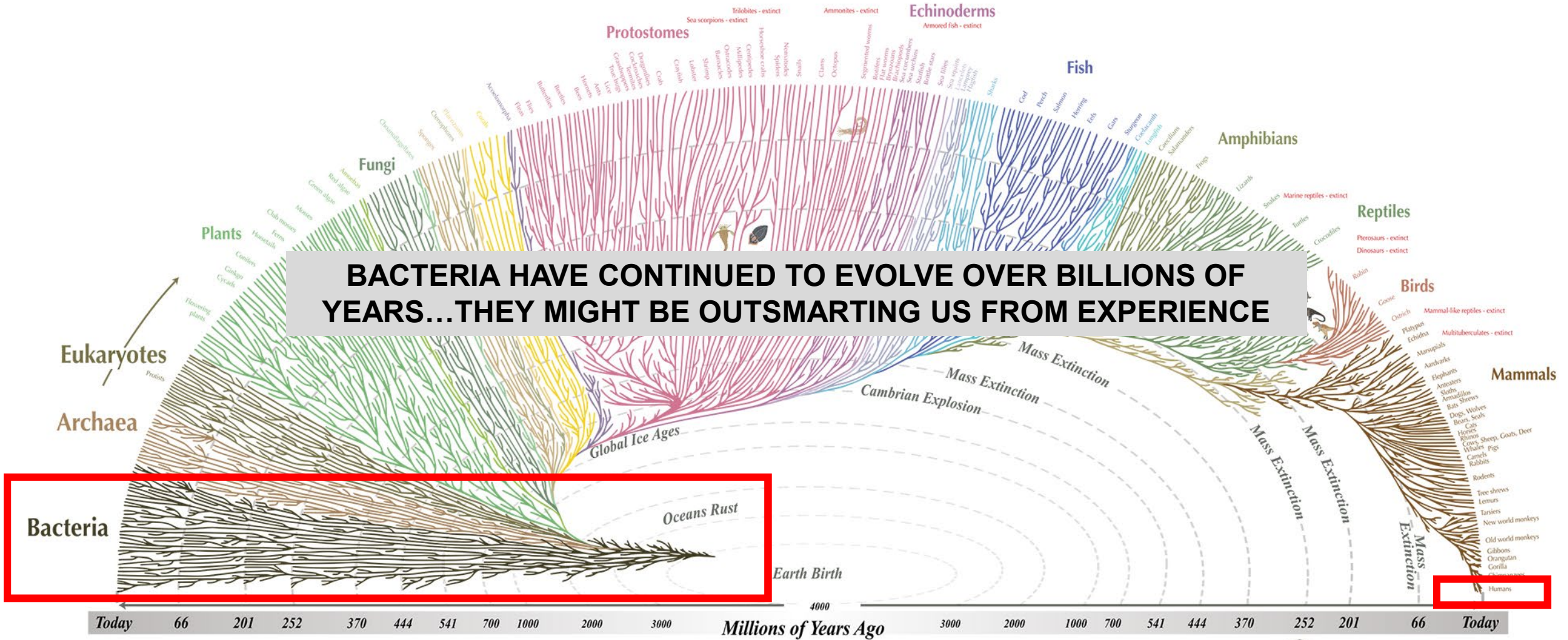



Microbiology Testing Methods

- **Bacteria Details**
 - How are they related?
 - Communities
- **Connecting the Methods**
 - Non-Specific Testing
 - ATP
 - Semi-Specific Testing
 - Indicator
 - Very-Specific Testing
 - Pathogen




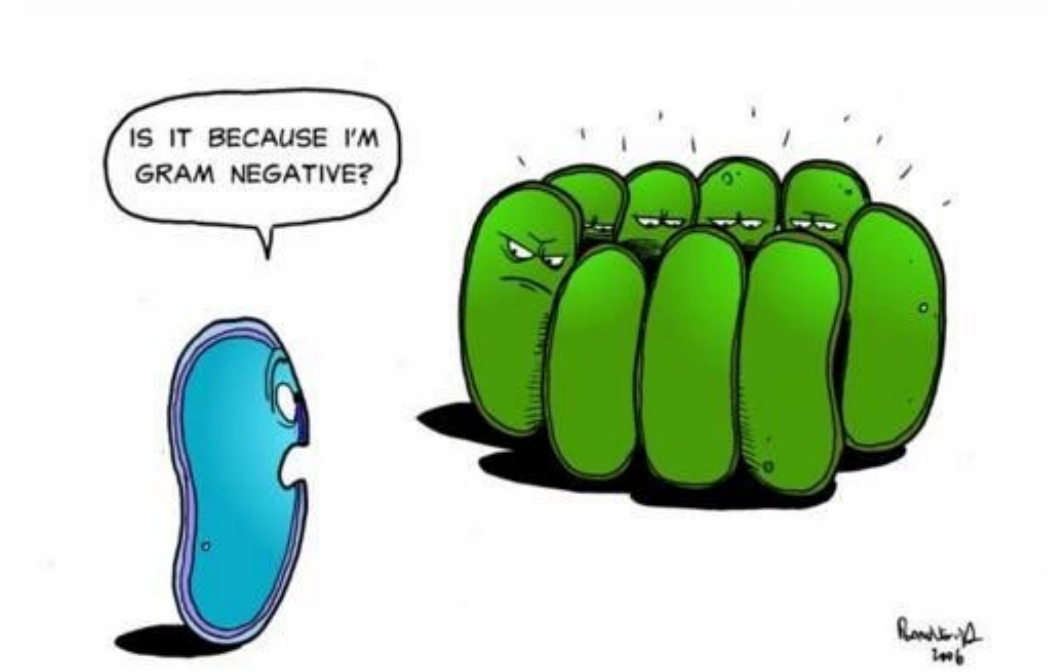
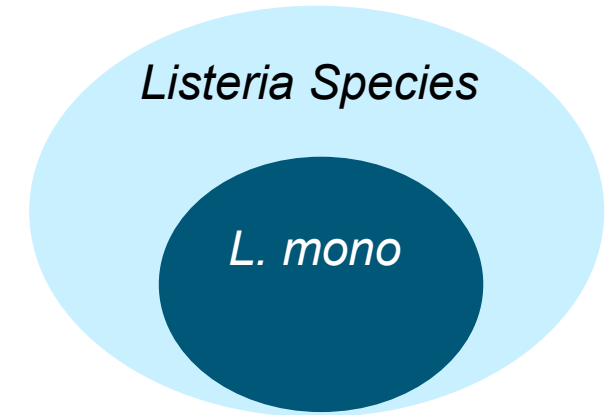
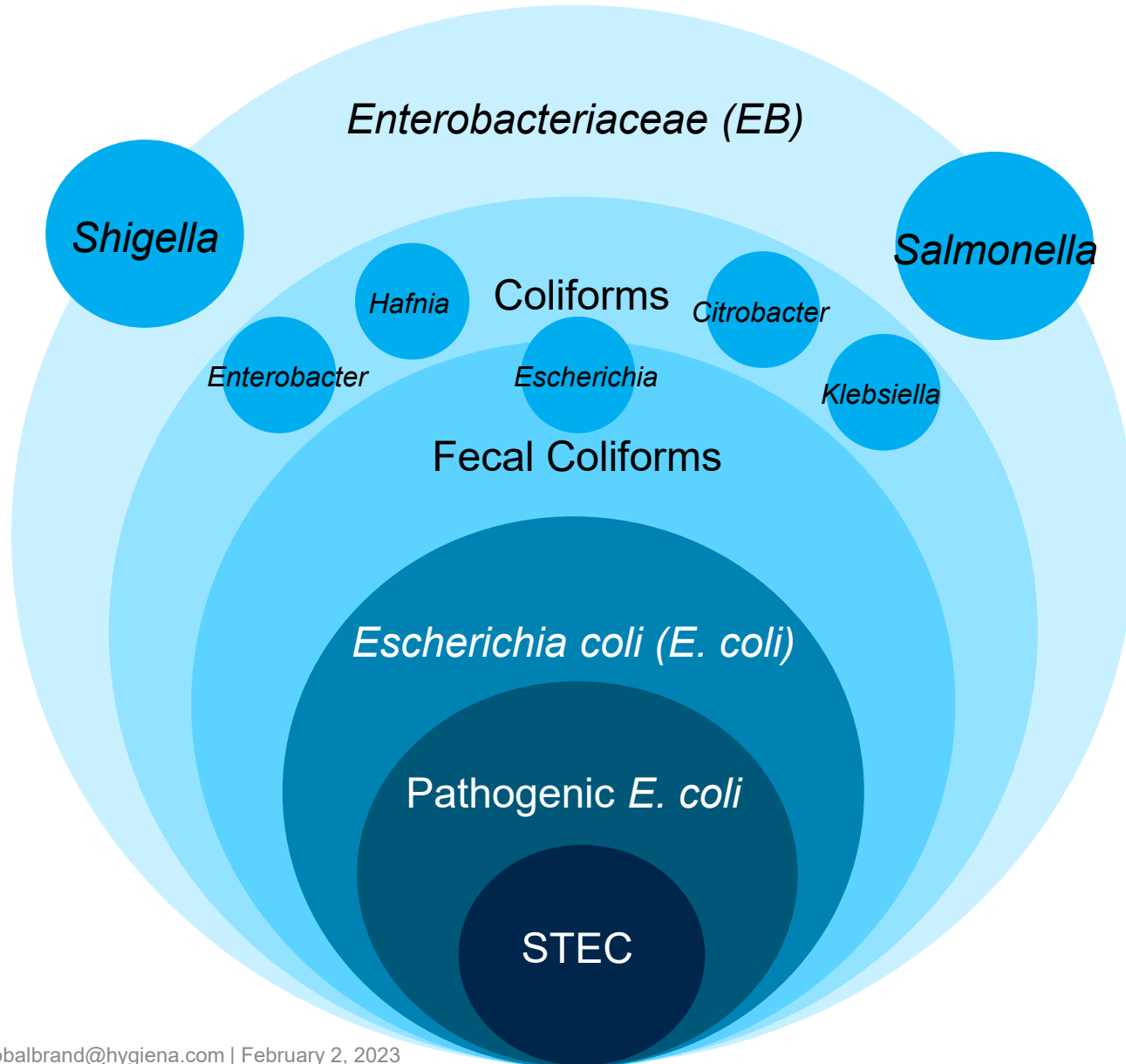
How are Bacteria Related?

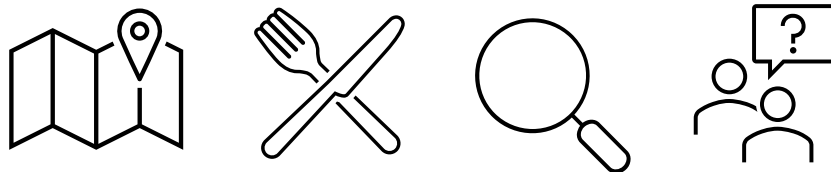
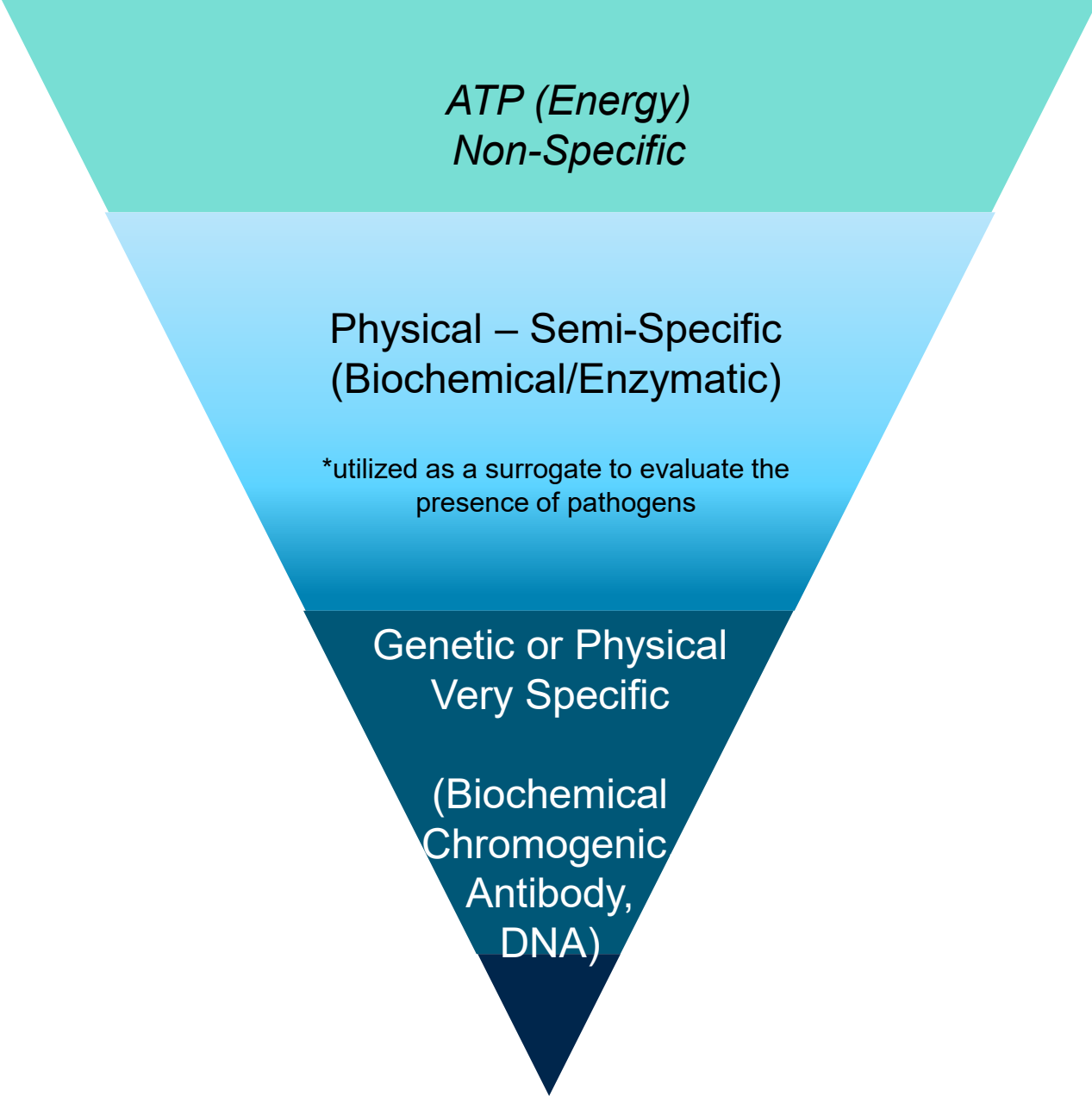
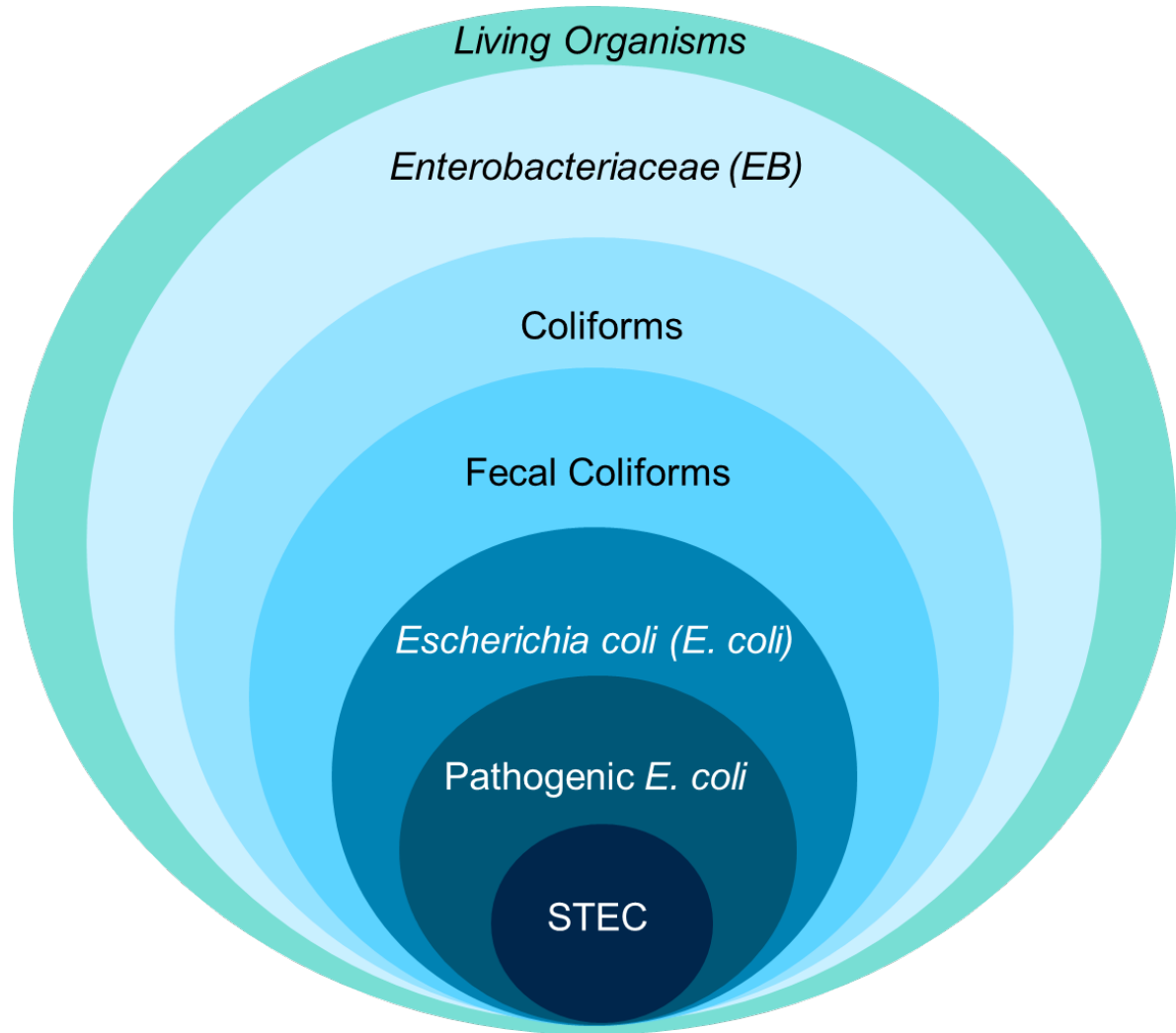


All the major and many of the minor living branches of life are shown on this diagram, but only a few of those that have gone extinct are shown. Example: **Dinosaurs - extinct**  © 2008, 2017 Leonard Eisenberg. All rights reserved. evogeneo.com

- Good:
Probiotics
- Bad:
Indicators
- Ugly:
Pathogens

- 
- A magnifying glass is positioned over a microscopic view of various bacteria. The bacteria are depicted in various colors (blue, green, red, orange) and shapes (rod-shaped, spherical, and some with flagella). The background is a soft-focus image of green leafy vegetables.
- Lactic Acid Bacteria
 - EB & Coliforms
 - *E. coli* O157:H7





TARGET ORGANISM

ALL ORGANISMS



X-RAY

PATHOGENS



MRI

INDICATORS



CT

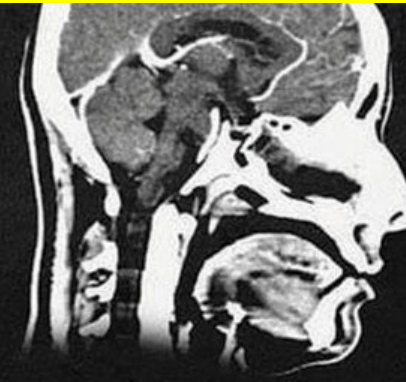
TESTING METHODS

ATP



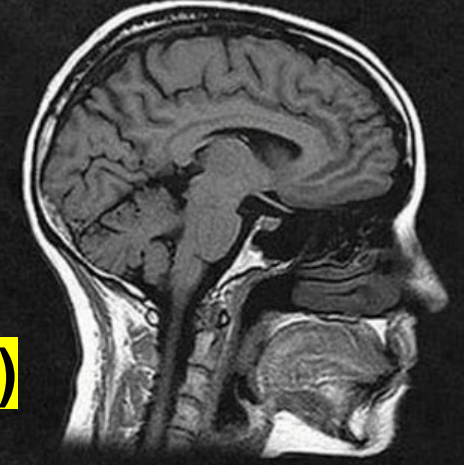
X-RAY

BIOCHEMICAL
(CONVENTIONAL)



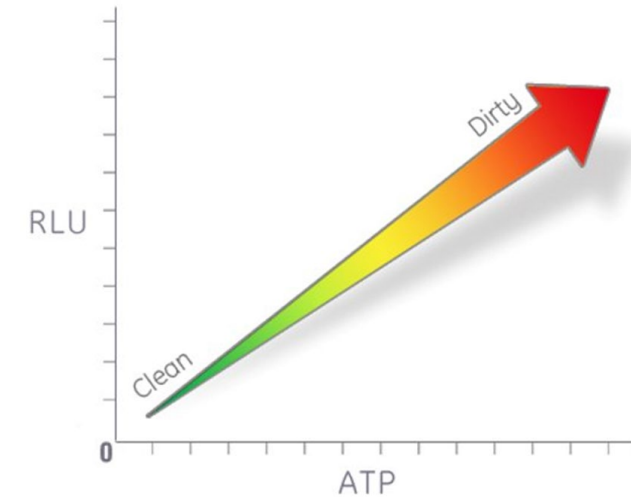
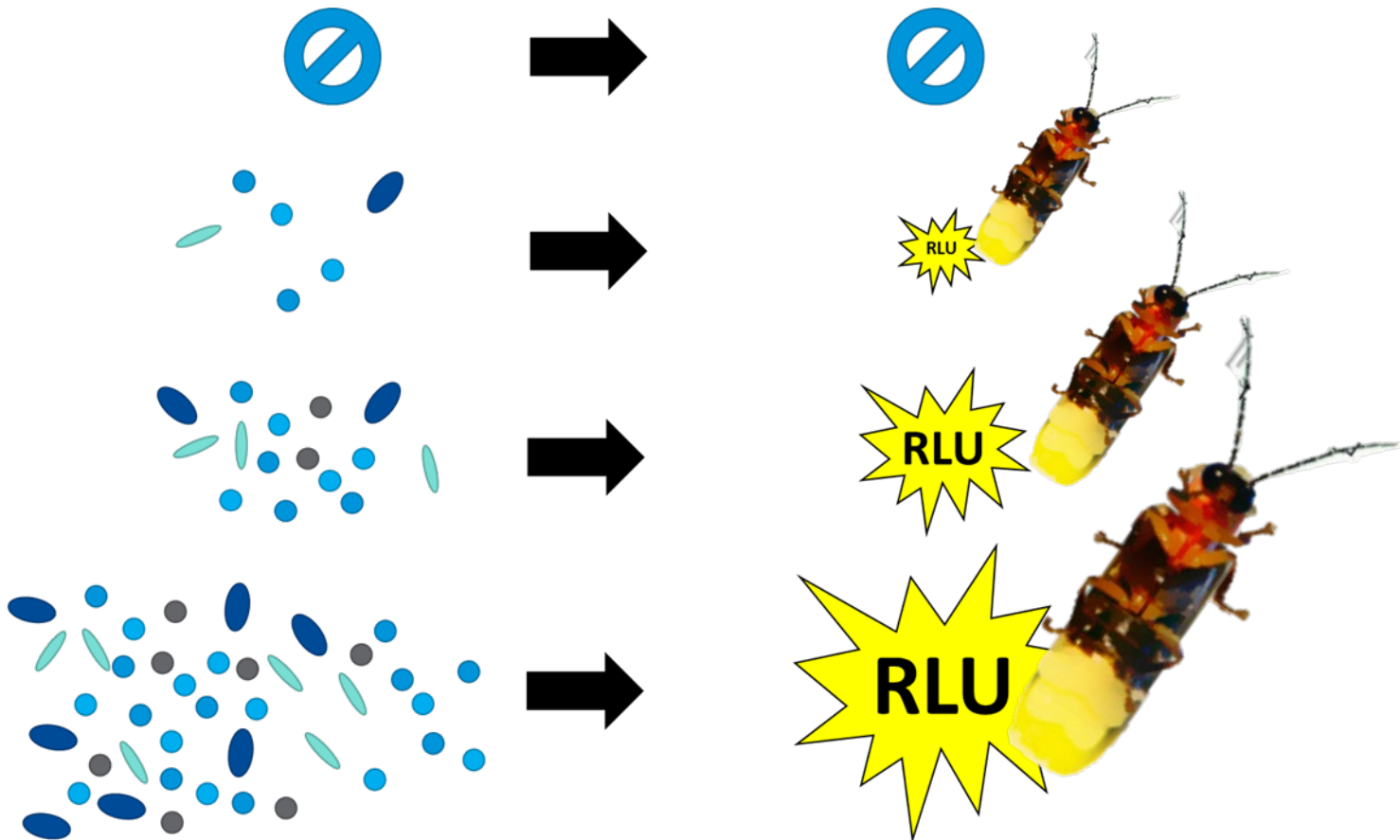
CT

PCR



MRI

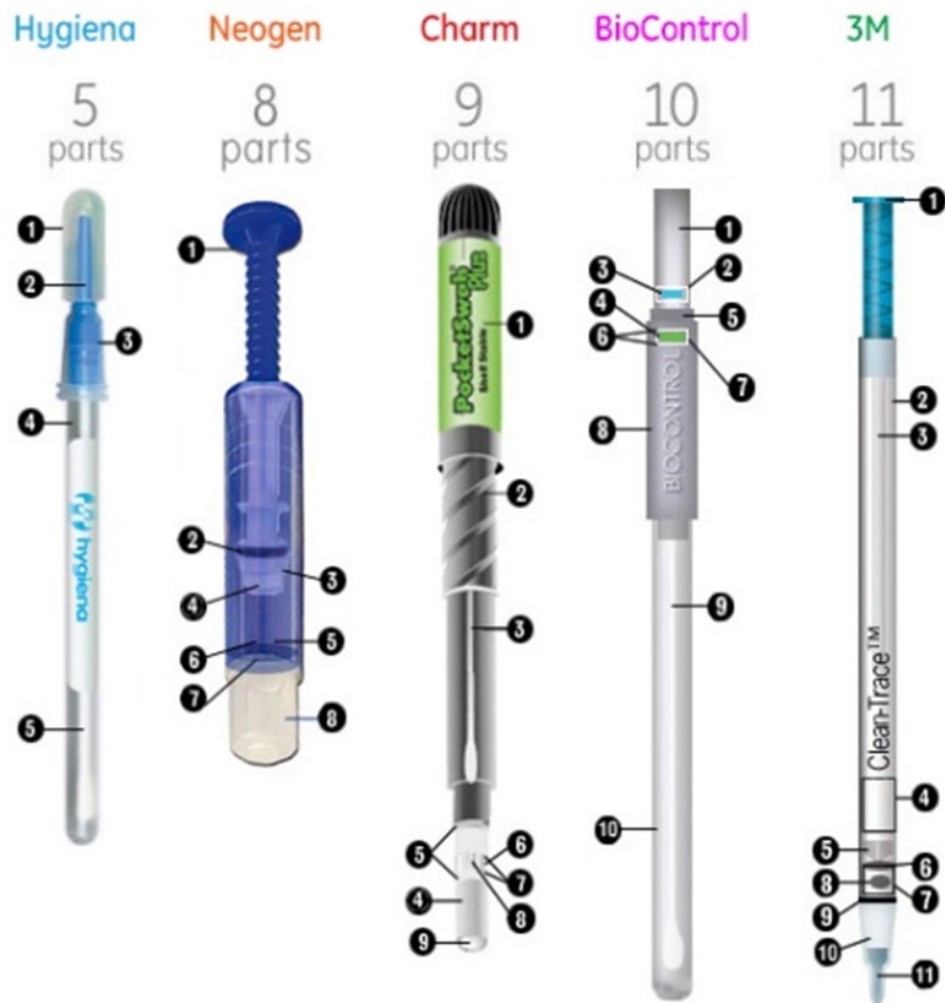
Organic Matter → ATP (Energy) + Luciferase (Fireflies) = Reaction



Pre-Harvest Uses:

- Equipment Cleaning Verification*
- Water Quality Verification*
- No lab or expertise needed (10 second tests)*

3 Components for ATP Testing: 1) Instrument, 2) Chemistry, 3) Device



Hygiena



[Learn more about ATP Systems](#)



Neogen Corp.



Charm Sciences

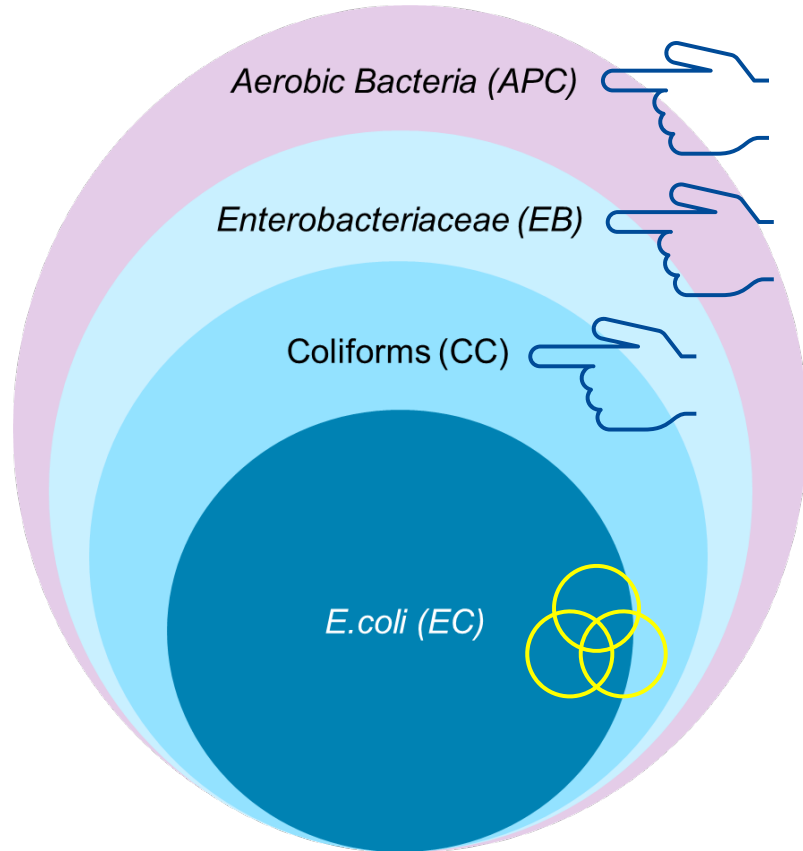


BioControl Systems



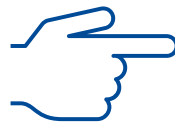
3M & Bio-Trace

Grouping Bacteria into Buckets – Process of Elimination



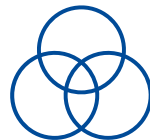
*Aerobic bacteria are **bacteria that can grow and live when oxygen is present**
(Example of Anaerobic = yeast & mold)

2 KEY WORDS:
- SELECTIVE
- DIFFERENTIAL



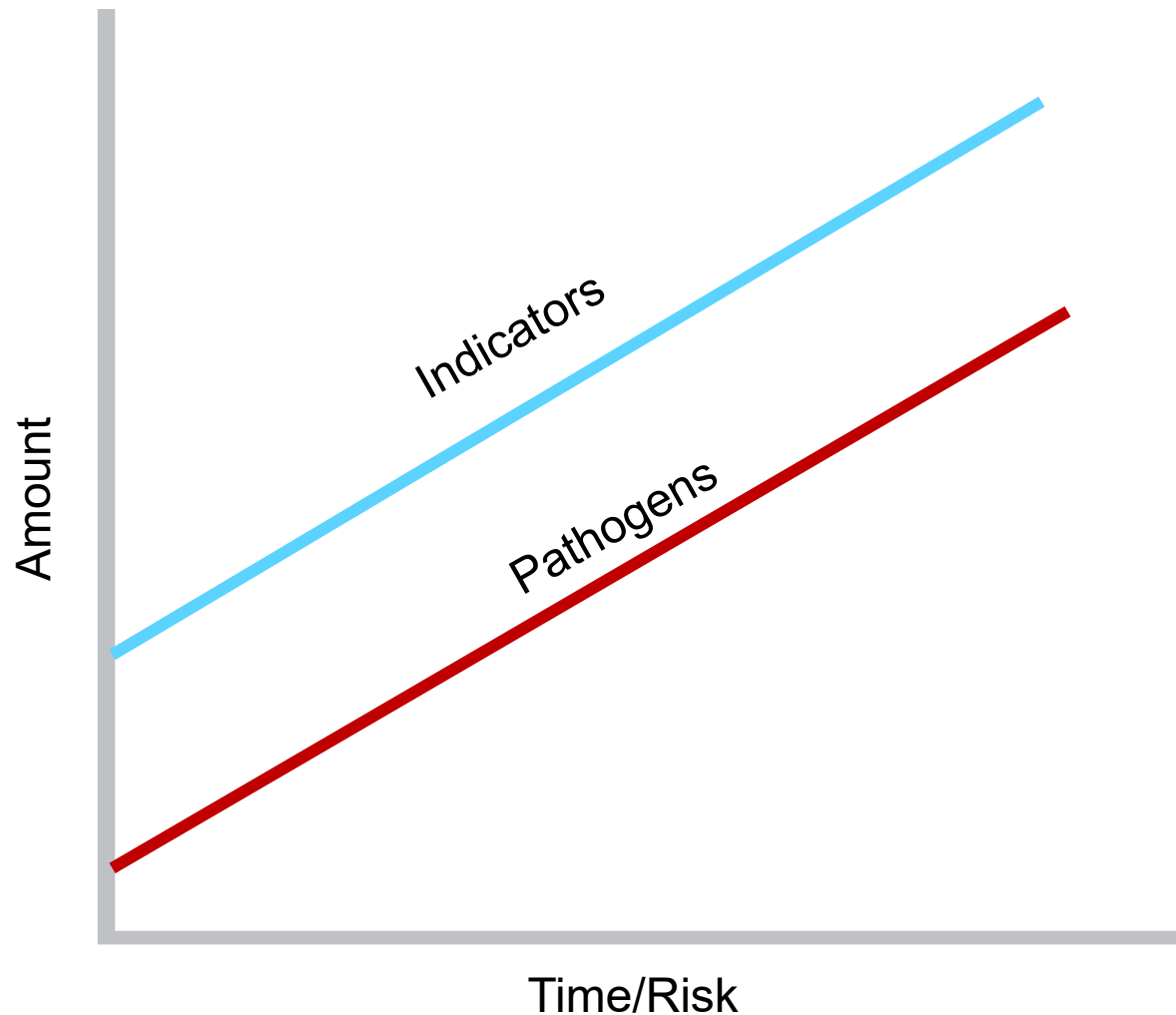
The Key Difference

Selective tests are used to grow and isolate a specific type of bacteria by suppressing the growth of other bacteria



Differential tests are used to visually or enzymatically distinguish microorganisms from one another

Utilizing generic bacteria that live and thrive in a similar environment as a pathogen to assess potential risk of contamination



Risk is Assessed with a Count!

Pre-Harvest Uses:

- Water Testing and Monitoring*
126 CFU/100 mL
- Soil Testing and Monitoring*
< 100 CFU/g

Conventional Biochemical Methods:

- Agar Plate Counts
- Most Probable Number (MPN)

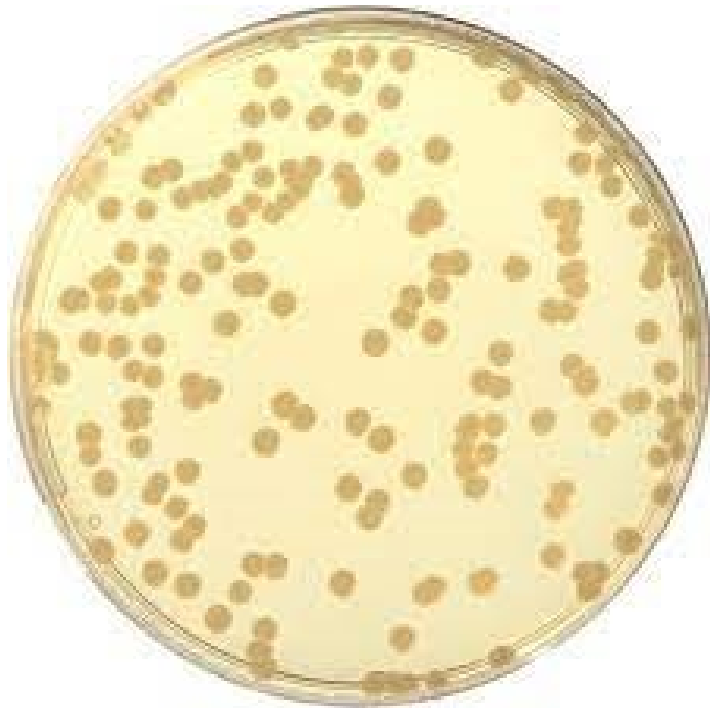
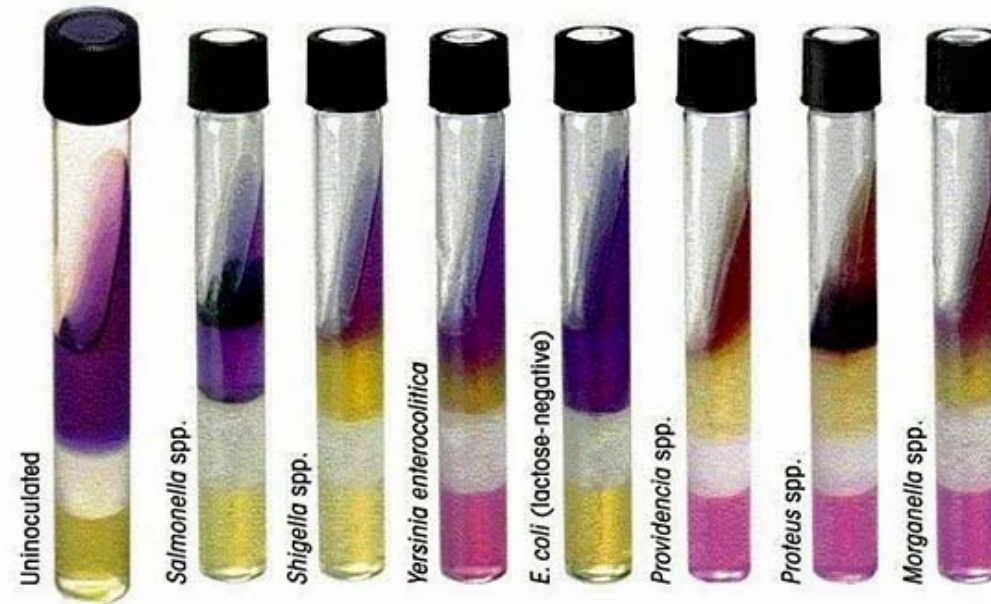
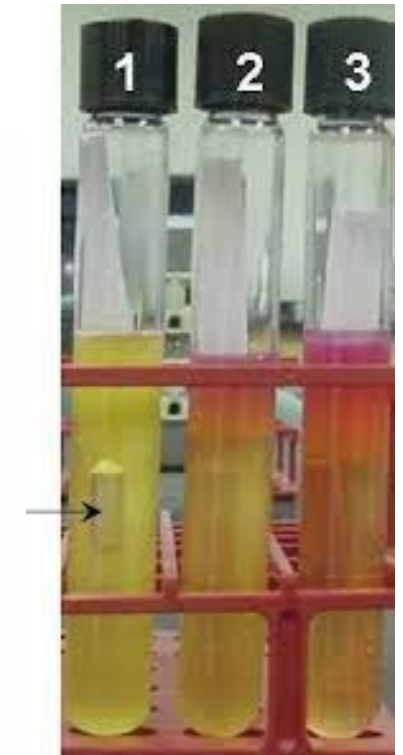


Plate Count Agar



LIA Slants (Biochemical Identification)



Coliform MPN

Ready to Use Biochemical:

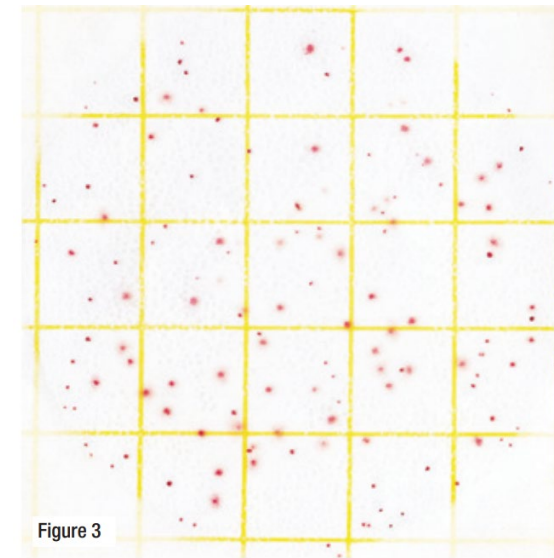
- Ready to Use Plate Counts (Petrifilm™)
- Analytical Profile Index Strip Tests (API)

API 20 E after incubation...Positive results for all tests :



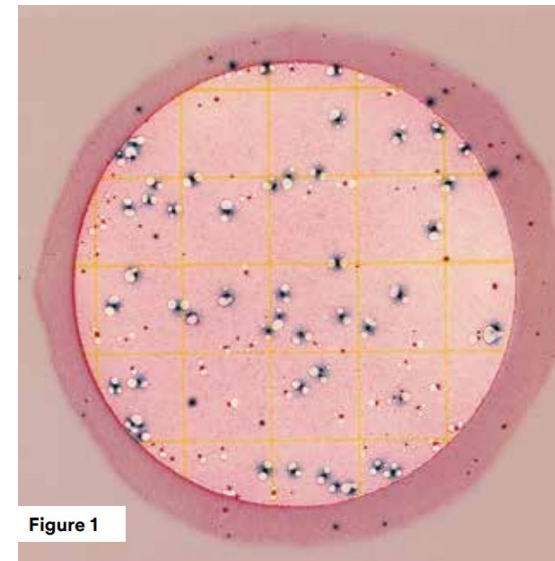
 Microbiology Info.com

API 20 E after incubation...Negative results for all tests :



APC Petrifilm
(Selective)

Figure 3



E. coli/Coliform Petrifilm
(Selective & Differential)

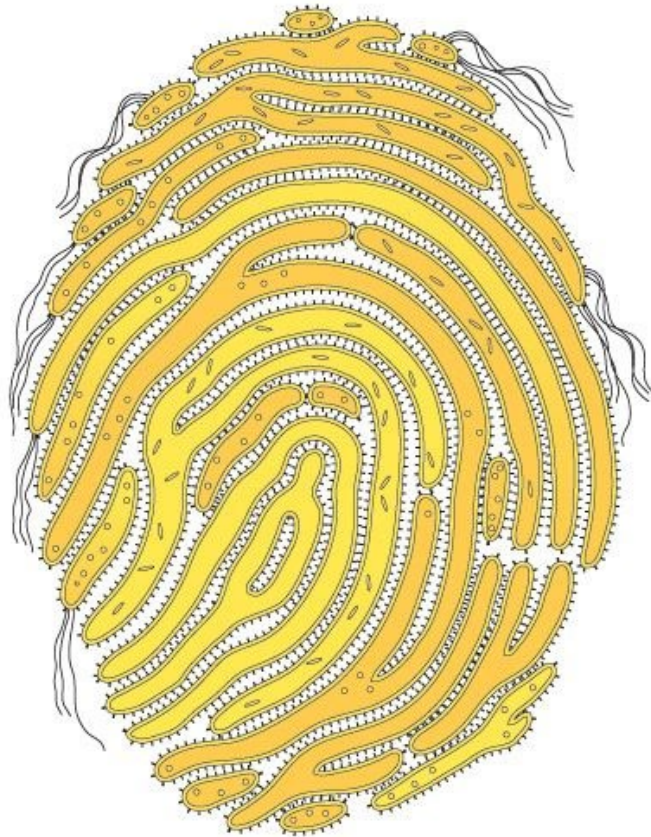
Figure 1

Bioluminescent

- MicroSnap™ - 6 to 8 hr results

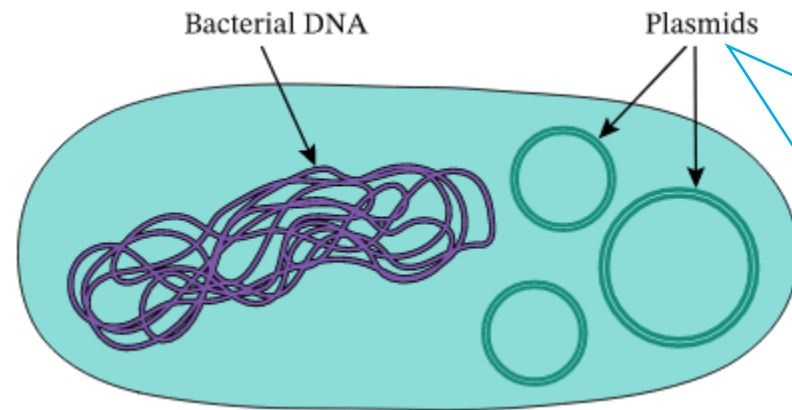


**Think of fingerprinting to catch a criminal,
PCR is similar by targeting very specific bacterial DNA**



Why is DNA a good source for a testing method?

Each bacterial cell only has ONE copy of DNA



Plasmids act as delivery vehicles, or vectors, to introduce foreign DNA into bacteria.

Plasmids carry genes that help bacteria to adapt to new niches and stresses, playing a key role in bacterial evolution!

5 FACTORS TO THINK ABOUT WHEN CHOOSING A PCR TESTING METHOD:

1

ENRICHMENT: CAN I GROW THE TARGET BACTERIA TO DETECTABLE LEVELS?

2

SENSITIVITY: DOES THE PCR ASSAY DETECT LOW LEVELS OF THE BACTERIA?

3

SPECIFICITY: DOES THE PCR ASSAY DETECT THE RIGHT BACTERIA AND EXCLUDE THE WRONG BACTERIA?

4

VALIDATION: DOES IT HAVE PROVEN PERFORMANCE FOR THE FULL METHOD FOR THE RIGHT PRODUCT?

5

REPEATABILITY: CAN DIFFERENT USERS, EXPERTISE, OR LABS ALL PERFORM THE TEST EASILY?

Pre-Harvest Uses:



Virulence (stx and eae)



Pathogen Prevalence & Identification



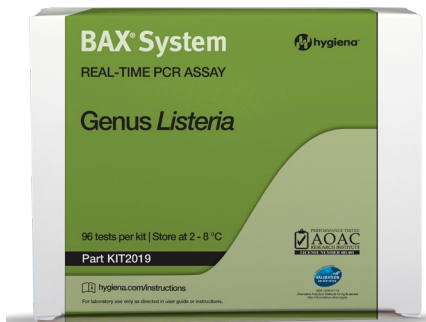
Water, Soil, Tissue Testing

BAX® Q7 Real-Time PCR Assays for the Produce Industry

Tissue



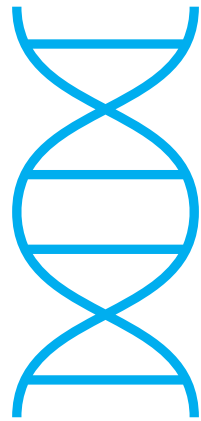
Environmental



All testing is optional at this time but...
YOU decide what results YOU need to understand and mitigate risk to consumers.

Majority of consumer illnesses have been traced back to *E. coli* O157:H7, however, all STECs have the possibility to make someone sick

Why does culture not match PCR sometimes?



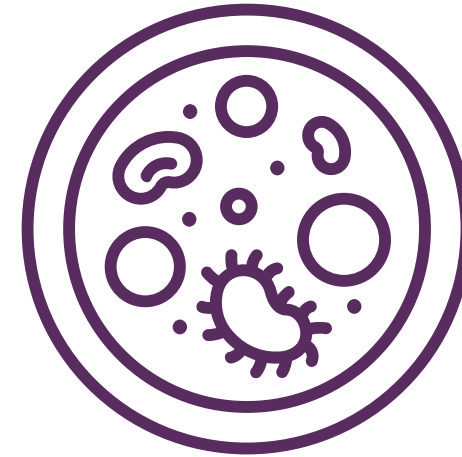
PCR

10,000 COPIES



NON-SELECTIVE ENRICHMENT

OBJECTIVE SELECTION (+ or – DNA Result)



CULTURE

1,000,000 COPIES

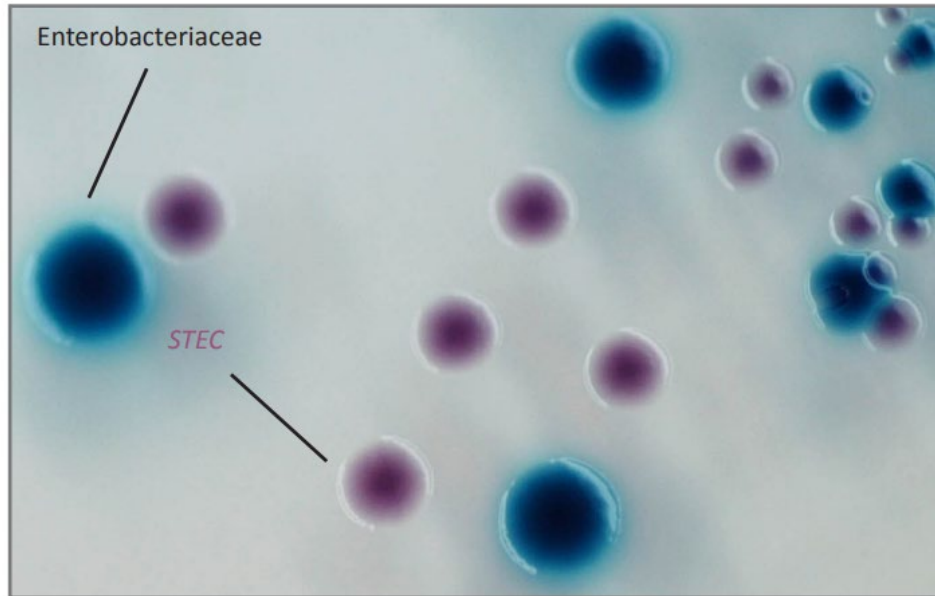


SELECTIVE & SECONDARY ENRICHMENTS

SUBJECTIVE SELECTION (COLONIES)

STEC O157	→ non fluorescent
STEC non O157	→ +/- fluorescent

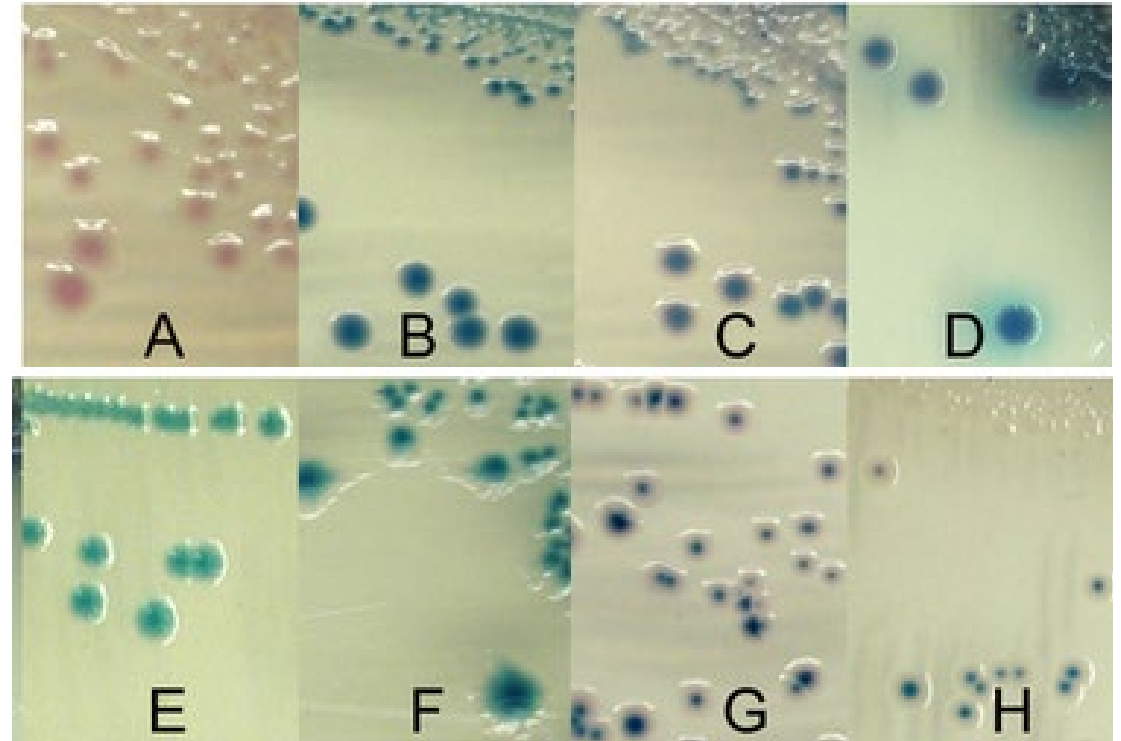
Typical colony appearance



The pictures shown are not contractual.

LIMITATIONS AND COMPLEMENTARY TESTS

- Some STEC could have a poor or no growth on the media.
- Some strains of non-STEC could appear as mauve colonies w/o fluorescence.
- Rare O157 are fluorescent positive.



Indicator agar	Colony morphology		Number of isolates by O-type ^a								
			O26	O45	O91	O103	O111	O113	O121	O145	O157
C-O157	pink	A ^b	1	0	0	0	0	0	0	0	37
C-O157	blue w/white perimeter	B	40 ^c	0	5	0	0	18	5	0	0
C-O157	blue w/pink perimeter	C	1	0	0	17	0	0	0	0	0
C-O157	blue, white perimeter, blue halo	D	0	10	0	0	0	0	0	0	0
C-O157	turquoise	E	0	0	0	0	0	1	1	4	0
C-O157	bluish turquoise, swarmy	F	0	0	0	0	0	6	0	0	0
C-O157	pink w/blue center	G	0	0	0	0	12	0	0	0	1
C-O157	white w/blue center	H	0	0	0	0	0	0	0	7	0

So...Which Test Do I Use?

Lots of things to consider to build a PROGRAM!

- Which bacteria should be tested for
- Time before decisions need to be made
- What action does the data trigger
- Repercussion of decision
- Product shelf-life
- Regulatory guidance
- Feasibility of test
- Expertise Needed
- Cost of technology



TRACEBACK & CORRECT

- It was *E. coli* O157:H7
- In the leafy greens
- From a water source



PROCESS OF ELIMINATION

- Need multiple tools
- View all data together



Thank You!

April Englishbey
aenglishbey@hygiena.com