Microbial Contamination and Control Conference

Horseshoe Crabs and Fireflies: A History of Innovating Science

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Who Am I?

- I am an 18+ year QC-Microbiologist, working in pharmaceutical manufacturing, contract lab, and now for technology innovator, Charles River Labs.
- I’m not here to sell you a product, I’m here to sell you an *idea*, that is how you can be an advocate for technological innovation and change, no matter what technology vendor you are interested in.
- For 4 years I have been dedicated to building the foundation of an alternative QC Microbiology technology to a reliable, traditional method.

History of an In Vivo Test

- “Parenteral” Industry emerged in the 1930’s as production of early injectable medicines became available in developed countries.
  - IV Infusions are commonly stored in vacuum-sealed glass containers.
  - Eureka! We discovered we can heat-sterilize at 121°C for at least 15 minutes to destroy any living microorganisms.
- Sterility tests confirm that products are free of microorganisms.
- Except…

A Very Unexpected Outcome

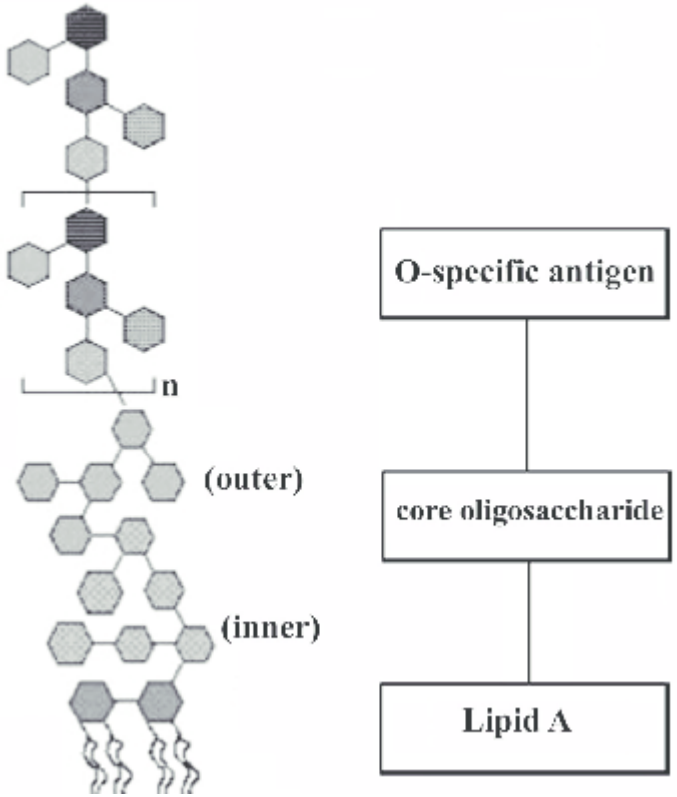
…Patients got sick anyway!

Patients exhibited signs of septicemic shock, indicating a terrible breach in sterility protocol.

Test samples were checked again, and still the sterility test passes.

Early physicians were confused – how does a sterile product result in septic shock?

A Very Unexpected Outcome



- Autoclave sterilization does not denature lipopolysaccharides from lysed Gram-negative microorganisms.
- Water used to produce IV-drug products was not sterile during production.
  - Non-sterile water is often full of Gram-negative microorganisms.
  - Gram-negative microorganisms are often unharmful in most environments but should not be injected into patients.
- Patient immune systems detected lipopolysaccharides and recognized them as bacteria in the blood stream.
  - Patients experienced endotoxemia, a deadly contamination event.

Meet our Model for the Pyrogen Test

The Pyrogen Test

1. Select 3 rabbits and fast them overnight with access to water before the test only.
2. Pin rabbits in a holder and fix a rectal thermometer; measure the temperature of animals 90 minutes prior to injection; should be between 38-39.8°C.
3. After 90 minutes, inject a calculated volume of test sample into the animal slowly into the marginal vein of the ear for a period of not more than 4 minutes.
4. Measure the rabbit's temperature at every half hour for 3 hours after injection.
5. Each individual rabbit must not experience a temperature increase >6°C.
6. The sum of increase for all rabbits must not increase >1.4°C.

Discovering a Better Way

1953: Dr. Frederick Bang publishes “The toxic effect of a marine bacterium on Limulus and formation of blood clots”
-Marine Biological Laboratory

1973: Baxter Travenol, largest producer of medial devices and LVP’s, validated LAL as an endotoxin test[1]. Baxter ran 143,196 LAL tests.

1983: USP publishes USP <85> to provide a standard method for use of LAL in lieu of the pyrogen test.

1987: FDA publishes the first LAL test guideline.

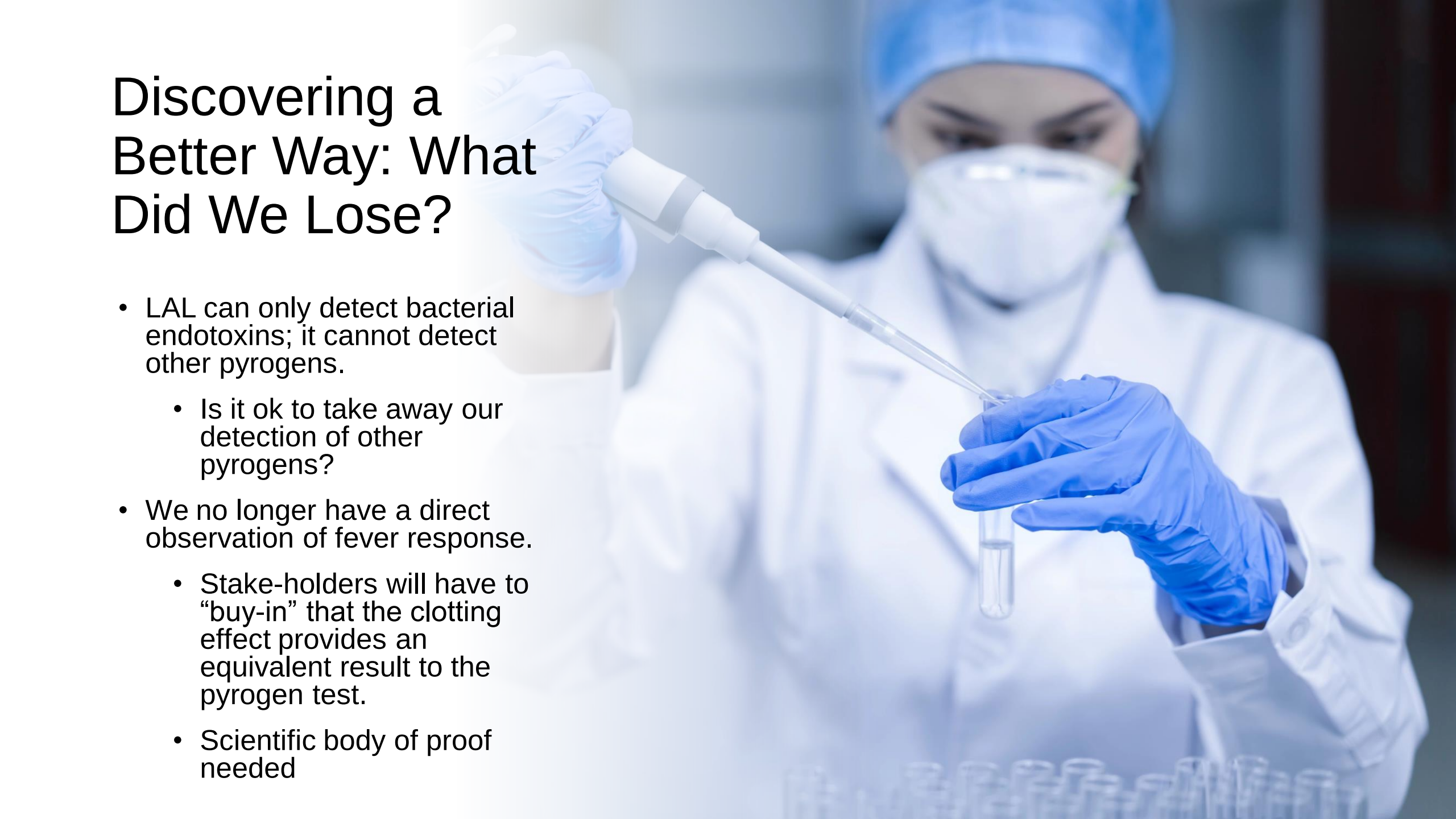
[1.] Source: Comparing the Established LAL Assay to Current Alternative Endotoxin Detection Methods; https://www.pda.org/pda-letter-portal/home/full-article/standing-guard

Meet our Model for the Limulus Test

Discovering a Better Way: What Did We Gain?

- More Animal-Friendly test method!!
- Semi-quantitation that is highly predictive, easier for setting limits.
- Reduction in cost of assay.
  - No animal maintenance.
  - No facilities for maintaining animals.
  - Smaller lab infrastructure.
- Higher through-put available.
- Later we gain the ability to quantify endotoxins

Discovering a Better Way: What Did We Lose?



- LAL can only detect bacterial endotoxins; it cannot detect other pyrogens.
  - Is it ok to take away our detection of other pyrogens?
- We no longer have a direct observation of fever response.
  - Stake-holders will have to “buy-in” that the clotting effect provides an equivalent result to the pyrogen test.
  - Scientific body of proof needed

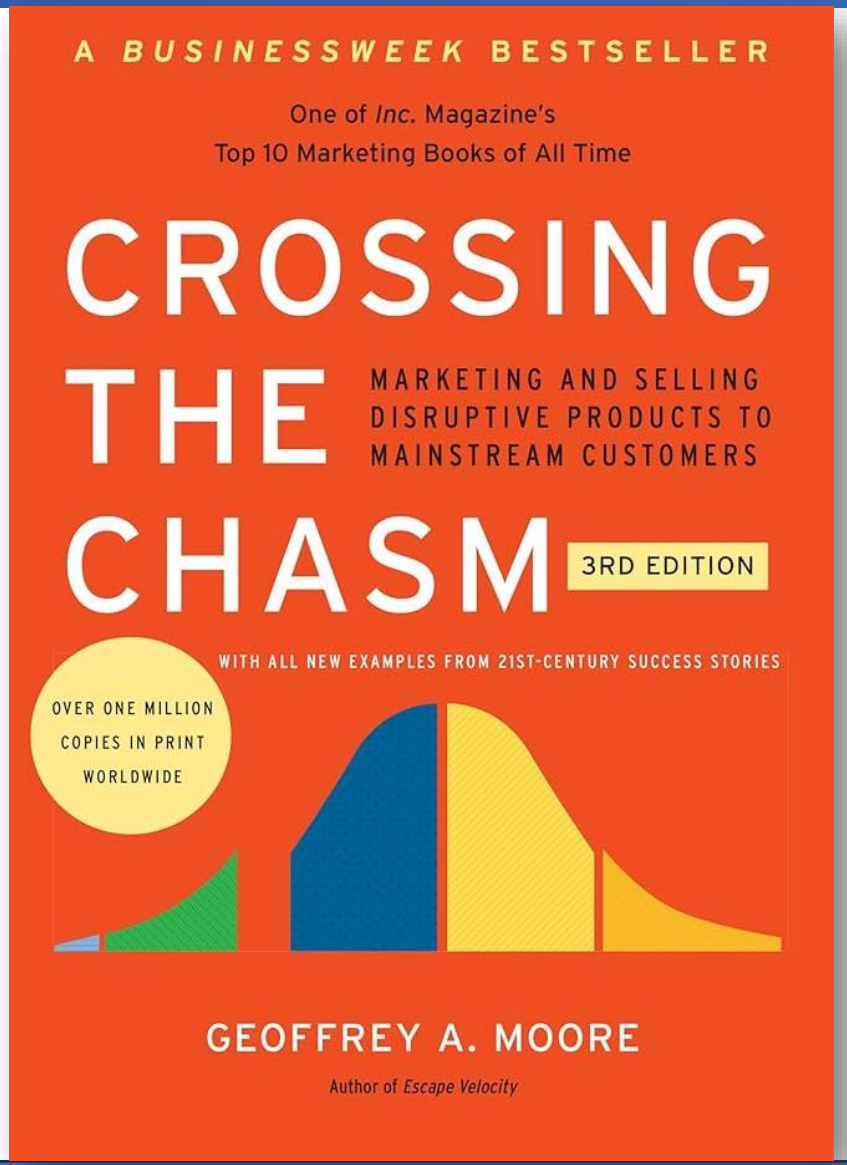
How to Bridge the Gap for Change

An early Scientific Body of Proof is not always enough to make change. Why not?

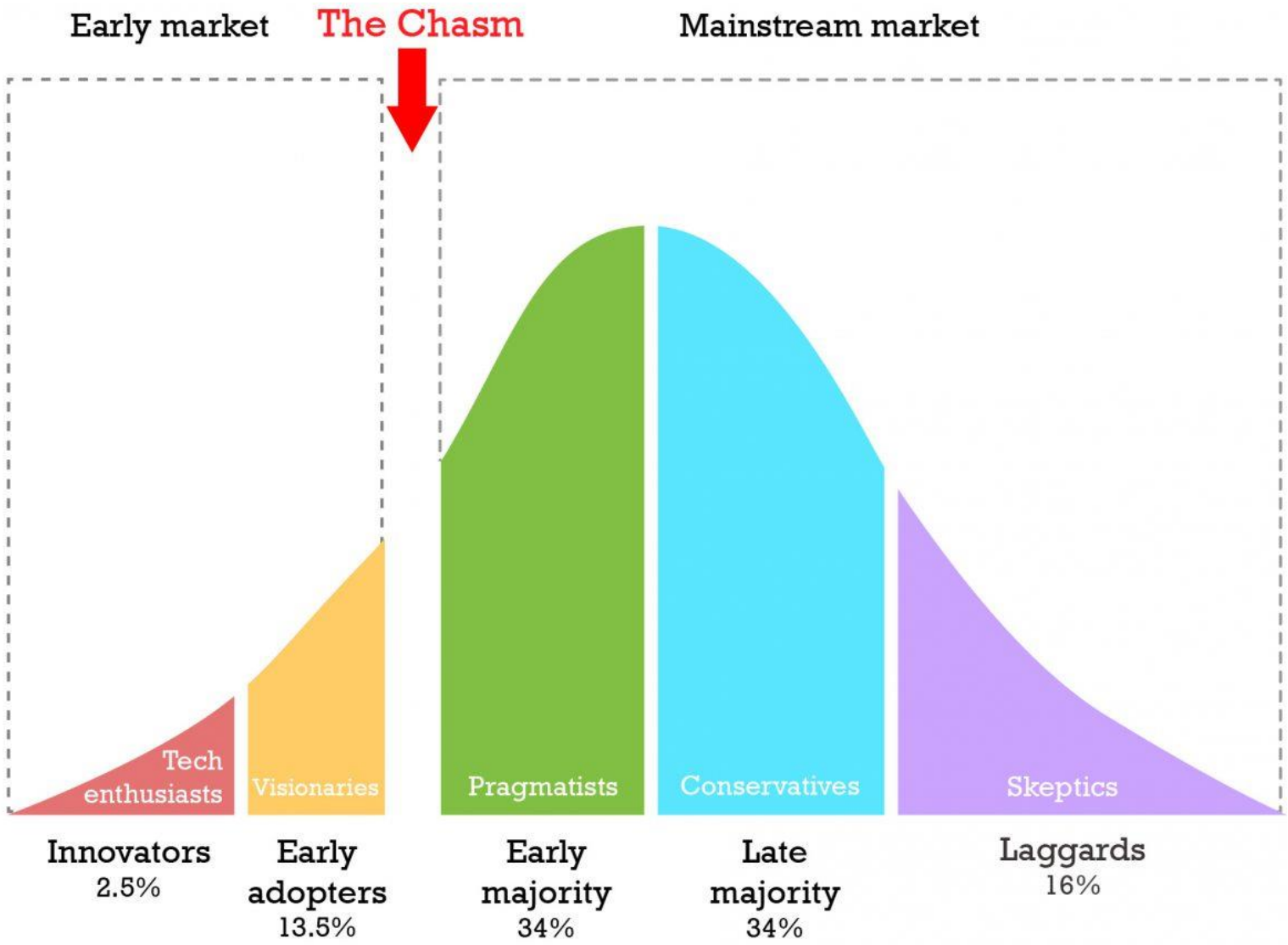
- Pharmaceutical industry is extremely risk averse.
- Manufacturers are profit driven, often focusing on today's profit over longer-term gains.
- Early adopters take a burden of risk that is not shared with later adopters.

The Chasm:

In our industry, the scary space where scientific concept is sound, but an idea has not yet been broadly applied. This is a space where a fear of things going wrong is greatest, since the body of knowledge is continuing to develop, but the consequences of such problems is great for both innovator and adopter.



The Chasm


Early market
The Chasm
Mainstream market
Tech enthusiasts
Visionaries
Pragmatists
Conservatives
Skeptics
Innovators 2.5%
Early adopters 13.5%
Early majority 34%
Late majority 34%
Laggards 16%

How to Bridge the Gap for Change

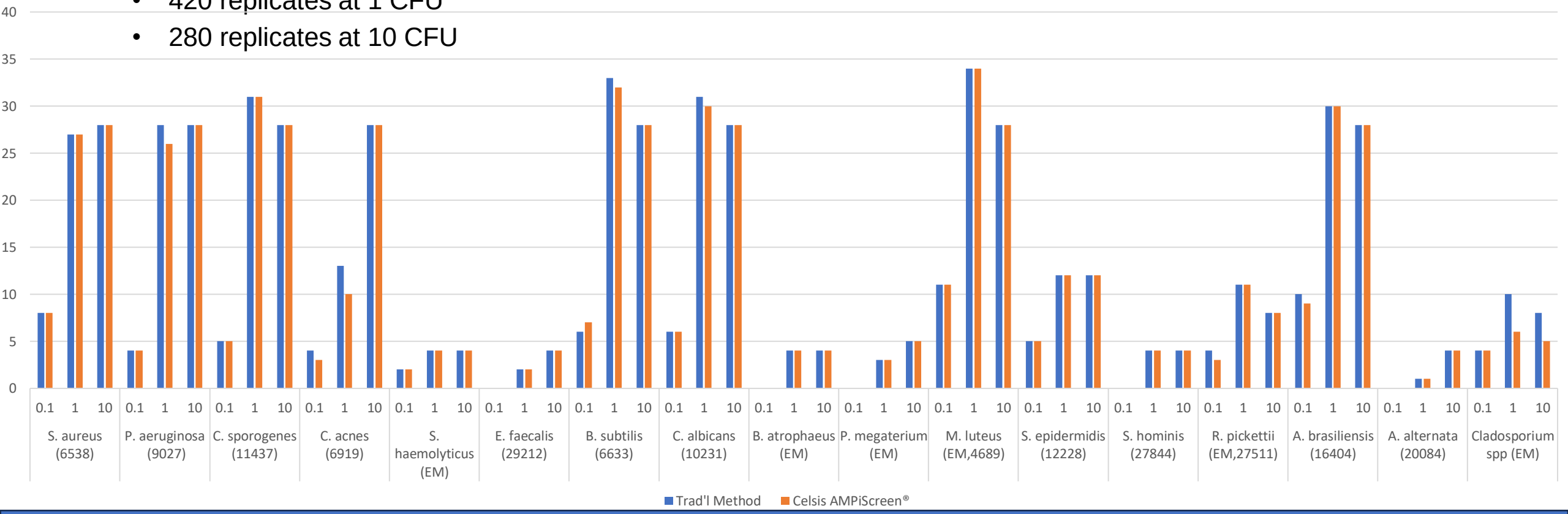
First, lay the scientific foundation by gathering a significant body of scientific evidence. For example, perform a statistical analysis comparing a traditional test to an alternative test:

- 12 microorganisms from a broad range
- 120 replicates tested at 10-100 CFU
- 336 replicates tested at 1-10 CFU
- 120 replicate tested at 0.1-1 CFU

CFU Level	Alternative Method	Traditional Method		Ratio of Detection Counts
		Positive	Negative	
10-100 CFU	Positive	105	0	105:105
	Negative	0	15	
1-10 CFU	Positive	227	0	227:235
	Negative	8	101	
0.1-1 CFU	Positive	30	2	32:31
	Negative	1	87	

Cross the Chasm! Repeat data collection across a broad range of circumstances, demonstrating repeatability and reliability:

- 2022-2023: 7 sites across the US/UK in the presence of various products.
- 17 microorganism species, including stressed and EM isolates.
  - 280 replicates at 0.1 CFU
  - 420 replicates at 1 CFU
  - 280 replicates at 10 CFU



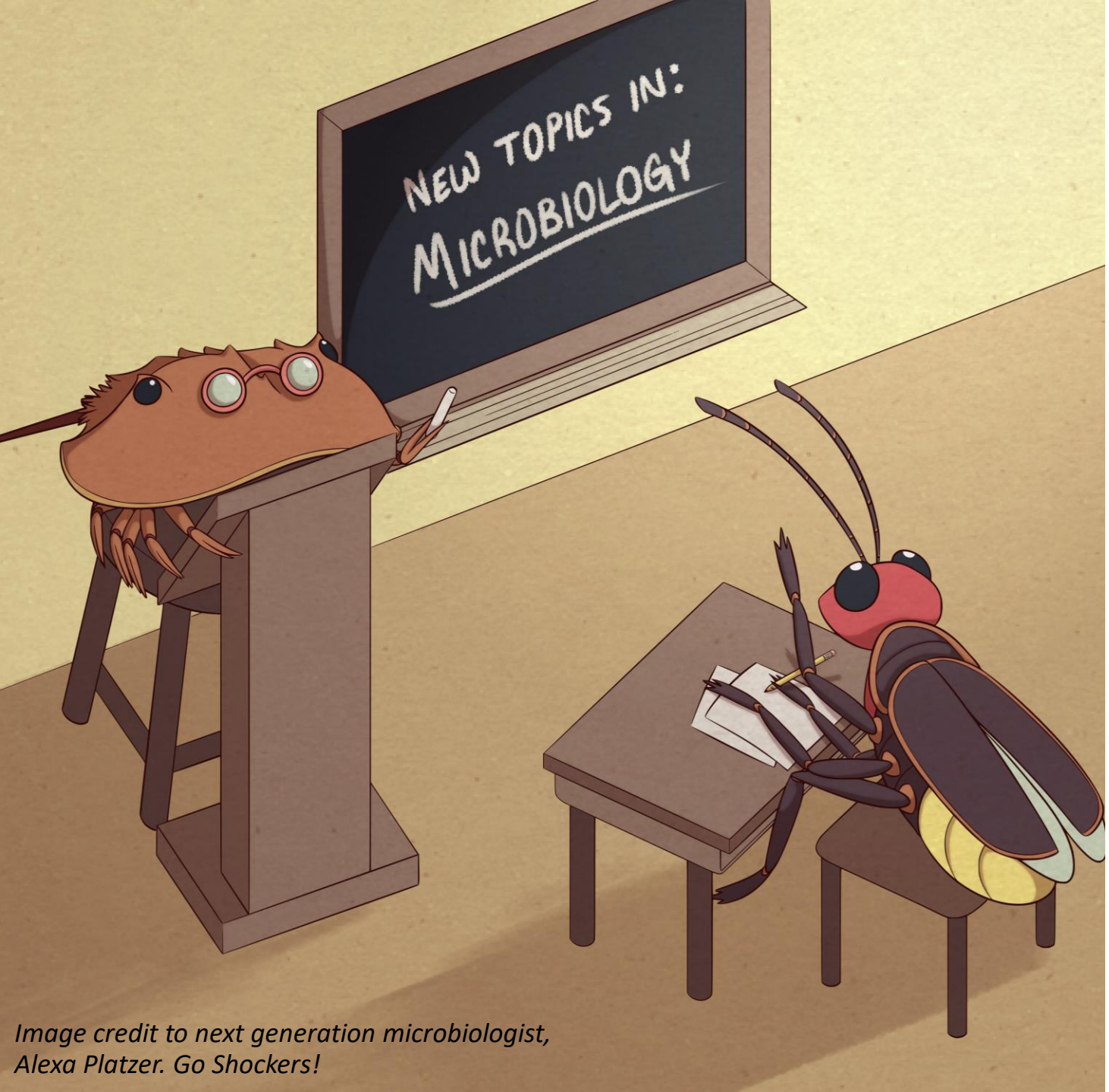


Image credit to next generation microbiologist, Alexa Platzer. Go Shockers!

How We Learn From History?

How We Learn From History

A roadmap exists today for innovating QC technology:

Step 1. Discover the Innovation.

Step 2. Gather a body of data, typically with the buy-in from an outside sponsor.

Step 3. Broadly apply the data to various circumstances; gather a greater body of data.

Step 4. Secure buy-in from stakeholders and regulators and cross the chasm.

But all along the way, *persist* in your endeavor for scientific discovery and change.