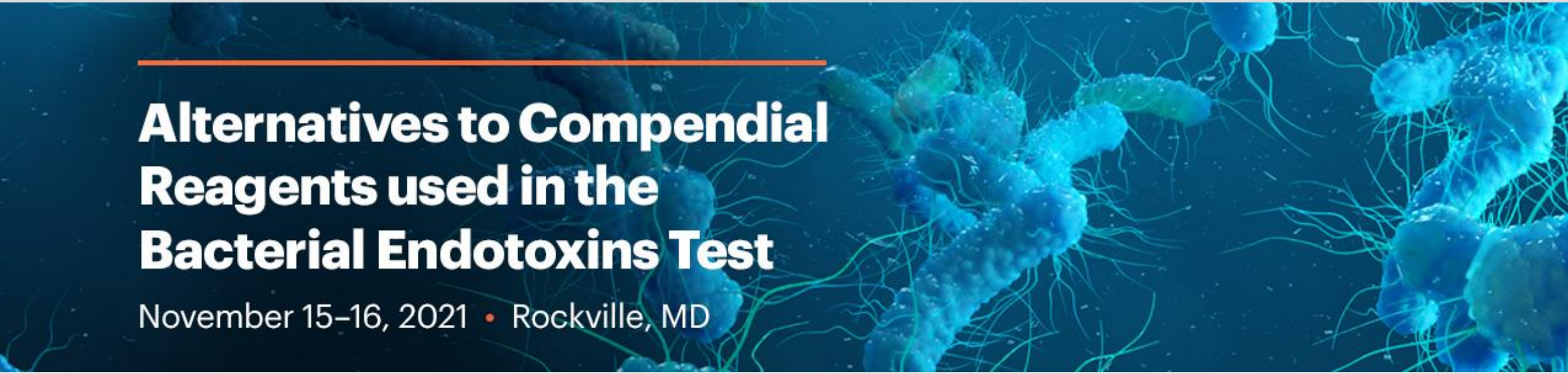


Setting the Stage:

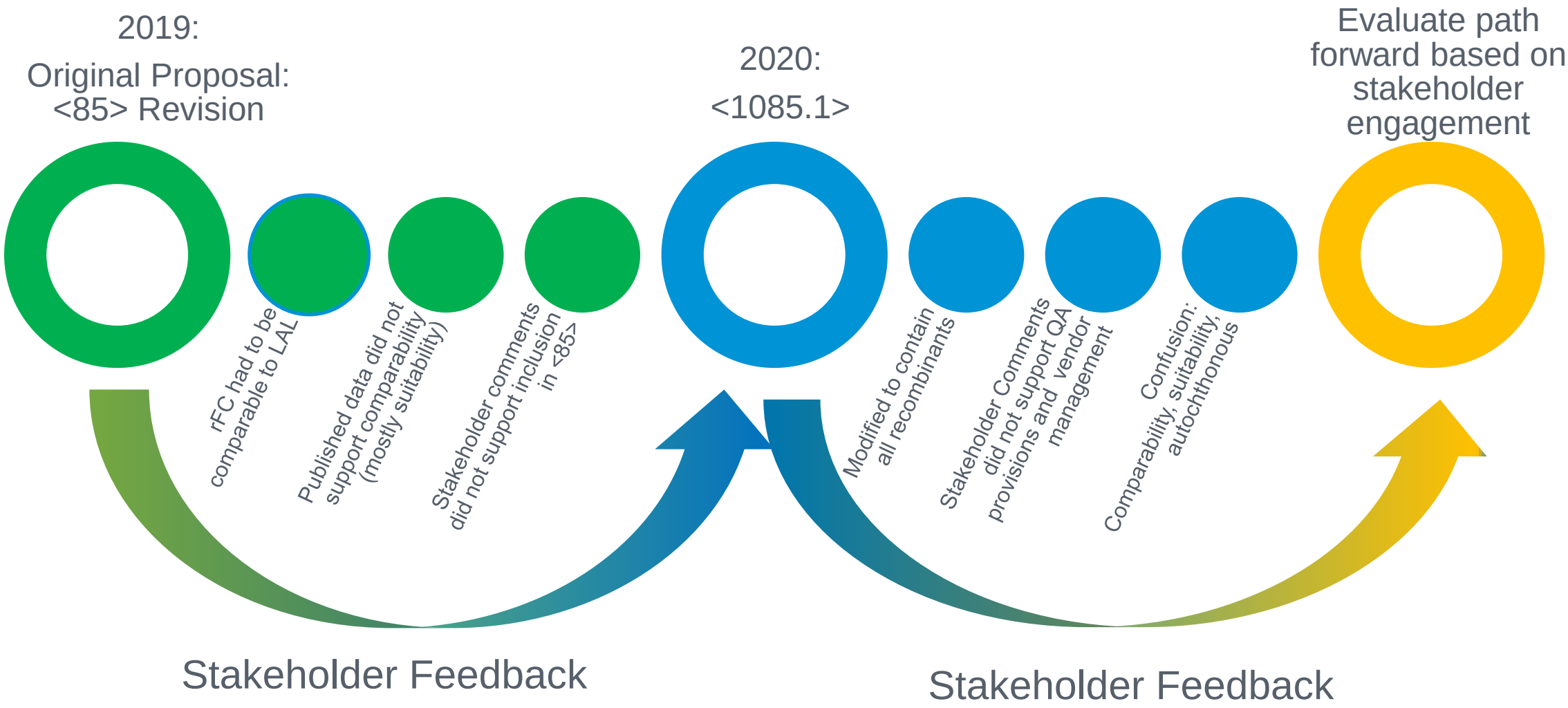
<1085.1>: Science and Stakeholders

USP Sub-Committee on Endotoxins and Pyrogens Testing

Karen McCullough, Subcommittee Chair

The standard of trust

Science and Stakeholders: History of <1085.1>



Stakeholder Comments on <85> and <1085.1>

Stakeholder Comments	
Did not support inclusion in <85>	Made an informational chapter <1085.1> as existing comparability data did not support inclusion into <85>
Did not support discussion of suggestions for QA support (e.g. questions for audit)	Eliminated that section
Did not support reference to “autochthonous”	Intent of <85> is to look for endotoxins from organisms autochthonous to the manufacturing environment that could contaminate products. Reference <1223>.
<1223> doesn’t apply because BET is a biological assay	Endotoxins are the product of the proliferation of Gram negative microorganisms = microbiology. <1223> is an excellent reference on the concept of comparability

Stakeholder Comments on <85> and <1085.1>

Stakeholder Comments	
Assertion that suitability = comparability	Suitability = PPC = demonstration that the validated test neither inhibits nor enhances. Part of the controls for the assay Comparability = demonstration that the same quality decisions would be made regarding product quality (equivalence in assay results)
EP has accepted rFC and there is a lot of peer reviewed literature	Although the current BET is harmonized, EP, JP and USP are separate organizations, each with its own approach, pressures, concerns. Peer reviewed data are mostly suitability data or “none detected” data. Lack of shared data and scientific discussion prompted this conference and planned future open forum events

What is the Purpose of ANY Bacterial Endotoxins Test?

- To detect and quantitate UNKNOWN levels of endotoxins activity from UNKNOWN sources
  - Current BET assays are CALIBRATED to a STANDARD, but are designed to detect UNKNOWNS
- Compare the level of recovered activity to the calculated or assigned endotoxin limit – DECISION on the endotoxins SAFETY of the product
  - ENDOTOXINS is a critical test for PATIENT SAFETY and endotoxins activity is a Critical Quality Attribute for any parenteral product
- Hence the importance of including materials with assayable levels of endotoxins from autochthonous microorganisms in any validation effort. WILL THE ALTERNATIVE FIND CONTAMININATING ENDOTOXINS?

Comparability and Validation

- USP General Notices 6.30:
  - The alternative method or procedure must be fully validated (see *Validation of Compendial Procedures* ⟨1225⟩) and must produce comparable results to the compendial method or procedure within allowable limits established on a case-by-case basis.
- FDA 2012 Guidance:
  - Firms may use alternative methods and/or procedures if they provide advantages in terms of accuracy, sensitivity, precision, selectivity, or adaptability to automation or computerized data reduction, and in other special circumstances. Such alternative procedures and methods should be validated as described in the USP General Chapter and should be shown to achieve equivalent or better results.

Comparability and Validation

- USP <1225>:
  - Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications
- Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics (2015).
  - The new method coupled with any additional control measures is equivalent or superior to the original method for the intended purpose (comparability)
  - The new analytical procedure is not more susceptible to matrix effects than the original procedure (suitability). Note: Products have specific, calculated endotoxin limits and associated MVDs. The alternative test may have different interference properties than the compendial test, but in any case, dilution may not exceed the MVD.

AUTOCHTHONOUS: Originating where it is found

- Sergei Winogradsky: a founder of modern microbiology and the first microbial ecologist (1924) *Sur la microflora autochtone de la terre arable*. Comptes rendus hebdomadaires des seances de l'Academie des Sciences (Paris) D, 178, 1236-1239.

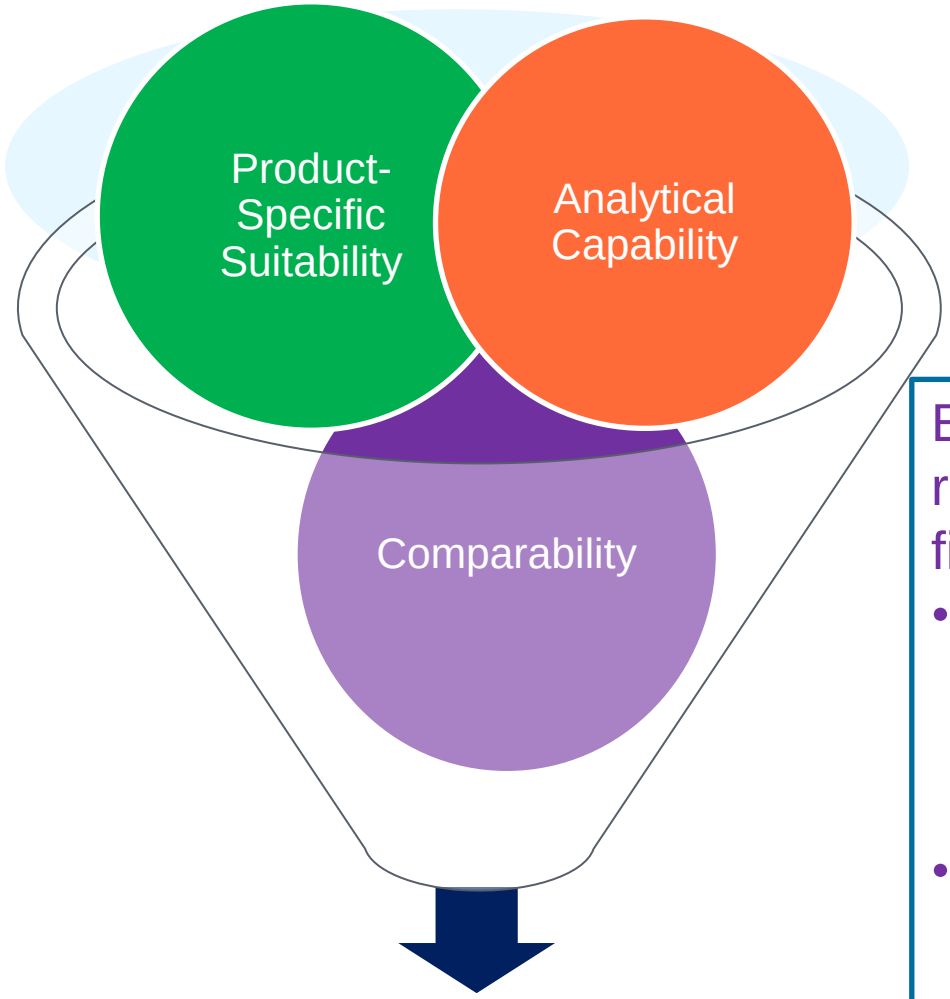
- For example,
  - An *Autochthonous Infection* is one caused by organisms *normally present* in the patient's body. It may occur when host defenses are compromised, or when resident flora are introduced into an abnormal site.
- Organisms autochthonous to a WFI system have adapted to and therefore originated as a result of the environment provided by the water purification system.

- Nature (2018): *Comparative assessment of autochthonous bacterial and fungal communities and microbial biomarkers of polluted agricultural soils of the Terra dei Fuochi*

- Environmental Science Water and Technology (2020): *Autochthonous tropical groundwater bacteria involved in manganese oxidation and removal*

Consistent Message: 3 Part Validation for a General Chapter on Alternative Endotoxin Methods

Reference USP <85>
- Use calibration standard to add a known level of endotoxins activity; recover quantitatively
- Product-specific



Reference relevant portions of USP <1225> to align with <85>

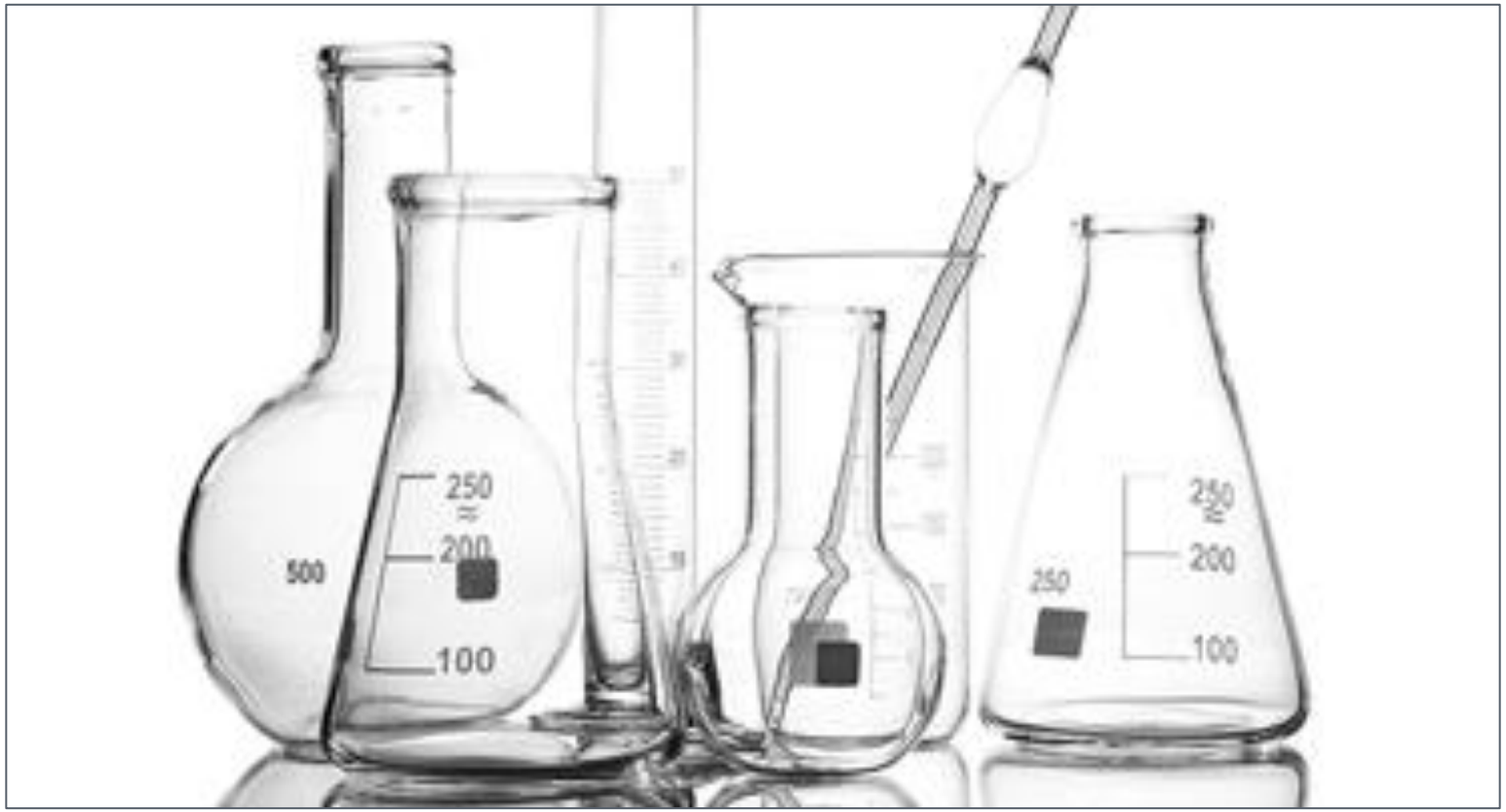
Endotoxins that could be in a raw material, intermediate, or finished product
- Assure that the test works across products (e.g. LVP, SVP, biologics, devices, cell/gene)
- Easiest performed by reagent manufacturer and confirmed in the laboratory

Biological, Chemical and Biochemical Alternatives: it's not only about rFC

- Already Commercialized and/or in Development
  - Recombinant reagents (rFC and recombinant cascade)
  - Monocyte Activation Tests (MAT)
  - HEK (TL4) assays
  - Flow cytometry
  - Fluorescent tagging
  - Aptasensors
  - Optical biosensor
  - LCMS/GCMS
- Not unreasonable to expect that others are in R&D

Discussion

- Data – Opportunity to Share and Learn from the Data
  - Need to evaluate what data is presented
  - What data is needed to adopt alternative procedures to LAL?
- Comparability
  - The use of the term, “autochthonous”
  - Alternative Endotoxin Detection/Quantitative Method
  - Regulatory and pharmacopeial documents on comparability and alternate tests
- Stakeholder Engagement
  - Industry, regulators, others



Trust the Science!

Thank You