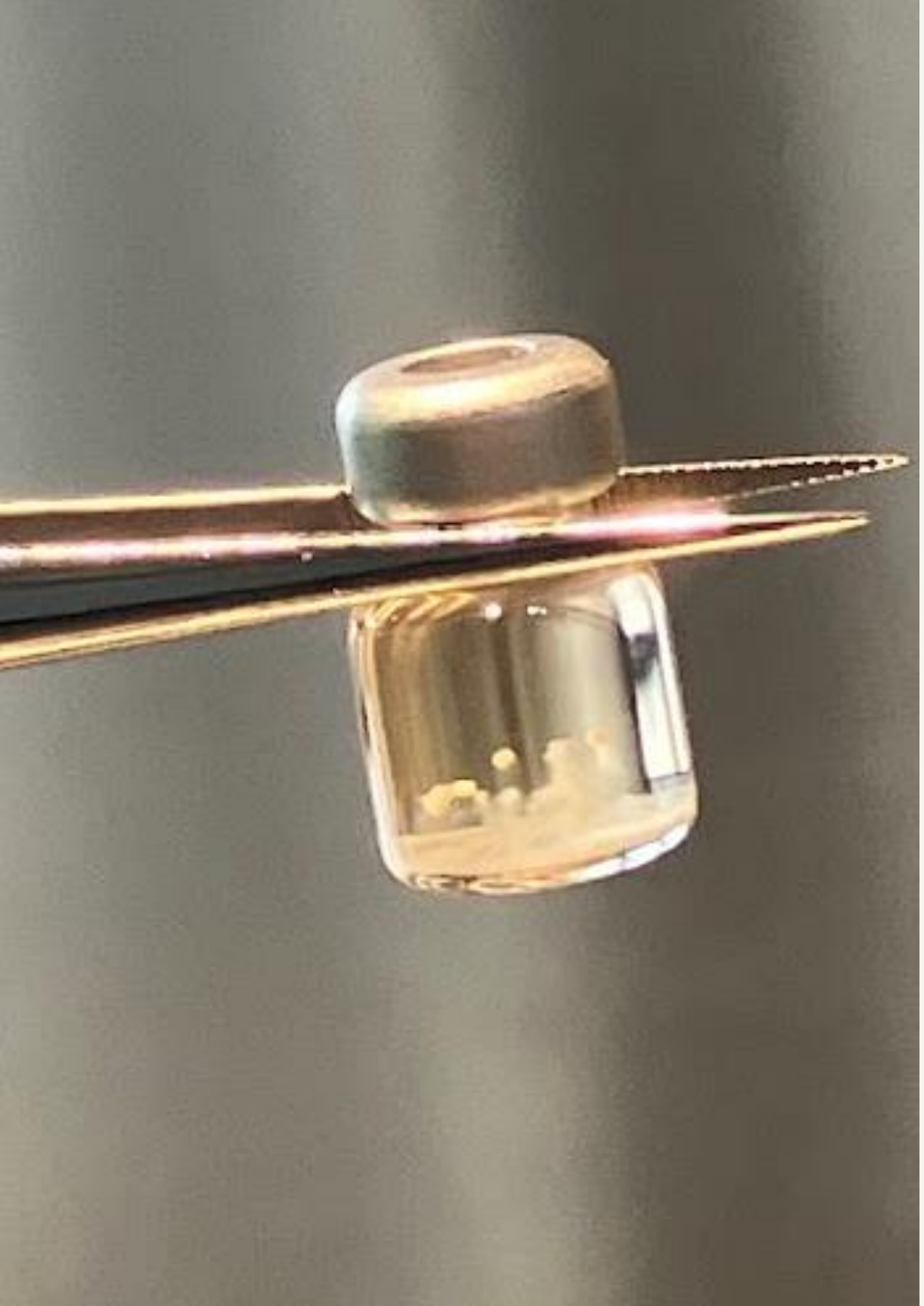


Evaluation of an alternative microbiological technique based on microcalorimetry

European Microbiological Conference

21- May-2025

Nathalie Vendur
Expert QC Biotesting

Evaluation of an alternative microbiological technique based on microcalorimetry

Introduction & Background Story

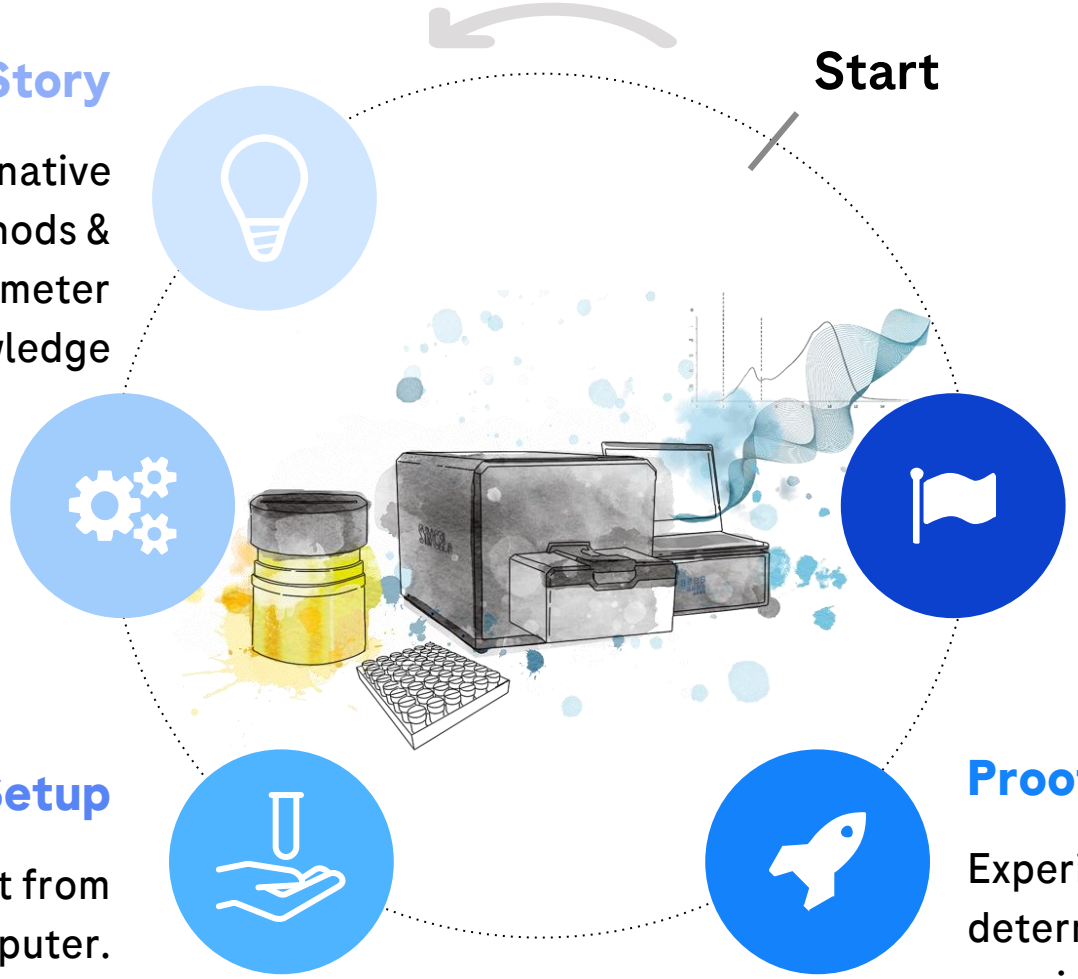
Introduction into alternative microbiological methods & Refreshing the microcalorimeter knowledge

Microcalorimeter Principle

Working principle in microbiological testing procedures.

General Experimental Setup

Heatflow signal measurement from vial to computer.



Conclusion & Outlook

Main conclusion, Method advantages and Consideration points.

Proof of concept

Experimental conduction of TTD determination, heat stress , media consideration and cellular material

Introduction

Sterility testing in the pharmaceutical industry

<u>Microbial Testing Methods (USP<71>), (EP 2.6.1), (JP 4.06):</u>

Direct Inoculation Technique:

- The volume of the product should not be more than 10% of the volume of the growth medium.

Membrane Filtration Technique:

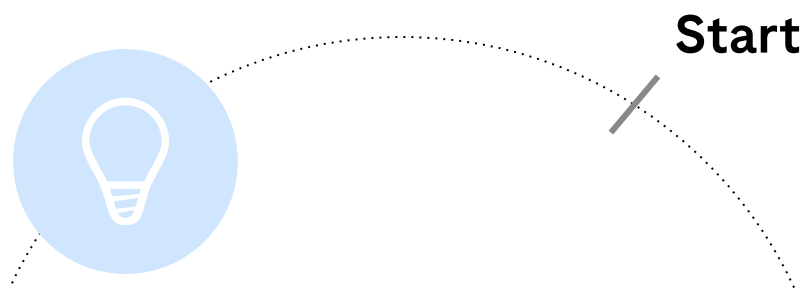
- Applied when the nature of product allows (e.g., filterable aqueous preparations, alcoholic or oily preparations and products miscible or soluble in aqueous or oily solvents.

Direct Inoculation

Membrane filtration

Limitations:

- Time-consuming
- Low sensitivity for very small contaminations
- Prones to human error/contamination



Introduction
Alternative microbiological methods

Alternative methods :

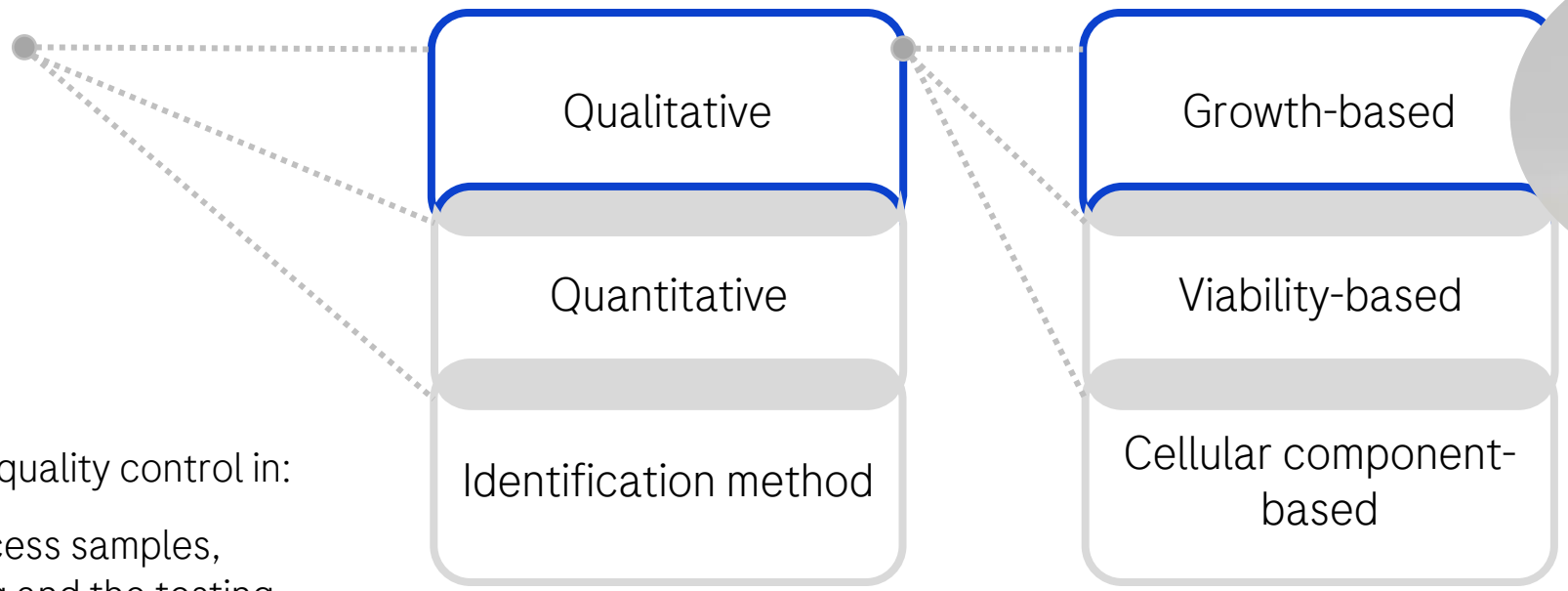
needs to be:

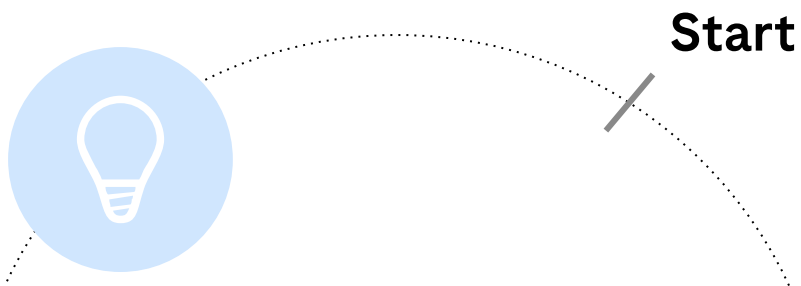
- equal
- or superior

to compendial methods.

Goal:
optimize microbiological quality control in:

sterility testing, in-process samples, environmental monitoring and the testing of industrial utilities (water&steam production and distribution).





Introduction

Alternative microbiological methods – Implementation as a 9 step approach

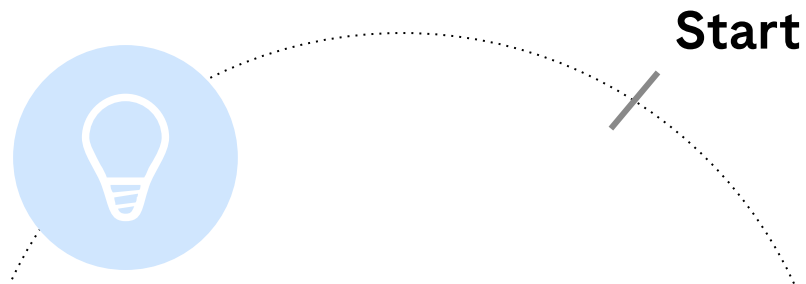
Implementation

- Faster analytical procedure (reduction of order lead time)
- Method compatible with recombinant proteins and/or Gen and Cell Therapy Products

- Sterility testing

- Qualitative method
- Fast
- Improvement in data integrity

1. Identify business need
2. Define application
3. Identify requirements
4. Compare technologies
5. Business case
6. Proof of concept
7. Validation at pilot site
8. Global deployment
9. Considerations for regulatory strategy

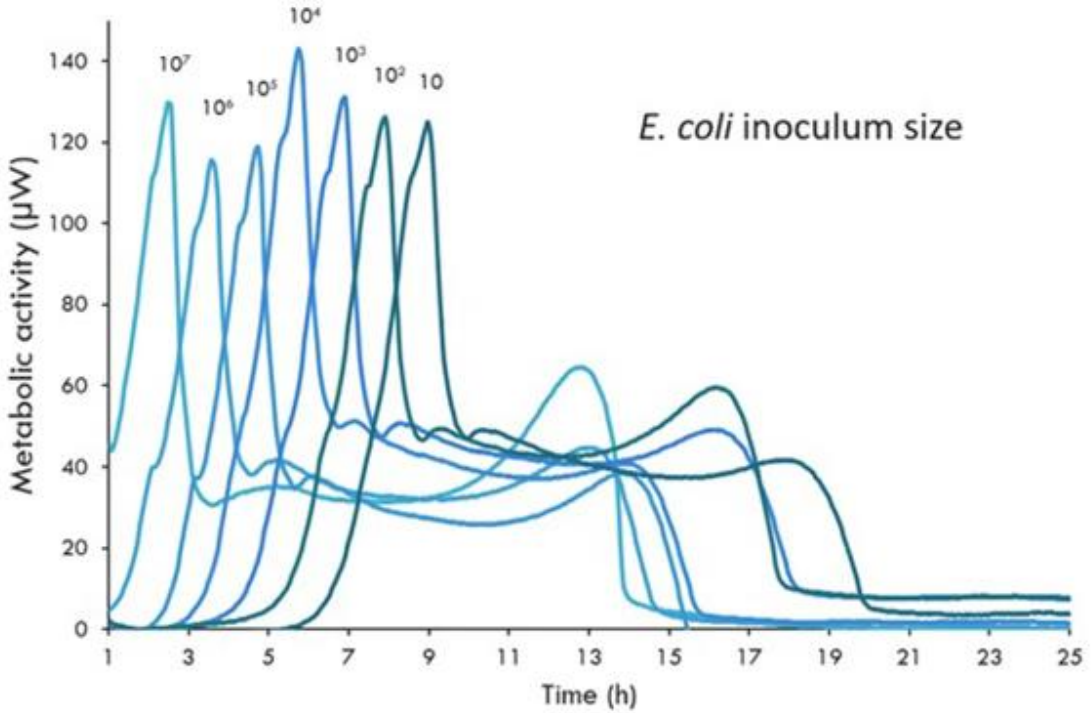


Ref.: S.Deutschmann et. Al. (2020), Alternative and rapid micro methods (ARMM)

Background Story

An alternative microbiological technique based on microcalorimetry

- History dates to **1789** and the potential still requires **further exploration**.
- Heat is a measurable form of energy that creats a **Thermogram**.
- Lactic acid fermentation or aerobic respiration produces **heat as a byproduct**.
- **Metabolic fingerprint** could be used for **identification** of organisms.
- The second law of **thermodynamics** stating: heat will naturally flow from a region with higher temperature to a region with lower temperature



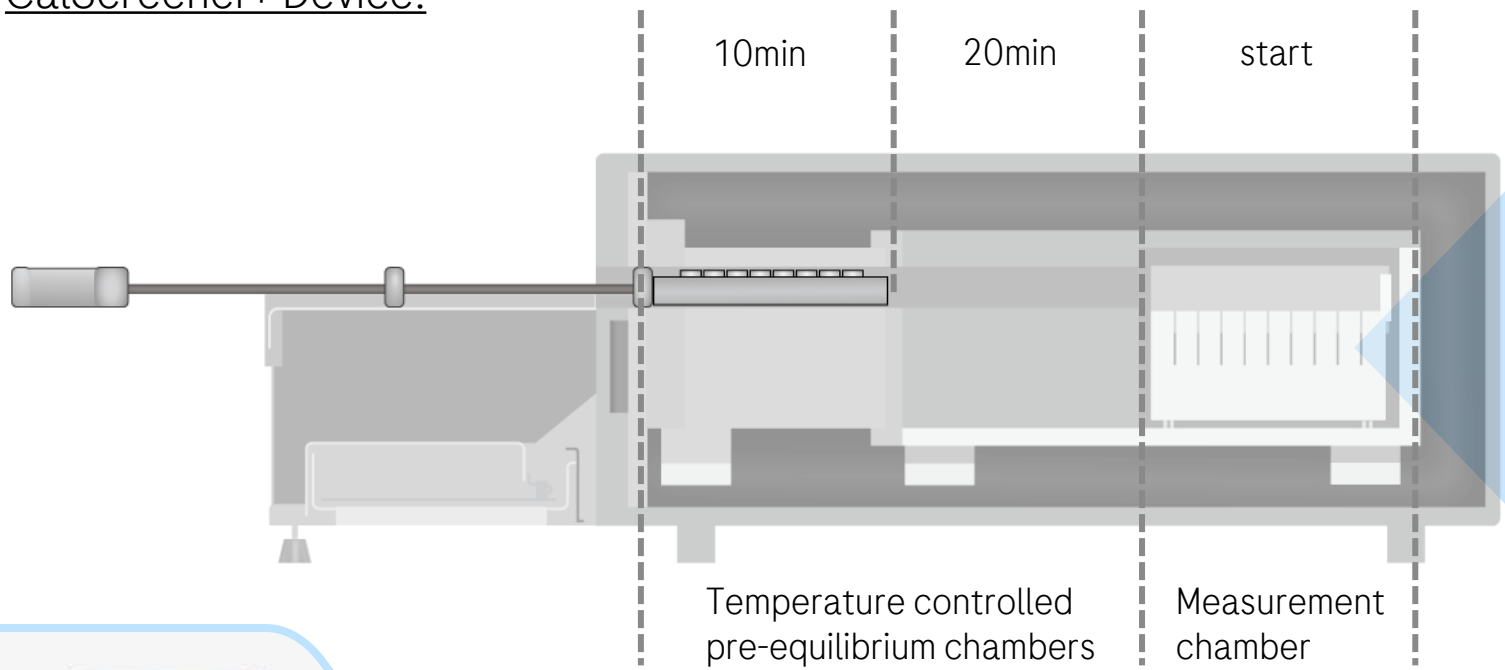
First calorimeter discovery by Lavoisier and Laplace in 1789.

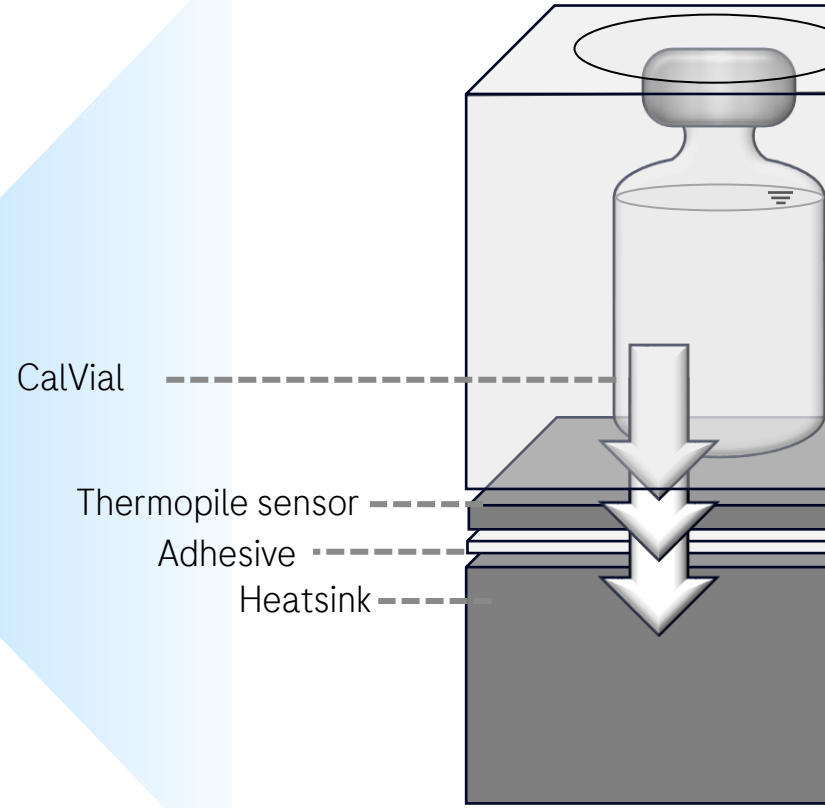
Start

Microcalorimeter Principle

Instrumentation from Symcel

CalScreener+ Device:



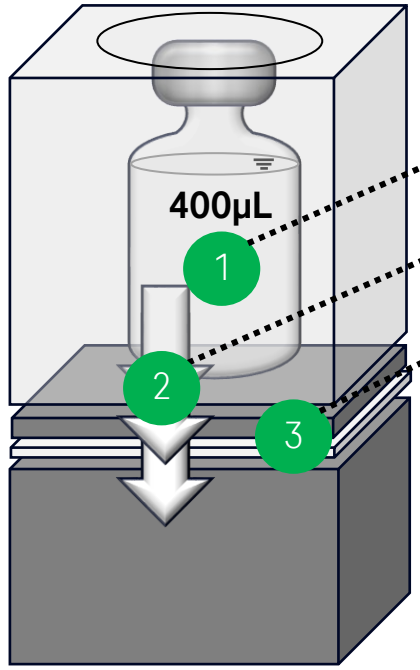


General Experimental Setup

Measurement principle of the CalScreener+ microcalorimetry

Pratical

1. Suspend Bioball® (bioMérieux) in Rehydrationbuffer
2. Inoculum control
3. Direct Inoculation:
   - Inject growth media
   - sample (10%) or/and
   - microorganism (50/5/0.5 CFU)
4. Crimp the vials
5. Insert in Microcalorimeter



Technical

1. Heat as byproduct of metabolic activity
2. Heat flows to the heat sink
3. Sensor measures change
4. Substraction of the References
5. Passing to the ADC* and Baseline adjustment
6. Data is illustration as Watt per time (Thermogram), with a threshold of $\mathbf{10^{-6}W}$.

*Analog-to-digital converter

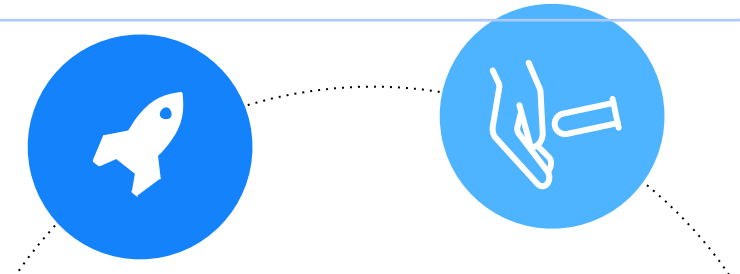
Proof of concept

Evaluation of an alternative microbiological technique based on microcalorimetry

Main focus of the feasability study can be divided in the following categories:

6 **Proof of concept**

Block 1 Robusstness	**Influence of growth media from different vendors**
Block 2 Robusstness	**Impact of Pre-Treatment Stress by Heat**
Block 3 LOD	**Determination of the Limit of detection**
Block 4 Specificity	**Product specific Test considering Monoclonal Antibodies and Eucaryotic Cell Solutions**



Microorganisms:

A.brasiliensis ATCC16404
B.subtilis ATCC 6633
C.acnes ATCC 6919
C.albicans ATCC 10231
C.sporogenes ATCC 19404
P.aeruginosa ATCC 9027
S.aureus ATCC 6538

S.epidermidis
B.cepacia

Proof of concept Robustness

Influence of growth media from different vendors on Microorganism Time to Detection

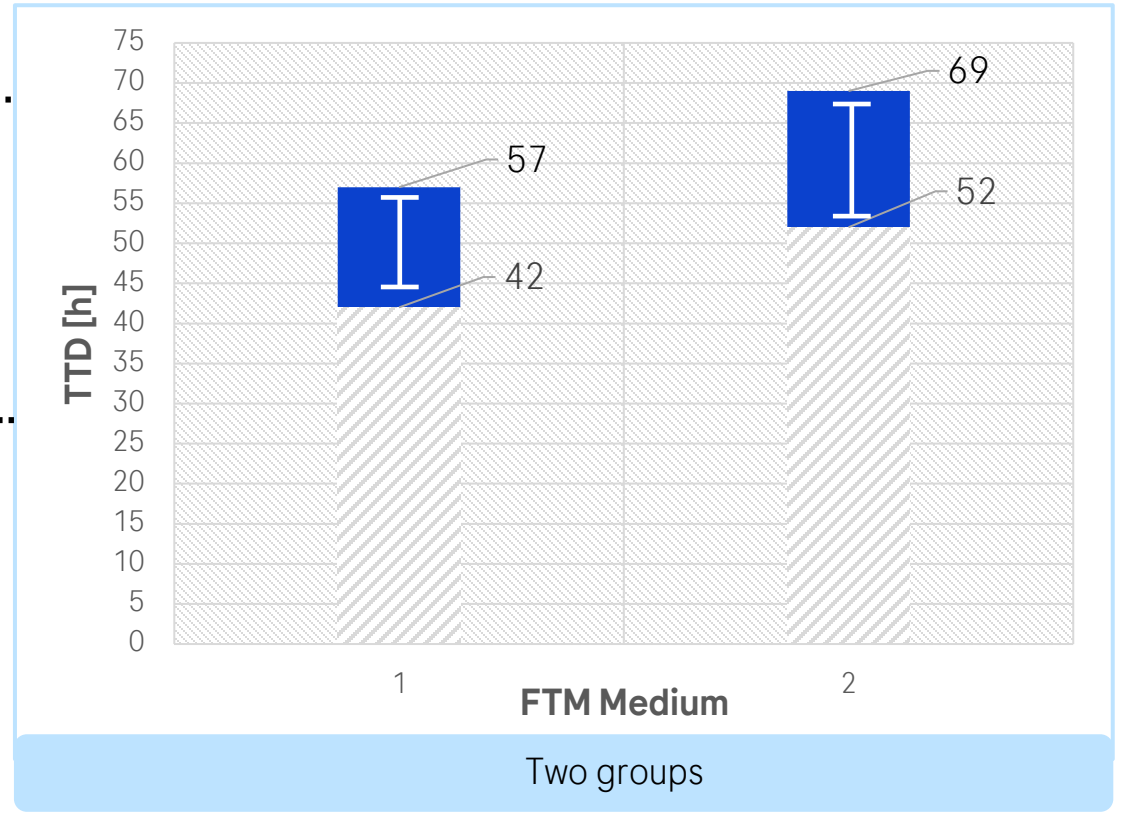
Measurement Results

Procaryotes

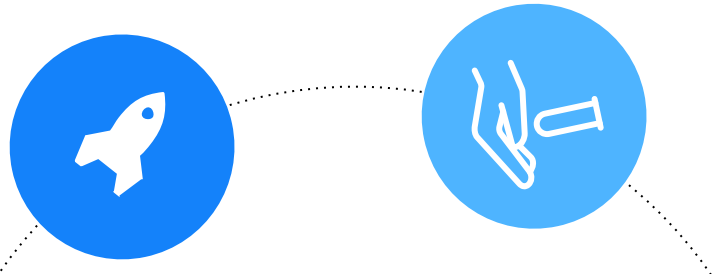
The media vendor has a crucial impact on the TTD.

Statistical analysis confirmed two media groups

Organism:	**Inoculum:**
C.acnes	50 CFU
Temperatur:	**Media vendor:**
35 °C	7 with 3 batches



Two groups



Proof of concept Robustness

Impact of Pre-Treatment Stress on Microorganisms Detection in Sterility Test considering Heat

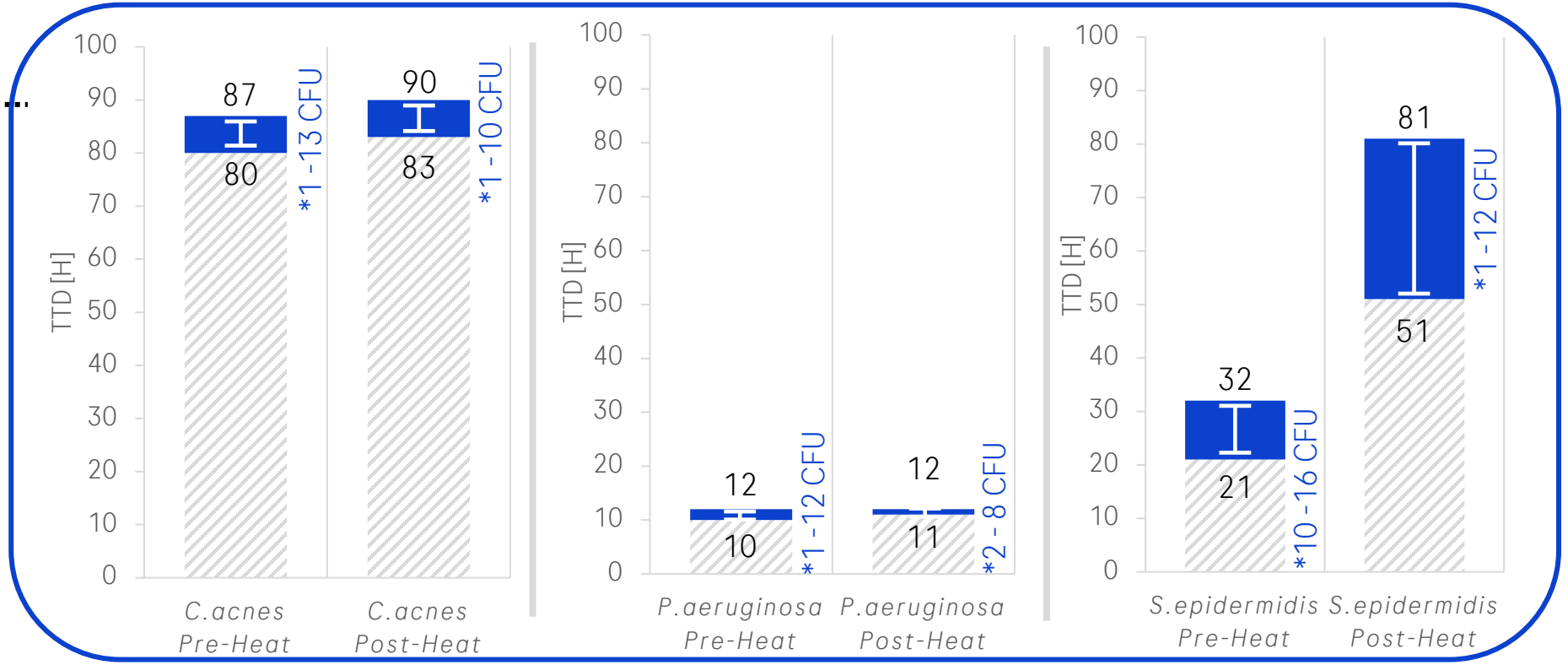
Measurement Results

Procaryotes

■ TTD [h]

Result:

Heat stressed organisms from culture plate are detected within 4 days.



Media from group 2

*Inoculum range

Proof of concept LOD

Determination of the Limit of detection for Microorganisms

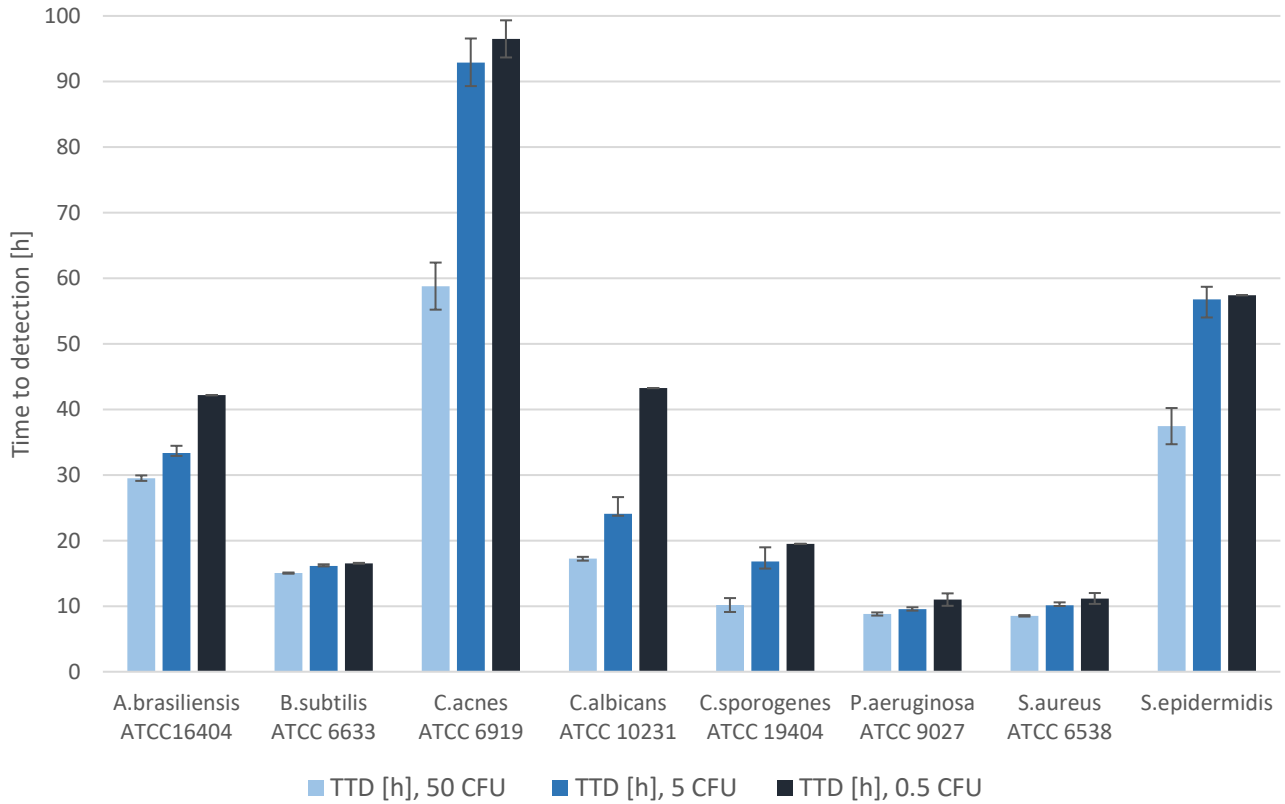
Results:

Measurement Results

Procaryotes

25°C:
A.brasiliensis, B.subtilis, C.albicans, P.aeruginosa, S.epidermidis

35°C:
P.aeruginosa, S.aureus, C.acnes, C.sporogenes



After ~max. 4 days all microorganisms were detected.

Media from group 2

Proof of concept Specificity

Product specific Test considering Monoclonal Antibodies

- Testing monoclonal antibodies, to evaluate the procaryotic detection:

Measurement Results

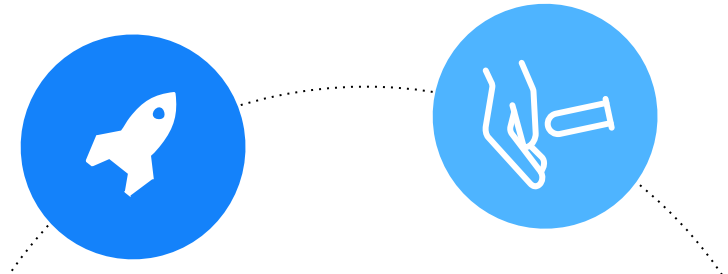
Inoculum:
50 CFU

Ranging of mAb concentration: 18mg – 2500mg

	TTD [h]						
Microorganism	Control	mAb1	mAb2	mAb3	mAb4	mAb5	mAb6
C.acnes ATCC 6919	58.8	57.1	57.4	56.1	59.9	61.7	62.9
P.aeruginosa ATCC 9027	8.1	7.7	7.0	N/A	N/A	N/A	N/A

Media from group 2

Microorganisms are detectable in presence of mAbs within 3 days .



Proof of concept Specificity

Product specific Test considering Eucaryotic Cell Solutions

- Testing eukyaryotic cells to evaluate the procaryotic detection :

Measurement Results

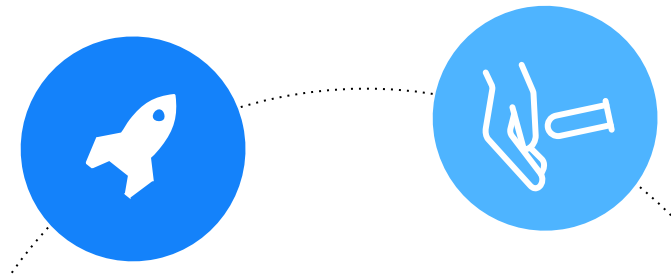
Procaryotes

Eucaryotes

Inoculum:
50 CFU

Microorganism	TTD [h], Control	TTD [h], HEK Cells (10^6cells/mL)	TTD [h], Killer cell (-)
A.brasiliensis ATCC16404	29.5	23.0	29.5
C.acnes ATCC 6919	58.8	75.4	51.8
C.albicans ATCC 10231	37.5	16.3	20.8
P.aeruginosa ATCC 9027	8.8	8.13	8.67

Media from group 2



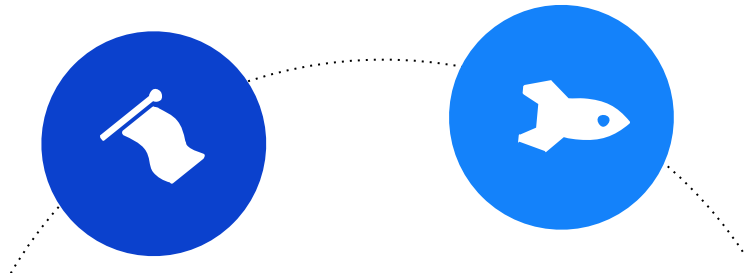
Microorganisms are detectable in presence of eucaryotic cells within 4 days .

AB = Antibiotic

Conclusion & Outlook

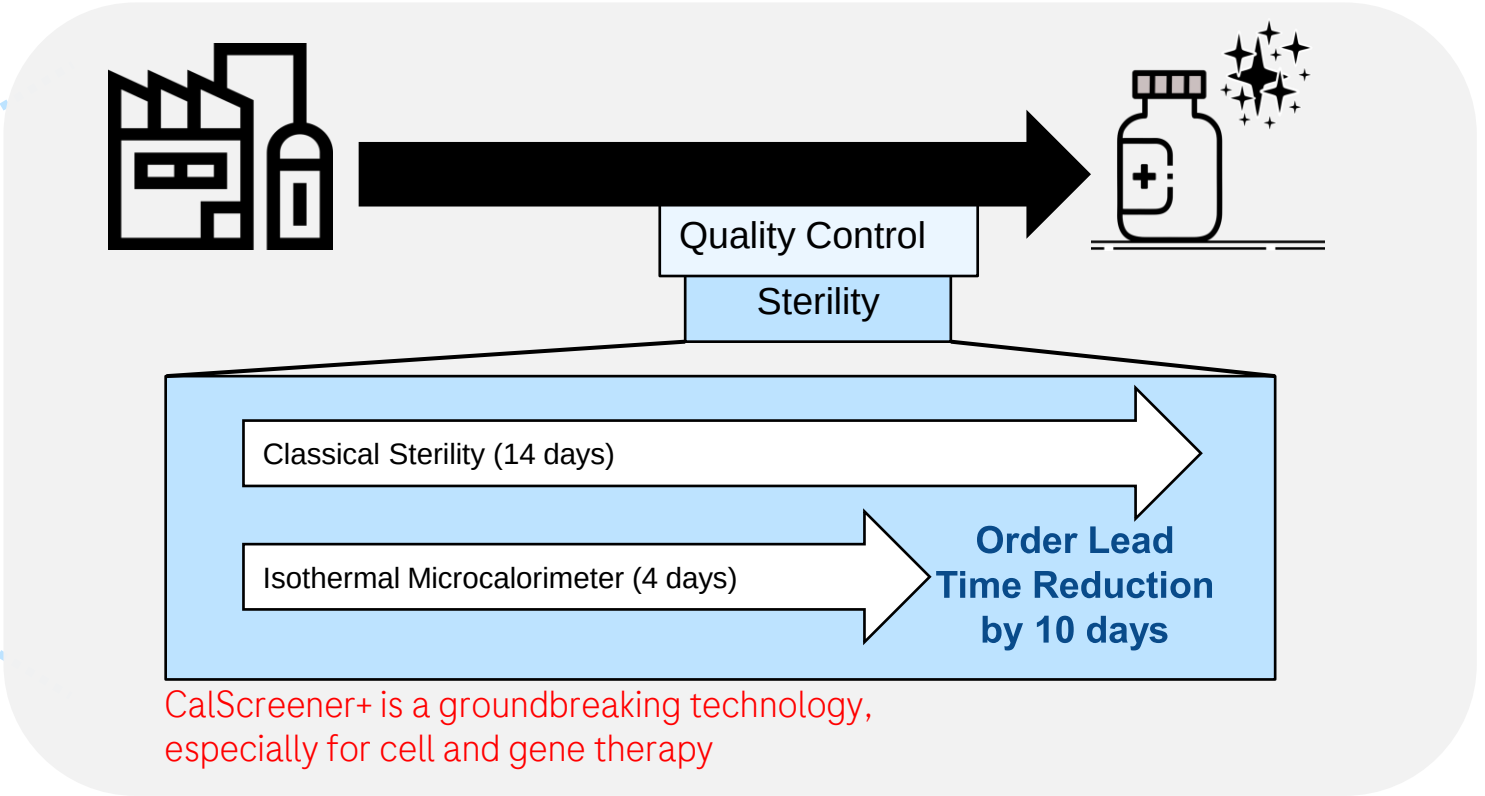
Evaluation of an alternative microbiological technique based on microcalorimetry

Block 1	**Growth media from different vendors can lead to big differences in TTDs, making it to an important parameter.**
Block 2	**The effect of heat is not drastically changing the TTD, reducing the sterility testing to 4 days.**
Block 3	**The slowest growing Microorganism was detected within 4 days, reducing time to detection for sterility.**
Block 4	**The effect of mAbs and eucaryotic cells is not drastically changing the TTD, reducing the sterility testing to 3 - 4 days.**

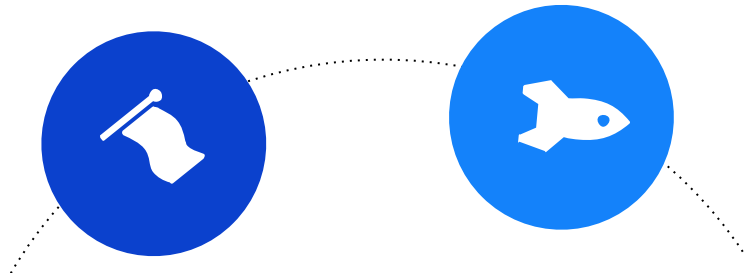


Conclusion & Outlook

Isothermal Microcalorimeter against the classical sterility testing method



CalScreener+ is a groundbreaking technology, especially for cell and gene therapy



Conclusion & Outlook

Evaluation of an alternative microbiological technique based on microcalorimetry

6 **Proof of concept**

Positive sites of this alternative method:

- Possibility to measure under anaerobic conditions, due to closed vial system.
- Sterility testing of Biologics, Cell Therapy, Gene Therapy with small volumes.
- Offers early real-time observation of metabolic activities and negative to date result as a release parameter.
- Simple and less time consuming.
- Isothermal microcalorimeter with higher volume is under development.

7 **Validation at pilot site**

Considerations for Validation:

- Growth media supplier and second supplier.
- Dilution effect of growth media.
- Added sample can have a different effect on the TTD.
- Antimicrobial properties of samples.
- Room with stable environmental temperature.

Acknowledgement

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Doing now what patients need next