Sterility Assurance & Quality Risk Management Conference

PDA®
Parenteral Drug Association
Midwest Chapter

PDA Parenteral Drug Association Midwest Chapter
Sterility Assurance & Quality Risk Management Conference
October 8th & 9th

Determination of Microbial Incubation Time when following USP<73> Guidance

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General Questions:

1. Who here works with short-life products?
   - Compounded sterile preparations (CSPs)
   - Positron emission tomographic (PET) products
   - Cell and gene therapies
2. Are rapid/alternative microbial detection methods utilized at your facility?
   - ATP-bioluminescence
   - Respiration-based methods
   - Solid phase cytometry
3. If rapid methods are not being utilized, why not?
   - Difficult to validate
   - Associated costs / resources

Why Consider Rapid Detection Methods?

- Faster time-to-result
- Objective result readings
- Reduced manufacturing costs
- Data integrity
- Earlier corrective actions
- Faster product release

Challenges with conventional sterility testing for short-life products

- Timing: Short-Life Products (ATMPs, Cell & Gene Therapies) & compounded products – At-risk release
- Sample Size: Small volumes and batch size for ONE personalized (autologous) therapy
- Matrices: May contain live cells (high cell densities) which may cause turbidity in culture media or interfere with microbial growth

Industry Guidance for Short-Life Products & RMMs

- Ph. Eur. 2.6.27 Microbiological Examinations of Cell-Based Preparation
- Ph. Eur. 5.1.6 Alternative Methods for Control of Microbiological Quality
- USP <1071> Rapid Microbial Tests for Release of Sterile Short-Life Products: A Risk-Based Approach
- USP <1223> Validation of Alternative Microbiological Methods (PDA TR 33, Ph. Eur. 5.1.6)

Official August 2025:

- USP <72> Respiration-Based Microbiological Methods for the Detection of Contamination in Short-Life Products
- USP <73> ATP Bioluminescence-Based Microbiological Methods for the Detection of Contamination in Short-Life Products

History of ATP-Bioluminescence

1963
Emmett Chappelle pioneers ATP-bioluminescence technology at NASA to detect life in space

1970
ATP Assay proposed in Food Monitoring

1978
Lumac produces the first luminometers for measuring ATP-bioluminescence

1990s
First use of ATP test for monitoring water systems

1994
Celsis® International studies recombinant luciferase for scaling to large market testing

1996
Celsis® International purchases Lumac

2000
PDA publishes the first guideline for validating RMMs, PDA TR 33

2006
US and European Pharmacopeia publish their first guideline chapters in support of RMMs

2015
Charles River Labs acquires Celsis International

2025
USP publishes <73>, the first general chapter in support of ATP-bioluminescence for releasing short shelf-life products

Millipore Milliflex® Rapid

Promicol Prenova®

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Regulatory Pathways for Short-Life Products

Previous Approach for Alternative Methods

- ✓ Feasibility/Compatibility
- ✓ Method Suitability
- ✓ Ruggedness/Repeatability
- ✓ Robustness
- ✓ Equivalence
- ✓ Limit of Detection
- ✓ Specificity

Revised Approach per USP <73>

- ✓ Feasibility/Compatibility
- ✓ Method Suitability
- ✓ Incubation Time Study

USP <73> could enable adoption by reducing validation time and costs associated with an extensive validation

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USP General Chapter <73>

Official Date: 01-Aug-2025

"ATP Bioluminescence-Based Microbiological Methods for the Detection of Contamination in Short-Life Products"

- Provides risk-based guidelines for alternative sterility testing with the use of ATP methods for rapid detection of viable microorganisms in short-life products
- Determination of the Incubation Time of the Product based on the slowest growing microorganism
- Compatible for both direct inoculation and membrane filtration methods

USP General Chapter <73>

- Generation Time (G)
  - Slowest-growing microorganism in the presence of product to establish the incubation time of microbial detection.
    - t = time during exponential growth phase.
    - N= the avg CFU at the end of the exponential growth phase
    - N_0=the avg CFU at the start of the exponential growth phase

$$G = \frac{t}{3.3 \, x \, log_{10}(N/N_0)}$$

- Incubation Time (T)
  - Calculated based on the time of detection with ATP-bioluminescence test (t_{ttd}) and the calc. G plus a safety margin.

$$T = t_{ttd} + log_2(10) \, x \, G$$

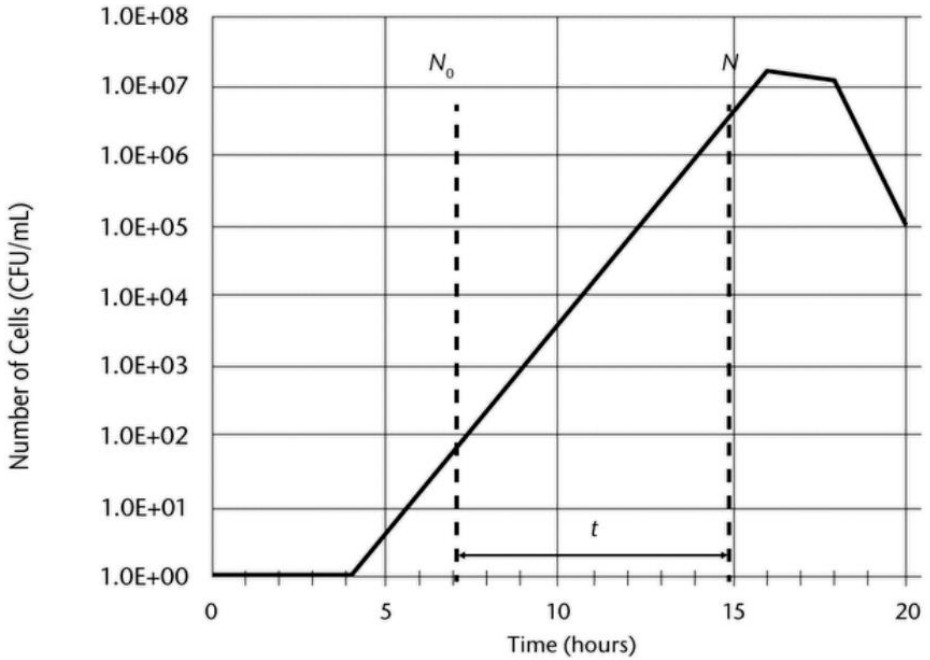

Figure 1. Growth phase curve.

In the figure above, t = 8 h, N_0 = 64 CFU/mL, N = 4.2 × 10^6 CFU/mL, and G would be calculated as 0.5 h.

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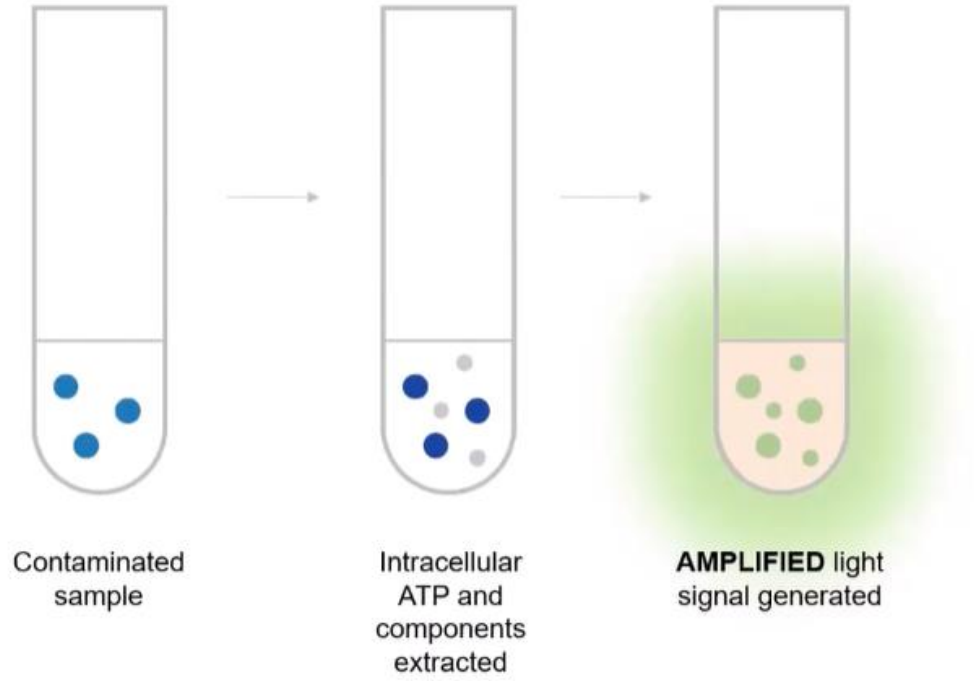
ATP-Bioluminescence Foundation

- Adenosine triphosphate (ATP) is present in all living cells, including bacteria, yeasts, and fungi.
- Standard ATP assays use enzyme luciferase to catalyse microbial ATP and produce light
- Light generated is measured using a luminometer (displayed as Relative Light Units)

- What about products that may contain their own ATP?



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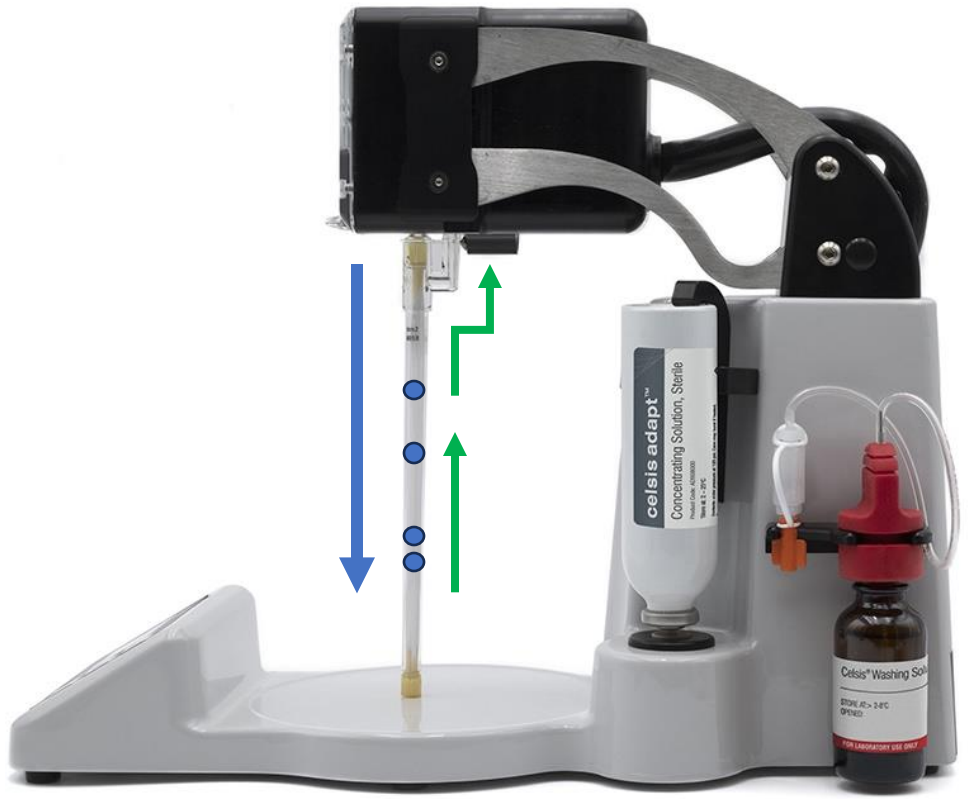
Cell-Based Sample Prep for ATP Testing

1. Prepare sample using compendial TSB/FTM and incubate accordingly
2. Vortex sample and transfer an aliquot to treatment solution to lyse the mammalian cells
3. The Celsis Adapt™ system draws the sample through a sterile, hollow fiber tip - **Physical removal of cellular debris and background ATP**
4. The microbial cells are captured on the hollow fibers
5. Separate concentration port allows sterile, pressurized concentrating solution to be pushed through tip and **removes microbial cells from the tip**
6. The sample is collected, which contains the **microbial cells**

If samples do not contain mammalian cells, this process can be skipped, and testing with ATP-bioluminescence can be completed directly from the incubated samples.



Celsis Adapt™ Instrument

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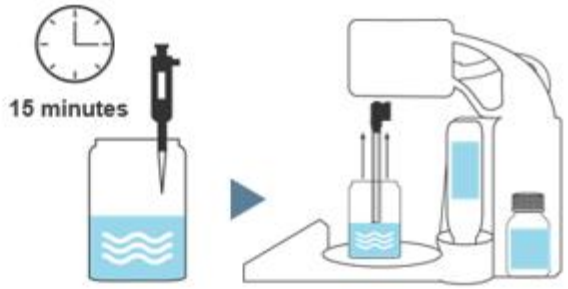
Workflow for Testing Cell-Based Samples

Prepare & Incubate

- Prepare sample using compendial method TSB/FTM media used

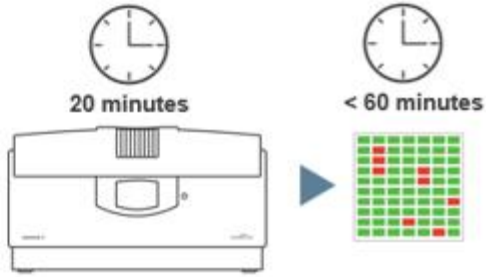


Lyse & Concentrate

- Aseptically remove sample aliquot and add to Treatment Solution
- Allow for a minimum dwell time of 15 minutes
- Concentrate using Celsis Adapt™

Pipette Concentrate + Celsis LuminASE®

- Combine aliquot of concentrate and reagent to sterile assay cuvettes.



Run on Celsis® instrument

- Load samples into Celsis Advance II™
- Allow 20-minute ATP depletion step
- Obtain rapid qualitative results reported in positive or negative

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Applying USP<73>

Using Celsis Adapt™ Cell for ATP-Bioluminescence

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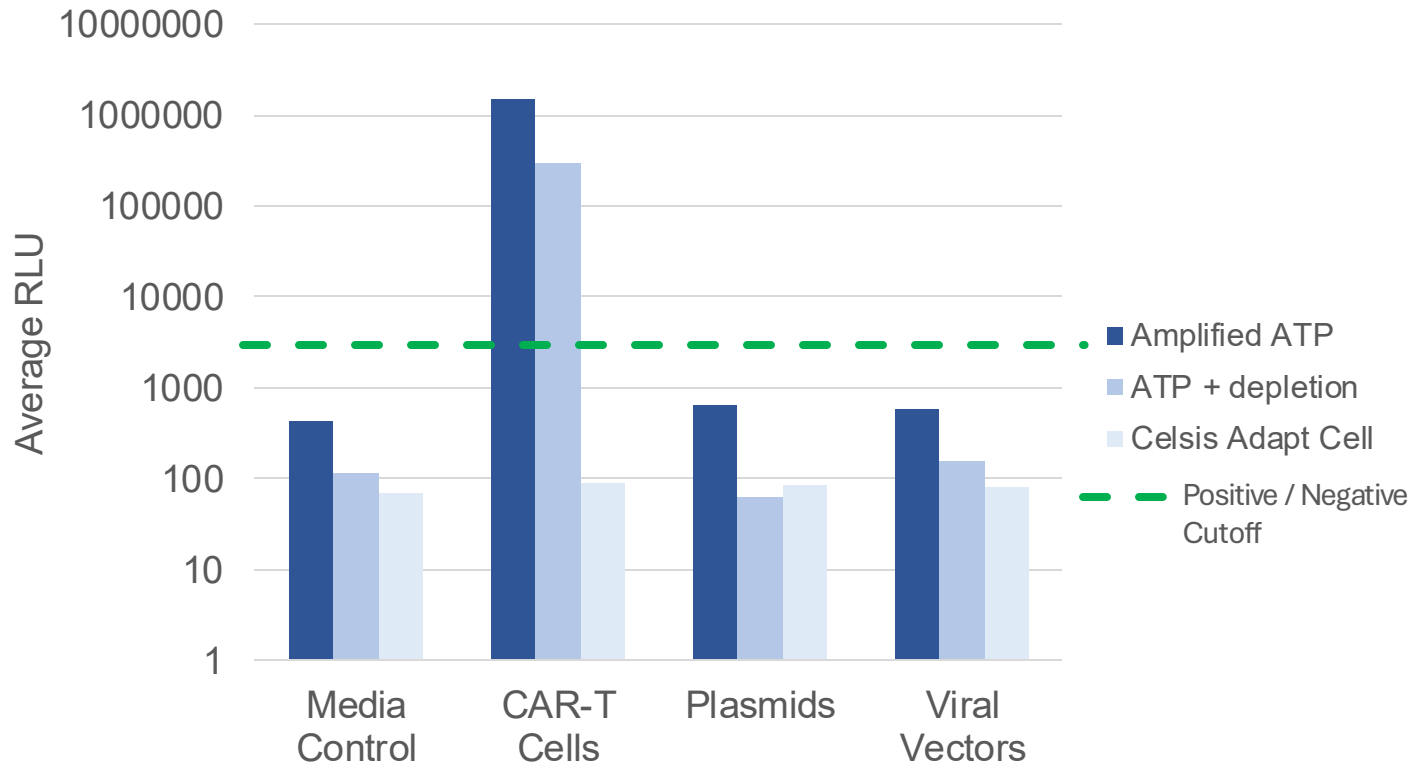
Sample Feasibility / Compatibility

- Are cell-based products compatible with ATP bioluminescence?



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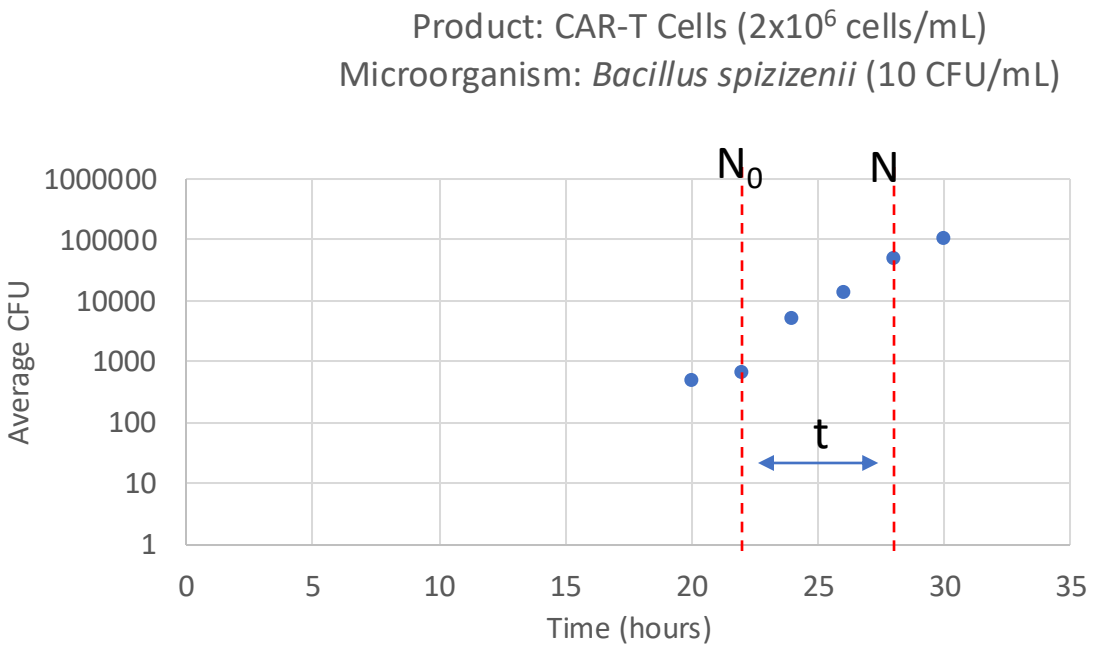
Method Suitability

- Products tested: CAR-T cells, viral vectors, plasmids
- Microorganisms tested in the presence of product as well as without product
  - *S. aureus, P. paraeruginosa, C. sporogenes, C. acnes* – FTM 30-35 °C
  - *B. spizizenii, C. albicans, A. brasiliensis* – TSB 20-25 °C
- Test microorganisms inoculated at not more than 10 CFU
- Test at multiple time points to determine exponential growth
- Calculate the generation time for the slowest growing microorganism and the incubation time for the products

Example of growth curve calculations - CFU



1. Identify the exponential phase
   - N= 52,200
   - N_0= 675
   - t= 6
2. Use the above variables to calculate the Generation Time (G):

$$G = \left(\frac{t}{3.3\ x\ log_{10}(N/N_0)}\right)$$

G = 0.96 hours

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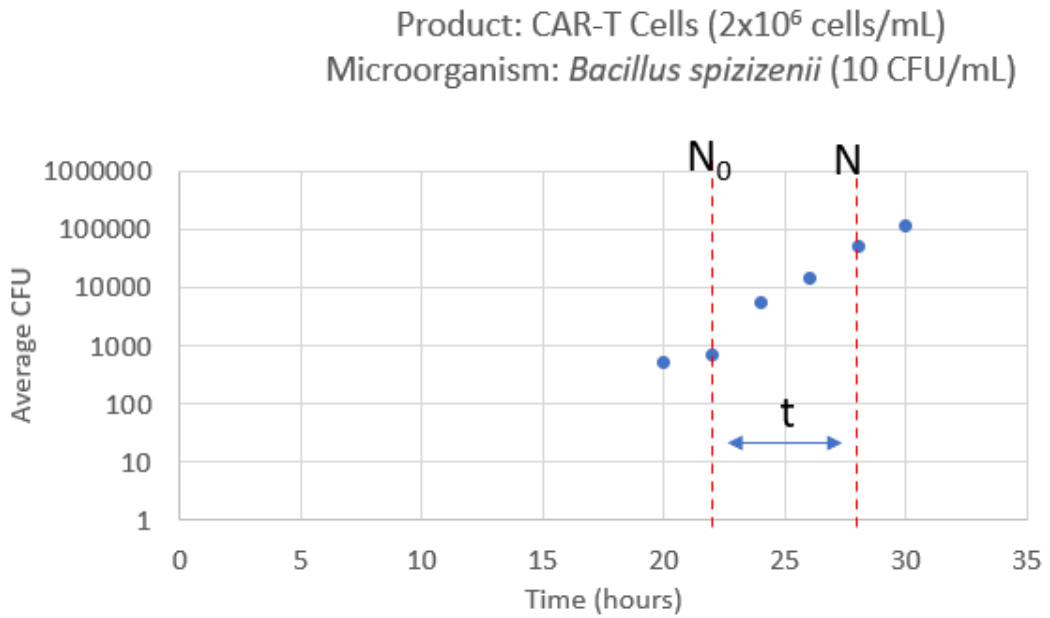
Example of growth curve calculations – CFU continued



1. Calculated G from previous slide

   G= 0.96 hours

2. Determine first timepoint in which rapid method detected the microorganism.

   t_{ttd} = 24 hours

3. Use the above variables to calculate Incubation Time (T)

$$T = t_{ttd} + log_2(10) \times G$$

T=27.2 hours / 1.13 days

Conclusion: The calculated Incubation Time is less than 2 days.

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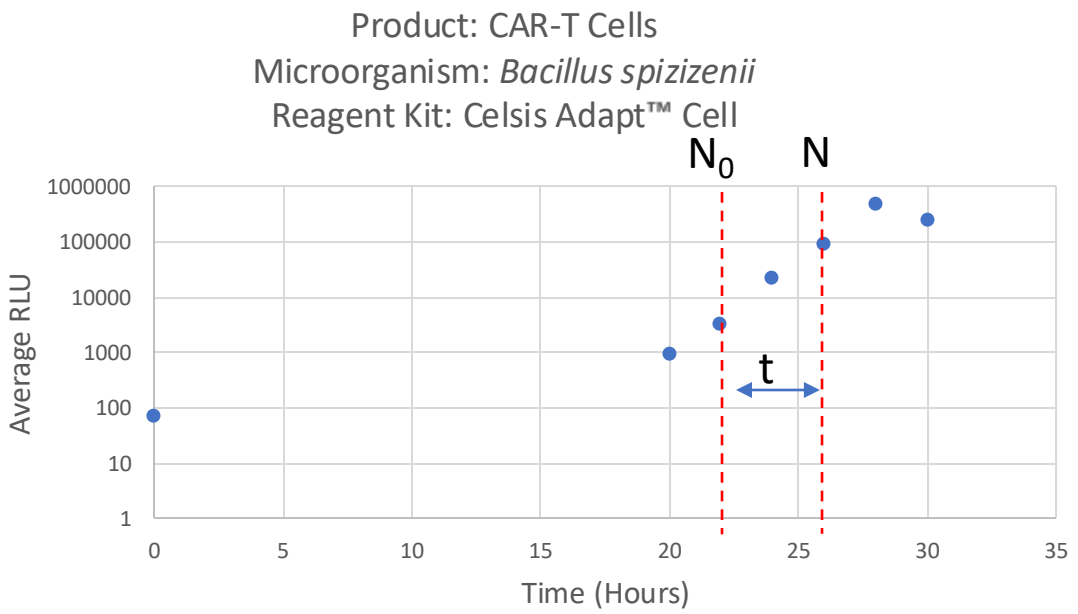
Growth Curve Calculations - RLU



1. Calculations based on RLU:
   - N = 491,968
   - N_0 = 3,277
   - t = 6
2. Generation Time (G) = 0.84 hours
3. Time point of ATP detection (t_{ttd})= 24
4. Incubation Time T = 26.8 hours / 1.12 days

Conclusion: Incubation Time based on CFU = **27.2 hours / 1.13 days**
Incubation Time based on RLU = **26.8 hours / 1.12 days**

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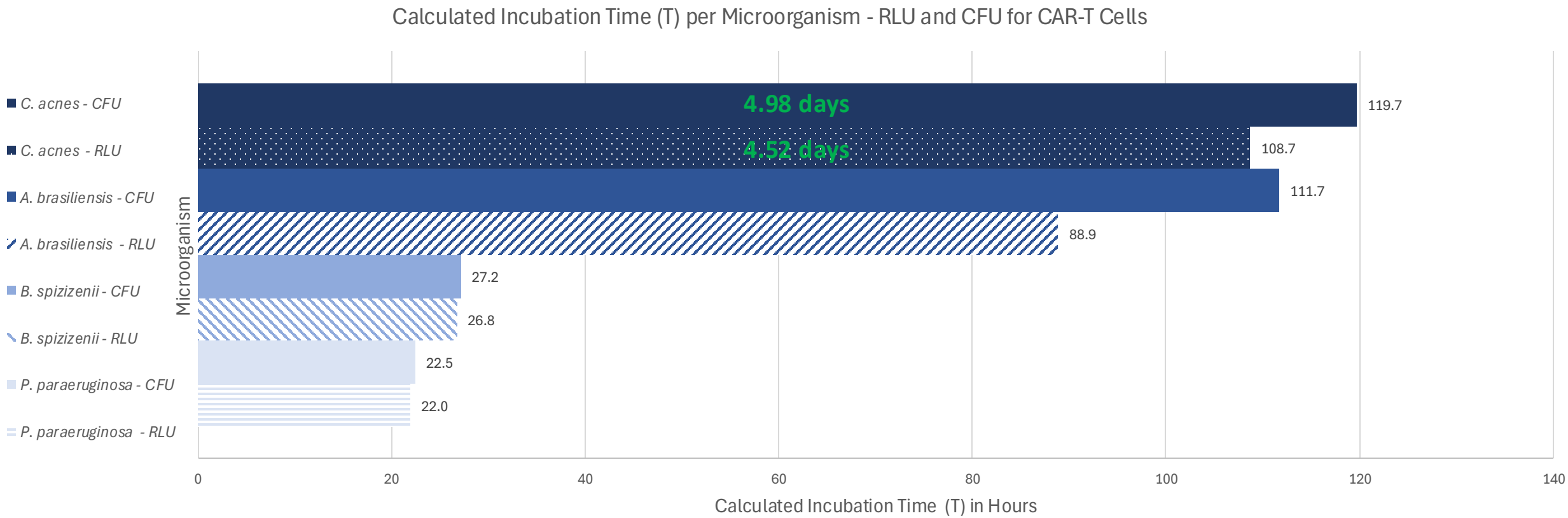
Product Incubation Time Summary



Slowest growing microorganism – *C. acnes* Calc. Incubation Time for CAR-T product = <5 days

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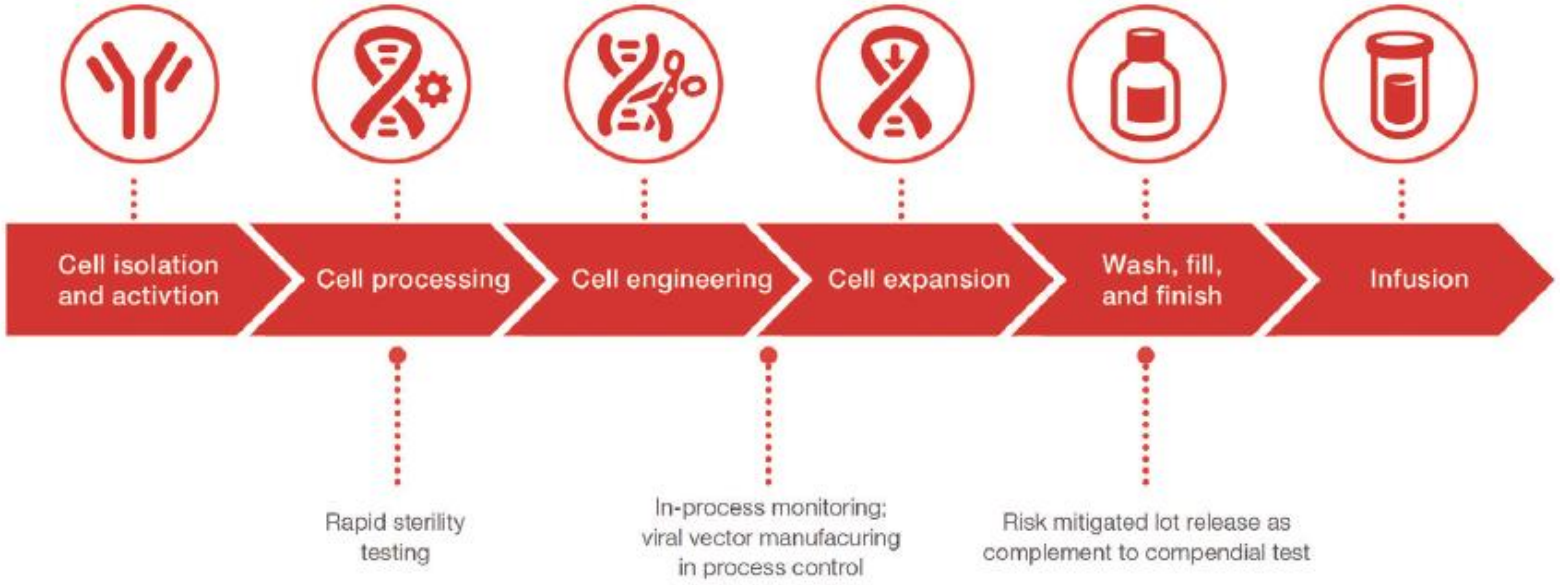
Compendial sterility test = Day 14, Celsis sterility test = Day 7, USP<73> = Day 5

CAR-T Production Workflow



www.cellgenetherapyreview.com

Observations

- Potential challenges with strict anaerobic cultures
  - When not using continuous monitoring
  - Slower growth observed
- Localized colony growth of molds in enrichment broth
- Challenges with achieving less than 10 CFU for inoculum
- Need to consider 8-hour shift and data collection requirements for robust growth curves
- Delayed results when needing to wait for colony growth versus collecting real time data with the rapid method

Conclusions

- USP <73> provides a clear compendial pathway for the use of ATP-Bioluminescence for rapid sterility testing

- New general chapters will pave the way for implementation of rapid detection methods for sterile manufacturing

Contact Information

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