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Identification and Qualification of CQAs for Live Biotherapeutic Products

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Abstract

The characterization of Live Biotherapeutic Products (LBPs) includes the examination of novel attributes outside of classical protein-based biotherapeutic properties. The FDA has set regulatory guidance for the evaluation of LBPs. Identification of novel attributes and the qualification of the release assays are critical to moving products into regulatory compliance. This presentation will discuss the development and qualification of assays used to dose LBPs.

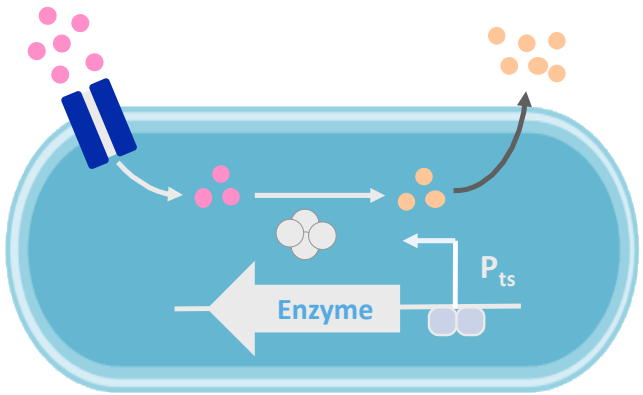
Overview

I. Synlogic Therapeutics
II. Live Biotherapeutic Product Attributes
    I. FDA requirements
    II. Analytical Methods for LBP Characterization and Release
III. Release Assay Qualification and Validation
    I. Live Cell Assay
    II. Qualification
    III. Results
    IV. Plan for Validation
IV. Lessons Learned
V. Questions
VI. References

Advancing a New Paradigm of Biotherapeutics

Synthetic Biotics

Programable, precision **genetic engineering** + Well-characterized **probiotic chassis**



Convert disease-causing targets to harmless metabolites

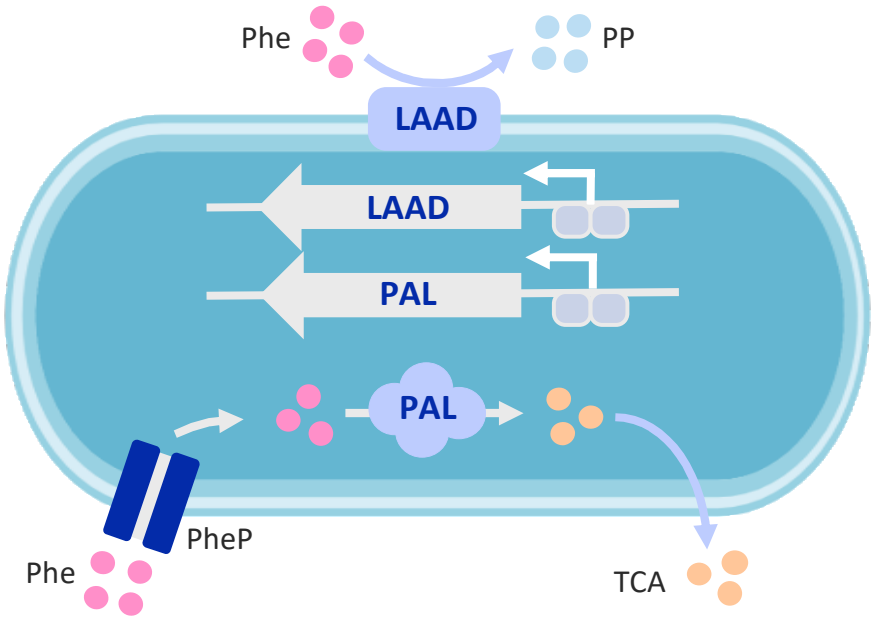
Differentiated Drug Candidates

- **Targets validated biology** in metabolic and immunological diseases
- **Safe** *chassis*, with >100 years of human experience, avoids systemic absorption
- **Convenient** oral delivery
- **Reversible** via rapid GI clearance
- Capable of addressing **rare and common** diseases

GI = gastrointestinal

PKU Strain

- Phenylketonuria (PKU) is a rare metabolic disease in which the body cannot breakdown Phenylalanine.
- Our PKU products have enzymes to metabolize Phe both on the cell membrane and inside the cell, as well as a cross-membrane transporter to increase Phe uptake.



PKU strain engineered to metabolize Phe.

Live Biotherapeutic Products

Guidance

FDA definition of a Live Biotherapeutic Product: “An LBP… is a biological product that: 1) contains live organisms, such as bacteria; 2) is applicable to the prevention, treatment, or cure of a disease or condition of human beings; and 3) is not a vaccine.” (Early Clinical Trials with LPBs, FDA)

The 5 Critical Attributes the FDA requires of LBPs:

- Safety
- Identity
- Strength
- Purity
- Quality

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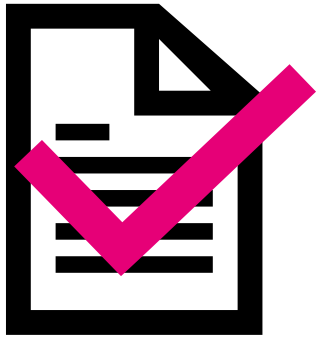
Live Biotherapeutic Products

What assays can be used to meet the attributes requested by the FDA in LBPs?

Safety	Identity	Strength	Purity	Quality
Endotoxin	Whole Genome Sequencing	CFU	Microbial enumeration	Genetic Stability
Water Activity	qPCR	Live Cell	Absence of Specified Organisms	pH
Antibiotic Sensitivity	Biological Transformation	Viability	Elemental Analysis	Appearance
		Activity		Water Content

Which methods need Qualification/Validation?

USP <1225>



USP Methods

Established and accepted by FDA

Only need feasibility work for qualification

- Microbial Enumeration USP <61>
- Absence of Specified Organisms USP <62>
- Elemental Analysis USP <232/233>
- Bacterial Endotoxin USP <85>
- Water Activity USP <922>
- pH Determination USP <791>

In-house methods

Potentially novel to the product

Needs development for qualification

- qPCR for Individual Strain
- Novel Technology
- CFU or Cell Counting
- Biological Transformations

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Live Cell Assay for Release

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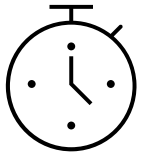
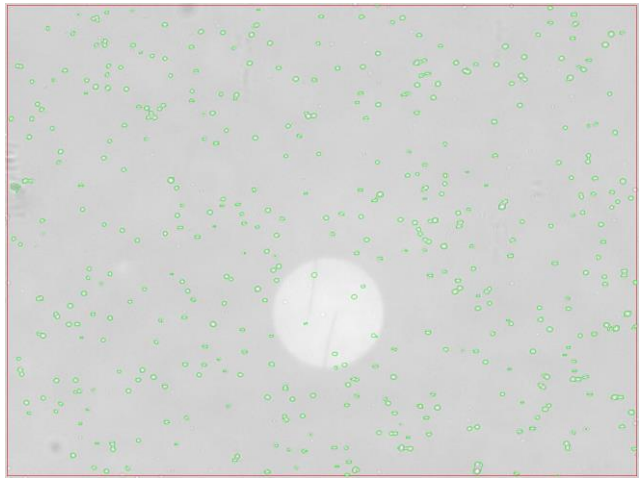
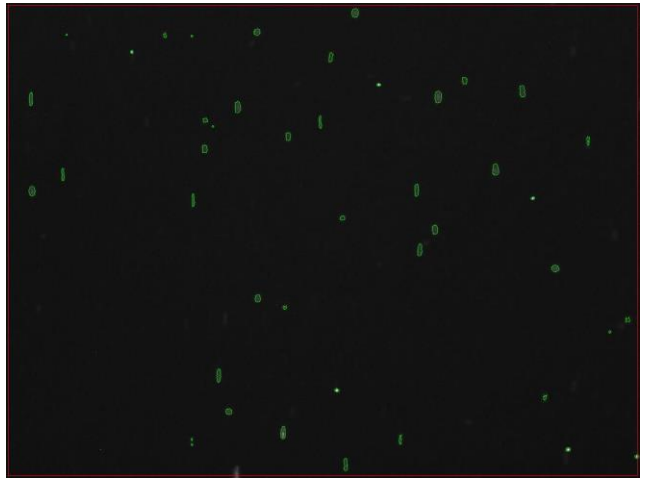
Live Cell Assay Method Overview





Bright Field



Fluorescent
(EX: 470 nm, EM: 535 nM)

Plan for Qualification

Design of Experiment (DoE)

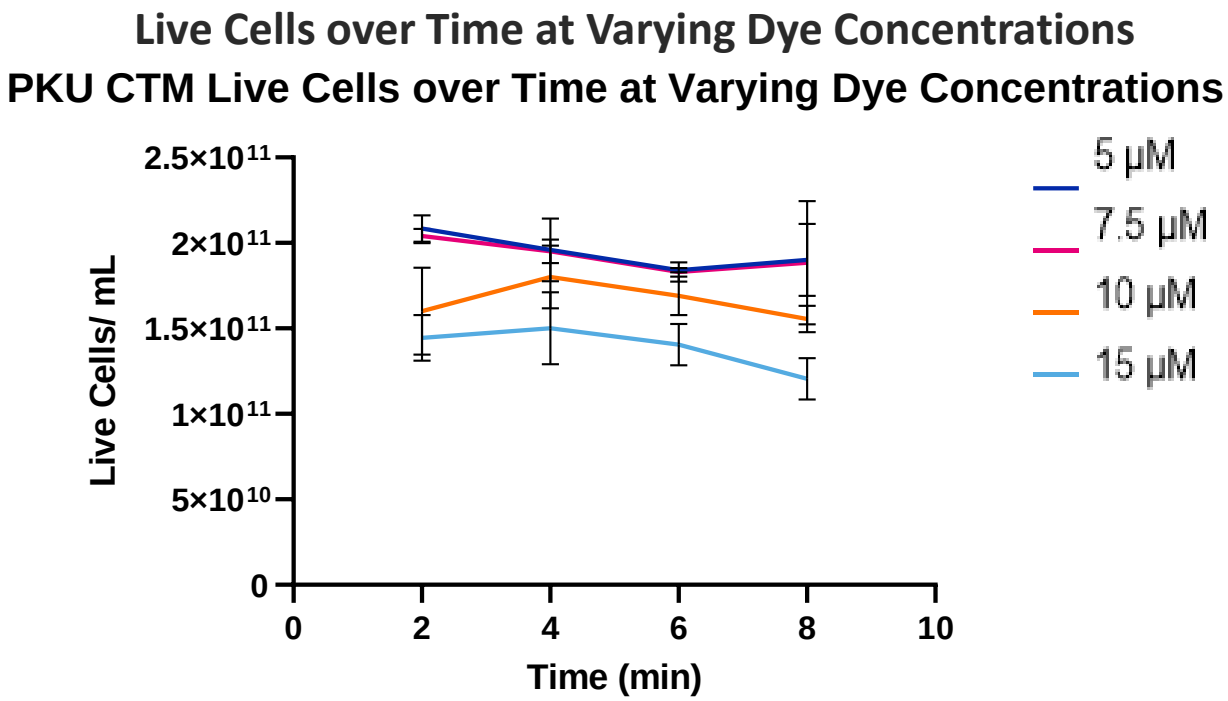
Sample Number	Replicates	Analysts	Equipment	Material Lots	Assay Parameters	Instrument Parameters

Outputs

Precision	Accuracy	Linearity	Limit of Detection	Limit of Quantification	Instrument Range of Accuracy

Viability Staining Over Time

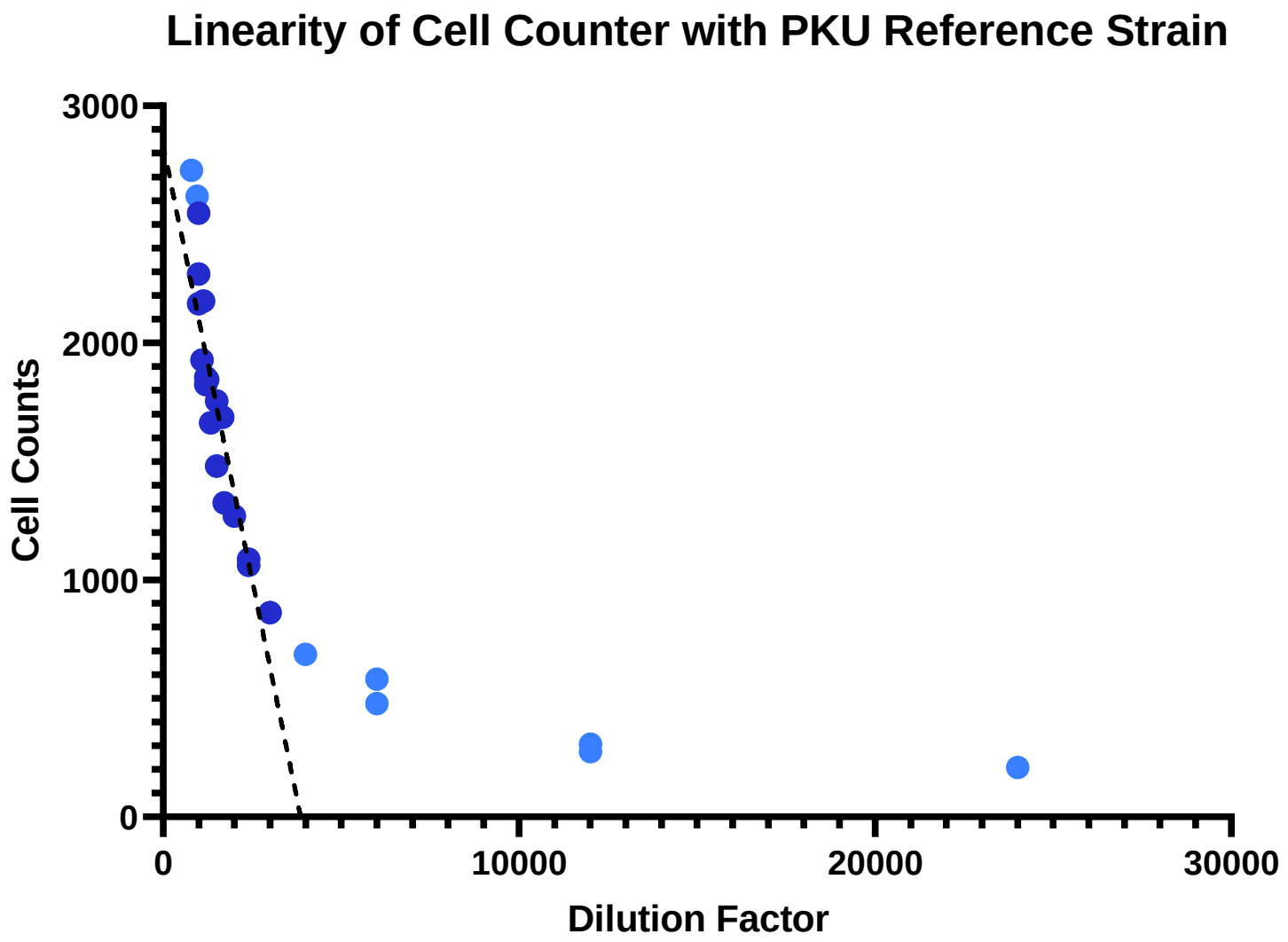
Staining Time and Concentration



Ideal Conditions for Strain:
Min Stain Time: 2 min
Final Concentration: 7.5 µM

Defining Counting Range on Cellometer X2

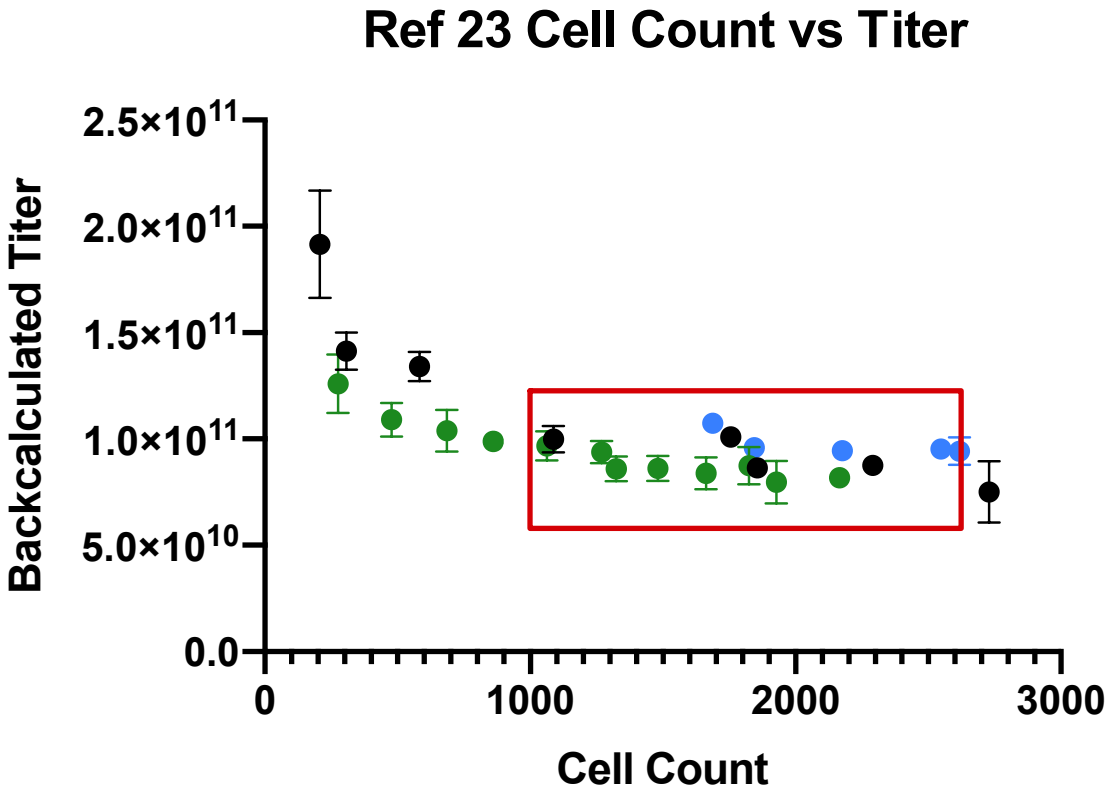
Linear Range

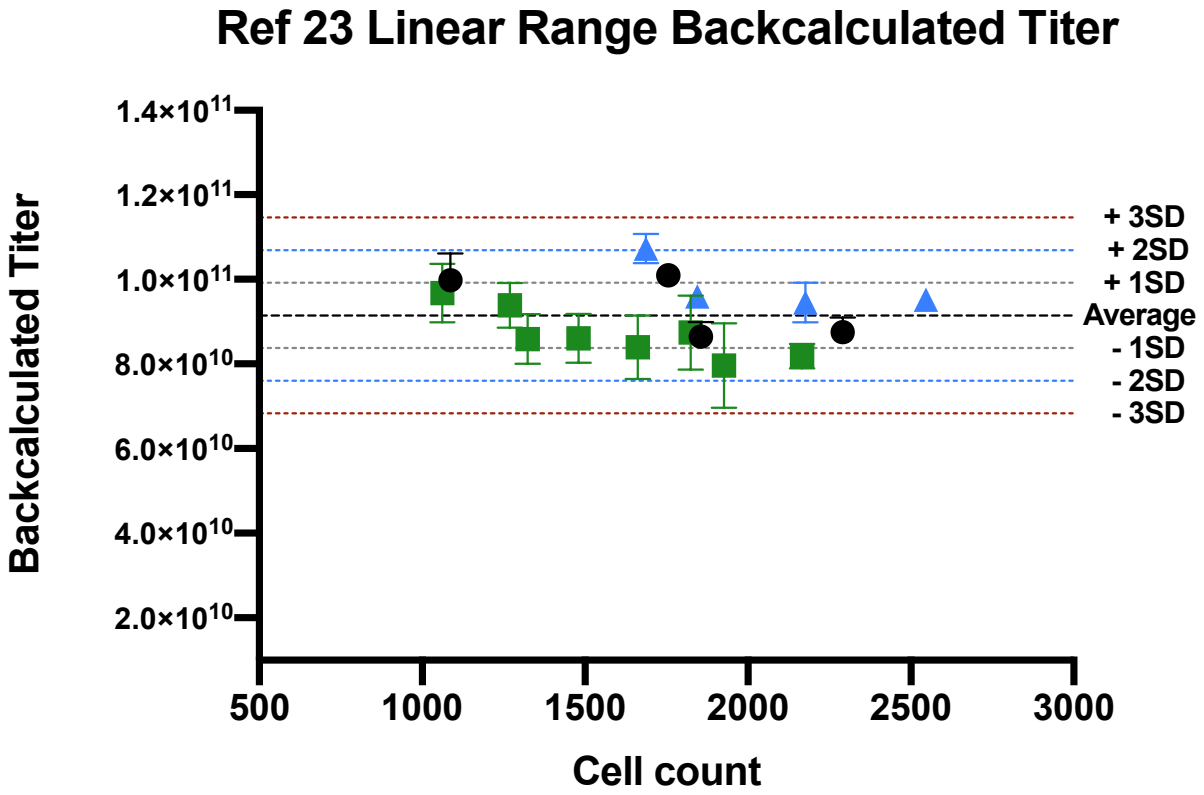


Results for a 1000-2600 range:
R^2=0.87, and CV=9.9%

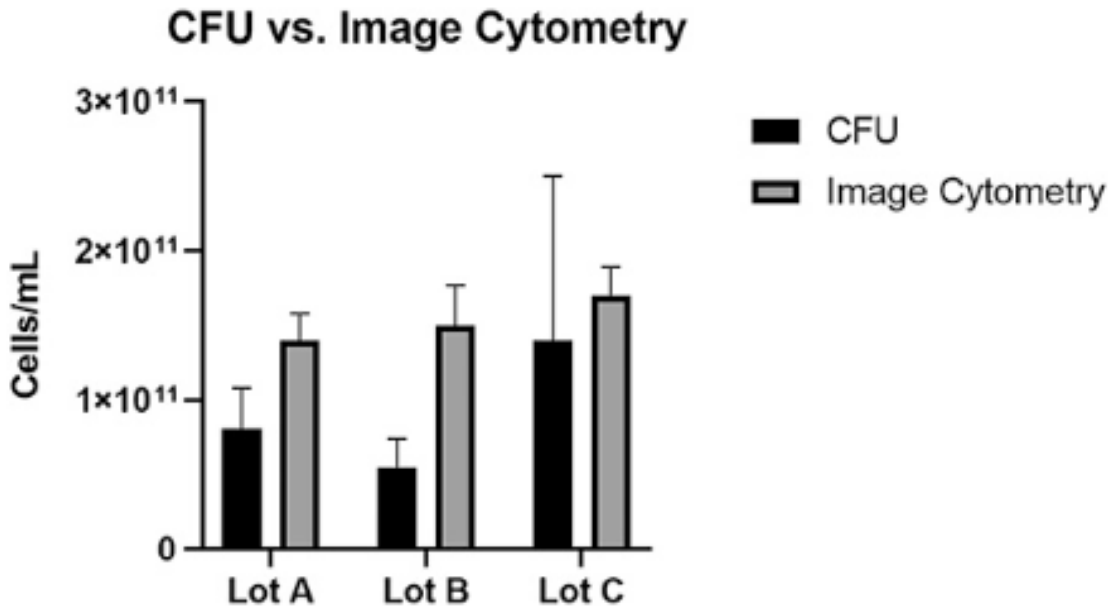
Cellometer Linear Range

Linear Range 1000-2600	
n	48
Average	9.2E+10
Stdev	7.7E+09
%CV	8.4%





Higher precision and accuracy than CFU method



(*Rapid Cell Counting and Viability Detection Method of Escherichia Coli NISSLE Using Image Cytometry.,* Perry, 2021.)

Method Comparison

CFU	Image Cytometry
• ~24 hrs • >30% CV • Culturable • Doesn't correlate to biomarker	• 15 minutes • <20% CV • VNBCs • Correlation to biomarker

Qualification Results

Parameters	**Acceptance Criteria**	**Results**
Precision	Precision: CV ≤25%	CV: <10.2%
Linearity	$R^2 \geq 0.98$	R2: ≥0.995
Limit of Detection	Lowest concentration of cells counted	1×10^6 live cells/mL
Limit of Quantitation	One dilution up from LOD	1×10^7 live cells/mL
Instrument Range of Accuracy	Samples must be diluted into this range for analysis	1×10^7 to 1×10^8 live cells/mL
Accuracy	Accuracy of the reference standard must fall within ± 25% of the expected cell count of 9.4×10^{10}	Accuracy: 17%

Plan for Validation

Validation is necessary before commercialization of the product

Master Plan:

- USP methods are validated for use upon feasibility studies with the product
- Validation Protocol for developed methods
  - Same tests as previously shown for linearity, precision, etc but target criteria now based off qualification results
  - Define number of replicates, lots, analysts, instruments in plan to reach statistically significant results
- Report to summarize that results meet criteria set forth

Lessons Learned

Live Cell Assay:	Instrument total range of detection is not the precise analysis range selected
	Stain time control needed
	Higher live cell count than CFU due to VBNCs, correlates to biomarker
	Better precision than CFU or OD
Platform Learnings:	Micro assays can be more variable than chemical assays
	New and emerging technology may be used for qualified and validated release assays
	Phase-based approach to analytical development was appropriate for our size and cost-needs
	Worked with the FDA guidance to find the best acceptance criteria for micro assays to release and characterize LBPs, then used our qualification data to lay the plan for validation acceptance criteria

Acknowledgements

- Synlogic:
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- PEGS Boston
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Questions?

If there are further questions,
please reach out to me at
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References

- Perry, Michele, McDonald, M., Lund, A., *et al.* "Rapid Cell Counting and Viability Detection Method of Escherichia Coli NISSLE Using Image Cytometry." *Journal of Microbiological Methods*, vol. 192, 2022, p. 106381., https://doi.org/10.1016/j.mimet.2021.106381.
- Isabella, V., Ha, B., Castillo, M. *et al.* "Development of a synthetic live bacterial therapeutic for the human metabolic disease phenylketonuria." *Nat Biotechnol* 36, 857–864 (2018). https://doi.org/10.1038/nbt.4222

