Production Assistance for Cellular Therapies

Educational Web Seminar

Early Phase Cell Therapy Product Development
-Potency Assays

Tuesday, 16 January 2018

12:00 noon – 1:00 PM ET

Speakers

Emily Hopewell, PhD
Assistant Technical Director, Cell Therapy Facility
Moffitt Cancer Center

Cheryl Cox, MT, ASCP
Manager, Experimental Therapies
Moffitt Cancer Center

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Unless otherwise noted, individuals did not indicate any relevant affiliations or financial interests

Faculty	Disclosure	Role	Name of Company
Emily Hopewell	None	Assistant Technical Director, Cell Therapy Facility, Moffitt Cancer Center	None
Cheryl Cox	None	Manager, Experimental Therapies Moffitt Cancer Center	None
Linda Kelley	None	Director, cGMP Cell Therapy Facility, Moffitt Caner Center	None
Debbie Wood	None	Project Director, The Emmes Corporation	None
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David McKenna	None	Medical Director, Molecular and Cellular Therapeutics, UMN	None
Aisha Khan	None	Executive Director of Laboratory Operation, University of Miami	None
Joseph Gold	None	Manufacturing Director, Center for Biomedicine and Genetics, City of Hope	None
Jodi Brenden Amir	None	Education Consultant, Office of Continuing Professional Development, UMN	None
Dasha Dobrinina	None	Education Coordinator, Office of Continuing Professional Development, UMN	None

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Each attendee must:

- Complete the online attendee roster w/in 72 hrs. of the web seminar
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Early Phase Cell Therapy Product Development:
Potency Assays
Introduction

Linda L. Kelley, PhD
Senior Member
Director, Cell Therapy Facility

What is Potency?

"The specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result."

2011 FDA Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products

FDA Requirements for Potency Assays

A validated potency assay with pre-defined acceptance/rejection criteria is required in the Biologics License Application (BLA) and must:

- Measure a relevant biological activity (mechanism of action) of the product
- Use appropriate reference standards and/or controls
- Be quantitative
- Establish accuracy, precision, specificity and range of test methods

2011 FDA Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products

21st Century Cures Act

The 21st Century Cures Act is a United States law enacted by the 114th United States Congress in December 2016. It authorized $6.3 billion in funding, mostly for the National Institutes of Health.

FDA drug approval process

The 21st Century Cures Act modified the FDA drug approval process by mandating new rules that direct the FDA to approve drugs and devices with greater urgency.

- RMAT (Regenerative Medicine Advanced Therapy)
- Fast Track
- Breakthrough Therapy
- Priority Review
- Accelerated Approval

When to Apply Potency Assays?

- Product characterization
- Comparability testing
- Assessing manufacturing changes
- Stability studies
- Lot release testing

Webinar Objectives

1. Acquire knowledge of the regulatory expectations for potency assay rigor in early or late phase clinical trials.
2. Observe relevant examples of potency assay application for a representative cell therapy product.
3. Determine when and what test methods to apply during cell therapy product characterization and validation.

Regulatory Publications

Source	Title
USP <1030>	Biological Assays Chapters-Overview and Glossary
USP <111>	Design and Analysis of Biological Assays
USP <1032>	Design and Development of Biological Assays
USP <1033>	Biological Assay Validation
USP <1034>	Analysis of Biological Assays
FDA Guidance for Industry	Potency Tests for Cellular and Gene Therapy Products

Early Phase Cell Therapy Product Development:
Potency Assays
Examples of Application

Emily Hopewell, PhD, MT
Assistant Technical Director,
Cell Therapy Facility

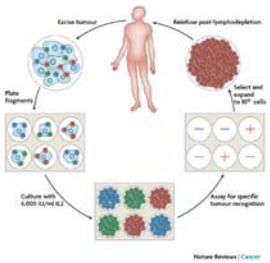
Potency assays in early phase cell therapy product development

Investigational New Drug (IND) Application

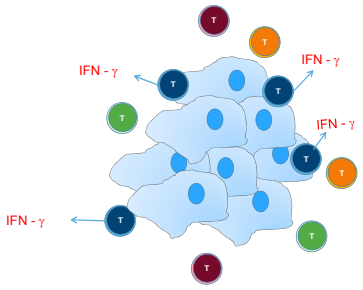
"Submit data to assure the identity, quality, purity, strength, stability of products used during all phases of clinical study."

- In early phase clinical investigations, it may not be possible to meet all of the requirements for licensed biological products
- The amount of information required will vary with the phase, duration and dosage of the investigation
- Products in pre-clinical, Phase 1 and early Phase 2 studies with limited quantitative information on relevant biological attributes may be sufficient
- Potency assays for early clinical studies are likely to have wider acceptance ranges than assays used in later phase investigations
- Develop and implement potency measurement(s) that quantitatively assess relevant biological product attribute(s) where and when possible
- Implore an incremental approach to the implementation of potency tests

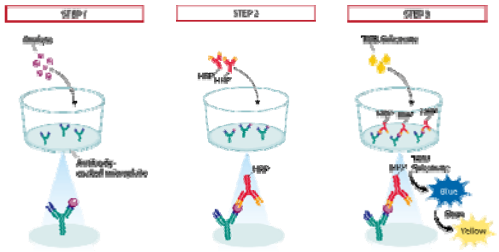
Manufacturing Protocol for Tumor Infiltrating Lymphocytes (TIL)



Mechanism of Action

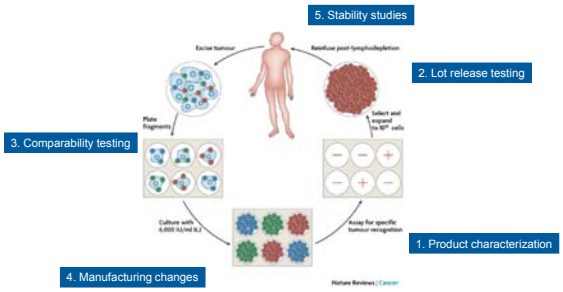


Enzyme-linked Immunosorbent Assay

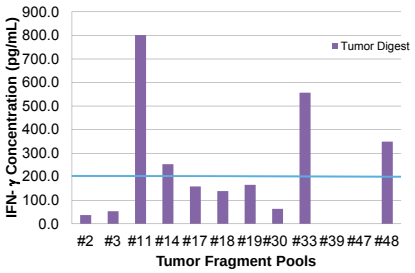


https://www.rndsystems.com/products/quantikine-colorimetric-sandwich-elisa-assay-principle

Examples of potency assay application

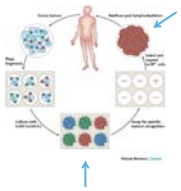


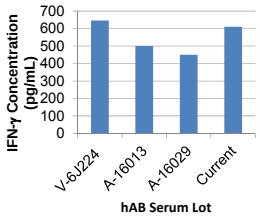
Product Characterization



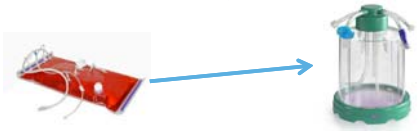
Comparability Testing

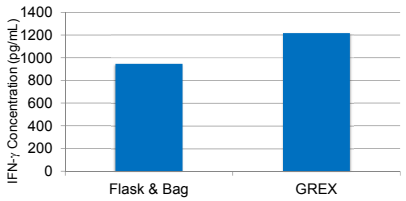
Lot-to-lot testing of hAB serum





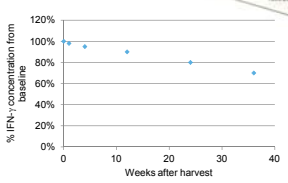
Manufacturing changes



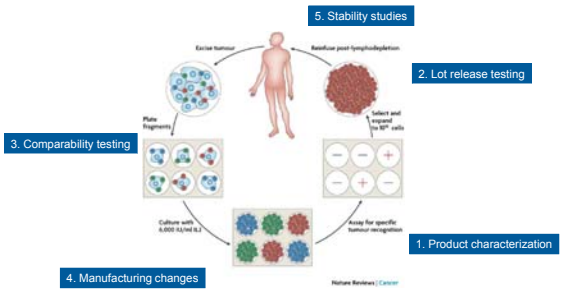


1996 FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-derived Products

Stability Studies



Summary



Early Phase Cell Therapy Product Development:

Potency Assays

Validation

Cheryl Cox, MT
Manager, Experimental Cell Therapies

The Validation Plan

Effectively establish the performance characteristics of the procedure.

- Number & types of samples to be studied
- Study design
- Acceptance criteria for each parameter
- Data & statistical analysis plan

The Validation Plan
Study Design

Effectively establish the performance characteristics of the procedure.

- ✓ Accuracy
- ✓ Precision
- ✓ Range
- ✓ Specificity
- ❖ Sensitivity
- ❖ Robustness

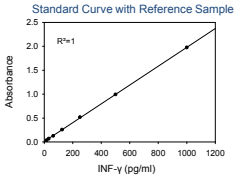
Accuracy

The degree of agreement between the measured (unknown) and actual (known)value.

- Dilutional linearity study: Construction of target concentrations by dilution of a standard reference material or a known test sample. A minimum of 3 dilutions is necessary but 5 are recommended.

INF-γ Potency Assay Accuracy

Standard Curve with Reference Sample



INF-γ Potency Assay Accuracy

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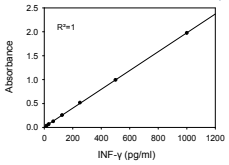

Table 1. INF-γ Positive Control [800 pg/ml] Dilutional Linearity

pg/ml	Run 1	Run 1	Run 1	Run 2	Run 2	Run 2	Mean	S.D.
800	858.2	935.8	858.9	944.0	943.9	954.0	915.8	44.7
400	361.8	290.6	327.2	426.8	449.6	460.7	386.1	69.9
200	184.3	199.5	156.3	181.6	216.9	194.3	188.8	20.3
100	92.1	87.2	90.9	91.3	88.4	92.5	90.4	2.1
50	56.0	48.1	47.6	56.0	48.1	47.6	50.6	4.2

INF-γ Potency Assay Accuracy

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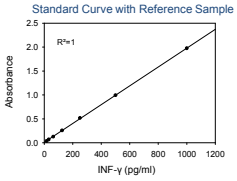

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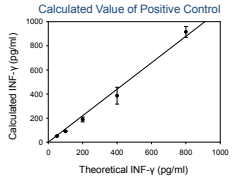
100	92.1	87.2	90.9	91.3	88.4	92.5	90.4	2.1
50	56.0	48.1	47.6	56.0	48.1	47.6	50.6	4.2

Calculated Value of Positive Control



INF-γ Potency Assay Accuracy

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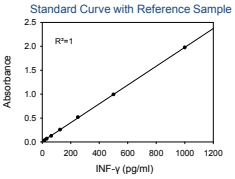

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Calculated Value of Positive Control

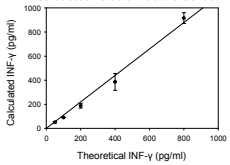


Table 2. % Recovery= ([calculated value/theoretical value] x 100)			
Theoretical Value	Average Calculated Value	Difference	% Recovery
800	915.8	+115.8	115
400	386.12	-13.88	97
200	188.82	-11.2	94
100	90.07	- 9.93	90
50	50.57	+0.57	101
Average			99

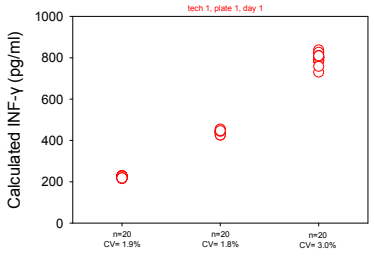
Expected Range= 80-120% Recovery PASS!

Precision

The degree to which repeated measurements show the same results under defined conditions (reproducibility and repeatability).

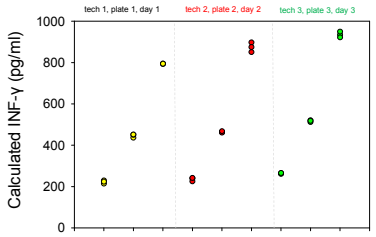
- Intra-run precision: To test for variations introduced by the analyst, reagents or instrument. Performed by a single analyst on the same day using the same samples, reagents, standards and instrument.
- Inter-run precision: To test for variations introduced by multiple analysts, different sources or lots of reagents or multiple instruments. Performed at at different times to assess comparability of analysts, reagents and/or instruments.

INF-γ Potency Assay Precision: Intra-run



Expected Range= Standard Deviation less than 5 % Mean PASS!

INF-γ Potency Assay Precision: Inter-run



Expected Range= Standard Deviation less than 10 % Mean PASS!

Range

The assay values for which it has been determined have suitable accuracy and precision in the analytical procedure.

- Dilutional linearity study: To minimally include the product specification range; to optimally include a broader range to be used for stability studies or to allow for hypo or hyper concentrations of samples.

Specificity

The degree to which only the true component is measured rather than a mistaken component (i.e., avoid false positives).

- To determine the lack of interference from components in the assay reagents or in the sample itself. Assessed by parallel dilution of the standard with and without the potentially interfering component.

Robustness

The degree to which the assay values remain unaffected by small but deliberate variations in method parameters.

- To determine the effect of pH, temperature, plate manufacturer, instruments, etc.

Lot Release Testing

TEST	Method	SPECIFICATIONS	RESULTS	Acceptable (Circle One)	Tech
Gross contamination	Gram stain	No organisms seen (NOS)		Yes No N/A	
Sterility	Sterility culture	No growth		Yes No N/A	
Endotoxin	Endosafe	<5 EU/kg		Yes No N/A	
Mycoplasma contamination	PCR or qPCR	Negative		Yes No N/A	
Viability	Dye Uptake	> 70% viable cells		Yes No N/A	
Cell Count	Dye Uptake	0.1 – 2 E 11 cells		Yes No N/A	
Interferon gamma production	ELISA	>200 pg/mL		Yes No N/A	

Thank You!

Early Phase Cell Therapy Product Development
-Potency Assays-

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