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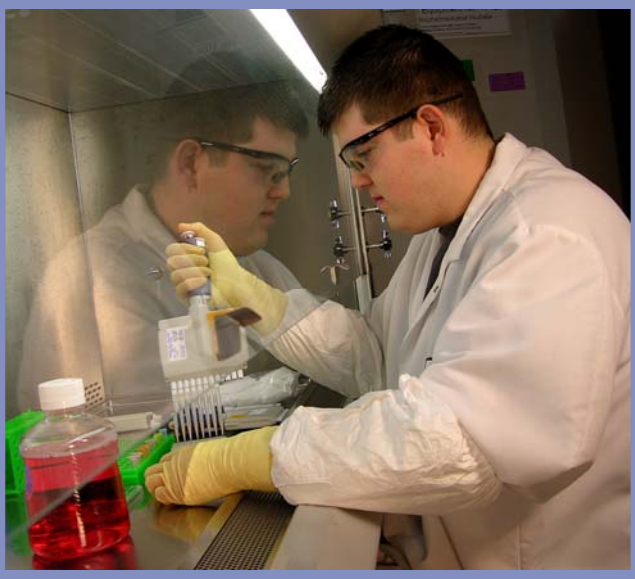
INTRODUCTION

A cell based potency assay measures the physiological response elicited by a given product. It is often the preferred format for determining the activity of biological products and is commonly employed for lot release as well as stability testing. Transfer of a cell based potency assay between laboratories can pose significant challenges due to the complexity of the assay. For a marketed product, method transfer is under direct scrutiny by regulatory agencies and therefore is an even more significant undertaking. This poster presents a case study to demonstrate how a cell-based potency assay can be successfully transferred to a third party contract testing laboratory.

BACKGROUND

A Request for Proposal was sent to multiple contract testing laboratories in order to identify a laboratory capable of executing the method on a routine basis. Eurofins Lancaster Laboratories, Inc. was selected as the receiving unit (RU) for the method transfer and for routine testing of release and stability samples.



SUCCESSFUL TRANSFER OF A CELL BASED POTENCY ASSAY FOR A BIOLOGICAL PRODUCT

DISCUSSION/RESULTS

Methodology 1

- Samples are extracted/diluted in phosphate buffer saline
- Cells are prepared in serum-free medium and added to assay plates containing serial dilutions of Reference standard, Control and extracted samples.
- Following incubation and staining, the response is measured by a colorimetric method.
- To obtain one potency result:
  - One weighing from each of six drug product (DP) samples is diluted
  - Two dilutions are tested in each run; a total of three runs must be performed
  - Each dilution is tested in triplicate within one run
  - The Log10Titer is calculated as the average of the triplicate results
  - The final reportable result is calculated as the average of the six DP samples
- Potency is calculated and Parallelism is tested using custom, validated Softmax ® Pro template

Assay Flow 2

- To generate one reportable result, six DP units must be tested.
- A maximum of 2 DP units may be tested in a single run. Therefore, a minimum of 3 runs must be performed to generate one reportable result.
- Weigh and extract an aliquot from each DP unit.
- Load each sample extract, one control, and 2 reference standards in Column 1 on 3 different assay plates.
- Serially dilute samples, control, and standard on each assay plate.
- Add cells and incubate.
- Stain cells and measure absorbance.

Method Transfer Logistics 3

- Qualified critical reagents were provided by the client through Sending Unit (SU)
- Data Analysis software template was provided to and subsequently validated at the Receiving Unit (RU)
- Method familiarity runs were performed at RU to confirm ability to execute
- Protocol driven method transfer involved testing the same set of multiple lots by SU and RU
- Regulatory submission and approval

Method Establishment at ELLI (SU) 4

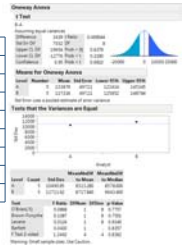
- Two trained analysts performed practice runs
- Method accuracy, linearity and range confirmed using mock samples prepared from Reference standard
- Assay performance was assessed based on system suitability criteria and control titers
- Actual sample lot was extracted, tested and compared to results generated at SU

Design of Method Transfer 5

- Demonstrate comparability of method performance between SU and RU
  - Total of 5 sample lots tested by both labs
- Demonstrate precision within RU
  - One sample lot tested 6 times (6 reportable results from a total of 36 individual sample units)
  - Each reportable result derived from individual intermediate results by two different analysts
- Appropriate statistical analysis

Comparability by different RU analysts 6

	Control Titer (IU/mL)	
	Analyst A	Analyst B
Run 1	119,905	125,306
Run 2	143,626	136,921
Run 3	132,052	127,933
Run 4	144,916	154,485
Run5	128,898	141,946
Mean	133,879	137,318
RSD	7.8%	8.5%



Two analysts at ELLI (RU) obtained comparable Control titer results well within spec (103,000 – 159,000 IU/mL)

Results for 1 lot x 6 Reportable Values 7

6 reportable values (IU/mg) from each lab	
SU	ELLI (RU)
860,000	900,000
910,000	880,000
940,000	880,000
970,000	900,000
990,000	900,000
940,000	930,000
Mean of 6 = 935,000	Mean of 6 = 898,000
RSD = 4.9%	RSD = 2.0%
Passed 2-sided, 2-sample t-test: Prob > |t| = 0.0995 Zero is within the 95% C.I. (ELLI-SU: LL = -81661; UL= 8327) Passed equal variance test F-test 2-sided P=0.0654	

Results for 5 lot x 1 Reportable Values 8

Lot	Titer by SU (IU/mg)	Titer by ELLI (IU/mg)	Δ(IU/mg) (SU-ELLI)	% Difference (|SU-ELLI|/SU)
A	930,000	900,000	+30,000	3.2%
B	930,000	920,000	+10,000	1.1%
C	840,000	860,000	-20,000	2.4%
D	830,000	890,000	-60,000	7.2%
E	830,000	890,000	-60,000	7.2%
Passed a paried t-test: Prob > |t| = 0.3327 Zero is within the 95% C.I. of mean D IU/mg (SU-ELLI: LL = -70437; UL= 30437)				

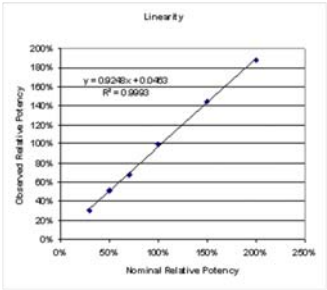
Additional Testing 9

- Method transfer was further substantiated by confirming assay linearity and range at Eurofins Lancaster Labs (RU)
  - 30%, 50%, 70%, 100%, 150%, and 200% nominal relative potency samples prepared in drug placebo using reference standard
  - Total of 4 independent assay runs by two analysts
  - 3 reportable results (9 intermediate results) for 30%, 50%, 70%, 150% and 200% samples
  - 7 reportable results (21 intermediate results) for 100%

Method Accuracy and Precision 10

Nominal Relative Potency	Reportable Result	Average Result	% RSD
30%	30.0%	31.0%	3.0%
	31.8%		
	31.3%		
50%	51.0%	51.4%	0.9%
	51.2%		
	51.9%		
70%	68.0%	68.4%	1.4%
	67.8%		
	69.5%		
100%	97.4%	99.5%	4.3%
	94.3%		
	102%		
	107%		
	101%		
	95.6%		
	99.1%		
150%	141%	144%	4.0%
	150%		
	152%		
200%	184%	188%	4.6%
	182%		
	198%		

Method Linearity 11



Additional Activities 12

- Qualification of critical reagents
  - Reference Standard, Control and Placebo
  - Critical media components (FBS etc)
  - Assay plates
- Assay trending and maintenance
  - Control chart

Evelyn Kilareski[1], Anthony Burkholder[1], Weihong Wang[1]*, Robert Donatelli[1], Miriam Franchini[2] and Larry Anderson[2]
[1] Eurofins Lancaster Laboratories, Inc
[2] Smith & Nephew, Inc

CONCLUSIONS

A cell based potency assay for a biological product was successfully transferred to Eurofins Lancaster Laboratories, Inc. Method transfer demonstrated comparability between sending unit and Eurofins Lancaster Laboratories. In addition, Eurofins Lancaster Laboratories performed partial method validation and demonstrated satisfactory accuracy, precision, linearity and range. Following a successful transfer and agency approval, Eurofins Lancaster Laboratories began routine testing of the drug product.