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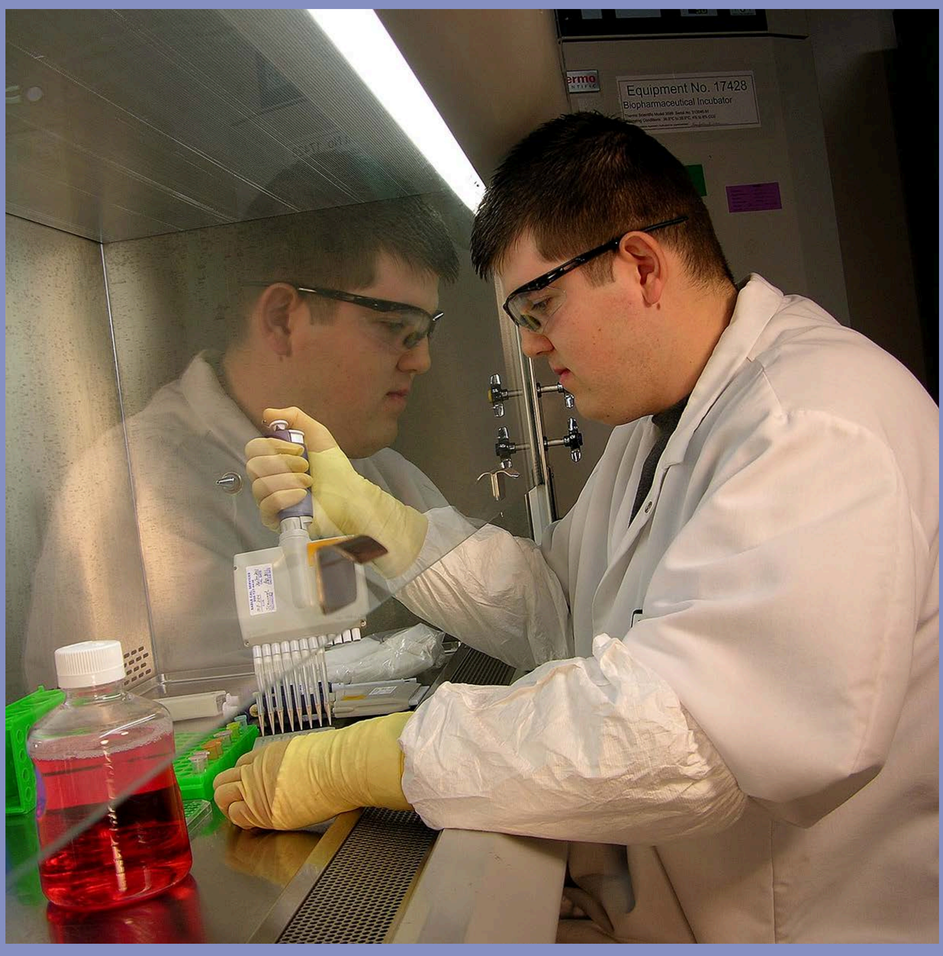
INTRODUCTION

As patents begin to expire on popular biologic medications, there is an emerging need for reliable testing capabilities in the biosimilar marketplace. Eurofins Lancaster Laboratories is currently focused on expanding its offerings of validated cell-based biopotency assays that are ready-to-use to test in-process, drug substance, and drug product biosimilar molecules. Here, methods to evaluate the biological activities of Filgrastim and Pegfilgrastim were validated according to ICH guidelines by Eurofins Lancaster Laboratories using an NFS-60 cell line. The methods are linear and accurate over the range of 50% to 150% of the nominal potency, and demonstrated acceptable specificity, precision, and robustness. These methods have been shown to be suitable for their intended applications for the measurement of the biological activity of both Filgrastim and Pegfilgrastim.



VALIDATION OF METHODS FOR THE MEASUREMENT OF FILGRASTIM AND PEGFILGRASTIM BIOPOTENCIES

DISCUSSION/RESULTS

Method Overview

- Methods originally developed at Eurofins BPT Munich and transferred to Eurofins Lancaster Laboratories for full validation
- Validation used innovator drug product as reference standard
- Comparable to compendial methods
- Cells are incubated with serial dilutions of Filgrastim or Pegfilgrastim. Induction of cell proliferations is then measured using MTT

Filgrastim Bioassay Linearity

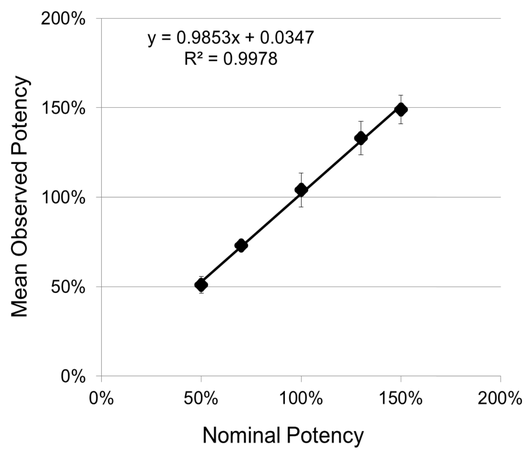


Figure 2. Linear regression of nominal vs mean observed potencies of Filgrastim over the range of 50% to 150% of the reference standard.

Representative Pegfilgrastim Dose Response Curves

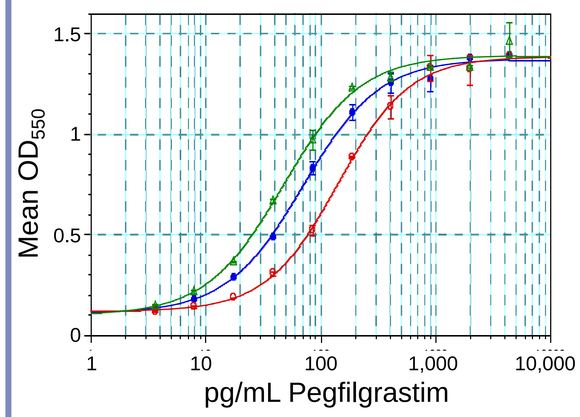


Figure 3. Representative dose response curve of the Pegfilgrastim reference standard (blue) and the reference standard at 50% nominal potency (red) and 150% nominal potency (green). Error bars represent standard deviation of triplicate absorbance values.

Suitability of Method for Testing Using USP Reference Standard

Nominal Potency	Average Observed Potency	% Recovery	% RSD
50%	49%	98%	5%
100%	99%	99%	6%
150%	151%	100%	1%

Table 5. Results of validation experiments for Pegfilgrastim bioassay using USP Pegfilgrastim reference standard

Representative Filgrastim Dose Response Curves

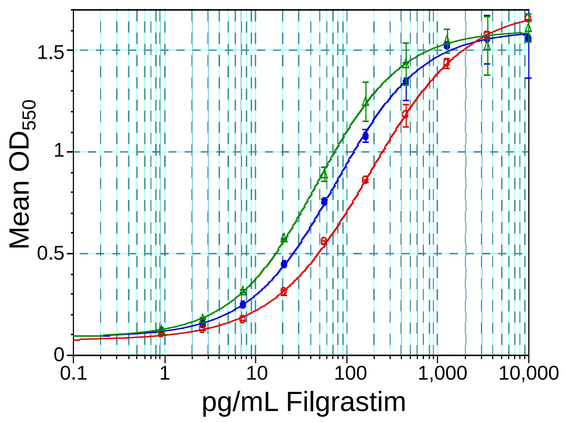


Figure 1. Representative dose response curve of the Filgrastim reference standard (blue) and the reference standard at 50% nominal potency (red) and 150% nominal potency (green). Error bars represent standard deviation of triplicate absorbance values.

Suitability of Method for Testing Using USP Reference Standard

Nominal Potency	Average Observed Potency	% Recovery	% RSD
50%	51%	102%	4%
100%	100%	100%	0%
150%	152%	101%	8%

Table 2. Results of validation experiments for Filgrastim bioassay using USP Filgrastim reference standard.

Pegfilgrastim Bioassay Accuracy and Precision

Nominal Potency	Average Observed Potency	% Recovery	% RSD
50%	50%	101%	2%
70%	68%	97%	3%
100%	100%	100%	6%
130%	126%	97%	3%
150%	145%	97%	3%

Table 4. Results of validation experiments for Pegfilgrastim bioassay. Nominal Potency indicates the nominal potency of the test samples. Four to seven preparations of each nominal potency were tested.

Pegfilgrastim Robustness Cell Density

Nominal Potency	Observed Potency	% Recovery	Max CV	(D-A) % Difference	Slope % Difference	Cell Density
50%	50%	100%	5%	4%	3%	50% Less Than Method
100%	96%	96%	6%	3%	5%	
150%	142%	95%	4%	1%	0%	
50%	50%	100%	6%	2%	6%	50% More Than Method
100%	96%	96%	5%	2%	4%	
150%	144%	96%	4%	3%	8%	

Table 3. Results of validation experiments for Pegfilgrastim bioassay using cell seeding densities from 50% to 150% of that detailed in the method.

Filgrastim Bioassay Accuracy and Precision

Nominal Potency	Average Observed Potency	% Recovery	% RSD
50%	51%	101%	9%
70%	73%	104%	3%
100%	104%	104%	9%
130%	133%	103%	7%
150%	149%	100%	5%

Table 1. Results of validation experiments for Filgrastim bioassay. Nominal Potency indicates the nominal potency of the test samples. Four to seven preparations of each nominal potency were tested.

Filgrastim Robustness Cell Density

Nominal Potency	Observed Potency	% Recovery	Max CV	(D-A) % Difference	Slope % Difference	Cell Density
50%	52%	103%	7%	4%	4%	50% Less Than Method
100%	92%	92%	8%	1%	9%	
150%	152%	101%	6%	3%	5%	
50%	48%	95%	5%	10%	14%	50% More Than Method
100%	104%	104%	5%	1%	6%	
150%	153%	102%	4%	3%	3%	

Table 3. Results of validation experiments for Filgrastim bioassay using cell seeding densities from 50% to 150% of that detailed in the method.

Pegfilgrastim Bioassay Linearity

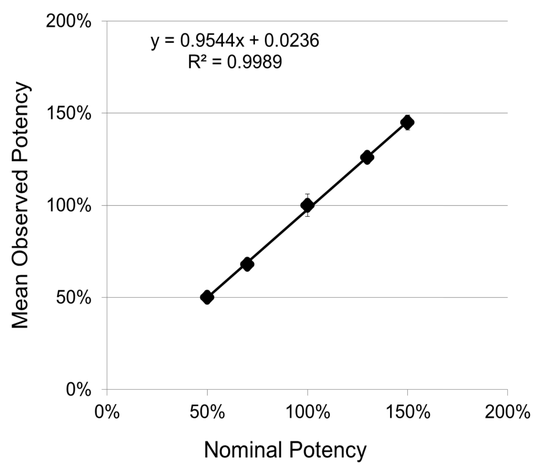


Figure 2. Linear regression of nominal vs mean observed potencies of Pegfilgrastim over the range of 50% to 150% of the reference standard.

Additional Services

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

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Eurofins Lancaster Laboratories, Inc

CONCLUSIONS

The bioassays developed at Eurofins Lancaster Laboratories for the measurement of Filgrastim and Pegfilgrastim biological activities are shown to be suitable for their purposes with acceptable levels of linearity, accuracy, specificity and precision. To this end, Eurofins Lancaster Laboratories is currently focused on expanding our offering of validated cell based potency assays that are ready-to-use to test biosimilar drug substance and drug product samples, as well as to assist process development and validation.