Comparison of *in vitro* and *in vivo* insulin bioidentity assays to monitor the quality of insulin products

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Abstract

Over 100 million individuals manage their diabetes with insulin products daily and rely on the pharmaceutical quality of these products to regulate their blood glucose. Since March 23rd, 2020, insulin products are regulated as biologics. Under the regulations for Biologics License Application review, the potency of the insulin products and of their associated biosimilars is expected to be assessed quantitatively in a cell-based assay or bioassay that, ideally, represents the product mechanism of action. To assess the biological activity or bioidentity of insulins, the USP recommends *in vivo* rabbit bioidentity test or an *in vitro* in-cell western assay (USP <121>). Reduction in animal experiments is a worldwide goal for many and it is likely that *in vitro* bioassays (based on USP <121> or other protocols) will be submitted for assessing the bioidentity of insulin products. This project aims to compare the sensitivity of several bioassays (both *in vitro* and *in vivo*) in their ability to measure the biological activity of insulin products. Using different bioassays, the USP <121> Rabbit assay, a real-time glucose sensing diabetic mouse model, and an in-cell western bioassay, we compared the potency of stressed and unstressed insulin products. Comparison of these bioassays will facilitate the transition of insulin bioassays from *in vivo* to *in vitro* and will support the assessment of prospective *in vitro* bioassays to monitor the quality of insulin products.

Introduction

Figure 1

>100 million individuals manage their diabetes with insulin products

March 23rd, 2020: insulin products became regulated as biologics

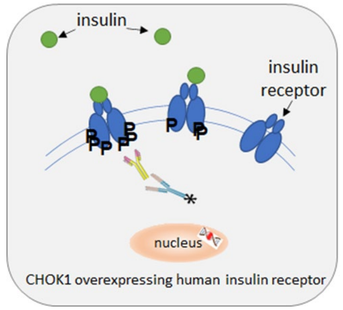
How to ensure adequate bioidentity of insulin products using *in vitro* approaches?

Compare multiple *in vitro* and *in vivo* assays

Methods

In-cell western in vitro bioassay (derived from USP <121>)

Figure 2



Real-time monitoring of blood glucose (mice)

Figure 3

Rabbit blood glucose assay (USP <121>)

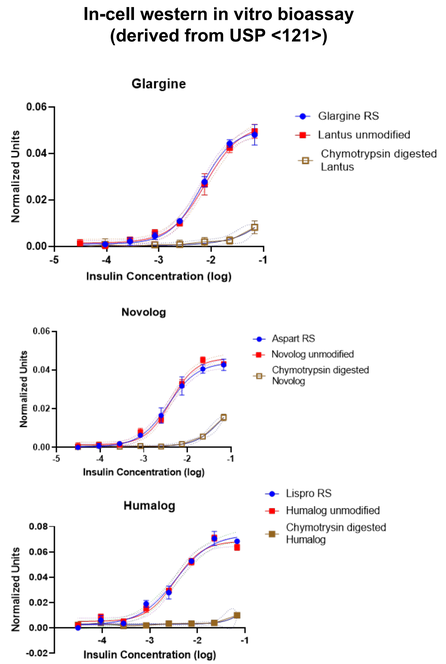
Figure 4

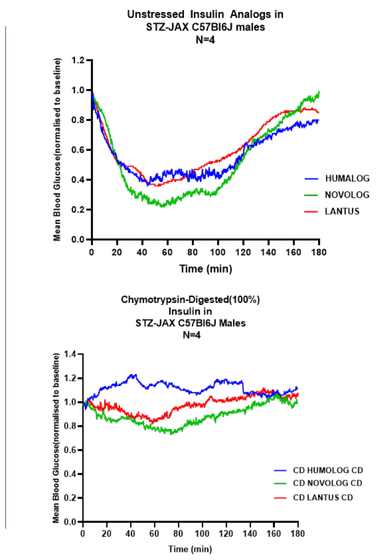
	First Injection	Second Injection
Group 1	Standard Solution 2	Standard Solution 1
Group 2	Standard Solution 1	Standard Solution 2
Group 3	Stressed Solution 2	Stressed Solution 1
Group 4	Stressed Solution 1	Stressed Solution 2

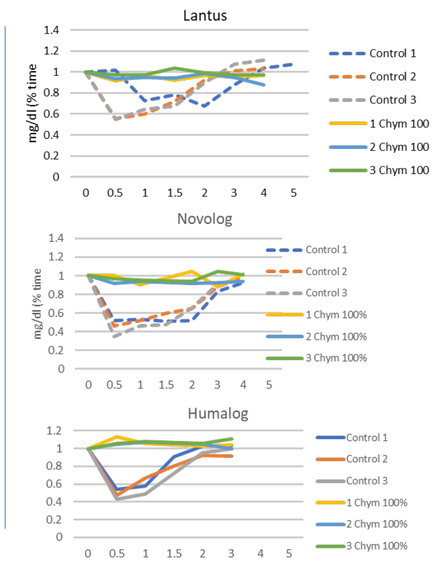
Results and Conclusions

Comparison of biological activity of chymotrypsin-digested insulin samples

Figure 5







Insulin products were either “unstressed” or digested with chymotrypsin for 30 minutes.
The results obtained using the *in vitro* and *in vivo* assays of unstressed and chymotrypsin-digested insulin samples were consistent across the 3 models:

- *In vitro*, using a validated in-cell western assay, unstressed lantus, novolog and humalog induced the phosphorylation of insulin receptor similarly to their respective reference standards, whereas chymotrypsin-digested insulins did not induce the phosphorylation of the receptor.
- *In vivo,* using a real-time glucose monitoring model in mice, lantus, novolog and humalog decreased temporally the blood glucose of mice, while chymotrypsin-digested insulin products did not significantly alter the blood glucose levels of the animals.
- Similar results were observed in an *in vivo* model using rabbits: unstressed lantus, novolog and humalog decreased the rabbits’ blood glucose, but not the chymotrypsin-digested samples.

Further studies with stressed insulin products will be performed to compare the ability of these models to detect insulin products’ impurities.

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Disclaimer

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