VALIDATION OF KRIBIOLISA™ Anti-Asparte (Novolog) ELISA KIT (CATALOG NO. KBI9003) AS PER FDA GUIDELINES FOR BIOANALYTICAL METHOD VALIDATION

This validation protocol has been adopted in line with the Methodology and Analytical Procedures Guideline recommended by FDA (guidelines May 2018)

Document History

First Codification	History	Date
Version#1	**VALIDATION DATA OF KRIBIOLISA™ Anti-Asparte (Novolog) ELISA (CATALOG NO. KBI9003)**	21.10.2024

Approved Quality Control	Approved Product Development	Approved Operations Head
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Introduction

This document presents a discussion of the characteristics of our **KRIBIOLISA™ Anti-Asparte (Novolog) ELISA (Catalog No KBI9003)** kit considered by us during the validation of this kit in accordance with ICH Q2 (R1) guidelines. The document is prepared based on tests run in our laboratory and does not necessarily seek to cover the testing that may be required at user's end for registration in, or regulatory submissions. The objective of this validation is to demonstrate that it is suitable for its intended purpose - detection of **Anti-Asparte.**

Validation characteristics considered by us in accordance with the guidelines are listed below:

- **Sensitivity**
- **Specificity / Cross reactivity**
- **Precision**
- **Recovery**
- **Traceability / Stability**

The degree of revalidation required depends on the nature of the changes. Certain other changes may require validation as well.

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

For any queries or support on the data and its performance, please contact us at sales1@krishgen.com

Background

Insulin Asparte is produced by recombinant DNA technology. It is an analogue of human insulin made by replacing the asparagine residue at position A21 of the A-chain with glycine and adding two arginine to the C-terminus (positions B31 and 32) of the B-chain. The resulting protein is soluble at pH 4 and forms micro precipitates at physiological pH 7.4. Small amounts of insulin Asparte are slowly released from micro precipitates giving the drug a long duration of action (up to 24 hours) and no pronounced peak concentration

1. Sensitivity:

Limit Of Detection It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD.
10 replicates of '0' standards were evaluated and the LOD was found to be less than 500 ng/ml

2. Specificity / Cross Reactivity:

Specificity:
The antibodies used in the kit are specific for insulin aspart.

3. Precision:

3.1 Inter/Intra Assay Precision:

Precision:

Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100.

Pool	Intra Assay %CV	Inter Assay %CV
Positive Control	<18%	<18%

4. Traceability and Stability:

4.1 Traceability:

The Controls provided in the kit are also calibrated against antibodies to Insulin Asparte.

4.2 Stability:

Shelf-Life Stability: An accelerated stability study set the shelf-life stability of KRIBIOLISA™ Anti-Asparte (Novolog) ELISA was performed and a shelf life of 12 months was assigned to the kit.

5. Serum/Plasma Spike Recovery:

Normal human serum was used to spike at 1000 ng/ml to check the recovery at different dilutions to observe optimal recoveries. It was seen that 1:1000 serum dilution reported the most optimal recoveries.

Serum spiked at 1000 ng/ml with Insulin Asparte antibody standard and dilution done for analysis at 1:10, 1:100 and 1:1000. Results were observed.

Standard Diluent	Standard (ng/ml)	Mean Abs @ 450nm
1:10 Human Serum	0	3.162
	1000	3.637
1:100 Human Serum	0	1.041
	1000	3.200
1:1000 Human Serum	0	0.485
	1000	4.062

Standard Diluent	Standard (ng/ml)	Mean Abs @ 450nm
1:10 Human Plasma	0	3.458
	1000	3.859
1:100 Human Plasma	0	1.215
	1000	3.857
1:1000 Human Plasma	0	0.587
	1000	4.500

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