# VALIDATION OF KRIBIOLISA™ ANTI-SEMAGLUTIDE (OZEMPIC) NEUTRALIZING ANTIBODY (CLBA) ELISA (CATALOG NO. KBN1930) 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-Semaglutide (OZEMPIC) Neutralizing Antibody (CLBA) ELISA** (Cat No #KBN1930) | 01.05.2024 |
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| Approved Quality Control | Approved Product Development | Approved Operations Head |
|---|---|---|
| Dr Prairna | Atul G | Dr Atahar |

## Introduction

This document presents a discussion of the characteristics of our KRIBIOLISA™ Anti-Semaglutide (OZEMPIC) Neutralizing Antibody (CLBA) ELISA 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 Neutralizing Antibodies to Semaglutide.

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

- **Sensitivity**
- **Specificity / Cross reactivity**
- **Precision**
- **Traceability / Stability**
- **Recovery**
- **Validation kit specific details**

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

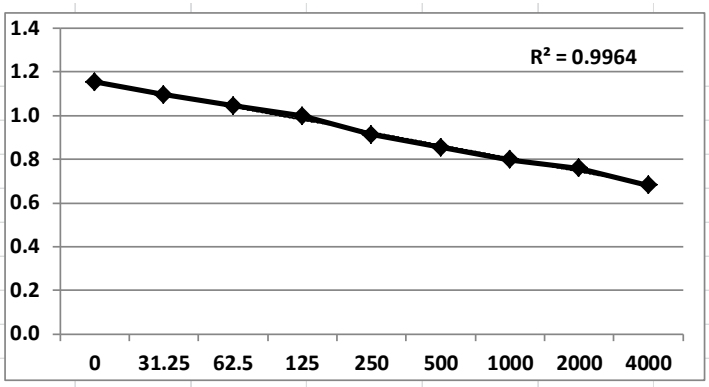
Semaglutide is an antidiabetic medication used for the treatment of type 2 diabetes and an anti-obesity medication used for long-term weight management. Semaglutide is the active ingredient in Ozempic, Wegovy, and Rybelsus. Semaglutide is used for weight loss in specific patients, and also to lower blood sugar levels, and to reduce the risk of major cardiovascular events such as heart attack or stroke in certain patients. Semaglutide is a GLP-1 agonist that works by increasing insulin release, lowering the amount of glucagon released, delaying gastric emptying, and reducing appetite. The KRIBIOLISA Neutralizing Antibodies to Semaglutide is a competitive ligand-binding assay that is specific for the detection of anti-Semaglutide NAbs in human serum or plasma.

### 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 50 ng/ml

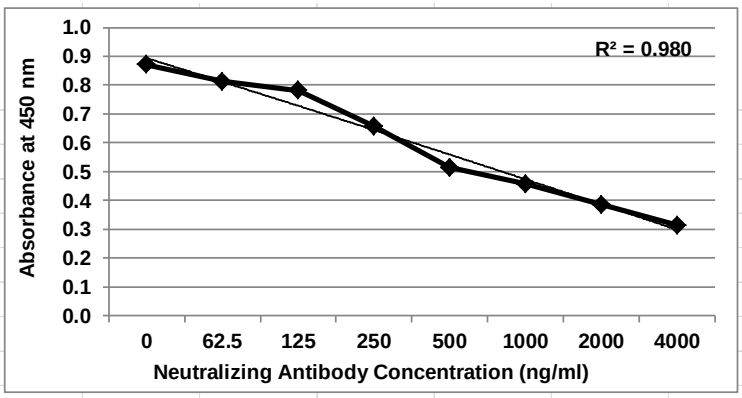
Additional experiments were conducted as under:
Mean of spike study was done using Semaglutide biosimilar grade spiked in normal human serum (n=5) and the absorbance were measured to assay the recovery and sensitivity of the kit. Results obtained indicated a sensitivity of ~50 ng/ml of the neutralizing antibody.

| Neutralizing Antibody Concentration (ng/ml) | Absorbance | Interpolated Concentration | % Recovery |
|---|---|---|---|
| 0 | 1.155 | 0.6 | -- |
| 31.25 | 1.095 | 25.2 | 80.8 |
| 62.5 | 1.045 | 63.5 | 101.7 |
| 125 | 0.999 | 116.8 | 93.4 |
| 250 | 0.913 | 293.4 | 117.4 |
| 500 | 0.855 | 523.0 | 104.6 |
| 1000 | 0.799 | 941.5 | 94.1 |
| 2000 | 0.759 | 1513.8 | 75.7 |
| 4000 | 0.681 | 5594.0 | 139.9 |




Mean of spike study was done using Ozempic™ Injection (Semaglutide) spiked in normal human serum (n=5) and the absorbance were measured to assay the recovery and sensitivity of the kit. Results obtained indicated a sensitivity of ~50 ng/ml of the neutralizing antibody.

| Neutralizing Antibody Concentration (ng/ml) | Absorbance | Interpolated Concentration | % Recovery |
|---|---|---|---|
| 0 | 0.871 | 6.2 | -- |
| 62.5 | 0.812 | 69.9 | 111.8 |
| 125 | 0.782 | 100.8 | 80.7 |
| 250 | 0.658 | 255.7 | 102.3 |
| 500 | 0.514 | 588.9 | 117.8 |
| 1000 | 0.458 | 850.2 | 85.0 |
| 2000 | 0.385 | 1626.5 | 81.3 |
| 4000 | 0.315 | 3269.2 | 81.7 |




## 2. Specificity / Cross Reactivity:

**Specificity:**
The Positive Control used in the kit has exhibited neutralizing properties and is well characterized. It is a mouse monoclonal antibody with immunogen being a synthetic peptide within Human GCG aa 50-150. The exact immunogen used to generate this antibody is proprietary information.
The Capture antibody used in the ligand binding assay is a recombinant Human GLP1-1R protein using a biotinylated Anti-GLP monoclonal antibody as detection antibody.

## 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. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (10 ng/ml), medium (40 ng/ml) and high (320 ng/ml) concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

| Pool | Intra Assay %CV | Inter Assay %CV |
|---|---|---|
| Low | <10% | <12% |
| Medium | <8% | <10% |
| High | <8% | <10% |

## 4. Traceability and Stability:

4.1 Traceability:
No known standard being established, the Positive Control is not validated against the same. We recommend each laboratory to establish its own Positive Control based on known clinical samples.

4.2 Stability:

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

## 5. Validation Kit Specific Details:

| Neutralizing Antibody Concentration (ng/ml) | Absorbance at 450nm |
|---|---|
| 0 | 1.15 |
| Positive Control | 0.68 |

Kit Lot No: NABS0824



*Validation Guide Ver1.0 dated 05.05.2024*