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# FINAL REPORT

## FINAL REPORT TITLE

Measurement of GLP-1 in Mouse Plasma by ELISA for Sponsor 177A

## FINAL ELECTRONIC DOCUMENTATION TITLE

Measurement of GLP-1 in Mouse Plasma by ELISA for Sponsor 177A

## PRINCIPAL INVESTIGATOR

Maloy Mangada, Research Scientist

## INITIATION DATE

07Oct2013

## COMPLETION DATE

Date the Final Report is approved by the Principal Investigator

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## 1.0 SUMMARY

Objective

The purpose of this report is to present the results and analysis of data from experiments conducted to measure GLP-1 in murine plasma samples as part of a time-course study conducted in Sponsor 177A facilities.

Study Personnel

Principal Investigator: Maloy Mangada, Research Scientist, Cambridge Biomedical Inc.

Analyst: Maloy Mangada, Research Scientist, Cambridge Biomedical Inc.

Analyst and Technical Reviewer: Douglas Wilson, Research Associate, Cambridge Biomedical Inc.

Management: Benjamin Bronfin MD, Director Clinical Research and Development, Cambridge Biomedical Inc.

Number of Samples

Two hundred ninety three (293) samples were received and analyzed to determine the concentration of GLP-1 in mouse plasma from four (4) time points during a three month period.

Analytical Methods

The analytical method used for this protocol is an ELISA with a colorimetric readout. This ELISA method quantitatively measures the active form of glucagon like peptide 1 (GLP-1 Active 7-36) in murine plasma. In this assay, murine plasma samples are added to microtiter wells pre-coated with Streptavidin together with a biotin/GLP1-specific antibody conjugate and an HRP-GLP1 antibody conjugate. After the first incubation period, a "sandwich" immuno-complex of Streptavidin – Biotin Antibody – GLP-1 (7-36) – HRP-conjugated antibody is formed and attached to the wells. An HRP substrate is added and the colorimetric reaction can be quantitatively measured using a spectrophotometer, where absorbance is measured at 450 nm. The intensity of the color produced is directly proportional to the amount of GLP-1 captured in the plate. The procedure is described in full in the document CB-001495 GLP-1 ELISA in Murine Plasma for Sponsor 177-005R.

## 2.0 INTRODUCTION

This document describes the results obtained for the determination of GLP-1 concentration in mouse plasma. The sample testing procedures were performed according academic research grade guidelines. The assay was optimized at Cambridge Biomedical Inc. The results of the method optimization can be found in the document CB-001454 GLP-1 Optimization Report. Testing was performed according to the document CB-001495 GLP-1 ELISA in Murine Plasma for Sponsor 177-005R. Platemaps and results templates are outlined in the Microsoft Excel documents 177A Lot A GLP-1 ELISA Sample Testing Results and 177A Lot B GLP-1 ELISA Sample Testing Results.

## 3.0 OBJECTIVE

The objective of this study was to determine GLP-1 levels in mouse plasma samples using ELISA. This analysis utilized the method detailed in Cambridge Biomedical Inc. CB-001495 GLP-1 ELISA in Murine Plasma for Sponsor 177-005R.

## 4.0 ABBREVIATIONS AND DEFINITIONS

| | |
|---|---|
| **HRP** | Horseradish peroxidase |
| **GLP-1** | Glucagon like peptide – 1 |
| **ELISA** | Enzyme linked immunosorbent assay |
| **RCF** | Relative centrifugal force |
| **HFD** | High fat diet |
| **CV** | Coefficient of variance |

## 5.0 REFERENCES

Cambridge Biomedical Inc. CB-001454 GLP-1 Optimization Report

Cambridge Biomedical Inc. CB-001495 GLP-1 ELISA in Murine Plasma for Sponsor 177-005R

## 6.0 DISCUSSION OF RESULTS

A total of two hundred ninety three (293) plasma samples were received in 2 separate lots from the Sponsor and stored at -80°C until GLP-1 analysis was performed. There were 149 samples in Lot A and 144 samples in Lot B.

One ELISA plate (or one batch) can accommodate thirty six samples. Therefore, the work plan was to perform 5 batches for Lot A and 5 batches for Lot B for GLP-1 analysis. Of the two hundred and ninety eight three samples tested, thirty one passed acceptance criteria, data from twenty seven samples were rejected due to high %CV, and two hundred thirty five samples gave data that were below the limit of detection of the assay. There were no failed runs for the duration of the project.

**REPEAT ANALYSES**

Due to the limited amount of plasma sample, repeat analysis was not performed for the samples that failed acceptance criteria.

## 7.0 ACCEPTANCE/REJECTION CRITERIA

The acceptance criteria was determined based on data obtained during the optimization phase of GLP-1 ELISA testing. If the calculated %CV for duplicate tests was less than or equal to 25% then the data is acceptable and was used for further analysis.

## 8.0 EXPERIMENTAL DESIGN

The analytical method used in this procedure is a sandwich ELISA. In this assay, murine plasma samples are added to microtiter wells pre-coated with Streptavidin together with a biotin/GLP1-specific antibody conjugate and an HRP-GLP1 antibody conjugate. After the first incubation period, a "sandwich" immunocomplex of Streptavidin – Biotin Antibody – GLP-1 (7-36) – HRP-conjugated antibody is formed and attached to the wells. An HRP substrate is then added and the colorimetric reaction can be quantitatively measured using a spectrophotometer where absorbance is measured at 450 nm. The intensity of the color produced is proportional to the amount of GLP-1 captured in the plate. Whenever applicable, the samples were spun down at 12000 RCF using a micro centrifuge to separate lipids from the aqueous part of the sample. The lipid layer was refrozen. The aqueous phase was used in the ELISA analysis.

**PROCEDURE SUMMARY**

Refer to CB-001495 GLP-1 ELISA in Murine Plasma for Sponsor 177-005R.

**SAMPLE, SPECIMEN, OR TEST/CONTROL ARTICLE REQUIREMENTS**

Refer to CB-001495 GLP-1 ELISA in Murine Plasma for Sponsor 177-005R.

**COMPUTERIZED SYSTEMS**

SoftMax Pro GXP v. 5.4.4.007 by Molecular Devices is used to control the spectrophotometer, to create and graph the standard curve and to calculate the concentration of GLP-1 from the measured OD readings.

A Microsoft Excel spreadsheet created and developed at Cambridge Biomedical is used to prepare the data in tabular and graphical formats.

Electronic data is stored on the Cambridge Biomedical Shared Files Drive>02 Clinical Diagnostics>Softmax gxp admin>SoftMax R&D>177A.

**EQUIPMENT AND CONDITIONS**

Refer to CB-001495 GLP-1 ELISA in Murine Plasma for Sponsor 177-005R.

**CHEMICALS, REAGENTS, SUPPLIES, AND REFERENCE MATERIALS**

Refer to CB-001495 GLP-1 ELISA in Murine Plasma for Sponsor 177-005R.

**STATISTICAL METHOD(S)**

The %CV of two replicates from each sample was calculated according to the following formula:

$$\%CV = \frac{standard\ deviation}{mean}\ x\ 100\%$$

## 9.0 LIST OF TABLES

**TABLE 1: PLASMA GLP-1 FINAL RESULTS**

| Accession # | Mouse ID # | Collection Data | GLP-1 (pg/ml) | Comments |
|---|---|---|---|---|
| 920747 | 6012001 | 31-Aug-13 | ND | N/A |
| 920747 | 6012001 | 28-Sep-13 | ND | N/A |
| 920747 | 6012001 | 26-Oct-13 | ND | N/A |
| 920747 | 6012001 | 16-Nov-13 | High CV | N/A |
| 920078 | 6012002 | 31-Aug-13 | ND | N/A |
| 920078 | 6012002 | 28-Sep-13 | ND | N/A |
| 920078 | 6012002 | 26-Oct-13 | ND | N/A |
| 920078 | 6012002 | 16-Nov-13 | ND | N/A |
| 920979 | 6012003 | 31-Aug-13 | ND | N/A |
| 920979 | 6012003 | 28-Sep-13 | ND | N/A |
| 920979 | 6012003 | 26-Oct-13 | ND | N/A |
| 920979 | 6012003 | 16-Nov-13 | ND | N/A |
| 920783 | 6012004 | 31-Aug-13 | High CV | N/A |
| 920783 | 6012004 | 28-Sep-13 | ND | N/A |
| 920783 | 6012004 | 26-Oct-13 | ND | N/A |
| 920783 | 6012004 | 16-Nov-13 | 1.915 | N/A |
| 921078 | 6012005 | 31-Aug-13 | ND | N/A |
| 921078 | 6012005 | 28-Sep-13 | ND | N/A |
| 921078 | 6012005 | 26-Oct-13 | ND | N/A |
| 921078 | 6012005 | 16-Nov-13 | ND | N/A |
| 920192 | 6012006 | 31-Aug-13 | ND | N/A |
| 920192 | 6012006 | 28-Sep-13 | 9.762 | N/A |
| 920192 | 6012006 | 26-Oct-13 | High CV | N/A |
| 920192 | 6012006 | 16-Nov-13 | ND | N/A |
| 920924 | 6012007 | 31-Aug-13 | ND | N/A |
| 920924 | 6012007 | 28-Sep-13 | ND | N/A |
| 920924 | 6012007 | 26-Oct-13 | ND | N/A |
| 920924 | 6012007 | 16-Nov-13 | ND | N/A |
| 920653 | 6012008 | 31-Aug-13 | ND | N/A |
| 920653 | 6012008 | 28-Sep-13 | ND | N/A |
| 920653 | 6012008 | 26-Oct-13 | ND | N/A |
| 920653 | 6012008 | 16-Nov-13 | ND | N/A |
| 920128 | 6012009 | 31-Aug-13 | ND | N/A |
| 920128 | 6012009 | 28-Sep-13 | ND | N/A |
| 920128 | 6012009 | 26-Oct-13 | ND | N/A |
| 920128 | 6012009 | 16-Nov-13 | ND | N/A |
| 920310 | 6012010 | 31-Aug-13 | ND | N/A |

| Accession # | Mouse ID # | Collection Data | GLP-1 (pg/ml) | Comments |
|---|---|---|---|---|
| 920310 | 6012010 | 28-Sep-13 | ND | N/A |
| 920310 | 6012010 | 26-Oct-13 | ND | N/A |
| 920310 | 6012010 | 16-Nov-13 | ND | N/A |
| 920385 | 6022001 | 31-Aug-13 | ND | N/A |
| 920385 | 6022001 | 28-Sep-13 | ND | N/A |
| 920385 | 6022001 | 26-Oct-13 | No Sample | N/A |
| 920385 | 6022001 | 16-Nov-13 | ND | N/A |
| 920616 | 6022002 | 31-Aug-13 | ND | N/A |
| 920616 | 6022002 | 28-Sep-13 | ND | N/A |
| 920616 | 6022002 | 26-Oct-13 | No Sample | N/A |
| 920616 | 6022002 | 16-Nov-13 | ND | N/A |
| 920569 | 6022003 | 31-Aug-13 | ND | N/A |
| 920569 | 6022003 | 28-Sep-13 | ND | N/A |
| 920569 | 6022003 | 26-Oct-13 | ND | N/A |
| 920569 | 6022003 | 16-Nov-13 | ND | N/A |
| 920293 | 6022004 | 31-Aug-13 | ND | N/A |
| 920293 | 6022004 | 28-Sep-13 | ND | N/A |
| 920293 | 6022004 | 26-Oct-13 | ND | N/A |
| 920293 | 6022004 | 16-Nov-13 | ND | N/A |
| 920215 | 6022005 | 31-Aug-13 | ND | N/A |
| 920215 | 6022005 | 28-Sep-13 | ND | N/A |
| 920215 | 6022005 | 26-Oct-13 | ND | N/A |
| 920215 | 6022005 | 16-Nov-13 | ND | N/A |
| 920813 | 6022006 | 31-Aug-13 | ND | N/A |
| 920813 | 6022006 | 28-Sep-13 | ND | N/A |
| 920813 | 6022006 | 26-Oct-13 | ND | N/A |
| 920813 | 6022006 | 16-Nov-13 | ND | N/A |
| 920196 | 6022007 | 31-Aug-13 | ND | N/A |
| 920196 | 6022007 | 28-Sep-13 | ND | N/A |
| 920196 | 6022007 | 26-Oct-13 | ND | N/A |
| 920196 | 6022007 | 16-Nov-13 | ND | N/A |
| 920834 | 6022008 | 31-Aug-13 | ND | N/A |
| 920834 | 6022008 | 28-Sep-13 | ND | N/A |
| 920834 | 6022008 | 26-Oct-13 | No Sample | N/A |
| 920834 | 6022008 | 16-Nov-13 | No Sample | N/A |
| 920581 | 6022009 | 31-Aug-13 | ND | N/A |
| 920581 | 6022009 | 28-Sep-13 | ND | N/A |
| 920581 | 6022009 | 26-Oct-13 | ND | N/A |
| 920581 | 6022009 | 16-Nov-13 | ND | N/A |

| Accession # | Mouse ID # | Collection Data | GLP-1 (pg/ml) | Comments |
|---|---|---|---|---|
| 920723 | 6022010 | 31-Aug-13 | ND | N/A |
| 920723 | 6022010 | 28-Sep-13 | ND | N/A |
| 920723 | 6022010 | 26-Oct-13 | ND | N/A |
| 920723 | 6022010 | 16-Nov-13 | ND | N/A |
| 920983 | 6032001 | 31-Aug-13 | ND | N/A |
| 920983 | 6032001 | 28-Sep-13 | High CV | N/A |
| 920983 | 6032001 | 26-Oct-13 | ND | N/A |
| 920983 | 6032001 | 16-Nov-13 | ND | N/A |
| 920317 | 6032002 | 31-Aug-13 | ND | N/A |
| 920317 | 6032002 | 28-Sep-13 | ND | N/A |
| 920317 | 6032002 | 26-Oct-13 | 1.043 | N/A |
| 920317 | 6032002 | 16-Nov-13 | 0.289 | N/A |
| 920683 | 6032003 | 31-Aug-13 | 15.138 | N/A |
| 920683 | 6032003 | 28-Sep-13 | 11.807 | N/A |
| 920683 | 6032003 | 26-Oct-13 | 0.699 | N/A |
| 920683 | 6032003 | 16-Nov-13 | No Sample | N/A |
| 920515 | 6032004 | 31-Aug-13 | High CV | N/A |
| 920515 | 6032004 | 28-Sep-13 | ND | N/A |
| 920515 | 6032004 | 26-Oct-13 | ND | N/A |
| 920515 | 6032004 | 16-Nov-13 | ND | N/A |
| 920645 | 6032005 | 31-Aug-13 | High CV | N/A |
| 920645 | 6032005 | 28-Sep-13 | ND | N/A |
| 920645 | 6032005 | 26-Oct-13 | ND | N/A |
| 920645 | 6032005 | 16-Nov-13 | ND | N/A |
| 920557 | 6032006 | 31-Aug-13 | ND | N/A |
| 920557 | 6032006 | 28-Sep-13 | ND | N/A |
| 920557 | 6032006 | 26-Oct-13 | ND | N/A |
| 920557 | 6032006 | 16-Nov-13 | ND | N/A |
| 920675 | 6032007 | 31-Aug-13 | ND | N/A |
| 920675 | 6032007 | 28-Sep-13 | ND | N/A |
| 920675 | 6032007 | 26-Oct-13 | ND | N/A |
| 920675 | 6032007 | 16-Nov-13 | ND | N/A |
| 920435 | 6032008 | 31-Aug-13 | ND | N/A |
| 920435 | 6032008 | 28-Sep-13 | ND | N/A |
| 920435 | 6032008 | 26-Oct-13 | ND | N/A |
| 920435 | 6032008 | 16-Nov-13 | High CV | N/A |
| 920203 | 6032009 | 31-Aug-13 | High CV | N/A |
| 920203 | 6032009 | 28-Sep-13 | High CV | N/A |
| 920203 | 6032009 | 26-Oct-13 | 5.686 | N/A |

| Accession # | Mouse ID # | Collection Data | GLP-1 (pg/ml) | Comments |
|---|---|---|---|---|
| 920203 | 6032009 | 16-Nov-13 | 4.950 | N/A |
| 920345 | 6032010 | 31-Aug-13 | High CV | N/A |
| 920345 | 6032010 | 28-Sep-13 | ND | N/A |
| 920345 | 6032010 | 26-Oct-13 | ND | N/A |
| 920345 | 6032010 | 16-Nov-13 | 2.388 | N/A |
| 920577 | 6112001 | 31-Aug-13 | ND | N/A |
| 920577 | 6112001 | 28-Sep-13 | ND | N/A |
| 920577 | 6112001 | 26-Oct-13 | ND | N/A |
| 920577 | 6112001 | 16-Nov-13 | ND | N/A |
| 920069 | 6112002 | 31-Aug-13 | High CV | N/A |
| 920069 | 6112002 | 28-Sep-13 | ND | N/A |
| 920069 | 6112002 | 26-Oct-13 | ND | N/A |
| 920069 | 6112002 | 16-Nov-13 | ND | N/A |
| 920487 | 6112003 | 31-Aug-13 | ND | N/A |
| 920487 | 6112003 | 28-Sep-13 | ND | N/A |
| 920487 | 6112003 | 26-Oct-13 | ND | N/A |
| 920487 | 6112003 | 16-Nov-13 | ND | N/A |
| 920558 | 6112004 | 31-Aug-13 | ND | N/A |
| 920558 | 6112004 | 28-Sep-13 | ND | N/A |
| 920558 | 6112004 | 26-Oct-13 | No Sample | N/A |
| 920558 | 6112004 | 16-Nov-13 | ND | N/A |
| 920939 | 6112005 | 31-Aug-13 | High CV | N/A |
| 920939 | 6112005 | 28-Sep-13 | ND | N/A |
| 920939 | 6112005 | 26-Oct-13 | ND | N/A |
| 920939 | 6112005 | 16-Nov-13 | ND | N/A |
| 920090 | 6112006 | 31-Aug-13 | ND | N/A |
| 920090 | 6112006 | 28-Sep-13 | ND | N/A |
| 920090 | 6112006 | 26-Oct-13 | 4.086 | N/A |
| 920090 | 6112006 | 16-Nov-13 | ND | N/A |
| 920889 | 6112007 | 31-Aug-13 | ND | N/A |
| 920889 | 6112007 | 28-Sep-13 | ND | N/A |
| 920889 | 6112007 | 26-Oct-13 | ND | N/A |
| 920889 | 6112007 | 16-Nov-13 | ND | N/A |
| 920759 | 6112008 | 31-Aug-13 | High CV | N/A |
| 920759 | 6112008 | 28-Sep-13 | ND | N/A |
| 920759 | 6112008 | 26-Oct-13 | ND | N/A |
| 920759 | 6112008 | 16-Nov-13 | 0.515 | N/A |
| 920043 | 6112009 | 31-Aug-13 | ND | N/A |
| 920043 | 6112009 | 28-Sep-13 | ND | N/A |

| Accession # | Mouse ID # | Collection Data | GLP-1 (pg/ml) | Comments |
|---|---|---|---|---|
| 920043 | 6112009 | 26-Oct-13 | ND | N/A |
| 920043 | 6112009 | 16-Nov-13 | 1.085 | N/A |
| 921085 | 6112010 | 31-Aug-13 | ND | N/A |
| 921085 | 6112010 | 28-Sep-13 | ND | N/A |
| 921085 | 6112010 | 26-Oct-13 | ND | N/A |
| 921085 | 6112010 | 16-Nov-13 | ND | N/A |
| 920471 | 6112011 | 31-Aug-13 | ND | N/A |
| 920471 | 6112011 | 28-Sep-13 | ND | N/A |
| 920471 | 6112011 | 26-Oct-13 | ND | N/A |
| 920471 | 6112011 | 16-Nov-13 | ND | N/A |
| 920388 | 6112012 | 31-Aug-13 | ND | N/A |
| 920388 | 6112012 | 28-Sep-13 | ND | N/A |
| 920388 | 6112012 | 26-Oct-13 | ND | N/A |
| 920388 | 6112012 | 16-Nov-13 | ND | N/A |
| 920636 | 6112013 | 31-Aug-13 | ND | N/A |
| 920636 | 6112013 | 28-Sep-13 | ND | N/A |
| 920636 | 6112013 | 26-Oct-13 | ND | N/A |
| 920636 | 6112013 | 16-Nov-13 | ND | N/A |
| 920998 | 6112014 | 31-Aug-13 | ND | N/A |
| 920998 | 6112014 | 28-Sep-13 | ND | N/A |
| 920998 | 6112014 | 26-Oct-13 | ND | N/A |
| 920998 | 6112014 | 16-Nov-13 | ND | N/A |
| 920286 | 6112015 | 31-Aug-13 | ND | N/A |
| 920286 | 6112015 | 28-Sep-13 | ND | N/A |
| 920286 | 6112015 | 26-Oct-13 | 2.939 | N/A |
| 920286 | 6112015 | 16-Nov-13 | ND | N/A |
| 920026 | 6122001 | 31-Aug-13 | ND | N/A |
| 920026 | 6122001 | 28-Sep-13 | ND | N/A |
| 920026 | 6122001 | 26-Oct-13 | High CV | N/A |
| 920026 | 6122001 | 16-Nov-13 | ND | N/A |
| 920274 | 6122002 | 31-Aug-13 | ND | N/A |
| 920274 | 6122002 | 28-Sep-13 | ND | N/A |
| 920274 | 6122002 | 26-Oct-13 | ND | N/A |
| 920274 | 6122002 | 16-Nov-13 | ND | N/A |
| 920884 | 6122003 | 31-Aug-13 | ND | N/A |
| 920884 | 6122003 | 28-Sep-13 | ND | N/A |
| 920884 | 6122003 | 26-Oct-13 | ND | N/A |
| 920884 | 6122003 | 16-Nov-13 | ND | N/A |
| 920943 | 6122004 | 31-Aug-13 | ND | N/A |

| Accession # | Mouse ID # | Collection Data | GLP-1 (pg/ml) | Comments |
|---|---|---|---|---|
| 920943 | 6122004 | 28-Sep-13 | 4.287 | N/A |
| 920943 | 6122004 | 26-Oct-13 | ND | N/A |
| 920943 | 6122004 | 16-Nov-13 | High CV | N/A |
| 920605 | 6122005 | 31-Aug-13 | ND | N/A |
| 920605 | 6122005 | 28-Sep-13 | ND | N/A |
| 920605 | 6122005 | 26-Oct-13 | ND | N/A |
| 920605 | 6122005 | 16-Nov-13 | ND | N/A |
| 920711 | 6122006 | 31-Aug-13 | ND | N/A |
| 920711 | 6122006 | 28-Sep-13 | ND | N/A |
| 920711 | 6122006 | 26-Oct-13 | ND | N/A |
| 920711 | 6122006 | 16-Nov-13 | ND | N/A |
| 920754 | 6122007 | 31-Aug-13 | High CV | N/A |
| 920754 | 6122007 | 28-Sep-13 | ND | N/A |
| 920754 | 6122007 | 26-Oct-13 | ND | N/A |
| 920754 | 6122007 | 16-Nov-13 | ND | N/A |
| 920640 | 6122008 | 31-Aug-13 | ND | N/A |
| 920640 | 6122008 | 28-Sep-13 | ND | N/A |
| 920640 | 6122008 | 26-Oct-13 | ND | N/A |
| 920640 | 6122008 | 16-Nov-13 | ND | N/A |
| 920565 | 6122009 | 31-Aug-13 | 3.430 | N/A |
| 920565 | 6122009 | 28-Sep-13 | High CV | N/A |
| 920565 | 6122009 | 26-Oct-13 | ND | N/A |
| 920565 | 6122009 | 16-Nov-13 | High CV | N/A |
| 920475 | 6122010 | 31-Aug-13 | ND | N/A |
| 920475 | 6122010 | 28-Sep-13 | High CV | N/A |
| 920475 | 6122010 | 26-Oct-13 | ND | N/A |
| 920475 | 6122010 | 16-Nov-13 | ND | N/A |
| 920931 | 6122011 | 31-Aug-13 | High CV | N/A |
| 920931 | 6122011 | 28-Sep-13 | ND | N/A |
| 920931 | 6122011 | 26-Oct-13 | ND | N/A |
| 920931 | 6122011 | 16-Nov-13 | 5.783 | N/A |
| 920746 | 6122012 | 31-Aug-13 | High CV | N/A |
| 920746 | 6122012 | 28-Sep-13 | ND | N/A |
| 920746 | 6122012 | 26-Oct-13 | ND | N/A |
| 920746 | 6122012 | 16-Nov-13 | ND | N/A |
| 921073 | 6122013 | 31-Aug-13 | ND | N/A |
| 921073 | 6122013 | 28-Sep-13 | ND | N/A |
| 921073 | 6122013 | 26-Oct-13 | ND | N/A |
| 921073 | 6122013 | 16-Nov-13 | ND | N/A |

| Accession # | Mouse ID # | Collection Data | GLP-1 (pg/ml) | Comments |
|---|---|---|---|---|
| 920376 | 6122014 | 31-Aug-13 | ND | N/A |
| 920376 | 6122014 | 28-Sep-13 | ND | N/A |
| 920376 | 6122014 | 26-Oct-13 | ND | N/A |
| 920376 | 6122014 | 16-Nov-13 | ND | N/A |
| 920687 | 6122015 | 31-Aug-13 | High CV | N/A |
| 920687 | 6122015 | 28-Sep-13 | ND | N/A |
| 920687 | 6122015 | 26-Oct-13 | ND | N/A |
| 920687 | 6122015 | 16-Nov-13 | ND | N/A |
| 920482 | 6132001 | 31-Aug-13 | ND | N/A |
| 920482 | 6132001 | 28-Sep-13 | ND | N/A |
| 920482 | 6132001 | 26-Oct-13 | ND | N/A |
| 920482 | 6132001 | 16-Nov-13 | ND | N/A |
| 920305 | 6132002 | 31-Aug-13 | ND | N/A |
| 920305 | 6132002 | 28-Sep-13 | ND | N/A |
| 920305 | 6132002 | 26-Oct-13 | High CV | N/A |
| 920305 | 6132002 | 16-Nov-13 | ND | N/A |
| 920664 | 6132003 | 31-Aug-13 | 18.898 | N/A |
| 920664 | 6132003 | 28-Sep-13 | High CV | N/A |
| 920664 | 6132003 | 26-Oct-13 | High CV | N/A |
| 920664 | 6132003 | 16-Nov-13 | ND | N/A |
| 920506 | 6132004 | 31-Aug-13 | ND | N/A |
| 920506 | 6132004 | 28-Sep-13 | ND | N/A |
| 920506 | 6132004 | 26-Oct-13 | ND | N/A |
| 920506 | 6132004 | 16-Nov-13 | ND | N/A |
| 920704 | 6132005 | 31-Aug-13 | ND | N/A |
| 920704 | 6132005 | 28-Sep-13 | ND | N/A |
| 920704 | 6132005 | 26-Oct-13 | ND | N/A |
| 920704 | 6132005 | 16-Nov-13 | ND | N/A |
| 920561 | 6132006 | 31-Aug-13 | ND | N/A |
| 920561 | 6132006 | 28-Sep-13 | ND | N/A |
| 920561 | 6132006 | 26-Oct-13 | ND | N/A |
| 920561 | 6132006 | 16-Nov-13 | ND | N/A |
| 921104 | 6132007 | 31-Aug-13 | 1.286 | N/A |
| 921104 | 6132007 | 28-Sep-13 | 26.285 | N/A |
| 921104 | 6132007 | 26-Oct-13 | 22.664 | N/A |
| 921104 | 6132007 | 16-Nov-13 | 18.347 | N/A |
| 920431 | 6132008 | 31-Aug-13 | ND | N/A |
| 920431 | 6132008 | 28-Sep-13 | ND | N/A |
| 920431 | 6132008 | 26-Oct-13 | ND | N/A |

| Accession # | Mouse ID # | Collection Data | GLP-1 (pg/ml) | Comments |
|---|---|---|---|---|
| 920431 | 6132008 | 16-Nov-13 | ND | N/A |
| 920608 | 6132009 | 31-Aug-13 | 7.058 | N/A |
| 920608 | 6132009 | 28-Sep-13 | 8.179 | N/A |
| 920608 | 6132009 | 26-Oct-13 | 13.433 | N/A |
| 920608 | 6132009 | 16-Nov-13 | ND | N/A |
| 920726 | 6132010 | 31-Aug-13 | 6.761 | N/A |
| 920726 | 6132010 | 28-Sep-13 | ND | N/A |
| 920726 | 6132010 | 26-Oct-13 | 1.070 | N/A |
| 920726 | 6132010 | 16-Nov-13 | ND | N/A |
| 920419 | 6132011 | 31-Aug-13 | ND | N/A |
| 920419 | 6132011 | 28-Sep-13 | 15.600 | N/A |
| 920419 | 6132011 | 26-Oct-13 | High CV | N/A |
| 920419 | 6132011 | 16-Nov-13 | ND | N/A |
| 920549 | 6132012 | 31-Aug-13 | ND | N/A |
| 920549 | 6132012 | 28-Sep-13 | ND | N/A |
| 920549 | 6132012 | 26-Oct-13 | ND | N/A |
| 920549 | 6132012 | 16-Nov-13 | ND | N/A |
| 920502 | 6132013 | 31-Aug-13 | 76.679 | N/A |
| 920502 | 6132013 | 28-Sep-13 | No Sample | N/A |
| 920502 | 6132013 | 26-Oct-13 | High CV | N/A |
| 920502 | 6132013 | 16-Nov-13 | 3.663 | N/A |
| 920187 | 6132014 | 31-Aug-13 | 1.451 | N/A |
| 920187 | 6132014 | 28-Sep-13 | ND | N/A |
| 920187 | 6132014 | 26-Oct-13 | ND | N/A |
| 920187 | 6132014 | 16-Nov-13 | ND | N/A |
| 920443 | 6132015 | 31-Aug-13 | ND | N/A |
| 920443 | 6132015 | 28-Sep-13 | ND | N/A |
| 920443 | 6132015 | 26-Oct-13 | ND | N/A |
| 920443 | 6132015 | 16-Nov-13 | ND | N/A |

Legend: ND – None detected; High CV – data point not accepted due to high %CV; No Sample – tube or vial for the specific mouse/time point was not received at Cambridge Biomedical

All data is stored on the Cambridge Biomedical Shared Files Drive>02 Clinical Diagnostics>Softmax gxp admin>SoftMax R&D>177A.

## 10.0 LIST OF FIGURES

### A. TIME COURSE OF PLASMA GLP-1 – ALL MOUSE SAMPLES BY GROUP

Note: There were no acceptable data points to be graphed for the Chow– 3R4F group.

**FIGURE 1 – CHOW SHAM**

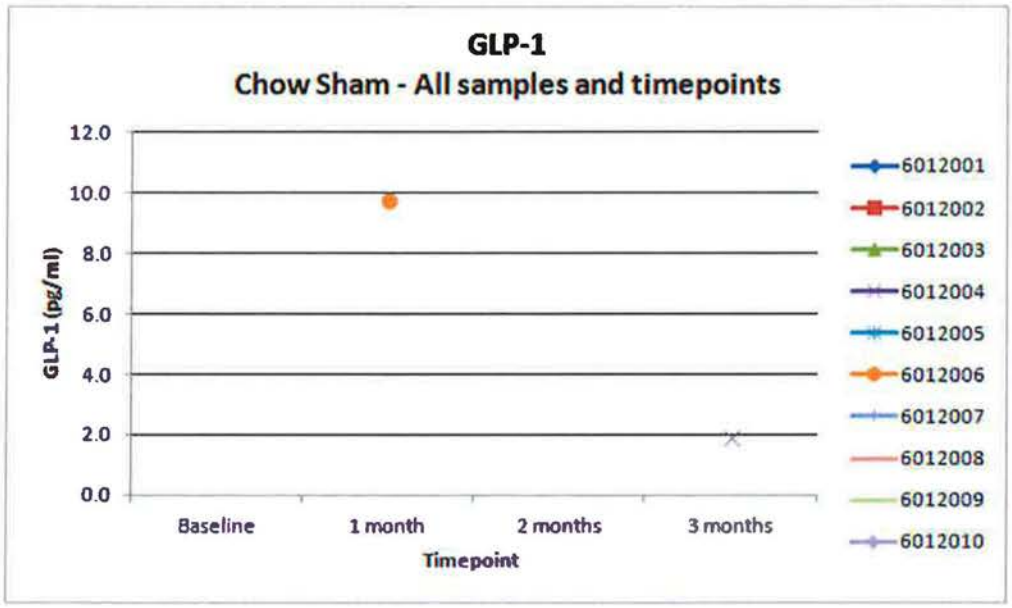


**FIGURE 2 – CHOW MRTP**

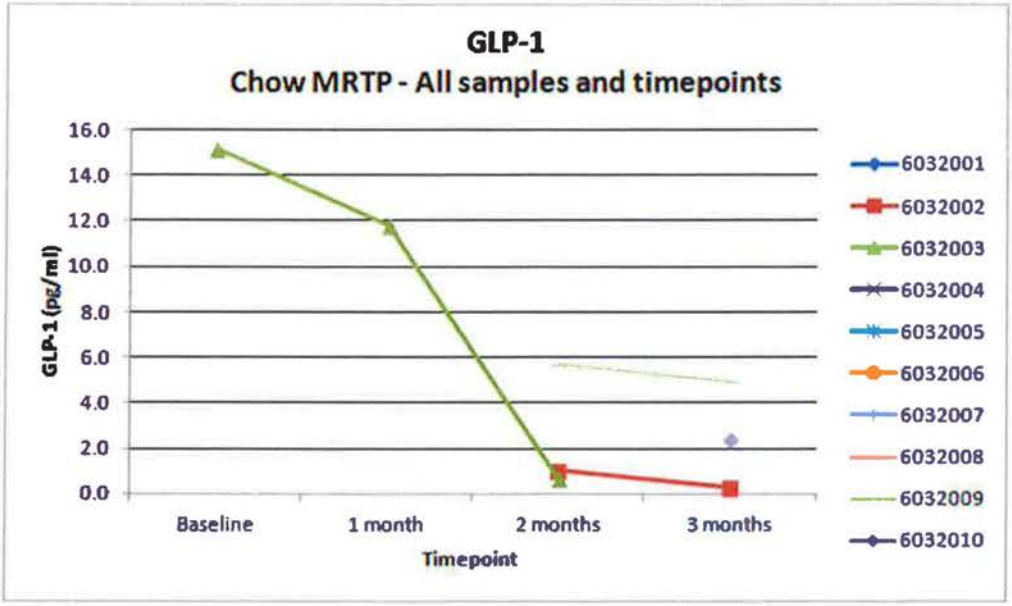


**FIGURE 3 – HFD SHAM**

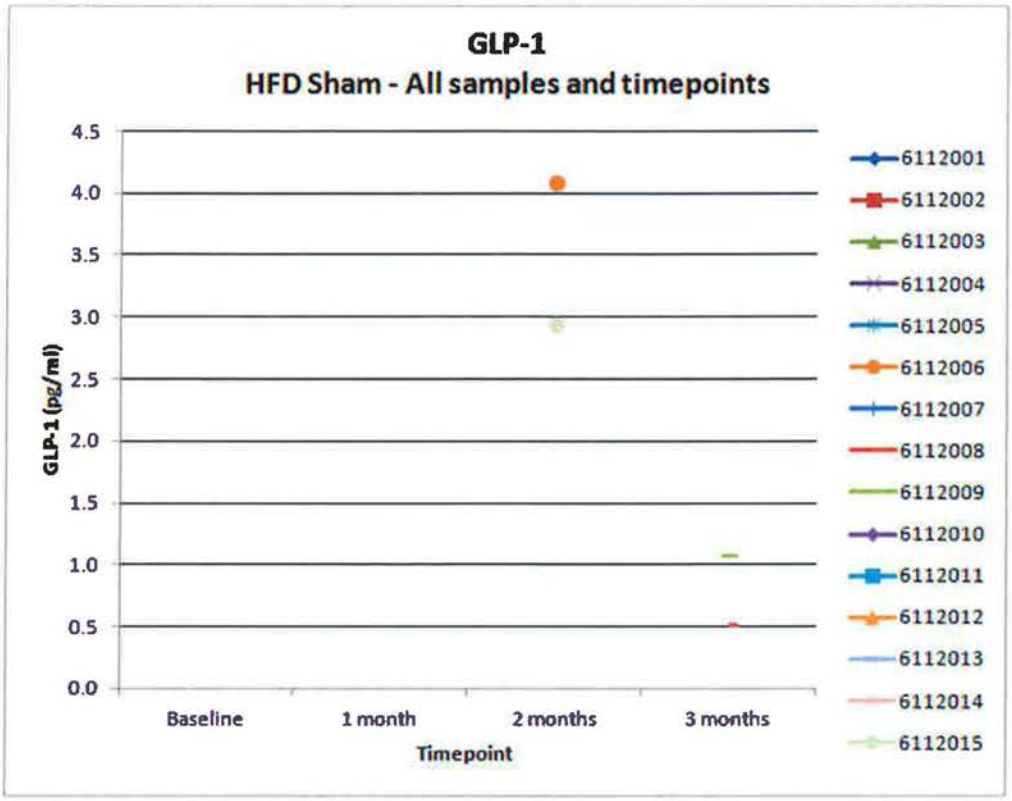


**FIGURE 4 – HFD 3R4F**

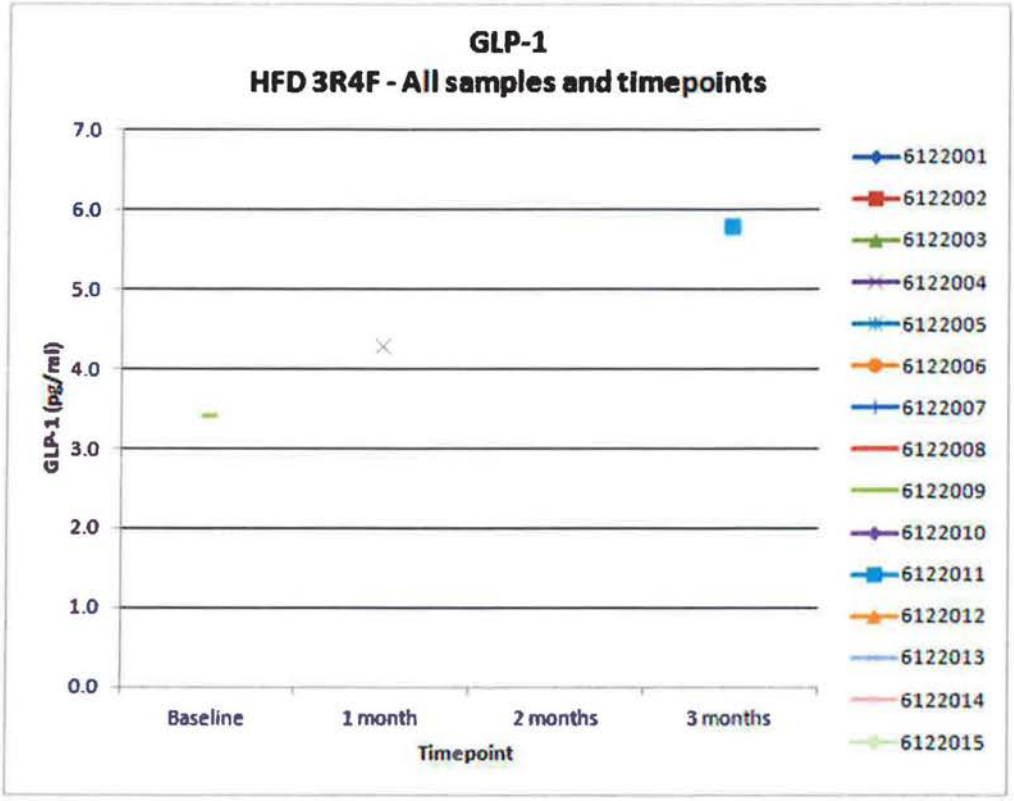


**FIGURE 5 – HFD MRTP**

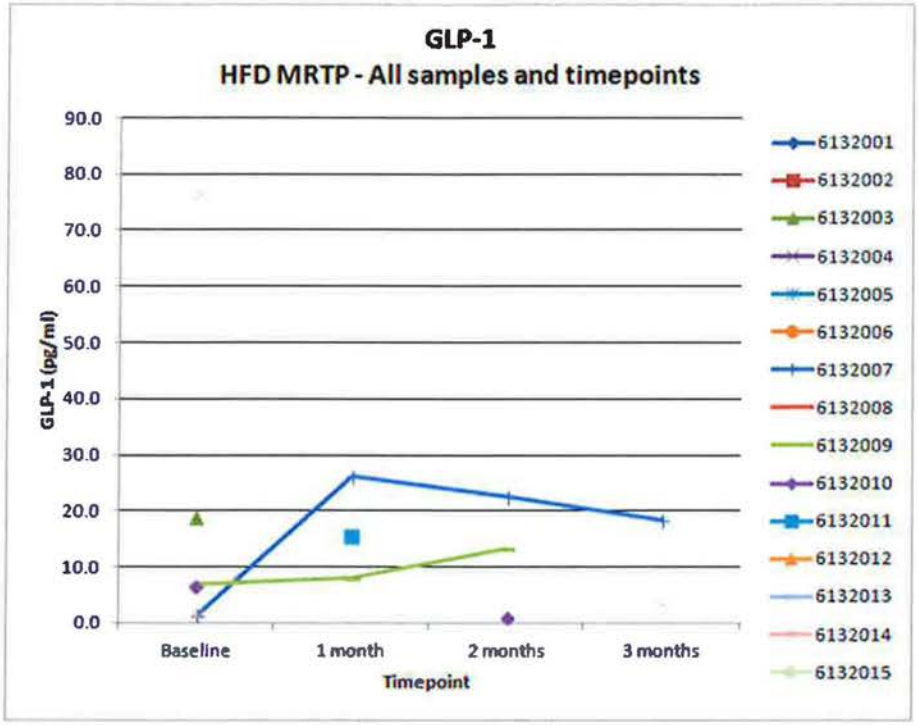
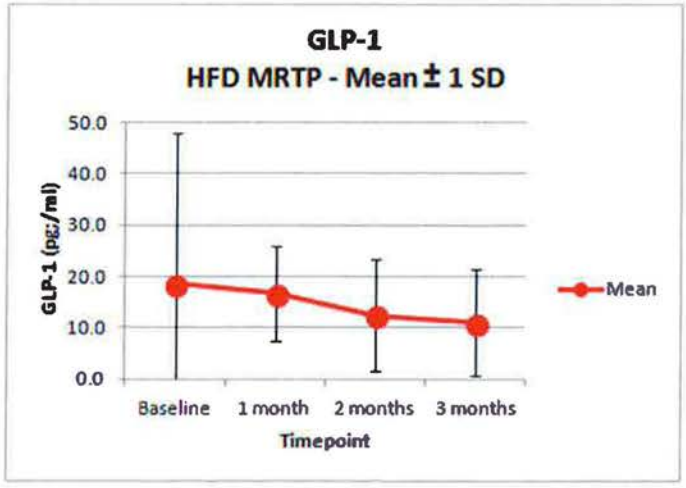



**FIGURE 6 – TIME COURSE OF MEAN VALUES FOR HFD – MRTP GROUP**




## 11.0 APPENDICES

There are no appendices for this report.

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# FINAL REPORT

## FINAL REPORT TITLE

Measurement of Insulin in Mouse Plasma by ELISA for Sponsor 177A

## FINAL REPORT ELECTRONIC DOCUMENTATION TITLE

Measurement of Insulin in Mouse Plasma by ELISA for Sponsor 177A

## PRINCIPAL INVESTIGATOR

Maloy Mangada, Research Scientist

## INITIATION DATE

07Oct2013

## COMPLETION DATE

Date the Final Report is approved by the Principal Investigator

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## 1.0 SUMMARY

Objective

The purpose of this report is to present the results and analysis of data from experiments conducted to measure insulin in mouse plasma samples as part of a time course study conducted in Sponsor 177A facilities.

Study Personnel

Principal Investigator: Maloy Mangada, Research Scientist, Cambridge Biomedical Inc.

Analyst: Maloy Mangada, Research Scientist, Cambridge Biomedical Inc.

Analyst and Technical Reviewer: Douglas Wilson, Research Associate, Cambridge Biomedical Inc.

Management: Benjamin Bronfin MD, Director Clinical Research and Development, Cambridge Biomedical Inc.

Number of Samples

Two hundred ninety eight (298) samples were received and analyzed to determine the concentration of insulin in mouse plasma from four (4) time points collected during a three month period.

Analytical Methods

The analytical method used for this protocol is an ELISA with a colorimetric readout. This ELISA method quantitatively measures insulin in murine plasma. In this assay, murine plasma samples are added to microtiter wells pre-coated with guinea pig derived anti-insulin antibody that in turn captures insulin in the plasma samples. An HRP linked anti-insulin antibody is then used to detect the captured insulin. An HRP substrate is added and the colorimetric reaction can be quantitatively measured using a spectrophotometer, where absorbance is measured at 450 nm. The intensity of the color produced is proportional to the amount of insulin captured on the plate. Details of the analytical methods can be found in the document CB-001371 Insulin ELISA in Murine Plasma for Sponsor 177-003R.

## 2.0 INTRODUCTION

This document describes the results obtained for the determination of insulin concentration in mouse plasma. The assay was optimized at Cambridge Biomedical Inc. The results of the method optimization can be found in the document CB-001373 Insulin Optimization Report. The testing procedures were performed according to the document CB-001371 Insulin ELISA in Murine Plasma for Sponsor 177-003R. Plate maps and results templates are recorded in the documents 177A Lot A Insulin ELISA Sample Testing Results and 177A Lot B Insulin ELISA Sample Testing Results.

## 3.0 OBJECTIVE

The objective of this study was to determine insulin levels in mouse plasma samples using ELISA. This analysis utilized the method detailed in Cambridge Biomedical Inc. CB-001371 Insulin ELISA in Murine Plasma for Sponsor 177-003R.

## 4.0 ABBREVIATIONS AND DEFINITIONS

| | |
|---|---|
| **HRP** | Horseradish peroxidase |
| **ELISA** | Enzyme linked immunosorbent assay |
| **RCF** | Relative centrifugal force |
| **HFD** | High Fat Diet |
| **CV%** | Coefficient of variance |

## 5.0 REFERENCES

Cambridge Biomedical Inc. CB-001373 Insulin Optimization Report

Cambridge Biomedical Inc. CB-001371 Insulin ELISA in Murine Plasma for Sponsor 177-003R

## 6.0 DISCUSSION OF RESULTS

A total of two hundred ninety eight (298) plasma samples were received in 2 separate lots from the Sponsor and stored at -80°C until insulin analysis was performed. There were 150 samples in Lot A and 148 samples in Lot B. Two samples were not received at Cambridge Biomedical.

One ELISA plate (or one batch) can accommodate thirty six samples. Therefore, the work plan was to perform 5 batches for Lot A and 5 batches for Lot B for insulin analysis. Of the two hundred and ninety eight plasma samples tested, two hundred and eighty passed acceptance criteria. There were no failed runs for the duration of the project.

**REPEAT ANALYSES**

Due to the limited amount of plasma sample, repeat analysis was not performed for the samples that failed acceptance criteria.

## 7.0 ACCEPTANCE/REJECTION CRITERIA

The acceptance criteria was determined based on data obtained during the optimization phase of insulin ELISA testing. If the calculated %CV for duplicate tests was less than or equal to 15% then the data is acceptable and was used for further analysis according to CB-001371 Insulin ELISA in Murine Plasma for Sponsor 177-003R.

## 8.0 EXPERIMENTAL DESIGN

The analytical method used in this procedure is a sandwich ELISA where murine plasma samples were added to microtiter wells pre-coated with guinea pig derived anti-insulin antibody that in turn captured insulin in the plasma samples. An HRP linked anti-insulin antibody was then used to detect the captured insulin. An HRP substrate was added and the colorimetric reaction was quantitatively measured using a spectrophotometer, where absorbance was measured at 450 nm. The intensity of the color produced is proportional to the amount of insulin captured in the plate. Whenever applicable, the samples were spun down at 12000 RCF using a micro centrifuge to separate lipids from the aqueous part of the sample. The lipid layer was refrozen. The aqueous phase was used in the ELISA analysis.

**PROCEDURE SUMMARY**

Refer to CB-001371 Insulin ELISA in Murine Plasma for Sponsor 177-003R.

**SAMPLE, SPECIMEN, OR TEST/CONTROL ARTICLE REQUIREMENTS**

Refer to CB-001371 Insulin ELISA in Murine Plasma for Sponsor 177-003R.

**COMPUTERIZED SYSTEMS**

SoftMax Pro GXP v. 5.4 by Molecular Devices is used to control the spectrophotometer, to create and graph the standard curve, and to calculate the concentration of insulin from the measured OD readings.

A Microsoft Excel spreadsheet created and developed at Cambridge Biomedical is used to calculate %CV, *p* values for a Student's t test comparison and to prepare the data in a tabular format.

Electronic data is stored on the Cambridge Biomedical Shared Files Drive>02 Clinical Diagnostics>SoftMax GxP Admin>SoftMax R&D>177A.

**EQUIPMENT AND CONDITIONS**

Refer to CB-001371 Insulin ELISA in Murine Plasma for Sponsor 177-003R.

**CHEMICALS, REAGENTS, SUPPLIES, AND REFERENCE MATERIALS**

Refer to CB-001371 Insulin ELISA in Murine Plasma for Sponsor 177-003R.

**STATISTICAL METHOD(S)**

A one-tailed distribution, two-sample equal variance Student's t-test was used to compare average insulin concentrations between the different groups. The %CV was calculated according to the following formula:

$$\%CV = \frac{standard\ deviation}{mean}$$

The Student's t test t values were calculated according to the following formula:

$$t = \frac{mean\ of\ first\ group - mean\ of\ second\ group}{pooled\ standard\ deviation \Big/ \sqrt{\frac{2}{sample\ size}}}$$

Using the t statistic calculated above, a table based on a Student's t-distribution was used to calculate *p* values.

## 9.0 LIST OF TABLES

**TABLE 1: PLASMA INSULIN FINAL RESULTS**

| Accession # | Mouse ID # | Collection Data | insulin (ng/ml) | Comments |
|---|---|---|---|---|
| 920747 | 6012001 | 31-Aug-13 | 0.295 | N/A |
| 920747 | 6012001 | 28-Sep-13 | 0.267 | N/A |
| 920747 | 6012001 | 26-Oct-13 | 0.773 | N/A |
| 920747 | 6012001 | 16-Nov-13 | 0.398 | N/A |
| 920078 | 6012002 | 31-Aug-13 | High CV | N/A |
| 920078 | 6012002 | 28-Sep-13 | High CV | N/A |
| 920078 | 6012002 | 26-Oct-13 | 0.403 | N/A |
| 920078 | 6012002 | 16-Nov-13 | 0.324 | N/A |
| 920979 | 6012003 | 31-Aug-13 | High CV | N/A |
| 920979 | 6012003 | 28-Sep-13 | 0.368 | N/A |
| 920979 | 6012003 | 26-Oct-13 | 0.392 | N/A |
| 920979 | 6012003 | 16-Nov-13 | High CV | N/A |
| 920783 | 6012004 | 31-Aug-13 | 0.915 | N/A |
| 920783 | 6012004 | 28-Sep-13 | 0.386 | N/A |
| 920783 | 6012004 | 26-Oct-13 | 0.448 | N/A |
| 920783 | 6012004 | 16-Nov-13 | 0.451 | N/A |
| 921078 | 6012005 | 31-Aug-13 | 0.415 | N/A |
| 921078 | 6012005 | 28-Sep-13 | 0.525 | N/A |
| 921078 | 6012005 | 26-Oct-13 | 0.309 | N/A |
| 921078 | 6012005 | 16-Nov-13 | 0.290 | N/A |
| 920192 | 6012006 | 31-Aug-13 | 0.371 | N/A |
| 920192 | 6012006 | 28-Sep-13 | 0.482 | N/A |
| 920192 | 6012006 | 26-Oct-13 | 0.343 | N/A |
| 920192 | 6012006 | 16-Nov-13 | 0.281 | N/A |
| 920924 | 6012007 | 31-Aug-13 | 0.222 | N/A |
| 920924 | 6012007 | 28-Sep-13 | 0.408 | N/A |
| 920924 | 6012007 | 26-Oct-13 | 0.469 | N/A |
| 920924 | 6012007 | 16-Nov-13 | 0.450 | N/A |
| 920653 | 6012008 | 31-Aug-13 | High CV | N/A |
| 920653 | 6012008 | 28-Sep-13 | 0.240 | N/A |
| 920653 | 6012008 | 26-Oct-13 | 0.125 | N/A |
| 920653 | 6012008 | 16-Nov-13 | 0.301 | N/A |
| 920128 | 6012009 | 31-Aug-13 | 0.317 | N/A |
| 920128 | 6012009 | 28-Sep-13 | 0.298 | N/A |
| 920128 | 6012009 | 26-Oct-13 | 0.534 | N/A |

| Accession # | Mouse ID # | Collection Data | insulin (ng/ml) | Comments |
|---|---|---|---|---|
| 920128 | 6012009 | 16-Nov-13 | 0.640 | N/A |
| 920310 | 6012010 | 31-Aug-13 | High CV | N/A |
| 920310 | 6012010 | 28-Sep-13 | 0.340 | N/A |
| 920310 | 6012010 | 26-Oct-13 | 0.364 | N/A |
| 920310 | 6012010 | 16-Nov-13 | 0.210 | N/A |
| 920385 | 6022001 | 31-Aug-13 | 0.444 | N/A |
| 920385 | 6022001 | 28-Sep-13 | 0.736 | N/A |
| 920385 | 6022001 | 26-Oct-13 | 0.610 | N/A |
| 920385 | 6022001 | 16-Nov-13 | 0.423 | N/A |
| 920616 | 6022002 | 31-Aug-13 | 0.321 | N/A |
| 920616 | 6022002 | 28-Sep-13 | 0.636 | N/A |
| 920616 | 6022002 | 26-Oct-13 | 0.992 | N/A |
| 920616 | 6022002 | 16-Nov-13 | 0.400 | N/A |
| 920569 | 6022003 | 31-Aug-13 | 0.333 | N/A |
| 920569 | 6022003 | 28-Sep-13 | 0.345 | N/A |
| 920569 | 6022003 | 26-Oct-13 | 0.466 | N/A |
| 920569 | 6022003 | 16-Nov-13 | 0.263 | N/A |
| 920293 | 6022004 | 31-Aug-13 | 0.205 | N/A |
| 920293 | 6022004 | 28-Sep-13 | 0.280 | N/A |
| 920293 | 6022004 | 26-Oct-13 | 0.579 | N/A |
| 920293 | 6022004 | 16-Nov-13 | 0.694 | N/A |
| 920215 | 6022005 | 31-Aug-13 | 0.494 | N/A |
| 920215 | 6022005 | 28-Sep-13 | 0.318 | N/A |
| 920215 | 6022005 | 26-Oct-13 | 0.672 | N/A |
| 920215 | 6022005 | 16-Nov-13 | 0.356 | N/A |
| 920813 | 6022006 | 31-Aug-13 | High CV | N/A |
| 920813 | 6022006 | 28-Sep-13 | 0.403 | N/A |
| 920813 | 6022006 | 26-Oct-13 | 0.317 | N/A |
| 920813 | 6022006 | 16-Nov-13 | 0.257 | N/A |
| 920196 | 6022007 | 31-Aug-13 | High CV | N/A |
| 920196 | 6022007 | 28-Sep-13 | 0.428 | N/A |
| 920196 | 6022007 | 26-Oct-13 | 0.410 | N/A |
| 920196 | 6022007 | 16-Nov-13 | 0.308 | N/A |
| 920834 | 6022008 | 31-Aug-13 | 0.906 | N/A |
| 920834 | 6022008 | 28-Sep-13 | High CV | N/A |
| 920834 | 6022008 | 26-Oct-13 | 0.887 | N/A |
| 920834 | 6022008 | 16-Nov-13 | 0.656 | N/A |
| 920581 | 6022009 | 31-Aug-13 | 0.250 | N/A |

| Accession # | Mouse ID # | Collection Data | insulin (ng/ml) | Comments |
|---|---|---|---|---|
| 920581 | 6022009 | 28-Sep-13 | 1.113 | N/A |
| 920581 | 6022009 | 26-Oct-13 | 0.740 | N/A |
| 920581 | 6022009 | 16-Nov-13 | 0.552 | N/A |
| 920723 | 6022010 | 31-Aug-13 | High CV | N/A |
| 920723 | 6022010 | 28-Sep-13 | 0.527 | N/A |
| 920723 | 6022010 | 26-Oct-13 | 0.265 | N/A |
| 920723 | 6022010 | 16-Nov-13 | 0.182 | N/A |
| 920983 | 6032001 | 31-Aug-13 | 0.495 | N/A |
| 920983 | 6032001 | 28-Sep-13 | 0.716 | N/A |
| 920983 | 6032001 | 26-Oct-13 | 0.377 | N/A |
| 920983 | 6032001 | 16-Nov-13 | 0.450 | N/A |
| 920317 | 6032002 | 31-Aug-13 | 0.526 | N/A |
| 920317 | 6032002 | 28-Sep-13 | 0.507 | N/A |
| 920317 | 6032002 | 26-Oct-13 | 0.707 | N/A |
| 920317 | 6032002 | 16-Nov-13 | 0.327 | N/A |
| 920683 | 6032003 | 31-Aug-13 | 1.264 | N/A |
| 920683 | 6032003 | 28-Sep-13 | 1.293 | N/A |
| 920683 | 6032003 | 26-Oct-13 | 1.006 | N/A |
| 920683 | 6032003 | 16-Nov-13 | High CV | N/A |
| 920515 | 6032004 | 31-Aug-13 | 0.554 | N/A |
| 920515 | 6032004 | 28-Sep-13 | 0.388 | N/A |
| 920515 | 6032004 | 26-Oct-13 | 0.342 | N/A |
| 920515 | 6032004 | 16-Nov-13 | 1.150 | N/A |
| 920645 | 6032005 | 31-Aug-13 | 0.341 | N/A |
| 920645 | 6032005 | 28-Sep-13 | 0.460 | N/A |
| 920645 | 6032005 | 26-Oct-13 | 0.160 | N/A |
| 920645 | 6032005 | 16-Nov-13 | 0.467 | N/A |
| 920557 | 6032006 | 31-Aug-13 | 0.777 | N/A |
| 920557 | 6032006 | 28-Sep-13 | 0.744 | N/A |
| 920557 | 6032006 | 26-Oct-13 | 0.369 | N/A |
| 920557 | 6032006 | 16-Nov-13 | 1.283 | N/A |
| 920675 | 6032007 | 31-Aug-13 | 0.878 | N/A |
| 920675 | 6032007 | 28-Sep-13 | 0.420 | N/A |
| 920675 | 6032007 | 26-Oct-13 | 0.472 | N/A |
| 920675 | 6032007 | 16-Nov-13 | 0.211 | N/A |
| 920435 | 6032008 | 31-Aug-13 | 0.769 | N/A |
| 920435 | 6032008 | 28-Sep-13 | 0.445 | N/A |
| 920435 | 6032008 | 26-Oct-13 | 0.309 | N/A |

| Accession # | Mouse ID # | Collection Data | insulin (ng/ml) | Comments |
|---|---|---|---|---|
| 920435 | 6032008 | 16-Nov-13 | 1.026 | N/A |
| 920203 | 6032009 | 31-Aug-13 | 0.581 | N/A |
| 920203 | 6032009 | 28-Sep-13 | 0.321 | N/A |
| 920203 | 6032009 | 26-Oct-13 | 0.332 | N/A |
| 920203 | 6032009 | 16-Nov-13 | 0.703 | N/A |
| 920345 | 6032010 | 31-Aug-13 | 0.761 | N/A |
| 920345 | 6032010 | 28-Sep-13 | 0.641 | N/A |
| 920345 | 6032010 | 26-Oct-13 | 0.517 | N/A |
| 920345 | 6032010 | 16-Nov-13 | 0.390 | N/A |
| 920577 | 6112001 | 31-Aug-13 | 0.646 | N/A |
| 920577 | 6112001 | 28-Sep-13 | 0.369 | N/A |
| 920577 | 6112001 | 26-Oct-13 | 0.390 | N/A |
| 920577 | 6112001 | 16-Nov-13 | 0.262 | N/A |
| 920069 | 6112002 | 31-Aug-13 | 0.874 | N/A |
| 920069 | 6112002 | 28-Sep-13 | 1.162 | N/A |
| 920069 | 6112002 | 26-Oct-13 | 0.560 | N/A |
| 920069 | 6112002 | 16-Nov-13 | 0.487 | N/A |
| 920487 | 6112003 | 31-Aug-13 | 0.519 | N/A |
| 920487 | 6112003 | 28-Sep-13 | 0.310 | N/A |
| 920487 | 6112003 | 26-Oct-13 | 0.555 | N/A |
| 920487 | 6112003 | 16-Nov-13 | 0.420 | N/A |
| 920558 | 6112004 | 31-Aug-13 | 0.592 | N/A |
| 920558 | 6112004 | 28-Sep-13 | 0.680 | N/A |
| 920558 | 6112004 | 26-Oct-13 | No sample | N/A |
| 920558 | 6112004 | 16-Nov-13 | No sample | N/A |
| 920939 | 6112005 | 31-Aug-13 | 0.323 | N/A |
| 920939 | 6112005 | 28-Sep-13 | 0.693 | N/A |
| 920939 | 6112005 | 26-Oct-13 | 0.586 | N/A |
| 920939 | 6112005 | 16-Nov-13 | 0.879 | N/A |
| 920090 | 6112006 | 31-Aug-13 | 0.556 | N/A |
| 920090 | 6112006 | 28-Sep-13 | 0.460 | N/A |
| 920090 | 6112006 | 26-Oct-13 | 0.718 | N/A |
| 920090 | 6112006 | 16-Nov-13 | 0.680 | N/A |
| 920889 | 6112007 | 31-Aug-13 | 0.714 | N/A |
| 920889 | 6112007 | 28-Sep-13 | 0.383 | N/A |
| 920889 | 6112007 | 26-Oct-13 | 0.483 | N/A |
| 920889 | 6112007 | 16-Nov-13 | 0.377 | N/A |
| 920759 | 6112008 | 31-Aug-13 | 0.600 | N/A |

| Accession # | Mouse ID # | Collection Data | insulin (ng/ml) | Comments |
|---|---|---|---|---|
| 920759 | 6112008 | 28-Sep-13 | 0.496 | N/A |
| 920759 | 6112008 | 26-Oct-13 | 0.606 | N/A |
| 920759 | 6112008 | 16-Nov-13 | 0.987 | N/A |
| 920043 | 6112009 | 31-Aug-13 | 0.344 | N/A |
| 920043 | 6112009 | 28-Sep-13 | 0.602 | N/A |
| 920043 | 6112009 | 26-Oct-13 | 0.862 | N/A |
| 920043 | 6112009 | 16-Nov-13 | 0.701 | N/A |
| 921085 | 6112010 | 31-Aug-13 | 0.760 | N/A |
| 921085 | 6112010 | 28-Sep-13 | 0.959 | N/A |
| 921085 | 6112010 | 26-Oct-13 | 0.456 | N/A |
| 921085 | 6112010 | 16-Nov-13 | 0.617 | N/A |
| 920471 | 6112011 | 31-Aug-13 | 0.424 | N/A |
| 920471 | 6112011 | 28-Sep-13 | 1.289 | N/A |
| 920471 | 6112011 | 26-Oct-13 | 1.202 | N/A |
| 920471 | 6112011 | 16-Nov-13 | 1.307 | N/A |
| 920388 | 6112012 | 31-Aug-13 | 0.650 | N/A |
| 920388 | 6112012 | 28-Sep-13 | 0.917 | N/A |
| 920388 | 6112012 | 26-Oct-13 | 0.473 | N/A |
| 920388 | 6112012 | 16-Nov-13 | 1.748 | N/A |
| 920636 | 6112013 | 31-Aug-13 | 0.787 | N/A |
| 920636 | 6112013 | 28-Sep-13 | 0.614 | N/A |
| 920636 | 6112013 | 26-Oct-13 | 0.590 | N/A |
| 920636 | 6112013 | 16-Nov-13 | 0.946 | N/A |
| 920998 | 6112014 | 31-Aug-13 | 0.629 | N/A |
| 920998 | 6112014 | 28-Sep-13 | 0.459 | N/A |
| 920998 | 6112014 | 26-Oct-13 | 0.275 | N/A |
| 920998 | 6112014 | 16-Nov-13 | 0.299 | N/A |
| 920286 | 6112015 | 31-Aug-13 | 0.460 | N/A |
| 920286 | 6112015 | 28-Sep-13 | 0.552 | N/A |
| 920286 | 6112015 | 26-Oct-13 | 0.456 | N/A |
| 920286 | 6112015 | 16-Nov-13 | 0.430 | N/A |
| 920026 | 6122001 | 31-Aug-13 | 0.715 | N/A |
| 920026 | 6122001 | 28-Sep-13 | 0.424 | N/A |
| 920026 | 6122001 | 26-Oct-13 | 0.450 | N/A |
| 920026 | 6122001 | 16-Nov-13 | 0.294 | N/A |
| 920274 | 6122002 | 31-Aug-13 | 0.797 | N/A |
| 920274 | 6122002 | 28-Sep-13 | High CV | N/A |
| 920274 | 6122002 | 26-Oct-13 | 1.231 | N/A |

| Accession # | Mouse ID # | Collection Data | insulin (ng/ml) | Comments |
|---|---|---|---|---|
| 920274 | 6122002 | 16-Nov-13 | 0.604 | N/A |
| 920884 | 6122003 | 31-Aug-13 | 1.223 | N/A |
| 920884 | 6122003 | 28-Sep-13 | 0.548 | N/A |
| 920884 | 6122003 | 26-Oct-13 | 0.457 | N/A |
| 920884 | 6122003 | 16-Nov-13 | 0.513 | N/A |
| 920943 | 6122004 | 31-Aug-13 | 0.671 | N/A |
| 920943 | 6122004 | 28-Sep-13 | 1.441 | N/A |
| 920943 | 6122004 | 26-Oct-13 | 0.918 | N/A |
| 920943 | 6122004 | 16-Nov-13 | 1.088 | N/A |
| 920605 | 6122005 | 31-Aug-13 | 0.712 | N/A |
| 920605 | 6122005 | 28-Sep-13 | 2.691 | N/A |
| 920605 | 6122005 | 26-Oct-13 | 0.322 | N/A |
| 920605 | 6122005 | 16-Nov-13 | 0.415 | N/A |
| 920711 | 6122006 | 31-Aug-13 | 0.496 | N/A |
| 920711 | 6122006 | 28-Sep-13 | 1.032 | N/A |
| 920711 | 6122006 | 26-Oct-13 | 0.497 | N/A |
| 920711 | 6122006 | 16-Nov-13 | 0.498 | N/A |
| 920754 | 6122007 | 31-Aug-13 | High CV | N/A |
| 920754 | 6122007 | 28-Sep-13 | 0.492 | N/A |
| 920754 | 6122007 | 26-Oct-13 | 0.600 | N/A |
| 920754 | 6122007 | 16-Nov-13 | 0.409 | N/A |
| 920640 | 6122008 | 31-Aug-13 | High CV | N/A |
| 920640 | 6122008 | 28-Sep-13 | 0.384 | N/A |
| 920640 | 6122008 | 26-Oct-13 | 0.477 | N/A |
| 920640 | 6122008 | 16-Nov-13 | 0.504 | N/A |
| 920565 | 6122009 | 31-Aug-13 | 1.364 | N/A |
| 920565 | 6122009 | 28-Sep-13 | 0.828 | N/A |
| 920565 | 6122009 | 26-Oct-13 | 0.180 | N/A |
| 920565 | 6122009 | 16-Nov-13 | 0.347 | N/A |
| 920475 | 6122010 | 31-Aug-13 | High CV | N/A |
| 920475 | 6122010 | 28-Sep-13 | 1.033 | N/A |
| 920475 | 6122010 | 26-Oct-13 | 0.264 | N/A |
| 920475 | 6122010 | 16-Nov-13 | 0.405 | N/A |
| 920931 | 6122011 | 31-Aug-13 | 0.532 | N/A |
| 920931 | 6122011 | 28-Sep-13 | 0.486 | N/A |
| 920931 | 6122011 | 26-Oct-13 | 0.494 | N/A |
| 920931 | 6122011 | 16-Nov-13 | 0.468 | N/A |
| 920746 | 6122012 | 31-Aug-13 | 0.438 | N/A |

| Accession # | Mouse ID # | Collection Data | insulin (ng/ml) | Comments |
|---|---|---|---|---|
| 920746 | 6122012 | 28-Sep-13 | High CV | N/A |
| 920746 | 6122012 | 26-Oct-13 | 0.086 | N/A |
| 920746 | 6122012 | 16-Nov-13 | 0.376 | N/A |
| 921073 | 6122013 | 31-Aug-13 | 0.886 | N/A |
| 921073 | 6122013 | 28-Sep-13 | 0.830 | N/A |
| 921073 | 6122013 | 26-Oct-13 | 0.758 | N/A |
| 921073 | 6122013 | 16-Nov-13 | 0.364 | N/A |
| 920376 | 6122014 | 31-Aug-13 | 1.229 | N/A |
| 920376 | 6122014 | 28-Sep-13 | 0.888 | N/A |
| 920376 | 6122014 | 26-Oct-13 | 0.570 | N/A |
| 920376 | 6122014 | 16-Nov-13 | 0.539 | N/A |
| 920687 | 6122015 | 31-Aug-13 | 1.077 | N/A |
| 920687 | 6122015 | 28-Sep-13 | 0.700 | N/A |
| 920687 | 6122015 | 26-Oct-13 | 0.952 | N/A |
| 920687 | 6122015 | 16-Nov-13 | 0.633 | N/A |
| 920482 | 6132001 | 31-Aug-13 | 0.853 | N/A |
| 920482 | 6132001 | 28-Sep-13 | 0.768 | N/A |
| 920482 | 6132001 | 26-Oct-13 | 0.871 | N/A |
| 920482 | 6132001 | 16-Nov-13 | 1.469 | N/A |
| 920305 | 6132002 | 31-Aug-13 | 1.138 | N/A |
| 920305 | 6132002 | 28-Sep-13 | 1.108 | N/A |
| 920305 | 6132002 | 26-Oct-13 | 0.908 | N/A |
| 920305 | 6132002 | 16-Nov-13 | 1.193 | N/A |
| 920664 | 6132003 | 31-Aug-13 | 0.749 | N/A |
| 920664 | 6132003 | 28-Sep-13 | 0.526 | N/A |
| 920664 | 6132003 | 26-Oct-13 | 0.544 | N/A |
| 920664 | 6132003 | 16-Nov-13 | 0.485 | N/A |
| 920506 | 6132004 | 31-Aug-13 | 0.359 | N/A |
| 920506 | 6132004 | 28-Sep-13 | 2.536 | N/A |
| 920506 | 6132004 | 26-Oct-13 | 0.339 | N/A |
| 920506 | 6132004 | 16-Nov-13 | 0.588 | N/A |
| 920704 | 6132005 | 31-Aug-13 | 0.385 | N/A |
| 920704 | 6132005 | 28-Sep-13 | High CV | N/A |
| 920704 | 6132005 | 26-Oct-13 | 0.267 | N/A |
| 920704 | 6132005 | 16-Nov-13 | 0.441 | N/A |
| 920561 | 6132006 | 31-Aug-13 | 0.564 | N/A |
| 920561 | 6132006 | 28-Sep-13 | 0.860 | N/A |
| 920561 | 6132006 | 26-Oct-13 | 0.547 | N/A |

| Accession # | Mouse ID # | Collection Data | insulin (ng/ml) | Comments |
|---|---|---|---|---|
| 920561 | 6132006 | 16-Nov-13 | 1.453 | N/A |
| 921104 | 6132007 | 31-Aug-13 | 1.151 | N/A |
| 921104 | 6132007 | 28-Sep-13 | 0.450 | N/A |
| 921104 | 6132007 | 26-Oct-13 | 0.477 | N/A |
| 921104 | 6132007 | 16-Nov-13 | 0.370 | N/A |
| 920431 | 6132008 | 31-Aug-13 | 0.382 | N/A |
| 920431 | 6132008 | 28-Sep-13 | 0.625 | N/A |
| 920431 | 6132008 | 26-Oct-13 | 0.337 | N/A |
| 920431 | 6132008 | 16-Nov-13 | 0.366 | N/A |
| 920608 | 6132009 | 31-Aug-13 | 2.218 | N/A |
| 920608 | 6132009 | 28-Sep-13 | 0.267 | N/A |
| 920608 | 6132009 | 26-Oct-13 | 0.704 | N/A |
| 920608 | 6132009 | 16-Nov-13 | 0.253 | N/A |
| 920726 | 6132010 | 31-Aug-13 | 0.890 | N/A |
| 920726 | 6132010 | 28-Sep-13 | 0.463 | N/A |
| 920726 | 6132010 | 26-Oct-13 | 1.012 | N/A |
| 920726 | 6132010 | 16-Nov-13 | High CV | N/A |
| 920419 | 6132011 | 31-Aug-13 | 0.759 | N/A |
| 920419 | 6132011 | 28-Sep-13 | 0.441 | N/A |
| 920419 | 6132011 | 26-Oct-13 | 0.426 | N/A |
| 920419 | 6132011 | 16-Nov-13 | 0.605 | N/A |
| 920549 | 6132012 | 31-Aug-13 | 0.858 | N/A |
| 920549 | 6132012 | 28-Sep-13 | 1.294 | N/A |
| 920549 | 6132012 | 26-Oct-13 | 0.297 | N/A |
| 920549 | 6132012 | 16-Nov-13 | 0.337 | N/A |
| 920502 | 6132013 | 31-Aug-13 | 2.066 | N/A |
| 920502 | 6132013 | 28-Sep-13 | 0.528 | N/A |
| 920502 | 6132013 | 26-Oct-13 | 0.558 | N/A |
| 920502 | 6132013 | 16-Nov-13 | 0.497 | N/A |
| 920187 | 6132014 | 31-Aug-13 | 2.736 | N/A |
| 920187 | 6132014 | 28-Sep-13 | 0.601 | N/A |
| 920187 | 6132014 | 26-Oct-13 | 0.569 | N/A |
| 920187 | 6132014 | 16-Nov-13 | 0.281 | N/A |
| 920443 | 6132015 | 31-Aug-13 | 0.851 | N/A |
| 920443 | 6132015 | 28-Sep-13 | 0.556 | N/A |
| 920443 | 6132015 | 26-Oct-13 | 0.393 | N/A |
| 920443 | 6132015 | 16-Nov-13 | 0.271 | N/A |

Legend: High CV – data point not accepted due to high %CV; No sample – tube or vial for the specific mouse/time point was not received at Cambridge Biomedical.

**TABLE 2: COMPARISON OF AVERAGE VALUES BETWEEN THE DIFFERENT GROUPS**

Student's t-test *p* values - CHOW

| | Baseline | 1 month | 2 months | 3 months |
|---|---|---|---|---|
| Sham vs 3R4F | 0.50 | 0.05 | 0.03 | 0.30 |
| Sham vs MRTP | 0.03 | 0.02 | 0.32 | 0.02 |

Student's t-test *p* values - HFD

| | Baseline | 1 month | 2 months | 3 months |
|---|---|---|---|---|
| Sham vs 3R4F | 0.01 | 0.09 | 0.36 | 0.03 |
| Sham vs MRTP | 0.01 | 0.23 | 0.34 | 0.25 |

All data is stored on the Cambridge Biomedical Shared Files Drive>02 Clinical Diagnostics>SoftMax GxP admin>SoftMax R&D>177A.

## 10.0 LIST OF FIGURES

### A. TIME COURSE OF PLASMA INSULIN IN CHOW FED MICE

All graphs show the mean of all data points ± 1 standard deviation

**FIGURE 1 – SHAM**

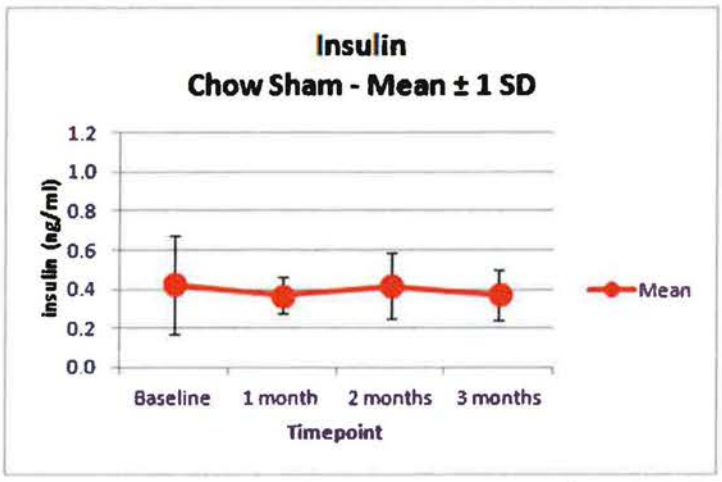


**FIGURE 2 – 3R4F**

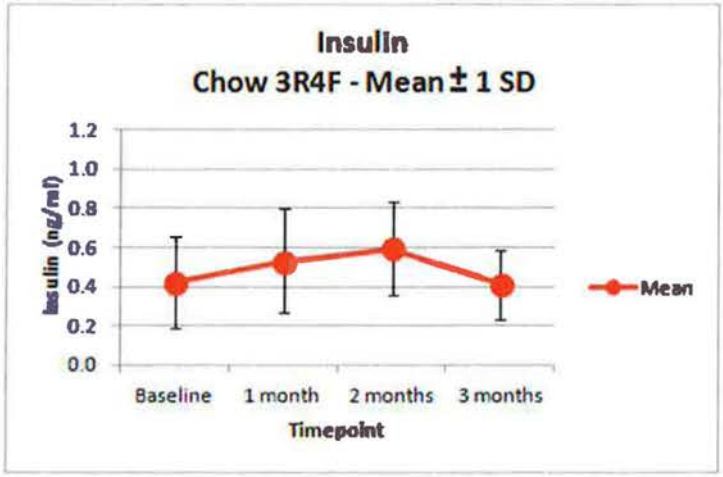
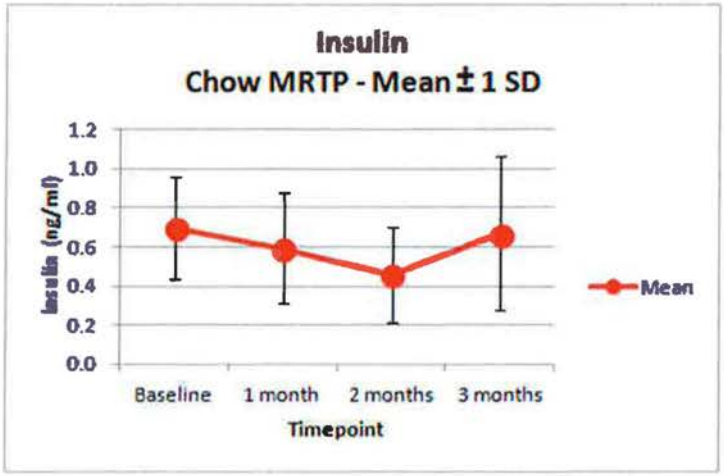



**FIGURE 3 – MRTP**



## B. TIME COURSE OF PLASMA INSULIN IN HIGH FAT DIET MICE

**FIGURE 4 – SHAM**

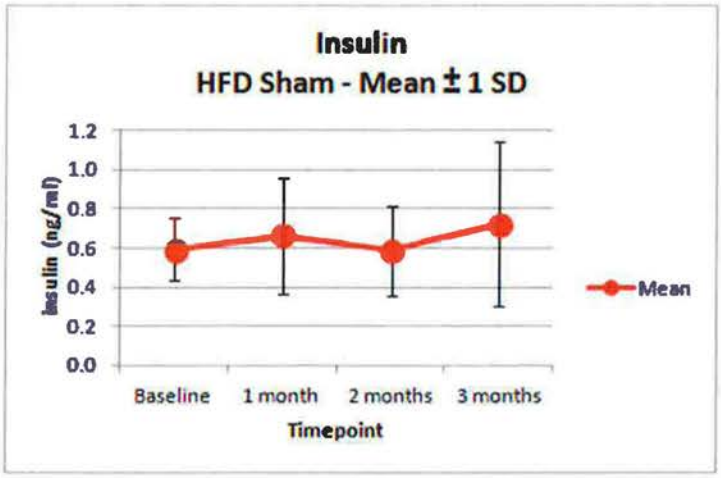



**FIGURE 5 – 3R4F**

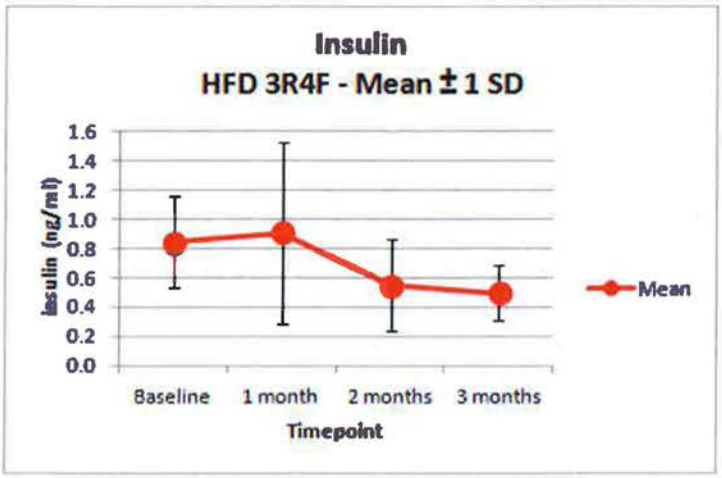
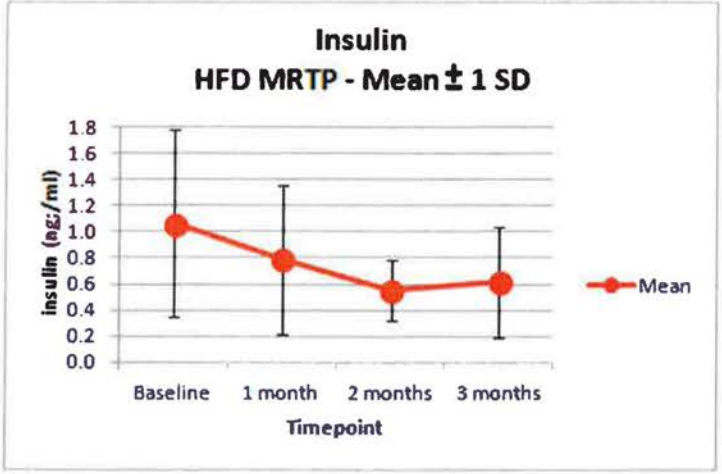


**FIGURE 6 – MRTP**




## C. TIME COURSE OF PLASMA INSULIN – ALL MOUSE SAMPLES BY GROUP

**FIGURE 7 – CHOW SHAM**

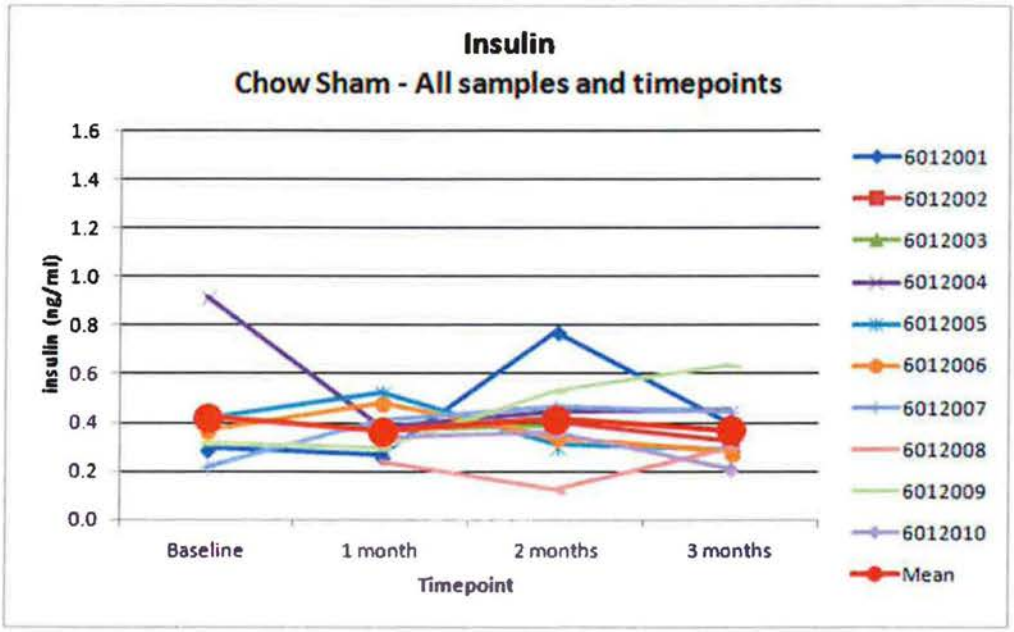


**FIGURE 8 – CHOW 3R4F**

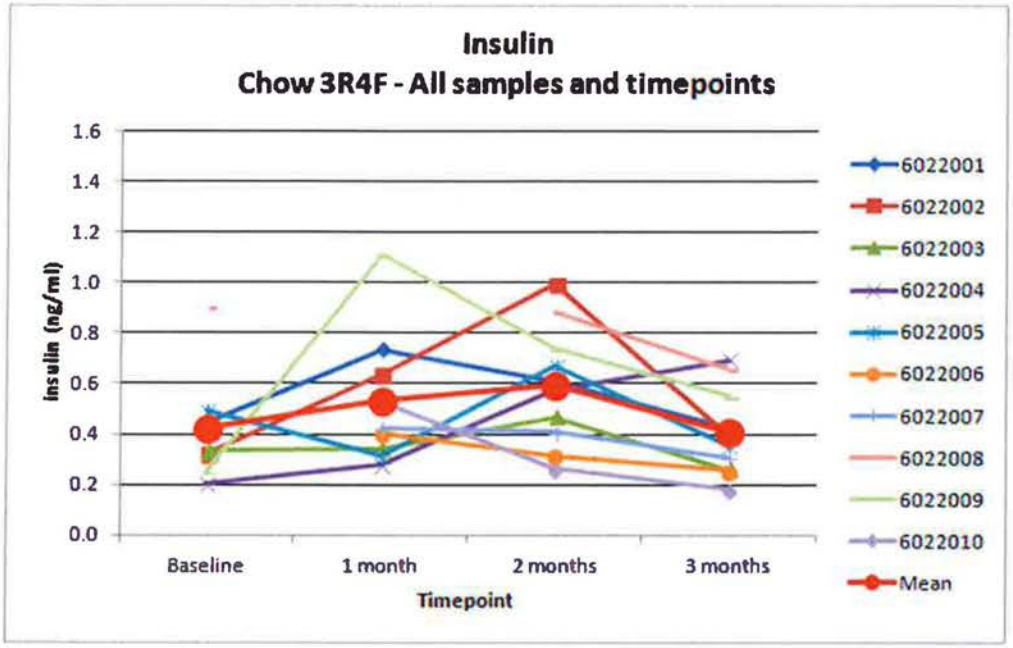



**FIGURE 9 – CHOW MRTP**

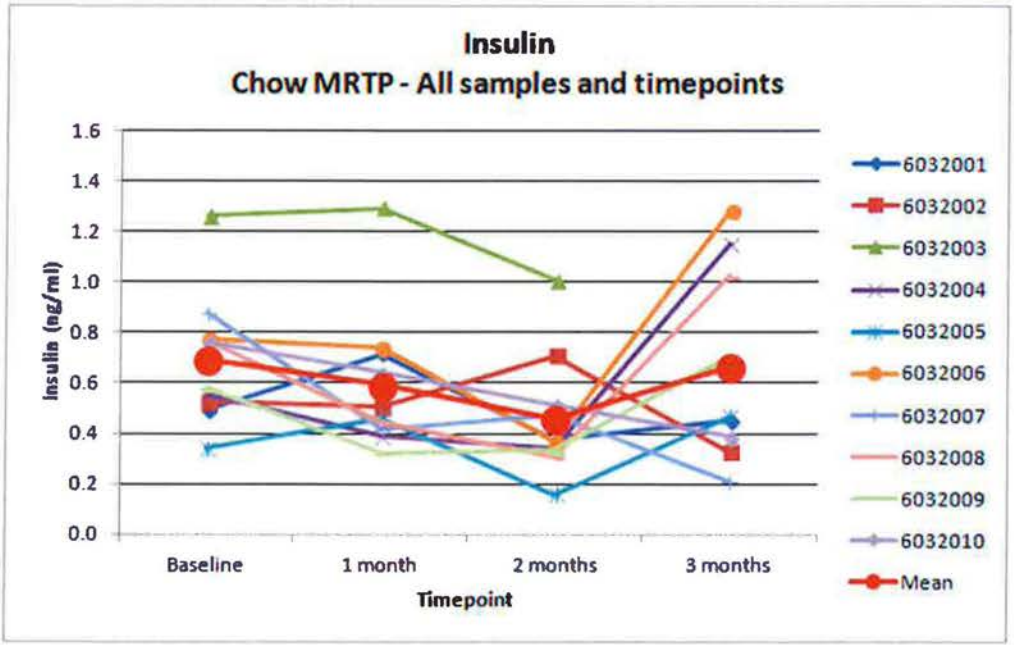


**FIGURE 10 – HFD SHAM**

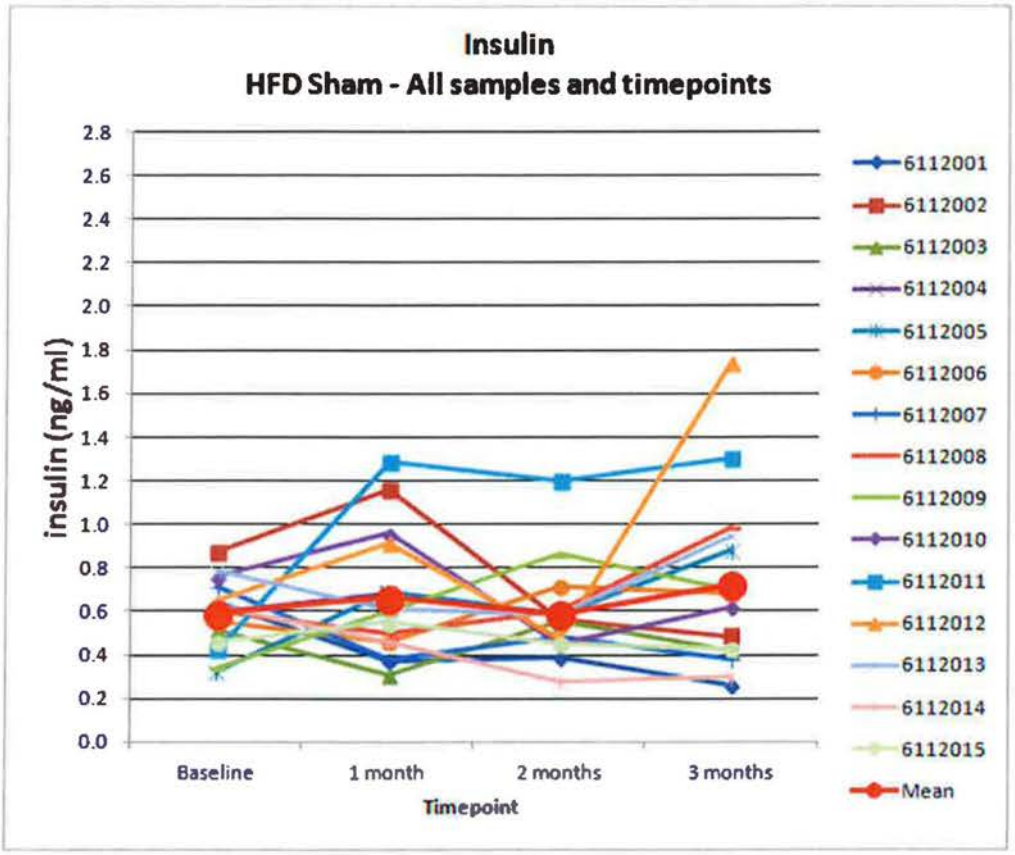


**FIGURE 11 – HFD 3R4F**

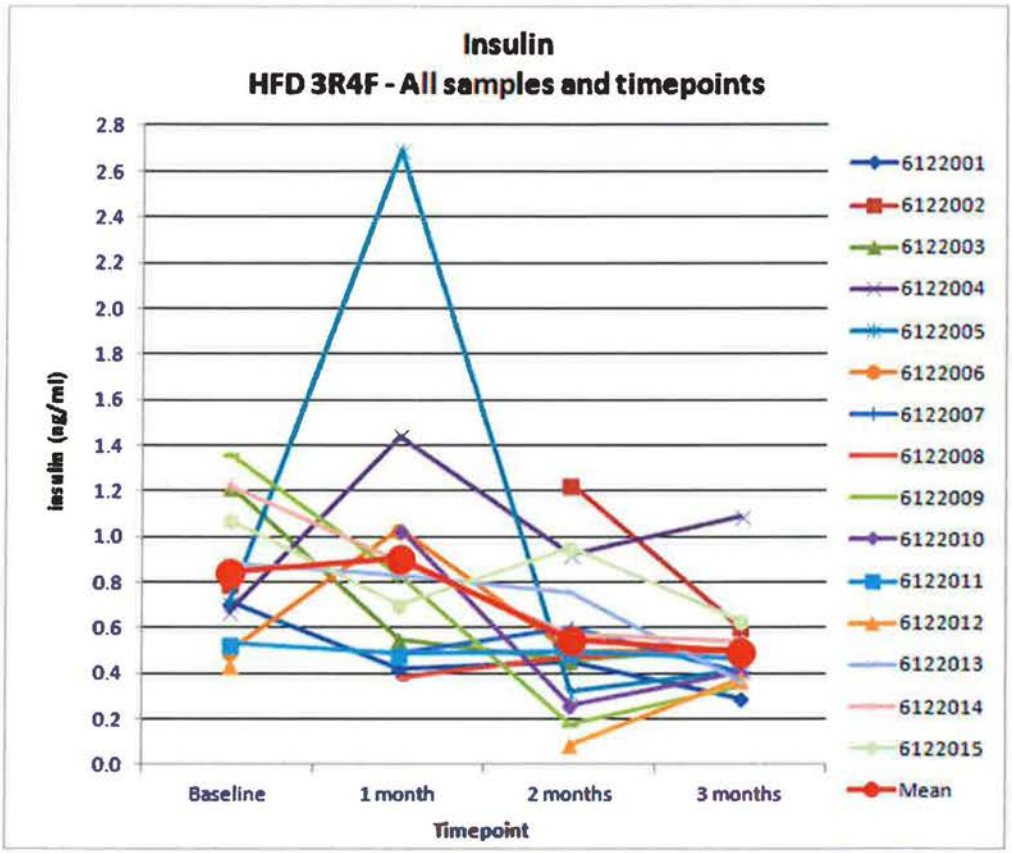
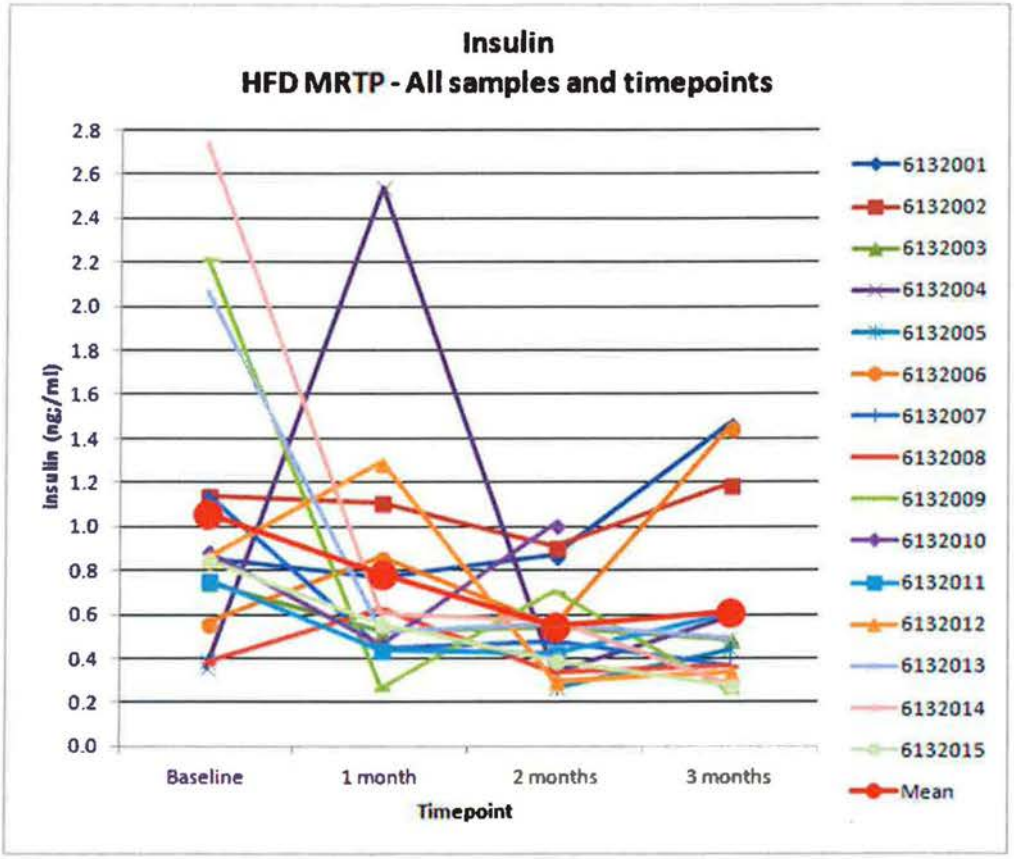


**FIGURE 12 – HFD MRTP**




## 11.0 APPENDICES

There are no appendices in this report.