

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

201849Orig1s000

**CLINICAL PHARMACOLOGY AND
BIOPHARMACEUTICS REVIEW(S)**

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA: 201849	Submission Date(s): 8/8/2014; 9/18/15; 12/19/14; 2/20/15
Generic Name	Glucagon for Injection
OCP Division	Clinical Pharmacology-2
OND Division	Metabolism and Endocrinology Products
Sponsor	Fresenius Kabi LLC
Submission Type, Code	NDA 505 (b) (2); Resubmission
Formulation; Strength(s)	Lyophilized powder for reconstitution; 1 mg
Proposed Indication	For use during radiologic examinations to temporarily inhibit movement of the gastrointestinal tract.
Clinical Pharmacology Reviewer	Jayabharathi Vaidyanathan, Ph.D
Clinical Pharmacology TL (Acting)	Manoj Khurana, Ph.D

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1 Executive Summary

1.1 Recommendation

The Office of Clinical Pharmacology (DCP-2) has reviewed the clinical pharmacology data submitted on 8/8/2014 under NDA 201849 and recommend approval.

1.2 Phase IV Commitments

None.

1.3 Summary of Important Clinical Pharmacology Findings

The proposed Glucagon for injection drug product is a sterile lyophilized powder intended for administration as a solution following reconstitution via intramuscular (IM), or intravenous (IV) injection. This application is a 505 (b) (2) application which relies on the Agency's previous finding of safety and effectiveness for the reference listed drug, Glucagen 1mg (1 IU) (NDA 20918, held by Novo Nordisk, and distributed by Bedford Laboratories). The proposed drug product and the reference listed drug are manufactured by different methods; Glucagen is a recombinant product, while the proposed glucagon product is a synthetic product. The reference Glucagen is approved for administration by subcutaneous (SC), IM or IV for the following indications:

- Emergency use for treatment in severe hypoglycemia
- As a diagnostic aid during radiologic examinations to temporarily inhibit gastrointestinal motility

The sponsor for the proposed glucagon for injection product is seeking approval for only the diagnostic indication (administration by IM and IV routes). (b) (4)

This application consists of a relative bioavailability study comparing the pharmacokinetics/pharmacodynamics of the proposed synthetic glucagon product to the reference product following SC administration.

Results indicate that the synthetic glucagon product met the bioequivalence criteria for the glucagon AUC_{inf} and C_{max} parameters (baseline uncorrected glucagon pharmacokinetic PK parameters) (Table 1).

Table 1: Statistical comparison of the PK parameters of synthetic glucagon and reference glucagon (baseline uncorrected) products following SC administration

Parameter	Test LS Means	Reference LS Means	Ratio	90% CI
C _{max} (pg/mL)	3532.87	3308.96	106.77	94.05 – 121.20
AUC _{0-t} (pg.h/mL)	3019.96	2462.14	122.66	111.38 – 135.07
AUC _{inf} (pg.h/mL)	3071.92	2759.31	111.33	102.14 – 121.35

In addition, the glucose (pharmacodynamic, PD; baseline corrected glucose) parameters met the bioequivalence (BE) criteria (Table 2).

Table 2: Statistical comparison of baseline corrected glucose parameters for the synthetic glucagon and reference glucagon products following SC administration

Parameter	Least-Square Means		Ratio	90% Confidence Intervals	
	Test	Reference		Lower CI	Upper CI
C _{max}	941.47	991.65	94.94	86.22	104.54
AUC ₀₋₂	921.54	927.54	99.35	85.64	115.26
AUC ₀₋₄	979.16	958.87	102.12	88.04	118.45

The proposed glucagon product is acceptable from a clinical pharmacology perspective based on the following:

- Meeting the bioequivalence criteria following SC administration for glucagon C_{max} and AUC_{inf} (PK) as compared to the reference glucagon.
- Glucose (PD) comparability was established in the current submission (Table 2). As shown the glucose AUC and C_{max} met the bioequivalence criteria.
- Additional supportive information comes from the PK data from the previous submission containing the bioequivalence study conducted for the proposed synthetic glucagon product as compared to the reference product following IM administration. As shown in the clinical pharmacology review (dated 8/27/2012 in DARRTS), the glucagon PK parameters met BE criteria following IM administration (Table 3):

Table 3: Statistical comparison of the PK parameters of baseline uncorrected synthetic glucagon and reference glucagon products following IM administration

Parameter	Test A (N=50)	Reference B (N=50)	Ratio	CI*	Intra-Subject %CV
AUC _{0-t} (pg·hr/mL)	2829.16	2959.26	0.9560	0.9078 - 1.0069	21.9512
AUC _{0-inf} (pg·hr/mL)	2947.78	3074.50	0.9588	0.9094 - 1.0108	21.5108
C _{max} (pg/ml)	3391.01	3817.62	0.8883	0.8198 - 0.9625	28.5482

- There were uncertainties in the PD (plasma glucose) data for the proposed glucagon product as compared to the reference product following IM administration. However, it is expected that the PD of the synthetic glucagon via IM route will be similar to the reference product based on the following:
 - Demonstration of PK and PD bioequivalence following SC administration with the proposed glucagon product.
 - Known PK/PD relationship of glucagon (see pages 7-8), which indicate that the glucose response appears to be saturated at very low doses of glucagon (e.g., 0.25 mg). Further, there were no differences in the glucagon PD profile following IM or SC route as demonstrated by Graf et. al., J. Pharm Sci, 1999;88(10). Therefore, the proposed 1 mg dose is expected to result in maximal clinical response.

2 Question-Based Review (QBR)

2.1 General Attributes of the Drug and Drug Product

Glucagon is a naturally occurring peptide hormone consisting of 29 amino acids that is secreted by the alpha cells of the pancreas. Glucagon for Injection in this submission is a synthetic human glucagon manufactured by a solid phase peptide method (b) (4)

The reference product, Glucagon for injection is produced by recombinant DNA technology.

2.1.1 What pertinent background or history contributes to the current assessment of the clinical pharmacology and biopharmaceutics of this drug?

The regulatory history for this application is summarized in the Table 4 below:

Table 4: Regulatory history for NDA 201849, Glucagon for injection

Date	Regulatory Action	Key comments
9/30/2010	Original NDA submitted by APP Pharmaceuticals	
12/3/2010	Refuse to file (RTF)	Nonclinical: Impurities above 1% limits were not qualified.
3/11/2011	Meeting to discuss RTF	Literature based PK bridging not acceptable. (b) (4)
11/30/2011	Resubmission after RTF	1 relative BA study (IM administration); (b) (4)
9/27/2012	Complete Response (CR)	Bioanalytical assay for glucose not validated
11/27/2012	Meeting to discuss CR	Sponsor agreed to conduct a new study with glucagon administration via SC route
8/8/2014	Resubmission class 2	1 relative BA study; SC route; seeking only diagnostic aid indication.

2.1.2 What are the highlights of the chemistry and physicochemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?

Glucagon is the active ingredient for the proposed product (b) (4). The inactive ingredients (lactose NF and sterile water for injection USP) are the same as those used in the reference drug, GlucaGen®. The two formulations use the same drug substance and excipients at the same concentration. The formulation of the product used in the current bioavailability study is the same as that used in the previous study submitted on 11/30/2011.

2.1.3 What is the mechanism of action and therapeutic indication?

The extrahepatic effects of glucagon include relaxation of the smooth muscle of the stomach, duodenum, small (b) (4) and colon. Glucagon increases plasma glucose levels

and causes smooth muscle relaxation and an inotropic myocardial effect because of the stimulation of adenylate cyclase to produce cyclic adenosine monophosphate (cAMP). The cAMP initiates a series of reactions that leads to the degradation of glycogen to glucose. Hepatic stores of glycogen are needed for glucagon to exert an antihypoglycemic effect.

Glucagon for Injection is a gastrointestinal motility inhibitor. The proposed indication is for use during radiologic examinations to temporarily inhibit movement of the gastrointestinal tract.

2.2 General Clinical Pharmacology

2.2.1 What is the known PK/PD relationship for glucagon?

Graf et. al., (J. Pharm Sci, 1999; vol. 88, No.10) compared the PK and PD parameters of recombinant glucagon and animal source glucagon. The PK and PD of recombinant glucagon was assessed following intravenous (IV) bolus administration of 0.25, 0.5, 1.0 and 2.0 mg dose with a 7-10 days interval between doses. The glucagon PK showed dose-proportional increase for C_{max} and AUC in this dose range. Mean maximal plasma glucagon concentrations ranging from 37 to 368 ng/mL occurred within 0.05 h following the IV bolus dose. Glucagon was rapidly eliminated, with mean half-lives ranging from 0.13 to 0.30 h. The mean clearance was similar between the treatments (~59 L/h).

Mean maximal blood glucose concentrations in this study were similar for each treatment (129 to 136 mg/dL) and occurred within 0.36 h after the IV bolus dose of glucagon. This shows that the maximum glucodynamic effect is seen even at the lowest glucagon dose. Blood glucose levels returned to baseline values by 1 h in most subjects (Figure 1).

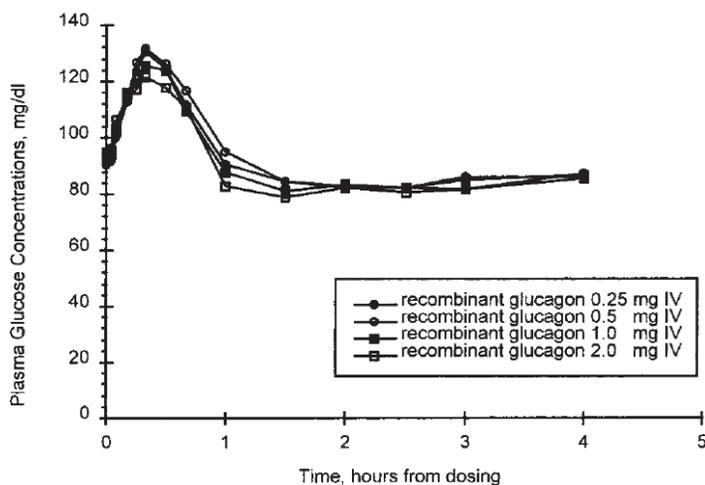


Figure 1: Mean blood glucose concentration versus time curves, all intravenous treatments.

Further the PK and PD was compared for the IM and SC route and the absolute bioavailability was evaluated as compared to the IV glucagon. The mean glucagon concentrations suggest rapid absorption with either of the route of administrations (IM and SC), with maximum concentrations attained approximately 0.21 and 0.35 after dosing. Slight differences in glucagon concentrations were noted between the injection routes with higher plasma concentrations occurring after SC administration. All glucagon formulations produced nearly identical glucose response curves after SC or IM administration (Figure 2).

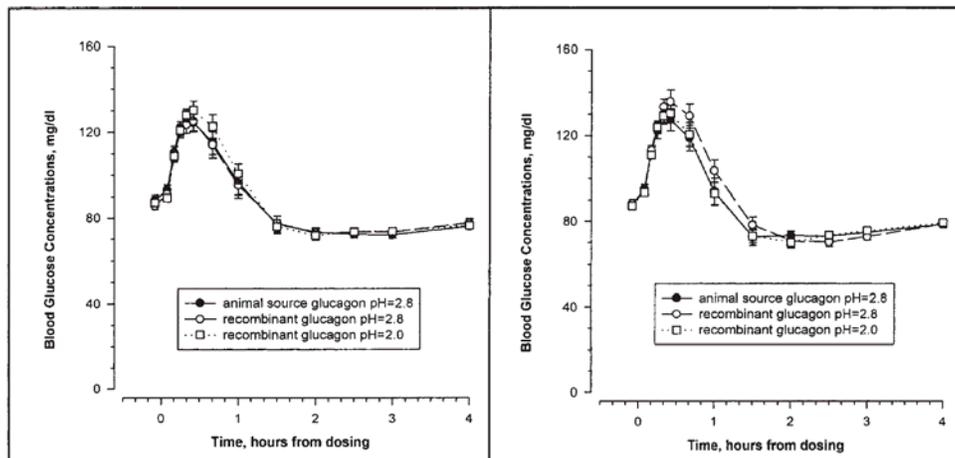


Figure 2: Mean blood glucose concentration versus time curves, all treatments. All glucagon doses were 1.0 mg. Left panel shows subcutaneous (SC) administrations; right panel shows intramuscular (IM) administrations

Overall, the authors demonstrated that there is PK dose-proportionality of glucagon and that the glucose response appears to be saturated with even low doses of glucagon. Additionally, there appears to be no differences in the glucose profiles following either IM or SC route. Therefore, the clinical dose of 1 mg is ensured to achieve the maximum glucose response regardless of the route of administration.

2.2.2 What are the PK/PD characteristics of the proposed synthetic glucagon for injection as compared to the reference Glucagon for Injection?

The pivotal clinical pharmacology study (GLUC-002-CP1) results demonstrated that the PK/PD profile of proposed synthetic glucagon for injection is similar to the reference Glucagon for injection after SC administration. This conclusion is based on the following observations:

- The comparison of the PK parameters C_{max} and AUC_{0-inf} for glucagon met the pre-specified BE criteria with LSM ratios and 90% CI within the 80 -125%.
- The comparison of the PD parameters C_{max} and AUC for glucose met the pre-specified BE criteria with LSM ratios and 90% CI within the 80 -125%.
- The time to peak glucagon and glucose were comparable. The median T_{max} for glucagon in the first and second treatment periods for the test product was 0.21 h and 0.17 h, respectively. While the median T_{max} for the reference

product was 0.25 h in both first and second replicate treatment periods. The median time for the maximum blood glucose for the test product was 0.83 h in the two replicate treatment periods, while it was 0.66 h and 0.83 h, respectively in the first and second replicate treatment period for the reference product.

- Collectively, this indicates similarity in the absorption characteristics (rate and extent) from the SC injection site of the test and reference glucagon for injection products. Similarity in the glucose response characteristics indicates that the in vivo PD activity of the glucagon is preserved with the synthetically manufactured glucagon.

The sponsor submitted the following clinical pharmacology study in this submission:

Study GLUC-002-CP1: “Bioequivalence of a Test Formulation of Glucagon for SC Injection Compared to Glucagon for Injection (Bedford Laboratories) Under Fasted Conditions”.

The primary objective of this study was to ascertain the PK and PD bioequivalence of an SC injection of 1 mg (1 IU) of Glucagon for Injection (Fresenius Kabi USA) in comparison to the reference product, GlucaGen (Novo Nordisk), 1 mg (1 IU), SC in healthy adult subjects. The primary endpoints in determining the bioequivalence of the proposed glucagon SC injection product was the baseline corrected PK (glucagon AUC and C_{max}) and PD (glucose AUC and BG_{max}) parameters. The sponsor also provided results for the uncorrected PK (glucagon AUC and C_{max}) and PD (glucose AUC and BG_{max}) parameters.

This study was a randomized, single-dose, single-blind, 2-treatment, 4-period, replicate crossover study design. During the course of this study, each subject received in each period either a single dose of 1 mg (1 IU) of glucagon (Fresenius Kabi USA) or a single dose of GlucaGen, 1 mg (1 IU), via SC injection. Blood samples were collected 3 times at 2, 1, and 0 hours prior to dosing and at intervals over 4 hours post-dose for the concentrations of glucagon and glucose. The washout of 7 days used in this study is considered to be sufficient to exclude any carryover effects based on the short elimination half-life of glucagon. All the subjects enrolled met the inclusion/exclusion criteria specified in the protocol. There were no protocol deviations. A total of 32 subjects entered the study and were randomized to study treatment. A total of 27 subjects completed the study (5 subjects discontinued early).

The products used in the study are as follows:

Test Product: A	1 mg (1 IU/mL) glucagon for injection (Fresenius Kabi USA), (Lot No: C113-002, Expiration Date: April 2015)
Reference Product: B	1 mg (1 IU/mL) of GlucaGen [®] for Injection (Bedford Laboratories), (Lot No: BW60511, Expiration Date: April 2014)

Two sets of PK/PD and statistical analyses were conducted for both glucagon and glucose. The first set of analyses was performed using uncorrected data. The mean of the 3 pre-dose

measurements (-2, -1, and 0 hours) was used as the 0-hour sample for analysis of AUC_{0-t} and AUC_{0-inf} . The second set of analyses was based on baseline corrected concentrations. All post-dose values were corrected for the mean of the 3 pre-dose measurements (-2, -1, and 0 hours) by subtracting the mean value from each of the post-dose values for each individual subject within the period. A similar analysis as described for AUC was also performed for C_{max} and BG_{max} .

The reviewer identified the following issues during review of this study:

- *Errors in batch information of products used and the route of administration in the Case Report Forms (CRF) used*
- *Outlier analysis*
- *Glucagon concentration: Baseline correction approach*
- *Normalization of potency/glucagon content in batches of test and reference glucagon product*

Errors in batch information of products used and the route of administration in the Case Report Forms (CRF) used: There was a discrepancy in the test and reference product Lot numbers and expiration dates between the Source Case Report Forms from clinical study GLUC-002-CP1 and the clinical study synopsis.

The information included in the Case Report Forms in submission dated 9/18/14 is included below:

“Test Product: 1 mg (1 IU/mL) Glucagon for injection (Synthetic) (APP Pharmaceuticals), Lot No.: C109-002. Expiration Date: 01 /2010, administered intravenously preceded by an overnight fast of 10 hours.

Reference Product: 1 mg (1 IU/mL) of Glucagon for Injection (rDNA origin) (Bedford Laboratories), Lot No.: vw60516, Expiration Date: 09/2010, administered intravenously preceded by an overnight fast of 10 hours.”

The information included in the synopsis of clinical study GLUC-002-CP1 (submission dated 8/8/14) is as follows:

“Test Product: 1 mg (1 IU/mL) Glucagon for Injection (Fresenius Kabi USA), (Lot No: C113-002, Expiration Date: April 2015).

Reference Product: 1 mg (1 IU/mL) of GlucaGen® (Bedford Laboratories), (Lot No: BW60511, Expiration Date: April 2014).”

The case report forms also indicate that the drug was administered intravenously to the subjects.

An information request was sent to the sponsor regarding these discrepancies. The sponsor responded that the information in the CRF was an error which they corrected subsequently. The sponsor’s claim was verified by the inspection conducted by OSI and was found to be acceptable (DARRT report dated 1/5/2015).

Outlier analysis: One of the subjects (Subject 7) had unusually high glucagon concentrations after the test product administration in the first replicate period (Figure 3). The protocol and the statistical analysis plan did not specify how to analyze such data from subjects. The sponsor used studentized residuals to decide whether this subject was an outlier. The studentized residuals for PK parameters C_{max} , AUC_t & AUC_{inf} were 4.99, 4.94 and 5, respectively for Subject 7 while for the other subjects it was < 4 . Therefore, this subject's data were considered to potentially bias the overall study results and the statistical analysis was, therefore, performed with and without the data from this subject.

The plasma glucagon and glucose concentration time profile for the Subject 7 is shown below. As shown the plasma glucagon levels following the first replicate of the test product is unusually high as compared to other replicates. The glucagon C_{max} for the subject 7 was 51300, 2320, 8830, and 2220 pg/mL, respectively in the four periods. In addition, the mean glucagon level for this subject was over 2 standard deviations of the other subjects. The reviewer agrees that this subject can be classified as an outlier and can be excluded from the analysis.

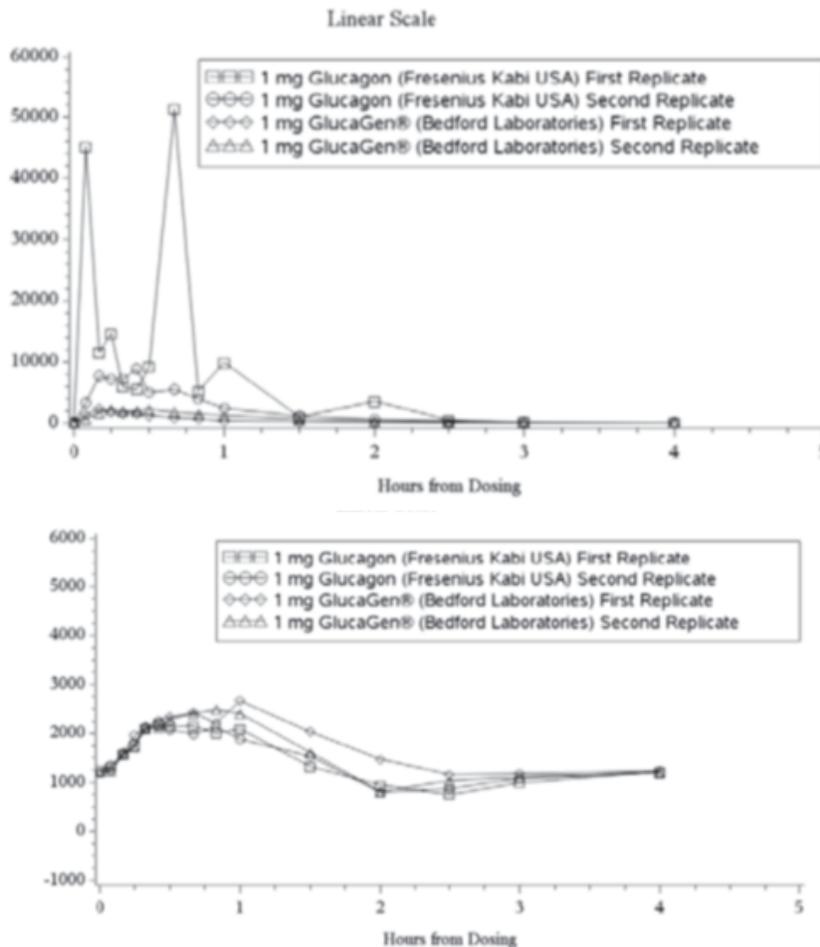


Figure 3: Glucagon plasma concentration (pg/mL) (top figure) and glucose concentrations (µg/mL) (bottom figure) versus time for Subject 7

Baseline correction for glucagon: The sponsor used the average of three pre-dose plasma glucagon concentrations (-2, -1 and 0 h) to obtain glucagon pre-dose concentrations. All pre-dose concentrations were below the limit of quantitation. The lower limit of quantitation (LLOQ) for glucagon assay was 100 pg/mL. As specified in the protocol, the sponsor used ½ of LLOQ (i.e., 50 pg/mL) as pre-dose concentrations and subtracted this from the concentrations at each post-dose time-point. Although pre-specified in the protocol, the reviewer does not agree with the sponsor’s approach of baseline correction, and will consider the glucagon parameters obtained from baseline uncorrected data as the primary analysis. To note is that in the previous bioavailability study (2011), there was no baseline levels detected as all concentrations were below LLOQ and uncorrected glucagon was used for PK evaluation.

Normalization of potency/glucagon content in batches of test and reference glucagon product: The two reference glucagon products are both produced from recombinant DNA technology. The USP Monograph for glucagon is as follows:

“Glucagon for Injection is a sterile lyophilized mixture of the hydrochloride of glucagon with one or more suitable buffering and stabilizing agents. It contains NLT 65% and NMT 110% of the labeled amount of glucagon.”

The sponsor states that although potency correction for glucagon was not discussed in the protocol or statistical analysis plan, the potency correction was deemed appropriate for plasma glucagon data based on the USP specifications for glucagon. According to the USP drug product monograph, the accepted criterion for the potency of Glucagon for Injection can vary by as much as 65% to 110% of label claim. Therefore, the sponsor states that if 2 different lots of glucagon for injection are administered from even the same reference drug product, the average difference in exposure between the 2 lots could be as great as (b) (4)%. In a BE study, if the difference in potencies between 2 different lots of the same product or 2 different products being compared is large (> (b) (4)%), according to the sponsor it will be impossible to meet the BE criteria if this difference is not taken into consideration. Therefore, they are of the opinion that due to this large potency acceptance criteria, potency correction should be applied when comparing the critical PK parameters for glucagon in a BE study.

The difference in potency between the 2 formulations used in the current study according to the sponsor was over 12%. The estimated potencies were 90% and 102.2% for the reference and test formulations, respectively. Therefore, potency corrections were applied to the ln-transformed PK parameters AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} . Correction for measured drug content was performed using the correction factor of 90.0% and 102.2%, respectively, for Treatments A and B as shown below:

Geometric Mean Ratio = $100 * (\text{test}/\text{reference}) * (0.900/1.022)$

Similar corrections were applied to confidence intervals.

The Agency has not relied on content/potency normalized data for bioequivalence assessment. This approach was also discussed with OCP-SLT (senior leadership team) and it was agreed to not use content normalization approach to support the primary endpoint. Therefore, sponsor’s approach of normalization is not acceptable.

Results: Based on the issues discussed above, the review will discuss the baseline uncorrected glucagon (PK) data. The glucose (PD) data presented is the baseline corrected data. As discussed above both PK and PD data will be presented following exclusion of Subject 7 (outlier). The content normalization approach was not acceptable and the PK results from this analysis are not presented in this review.

Pharmacokinetics

Glucagon (PK): Figure 4 shows the mean plasma glucagon concentrations versus time profile following administration of the test and reference glucagon products. As shown, the mean glucagon concentrations were higher in the second replicates compared to the first replicate for each of the test and reference products. Both test and reference products appear to have similar shapes of mean plasma glucagon concentration-time profiles (Figure 4).

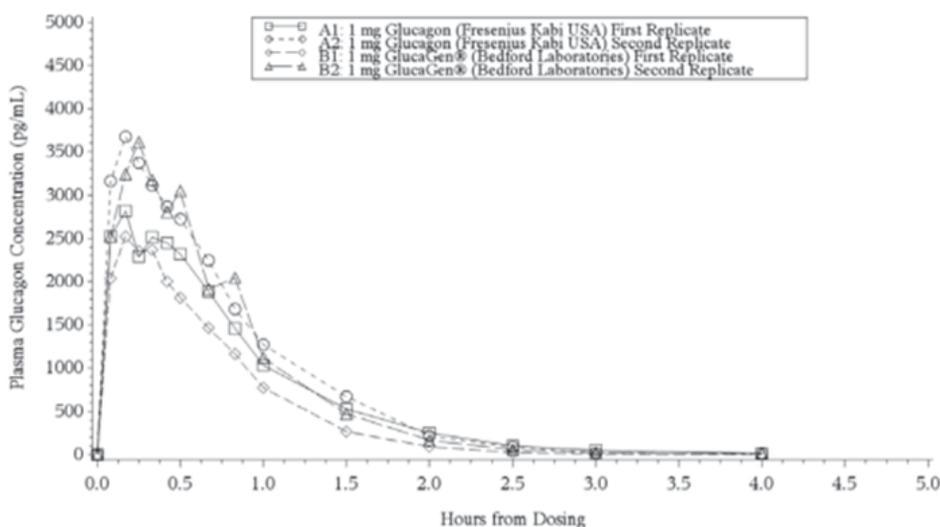


Figure 4: Baseline uncorrected Mean Plasma Glucagon Concentrations Versus Time Following Test Product A and Reference Product B (Linear Scale) (without subject 7)

Exposure (C_{max} , AUC_{0-t} , and $AUC_{0-\infty}$) to glucagon was higher in the second replicates compared to the first replicate for each of the test and reference products (Table 5).

Table 5: Summary of the Mean (\pm SD) Baseline uncorrected Plasma Glucagon Pharmacokinetic Parameters Following Test Product A and Reference Product B (excluding Subject 7)

PK Parameter	Treatment A First replicate	Treatment A Second replicate	Treatment B First replicate	Treatment B Second replicate
C_{max} (pg/mL)	3643 \pm 2675.6	4232 \pm 1468.2	3315 \pm 1713.8	4318 \pm 2991.2
AUC_{0-t} (pg.h/mL)	2928 \pm 1010.4	3480 \pm 1172.9	2271 \pm 1012.4	3261 \pm 1446.9
AUC_{inf} (pg.h/mL)	3038 \pm 980.33	3524 \pm 1232	2648 \pm 1106.2	3416 \pm 1433.2
T_{max} (h)	0.21 (0.08 – 0.53)	0.17 (0.08 – 1.5)	0.25 (0.08 – 0.5)	0.25 (0.08 – 0.83)

T_{max} is presented as median (Minimum, Maximum)

The statistical analysis of the PK parameters is shown in the Table 6 below. As shown, although the glucagon exposure was slightly higher (6-11%), the BE criteria is met for the glucagon C_{max} and AUC_{inf} parameters. It is noted that the AUC_{0-t} did not meet the regulatory criteria (Table 6). While the cause of this observation is not clear, however as the percent extrapolation of the AUC_{inf} values were <10% for both the reference and test product the exposure from the two products is considered to be bioequivalent.

Table 6: Statistical comparisons of baseline uncorrected plasma glucagon PK parameters (excluding Subject 7):

Parameter	Test LS Means	Reference LS Means	Ratio	90% CI
C _{max} (pg/mL)	3532.87	3308.96	106.77	94.05 – 121.20
AUC _{0-t} (pg.h/mL)	3019.96	2462.14	122.66	111.38 – 135.07
AUC _{inf} (pg.h/mL)	3071.92	2759.31	111.33	102.14 – 121.35

Pharmacodynamics (PD)

OSI inspection was requested for this pivotal bioavailability study. At the conclusion of the inspection, a Form 483 dated (b) (4) was issued pertaining to an observation in Study AA98483-02 (DARRT report dated 1/5/2015). The comment was that (b) (4) failed to accurately calculate the concentrations of the analyte glucose for calibration standards, quality control samples and study plasma samples for the assays in this study. Specifically, (b) (4) failed to take into account the specific gravity of the glucose solution for calculation of primary stock, sub-stock, calibration standard and quality control sample concentrations. It was suggested by OSI that (b) (4) measure the specific gravity of the glucose solution and re-regress data using updated concentrations. Based on the Agency audit of the bioanalytical site (b) (4) the site recalculated the concentrations of glucose for calibration standards, quality control standards and study samples for the study AA98483-02. This resulted in the submission of a new amended clinical study report on Dec 19, 2014. The submission was considered as a major amendment and resulted in extension of the PDUFA goal date. The results from the recalculation are discussed below.

Glucose (PD): The plasma concentration time profile for glucose is shown below and the Table 7 shows the summary of the PD parameters. Both test and reference products display similar shapes for the mean glucose concentration-time profiles. Statistical analysis shows that the BE criteria is met for all the PD parameters (Table 8). The median time to reach BG_{max} was 50 minutes for Test Product A and 40 - 50 minutes for Reference Product B. The 90% confidence interval for the point estimate ratio of the test and reference for all the glucose parameters (BG_{max}, AUC₀₋₂, AUC₀₋₄) met the 80-125% BE criteria, demonstrating the PD comparability between the two products.

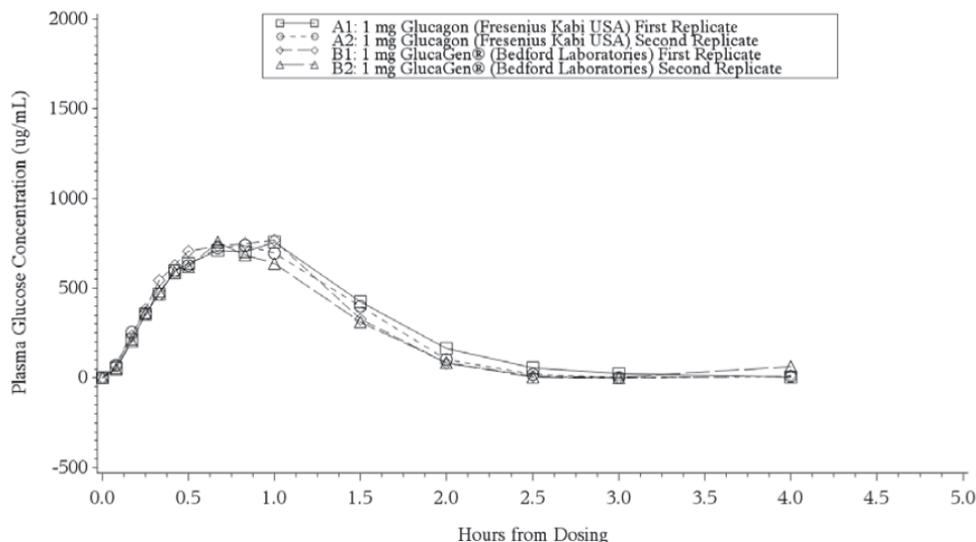


Figure 5: Baseline Corrected Mean Plasma Glucose Concentrations Versus Time Following Test Product A and Reference Product B (Linear Scale)

Table 7: Summary of the Mean (\pm SD) Baseline Corrected Plasma Glucose Pharmacodynamic Parameters Following Test Product A and Reference Product B

Pharmacokinetic Parameters	Treatment A First Replicate Mean \pm SD	Treatment A Second Replicate Mean \pm SD	Treatment B First Replicate Mean \pm SD	Treatment B Second Replicate Mean \pm SD
BG _{max} (ug/mL)	852.9 \pm 283.66	833.6 \pm 282.52	918.5 \pm 310.52	836.5 \pm 252.84
TBG _{max} (hr)	0.8333 (0.250, 1.50)	0.8333 (0.167, 1.00)	0.8333 (0.333, 1.00)	0.6667 (0.333, 2.00)
AUC ₀₋₂ (ug*hr/mL)	962.0 \pm 437.26	923.6 \pm 449.27	931.2 \pm 357.61	846.5 \pm 364.46
AUC ₀₋₄ (ug*hr/mL)	971.2 \pm 487.71	897.3 \pm 453.99	966.6 \pm 377.78	896.5 \pm 473.98
Treatment A: 1 mg Glucagon (Fresenius Kabi USA) Treatment B: 1 mg GlucaGen® (Bedford Laboratories) T _{max} is presented as Median (Minimum, Maximum) SD = standard deviation Subject 7 was excluded from summary statistics, N=26 Source: Tables 14.2.2.5 through 14.2.2.8				

Table 8: Statistical Comparisons of Baseline Corrected Plasma Glucose Log-Transformed Pharmacodynamic Parameters: Glucagon (Fresenius Kabi USA) Versus GlucaGen® (Bedford Laboratories) (Test Product A Versus Reference Product B)

Parameter	Geometric LS Means		% Mean Ratio	Confidence Intervals
	Treatment A	Treatment B		90% Confidence
AUC ₀₋₂ (ug*hr/mL)	812.966	804.555	101.05	87.24 - 117.04
AUC ₀₋₄ (ug*hr/mL)	814.628	812.203	100.30	85.40 - 117.79
BG _{max} (ug/mL)	792.959	835.253	94.94	86.10 - 104.67
Treatment A: 1 mg Glucagon (Fresenius Kabi USA) (test) Treatment B: 1 mg GlucaGen® (Bedford Laboratories) (reference) Subject 7 was excluded from statistical analysis. Parameters were ln-transformed prior to analysis. Values for Treatment A and Treatment B LS means are the (back transformed) LS means from the ANOVA. Geometric Mean Ratio = 100*(test/reference) Source: Table 14.2.2.9				

As stated previously, glucose concentrations were subject to additional analysis following an FDA audit of the bioanalytical method applied for glucose analysis. As a result of this reanalysis, the AUC₀₋₄ parameter could not be calculated for Subjects 1, 2, 3, 4, 6, 7, 8, and 11 as the 4 hour glucose concentration data and were subsequently determined to be “not reportable”. Therefore, these missing AUC₀₋₄ for these 8 subjects were not included in the statistical analysis. The results based on the remaining subjects demonstrate that AUC₀₋₄ still meets the BE criteria (Table 8).

As noted in Table 8 above, the LSM means between baseline-corrected AUC₀₋₂ and AUC₀₋₄ for both formulations are less than 1% different. The individual profiles indicate that most baseline-corrected concentrations were zero after Hour 2. Therefore, AUC₀₋₄ and AUC₀₋₂ are almost identical, and the missing values at Hour 4 have very minimal impact on the estimation of AUC₀₋₄ and on the glucose analysis conclusions that were based on this parameter. Literature data also indicate that the glucose levels reach baseline values within 2 h (Figure 2). Overall, the 90% CIs of the GMRs for each PD parameter were within the 80.00% to 125.00% range. Therefore, the test and the reference products are considered to be bioequivalent from the PD perspective.

3 Analytical

Glucagon assay: The analytical method was developed at [REDACTED] (b) (4) and validated according to the standard operating procedures (SOPs) in effect during the conduct of the validation. The concentration of glucagon in human plasma was determined using high performance liquid chromatography (HPLC) with mass spectrometric detection.

A set of 9 non-zero calibration standards, ranging from 100 pg/mL to 10000 pg/mL was prepared and subsequently stored at a nominal temperature of -80°C.

QC samples at 5 different concentrations: 300 pg/mL, 1000 pg/mL, 3000 pg/mL, 7500 pg/mL, and 25000 pg/mL were prepared and subsequently stored at a nominal temperature of -80°C.

Validation summary for glucagon

Validation Summary	(b) (4) Validation Study ZZ17705-05
Analyte	Glucagon
Internal Standard (IS)	(Des-Thr ⁷)-Glucagon
Method Description	Solid phase extraction with analysis/detection by LC-MS/MS
Limit of Quantitation (pg/mL)	100 pg/mL
Average Recovery of Drug (% Mean)	46% at 300 pg/mL 46% at 1000 pg/mL 50% at 7500 pg/mL
Average Recovery of IS (% Mean)	65%
Standard Curve Concentrations (pg/mL)	100, 150, 250, 500, 1000, 2500, 5000, 8000, and 10,000 pg/mL
QC Concentrations (pg/mL)	LLOQ QC, 300, 1000, and 7500 pg/mL
QC Intra-Batch Precision Range (% CV)	1.5 to 11.5%
QC Intra-Batch Accuracy Range (% Bias)	-2.6 to 14.0%
QC Inter-Batch Precision Range (% CV)	2.2 to 10.7%
QC Inter-Batch Accuracy Range (% Bias)	3.0 to 6.0%
Bench-Top Stability (Hrs)	Short-Term Stability: 3 hours in polypropylene tubes in an ice water bath under white light Cumulative Short-Term Stability: 5 hours in polypropylene tubes in an ice water bath under white light (total of all thaw cycles)
Stock Stability (Days)	Long-Term Stability for Stock Solutions (Stock): 275 days at approximately 400 µg/mL in 25:75:0.1 acetonitrile:water:formic acid in a BSA-treated polypropylene container at -80°C
Processed Stability (Hrs)	Post-Preparative Stability: 129 hours in a polypropylene 96 well plate at 5°C Processed Sample Integrity: 128 hours in a polypropylene 96 well plate at 5°C
Freeze-Thaw Stability (Cycles)	2 freeze (-80°C)-thaw (ice water bath) cycles in polypropylene tubes under white light
Long-Term Storage Stability (Days)	Long-Term Stability: 420 days in polypropylene tubes at -80°C
Dilution Integrity	up to 25,000 pg/mL, diluted 5-fold
Selectivity	No significant interference at the retention time and mass transition of glucagon was observed from endogenous components in any of the 6 human plasma (EDTA) lots screened or of (des-Thr ⁷)-glucagon (IS) in any of the 6 human plasma (EDTA) lots screened

Glucose assay: (b) (4) has determined the concentrations of glucose in human plasma using an enzyme method. An aliquot of human plasma (EDTA) containing glucose was analyzed using an enzyme assay method. A kit from (b) (4) was used. The signal

absorbance was read on a spectrophotometric microplate reader. A weighted linear regression curve ($1/\text{concentration}^2$) was used to determine the concentration of glucose. The assay is a direct measurement of glucose in biological samples using hexokinase enzyme. Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate (G6P) using adenosine triphosphate (ATP). G6P is then oxidized to 6-phosphogluconate in the presence of oxidized nicotinamide adenine dinucleotide (NAD^+) in a reaction catalyzed by G6P dehydrogenase (G6PDH). During this oxidation an equimolar amount of NAD^+ is reduced to NADH. The rate of NADH formation is directly proportional to the glucose concentration in the sample and can be measured spectrophotometrically at 340 nm.

A set of 8 non-zero calibration standards, ranging from 50.6 $\mu\text{g/mL}$ to 1520 $\mu\text{g/mL}$ was prepared daily and discarded after each use. Standards were prepared in calibrator diluent.

Validation summary for glucose:

Information Requested	Data
Validation Summary	(b) (4) Validation Study AA99722-01
Analyte	Glucose
Method Description	Analysis using enzyme assay
Limit of Quantitation ($\mu\text{g/mL}$)	50.8 $\mu\text{g/mL}$
Standard Curve Concentrations ($\mu\text{g/mL}$)	50.8, 102, 212, 423, 760, 1010, 1260, and 1520 $\mu\text{g/mL}$
QC Concentrations ($\mu\text{g/mL}$)	LLOQ QC, 409, 817, 3340, and ULOQ QC $\mu\text{g/mL}$
QC Intra-Batch Precision Range (% CV)	0.7 to 22.7%
QC Intra-Batch Accuracy Range (% Bias)	-22.1 to 8.7%
QC Inter-Batch Precision Range (% CV)	3.0 to 14.9%
QC Inter-Batch Accuracy Range (% Bias)	-11.2 to 5.1%
Bench-Top Stability (Hrs)	Short-Term Stability: 16 hours in polypropylene tubes at ambient temperature under white light Cumulative Short-Term Stability: 26 hours in polypropylene tubes at ambient temperature under white light (total of all thaw cycles)
Stock Stability (Days)	Long-Term Stability for Stock Solutions (Stock): 109 days at approximately 250,000 $\mu\text{g/mL}$ in water in a polypropylene container at 5°C
Freeze-Thaw Stability (Cycles)	6 freeze (-80°C)-thaw (ambient temperature) cycles in polypropylene tubes under white light
Long-Term Storage Stability (Days)	Long-Term Stability: 647 days in polypropylene tubes at -80°C
Dilution Integrity	Samples diluted up to 90-fold can be quantified
Selectivity	Human plasma (EDTA) was screened for basal levels of glucose

4 Detailed labeling recommendation

Labeling comments will be included in a separate review.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JAYABHARATHI VAIDYANATHAN
04/01/2015

MANOJ KHURANA
04/01/2015

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA: 201-849	Submission Date(s): 11/30/2011
Brand Name	TBD
Generic Name	Glucagon (synthetic) for injection
Clinical Pharmacology Reviewer	Immo Zadezensky, Ph.D.
Clinical Pharmacology Team Leader (Acting)	Jaya Vaidyanathan , Ph.D.
OCP Division	Clinical Pharmacology II
OND Division	Metabolism and Endocrinology Products
Sponsor	APP Pharmaceuticals Inc.
Submission Type	505 (b)(2)
Formulation	Lyophilized powder for injection; 1 mg/vial
Indication	gastrointestinal motility inhibitor as diagnostic aid

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1. Executive Summary

The sponsor, APP Pharmaceuticals Inc. submitted a 505(b)(2) new drug application (NDA201-849) seeking marketing application approval for glucagon lyophilized powder for injection (1 mg/vial), referencing Novo Nordisk's GlucaGen® (NDA20-918). APP Pharmaceuticals Inc. is proposing to use glucagon as a gastrointestinal motility inhibitor to be indicated as a diagnostic aid. In this submission, the drug substance is (b) (4) whereas the drug substance of the reference product is manufactured (b) (4)

1.1 RECOMMENDATIONS

The Office of Clinical Pharmacology / Division of Clinical Pharmacology 2 (OCP/DCP-2) has reviewed the Clinical Pharmacology information submitted by the sponsor for NDA 201-849 for glucagon for injection. The results of the OSI consult for glucagon analytical site audit and clinical site audit are still pending. OCP/DCP2 makes the final recommendation that NDA 201-849 is not acceptable for the following reasons:

- In collaboration with OSI we found significant deficiencies in the bioanalytical assay for glucose. Thus the glucose measurements are not reliable. Due to these deficiencies, the pivotal bioavailability study (200090101) is not acceptable.

Until the deficiencies identified are corrected, NDA201-849 will be considered as not acceptable. The sponsor mentioned in an email communication with the Agency that no samples were retained. Thus, to address this deficiency, another bioequivalence study will need to be conducted.

1.2 PHASE IV REQUIREMENT

None

1.3 SUMMARY OF IMPORTANT CLINICAL PHARMACOLOGY FINDINGS

Bioequivalence study 20090101: The bioequivalence of APP Pharmaceuticals Inc. Glucagon for Injection (test product) was compared to the reference product glucagon for injection (GlucaGen®, NovoNordisk), in a randomized, single-dose, single-blind, two- treatment, four-period, replicate-design, crossover study, conducted under fasting conditions in 25 healthy volunteers (completed). Test and reference product were administered via the intramuscular route at a dose of 1 mg into the upper deltoid muscle.

Results from study 20090101 demonstrate that the geometric mean ratio for both rate (Cmax) and extent (AUC) of exposure for non-baseline corrected glucagon concentrations and the 90 % confidence interval falls within the 80-125% limit (Table 3).

Table 1 Geometric Means, Ratio of Means, and 90% Confidence Intervals Based on ANOVA of Ln-Transformed Data Analyte: Non-Baseline-Corrected Glucagon Test A vs Reference B

Parameter	Test A (N=50)	Reference B (N=50)	Ratio	CI*	Intra-Subject %CV
AUC0-t (pg·hr/mL)	2829.16	2959.26	0.9560	0.9078 - 1.0069	21.9512
AUC0-inf (pg·hr/mL)	2947.78	3074.50	0.9588	0.9094 - 1.0108	21.5108
Cmax (pg/ml)	3391.01	3817.62	0.8883	0.8198 - 0.9625	28.5482

2. Question Based Review

2.1. GENERAL ATTRIBUTES OF THE DRUG

2.1.1 What is the regulatory background for this application?

There was no regulatory interaction with APP Pharmaceuticals Inc. prior to the submission of this NDA.

2.1.2 What is the proposed mechanism of action and therapeutic indication?

The sponsor proposes the following mechanism of action:

Gastrointestinal Motility Inhibition: Extra hepatic effects of glucagon include relaxation of the smooth muscle of the stomach, duodenum, small bowel, and colon.

The proposed indication is for use as diagnostic aid as a gastrointestinal motility inhibitor.

2.1.3 What is the proposed dose and dosage form?

Glucagon is supplied in a vial, alone, (b) (4)

(b) (4) When the glucagon powder is reconstituted with Sterile Water for Injection, USP, it forms a solution of 1 mg/mL (1 unit/mL) glucagon for intramuscular or intravenous injection.

The sponsor state that the usual diagnostic dose for relaxation of the stomach, (b) (4) and small bowel is 0.2 mg to 0.5 mg given intravenously or 1 mg given intramuscularly; the usual dose to relax the colon is 0.5 mg to 0.75 mg intravenously and 1 mg to 2 mg intramuscularly. Additionally, the following table is provided to guide dosing:

Table 2 Sponsor dosing recommendation (same as reference product, GlucaGen®)

Route of Administration	Dose*	Time of Maximal Glucose Concentration	Time of Onset of Action for GI Smooth Muscle Relaxation	Duration of Smooth Muscle Relaxation ¹
IV	0.25 to 0.5 mg (0.25 to 0.5 units)	5 to 20 minutes	45 seconds	9 to 17 minutes
	2 mg (2 units)	5 to 20 minutes	45 seconds	22 to 25 minutes
IM	1 mg (1 unit)	30 minutes	8 to 10 minutes	12 to 27 minutes
	2 mg (2 units)	30 minutes	4 to 7 minutes	21 to 32 minutes

2.1.4 What is the relative bioavailability APP's Glucagon for Injection compared to Novo Nordisk's GlucaGen®?

Pharmacokinetics:

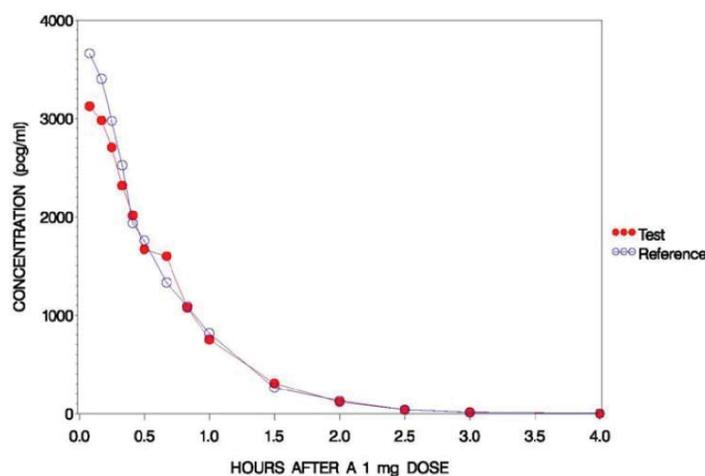
Results from study 20090101 demonstrate that the geometric mean ratio for both rate (C_{max}) and extent (AUC) of exposure fro non-baseline corrected glucagon concentrations and the 90 % confidence interval falls within the 80-125% limit (Table 3). The test product APP Pharmaceutical Glucagon for

Injection is thus bioequivalent to the reference product GlucaGen[®] (Novo Nordisk) with regards to glucagon concentrations. A concentration time profile of non-baseline corrected plasma concentrations is illustrated in Figure 1.

Table 3 Geometric Means, Ratio of Means, and 90% Confidence Intervals Based on ANOVA of Ln-Transformed Data Analyte: Non-Baseline-Corrected Glucagon Test A vs Reference B

Parameter	Test A (N=50)	Reference B (N=50)	Ratio	CI*	Intra-Subject %CV
AUC _{0-t} (pg·hr/mL)	2829.16	2959.26	0.9560	0.9078 - 1.0069	21.9512
AUC _{0-inf} (pg·hr/mL)	2947.78	3074.50	0.9588	0.9094 - 1.0108	21.5108
C _{max} (pg/ml)	3391.01	3817.62	0.8883	0.8198 - 0.9625	28.5482

Figure 1 Non-Baseline-Corrected Glucagon Plasma Concentrations, LS Mean Plasma Concentrations (N=25)



The sponsor did not report analysis of baseline corrected glucagon concentrations. The sponsor reported that no concentrations were detected at pre-dose at the lower limit of quantification; therefore all of the concentrations at baseline were set to zero. The results for the DSI inspection for the analytical method for glucagon are still pending.

Pharmacodynamics:

The sponsor reported that the geometric mean ratio for both rate (C_{max}) and extent (AUC) of exposure for non-baseline corrected glucose (Table 3) concentrations and baseline corrected glucose concentrations (Table 5) and the 90 % confidence interval falls within the 80-125% limit. However, these results are not reliable since no adequately validated method was used for the determination of glucose concentrations.

Table 4 Geometric Means, Ratio of Means, and 90% Confidence Intervals Based on ANOVA of Ln-Transformed Data Analyte: Non-Baseline-Corrected Glucose Test A vs Reference B

Parameter	Test A (N=50)	Reference B* (N=50)	Ratio	CI	Intra-Subject %CV
AUC0-t (mg·hr/ml)	596.62	591.46	1.0087	0.9973 - 1.0203	5.2621
AUC0-inf (mg·hr/ml)	1028.11	1000.77	1.0253	0.9515 - 1.1048	22.0607
Cmax (mg/ml)	165.54	162.90	1.0162	0.9916 - 1.0414	8.2685

*N=49 for AUC0-inf for Reference Product B.

Table 5 Geometric Means, Ratio of Means, and 90% Confidence Intervals Based on ANOVA of Ln-Transformed Data Analyte: Glucose-Baseline-Corrected Test A vs Reference B

Parameter	Test A (N=50)*	Reference B (N=50)**	Ratio	CI***	Intra-Subject %CV
AUC0-t (mg·hr/mL)	53.49	52.51	1.0186	0.9130 – 1.1364	35.8676
AUC0-inf (mg·hr/mL)	68.55	69.00	0.9895	0.8505 – 1.1512	51.6773
Cmax (mg/mL)	69.90	67.70	1.0324	0.9709 – 1.0979	19.2869

*N=48 for AUC0-inf for Test Product A.

**N=49 for AUC0-inf for Reference Product B.

***Bioequivalent if confidence intervals are within 0.8000 – 1.2500 (80.00 to 125.00%).

2.6 ANALYTICAL SECTION

2.6.1 Have the bioanalytical assays used in study 20090101 been adequately validated?

Yes, the glucagon assay was adequately validated, however the glucose assay was not adequately validated. However, the results of the OSI inspection for the glucagon assay are still pending.

Glucagon

The validation titled “Validation of an LC-MS/MS method for the determination of glucagon in human plasma (EDTA)” was conducted at (b) (4)

An aliquot of human plasma (EDTA) containing the analyte and internal standard was extracted using a solid phase extraction procedure. The extracted samples were analyzed by an HPLC equipped with an AB | MDS Sciex API 4000 mass spectrometer. Positive ions were monitored in

the multiple reaction monitoring (MRM) mode. Quantitation was determined using a weighted quadratic regression analysis ($1/\text{concentration}^2$) of peak area ratios of the analyte and internal standard. Long term stability at -80°C was 37 days and samples were stable for 2 freeze thaw cycles. Standard curve concentrations were 100, 150, 250, 500, 1000, 2500, 5000, 8000, and 10,000 pg/mL. Quality control concentrations were LLOQ QC, 300, 1000, and 7500 pg/mL.

Table 6 Results of Quality Control from the bioanalytical method validation

Analyte / Parameter	Curve range (pg/mL)	Calibration		Quality control (between batch)	
		LLOQ (pg/mL)	%CV	%CV	%Bias
Glucagon	100 to 10,000 pg/mL	100 pg/mL	1.5% to 11.5%	3.3% to 10.7%	3.0% to 6.0%

Glucose

The sponsor used a conventional test diagnostic test procedure, with single concentration calibration for the glucose measurement. This is not a 21CFR320.29(a)-compliant and Bioanalytical Method Validation Guidance-recommended methodology(Attachment 1).

The sponsor mentioned in an email communication with the Agency, that no samples were retained (Attachment 2), thus reanalysis of the samples for glucose assay is not possible.

3. Preliminary Labeling Recommendations

Labeling statements to be removed are shown in ~~red strikethrough~~ and suggested labeling to be included is shown in underline blue font. The following main labeling recommendations based on this submission should be considered during labeling negotiations:

Since this application is not acceptable, no labeling recommendations will be made at this stage.

4. Individual Study Reviews

4.1. *Bioequivalence Study: 20090101*

The study was a bioequivalence study titled: “Bioequivalence of a Test Formulation of Glucagon for Injection, 1 mg (1 IU/mL) (manufactured by APP Pharmaceuticals) compared to GlucaGen® 1 mg (1 IU/mL) Manufactured by Bedford Laboratories Under Fasted Conditions”. The primary objective of this study was to determine the pharmacokinetic bioequivalence of glucagon for injection, 1 mg (1 IU/mL) (manufactured by APP Pharmaceuticals) in comparison to the reference formulation, GlucaGen® 1 mg (1 IU/mL) (manufactured by Bedford Laboratories), via intramuscular route, in healthy adult subjects.

STUDY DESIGN

The study was a Randomized, Single-Dose, Single-Blind, Two- Treatment, Four-Period, Replicate-Design, Crossover Study, conducted under fasting conditions.

SAMPLE COLLECTION

- Blood samples were collected at 2, 1, and 0.5 hours prior to dosing and at 10, 20, 25, 30, 40, 50 minutes and 1, 1.5, 2, 2.5, 3, and 4 hours post-dose.

CHANGES IN THE CONDUCT OF THE STUDY

The sponsor reported the following changes in the conduct of the study:

- The protocol (Protocol No. 20090101, Date: 12/16/08) for this study in Section 9.7.1 states that “Primary determination of bioequivalence will be based on the baseline-adjusted glucagon results. The uncorrected glucagon analysis and both sets of glucose analysis will be used as supporting evidence.”
- However, the analytical method utilized for determining the concentration of glucagon in blood, did not detect any pre-dose concentrations at the lower limit of quantification and therefore all of the concentrations at baseline were zero. As all the pre-dose concentrations had values of zero, baseline corrected data were unnecessary and not applicable

Reviewer comment:

This is acceptable.

PROTOCOL VIOLATIONS

No protocol deviations were noted during the course of this study.

RESULTS

Demographics:

A total of 32 subjects were entered into this study, and 25 subjects completed the study. 7 subjects did not return for subsequent period check-in and were considered non-compliant and excluded from the PK analysis. A demographic profile for the subjects included in the bioequivalence analysis for each drug is provided below:

Table 7 Study 20090101 Demographics

Subjects Included in the Bioequivalence Analysis (N =25)	
Gender	
Males	13 (52.00%)
Females	12 (48.00%)
Race	
American Indian	0 (0.00%)
Asian	0 (0.00%)
Black	0 (0.00%)
Pacific Islander	0 (0.00%)
White	0 (0.00%)
Other	25 (100.00%)
Ethnicity	
Hispanic/Latino	25 (100.00%)
Not Hispanic/Latino	0 (0.00%)
Age (years)	
Mean ± SD	38.24 ± 11.13
Median	39.00
Minimum	18
Maximum	58
Age Groups	
<18	0 (0.00%)
18-40	13 (52.00%)
41-64	12 (48.00%)
65-75	0 (0.00%)
>75	0 (0.00%)
Weight (Kg)	
Mean ± SD	159.40 ± 23.13
Median	162.00
Minimum	122.0
Maximum	209.0
BMI (Kg/m²)	
Mean ± SD	26.40 ± 2.22
Median	27.00
Minimum	21.0
Maximum	29.0
Tobacco User	
Yes	0 (0.00%)
No	25 (100.00%)

Pharmacokinetics:

The pharmacokinetic parameters for non baseline corrected glucagon plasma concentrations are presented in **Table 8**.

Table 8 Summary of the Pharmacokinetic Parameters of Non-Baseline Corrected Glucagon concentrations.

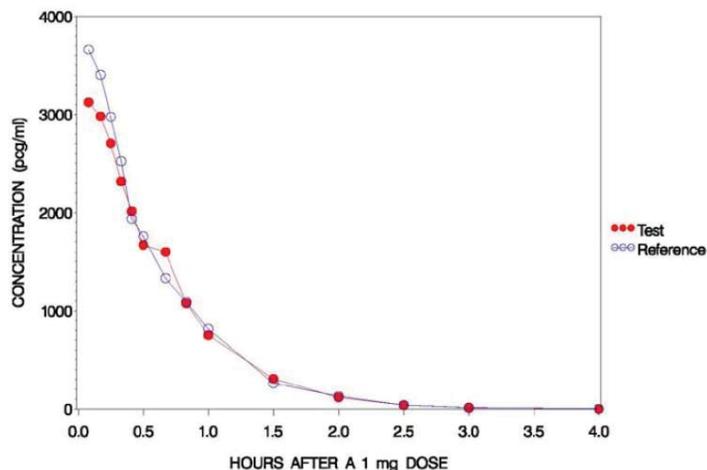
Pharmacokinetic Parameter	Arithmetic mean \pm SD (%CV)	
	Test A (N=50)	Reference B (N=50)
AUC0-t (pg·hr/mL)	3009.0651 \pm 1062.9534 (35.3250)	3246.7540 \pm 1311.6178 (40.3978)
AUC0-inf (pg·hr/mL)	3123.2892 \pm 1052.7585 (33.7067)	3349.0712 \pm 1298.7138 (38.7783)
C _{max} (pg/mL)	3667.8000 \pm 1433.6215 (39.0867)	4300.6000 \pm 1891.7020 (43.9869)
T _{max} (hr)	0.1686 \pm 0.1197 (70.9927)	0.1391 \pm 0.0865 (62.1884)
Ke (1/hr)	1.7517 \pm 0.5507 (31.4367)	1.9685 \pm 0.6594 (33.4988)
T _{1/2} (hr)	0.4343 \pm 0.1338 (30.8013)	0.3961 \pm 0.1431 (36.1347)

Results from study 20090101 demonstrate that the geometric mean ratio for both rate (C_{max}) and extent (AUC) of exposure from non-baseline corrected glucagon concentrations and the 90 % confidence interval falls within the 80-125% limit (Table 3). The test product APP Pharmaceutical Glucagon for Injection is thus bioequivalent to the reference product GlucaGen[®] (Novo Nordisk) with regards to glucagon concentrations. A concentration time profile of non-baseline corrected plasma concentrations is illustrated in *Figure 2*.

Table 9 Geometric Means, Ratio of Means, and 90% Confidence Intervals Based on ANOVA of Ln-Transformed Data Analyte: Non-Baseline-Corrected Glucagon Test A vs Reference B

Parameter	Test A (N=50)	Reference B (N=50)	Ratio	CI*	Intra-Subject %CV
AUC0-t (pg·hr/mL)	2829.16	2959.26	0.9560	0.9078 - 1.0069	21.9512
AUC0-inf (pg·hr/mL)	2947.78	3074.50	0.9588	0.9094 - 1.0108	21.5108
C _{max} (pg/ml)	3391.01	3817.62	0.8883	0.8198 - 0.9625	28.5482

Figure 2 Non-Baseline-Corrected Glucagon Plasma Concentrations, LS Mean Plasma Concentrations (N=25)



The sponsor did not report analysis of baseline corrected glucagon concentrations. The sponsor reported that no concentrations were detected at pre-dose at the lower limit of quantification; therefore all of the concentrations at baseline were set to zero.

ANALYTICAL METHOD:

The extracted samples were analyzed by an HPLC equipped with an AB | MDS Sciex API 4000 mass spectrometer. Positive ions were monitored in the multiple reaction monitoring (MRM) mode. Quantitation was determined using a weighted quadratic regression analysis (1/concentration²) of peak area ratios of the analyte and internal standard.

Table 10 Results of Quality Control from the bioanalytical method

Analyte / Parameter	Curve range (ng/mL)	Calibration		Quality control (between batch)	
		LLOQ (ng/mL)	%CV	%CV	%Bias
Glucagon	100 – 10,000 pg/mL	100 pg/mL	1.9 to 5.9%	5.0% to 6.2 %	2.0% to 4.0%

CONCLUSIONS

The study is not acceptable since the glucose bioanalytical method was not adequately validated.

10 Pages of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page.

Attachment 1

Zadezensky, Immo

From: Jairath, Meghna
Sent: Friday, June 01, 2012 10:09 AM
To: CDER OSI BEQ; Zadezensky, Immo
Cc: Taylor, William (CDER); Haidar, Sam H
Subject: RE: Finalized - NDA-201849 DSI Bioequivalence Audit Request (FRM-CONSULT-09)

Hello,
I will let Immo address your concern.

Thanks,
Meghna

From: CDER OSI BEQ
Sent: Friday, June 01, 2012 9:25 AM
To: Jairath, Meghna; Zadezensky, Immo
Cc: Taylor, William (CDER); Haidar, Sam H
Subject: FW: Finalized - NDA-201849 DSI Bioequivalence Audit Request (FRM-CONSULT-09)

Meghna:

Please confirm what needs inspecting. The request form has a check next to the clinical site (not identified, but West Houston Clinical Research Services, 2026 Wirt Rd., Houston, TX 77055), and it refers to plural inspections, leading me to suspect you want audits at both WHCR and the bioanalytical site (b) (4) for glucagon pharmacokinetic measures.

(b) (4) in Houston performed the glucose pharmacodynamic ("acute pharmacologic effect") measures, using a conventional diagnostic test procedure (single concentration calibration, etc.; see 5.3.1.4 "Glucose Analytical Testing" in eCTD) rather than 21CFR320.29(a)-compliant and Bioanalytical Method Validation Guidance-recommended methodology. If you want an inspection there (or only there), the outcome would likely be that they didn't comply with 320.29(a).

We'll initiate our paperwork as soon as we get confirmation.

Thanks,
/Mike

Michael F. Skelly, Ph.D.
Pharmacologist
Division of Bioequivalence and GLP Compliance
Office of Scientific Investigations
Office of Compliance/CDER
Food and Drug Administration
Bldg. 51 Room 5312
10903 New Hampshire Ave
Silver Spring, MD 20993
TEL 1-301-796-3375
FAX 1-301-847-8750

Attachment 2

Zadezensky, Immo

From: Jairath, Meghna
Sent: Friday, June 15, 2012 3:59 PM
To: Zadezensky, Immo; Calis, Karim; Parks, Mary H; Aljuburi, Lina
Subject: Glucagon for Injection NDA 201849 Sample Availability
Importance: High

Hello,
I finally got a response from the sponsor from the phone conversation I had with them on Tuesday.

Let me know how to proceed at this point. I assume this will definitely mean they have to redo their BE study and will be CR them.

Thanks,
Meghna

From: Heidi.Guzalo@fresenius-kabi.com [mailto:Heidi.Guzalo@fresenius-kabi.com]
Sent: Friday, June 15, 2012 2:43 PM
To: Jairath, Meghna
Subject: Glucagon for Injection NDA 201849 Sample Availability

Hi Meghna,

I received your voice message regarding Glucagon for Injection NDA 201849. The glucose samples that were analyzed by (b) (4) were not retained.

Please let me know if you have any other concerns.

Kind regards,

Heidi Guzalo
Regulatory Affairs
1501 East Woodfield Road
Suite 300 East
Schaumburg, IL 60173
Phone: 847-517-5772

@

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

IMMO ZADEZENSKY
08/27/2012

JAYABHARATHI VAIDYANATHAN
08/27/2012

**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
FILING FORM/CHECKLIST FOR NDA/BLA or Supplement**

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA/BLA Number	201949	Brand Name	Glucagon for injection 1 mg (1 IU/mL)
OCP Division (I, II, III, IV, V)	DCP2	Generic Name	Glucagon
Medical Division	DMEP	Drug Class	Anti-hypoglycemic
OCP Reviewer	Immo Zadezensky, Ph.D.	Indication(s)	as diagnostic aid (gastrointestinal motility inhibitor)
OCP Team Leader	Jaya Vaidyanathan, Ph.D. (acting)	Dosage Form	Solution for Intravenous/Intramuscular injection
Pharmacometrics Reviewer		Dosing Regimen	Single dose diagnostic aid
Date of Submission	11/30/2011	Route of Administration	Intravenous / intramuscular
Estimated Due Date of OCP Review	08/28/2012	Sponsor	APP Pharmaceuticals
Medical Division Due Date	09/28/2012	Priority Classification	Standard
PDUFA Due Date	09/28/2012		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies				
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X			
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:				
multiple dose:				
Patients-				
single dose:				
multiple dose:				
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				

File name: 5_Clinical Pharmacology and Biopharmaceutics Filing Form/Checklist for NDA_BLA or Supplement 090808

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:				
hepatic impairment:				
PD -				
Phase 2:				
Phase 3:				
PK/PD -				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:	X	1		Study No. 20090101 (PK – Primary, PD- Secondary)
Food-drug interaction studies				
Bio-waiver request based on BCS				
BCS class				
Dissolution study to evaluate alcohol induced dose-dumping				
III. Other CPB Studies				
Genotype/phenotype studies				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		1		
Filability and QBR comments				
	“X” if yes	Comments		
Application filable?	X	Yes, it is filable.		
Comments sent to firm?				
QBR questions (key issues to be considered)		<ul style="list-style-type: none"> Is the proposed to-be-marketed glucagon injection formulation bioequivalent to the reference GlucaGen® formulation? Are analytical methods adequate? 		

File name: 5_Clinical Pharmacology and Biopharmaceutics Filing Form/Checklist for NDA_BLA or Supplement 090808

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Other comments or information not included above	<ul style="list-style-type: none"> Please send an OSI consult for inspection of the clinical and bioanalytical site. Address of Bioanalytical Site: (b) (4) Principal Investigator and Clinical Facility Address: Oscar De Valle, M.D. West Houston Clinical Research Services, 2026 Wirt Road, Houston Texas 77055, Telephone: 281-738-2642, Fax: 713-344-0634
Primary reviewer Signature and Date	Immo Zadezensky, Ph.D.
Secondary reviewer Signature and Date	Jaya Vaidyanathan, Ph.D.

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?	X			
2	Has the applicant provided metabolism and drug-drug interaction information?		X		
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?			X	
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	X			
5	Has a rationale for dose selection been submitted?			X	
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?			X	
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	X			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	X			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
Data					
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?	X			
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			X	
Studies and Analyses					
11	Is the appropriate pharmacokinetic information submitted?	X			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?			X	

File name: 5_Clinical Pharmacology and Biopharmaceutics Filing Form/Checklist for NDA_BLA or Supplement 090808

**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
FILING FORM/CHECKLIST FOR NDA/BLA or Supplement**

13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?			X	
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?			X	
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			X	
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			X	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?			X	
General					
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	X			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?			X	

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?

 Yes

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Immo Zadezensky, Ph.D.

Reviewing Clinical Pharmacologist

Date

Jaya Vaidyanathan, Ph.D.

Team Leader/Supervisor (acting)

Date

File name: 5_Clinical Pharmacology and Biopharmaceutics Filing Form/Checklist for NDA_BLA or Supplement 090808

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

The purpose of this document is to identify refuse to file and special issues, describe the materials needed for review but not included in the application, and summarize the application relevant to clinical pharmacology.

1. Identify refuse to file issues

Are there any refuse to file issues?

No, the application is filable from the Clinical Pharmacology perspective.

Does the applicant provide sufficient data to support the labeling claims?

Yes, from a clinical pharmacology perspective, sufficient data is provided to perform appropriate evaluation of the label claims.

2. Identify special issues

What are the specific issues regarding this application?

- Is the proposed to-be-marketed glucagon injection formulation bioequivalent to the reference GlucaGen® formulation?
- Are analytical methods adequate?

3. Identify materials needed for review but not included in the application

What are the materials needed for review but not included in the application?

None.

4. Summary of the application relevant to clinical pharmacology

The sponsor, APP Pharmaceuticals LLC (APP), is submitting a 505 (b)(2) new drug application (NDA 201-849) seeking a marketing approval for glucagon. Glucagon is indicated for the treatment of severe hypoglycemic reactions that may occur in patients with diabetes treated with insulin; as well as a diagnostic aid during radiologic examinations to temporarily inhibit movement of the GI tract. The sponsor is only seeking the indication as diagnostic aid via the intramuscular or intravenous route of administration after the Agency's refuse to file (DARRT date 09/30/2010). APP relies on the Agency's previous finding of safety and effectiveness for the reference listed drug (RLD), GlucaGen® 1 mg (1 IU), held by NOVO NORDISK (NDA 020918).

APP proposes the following dosing regimen for glucagon as diagnostic aid:

File name: 5_Clinical Pharmacology and Biopharmaceutics Filing Form/Checklist for NDA_BLA or Supplement 090808

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Glucagon for Injection should be reconstituted with 1 mL of Sterile Water for Injection, USP. Using a syringe, withdraw 1 mL Sterile Water for Injection, USP and inject into the Glucagon for Injection vial.

The usual diagnostic dose for relaxation of the stomach, [REDACTED] ^{(b) (4)} and small bowel is 0.2 mg to 0.5 mg given intravenously or 1 mg given intramuscularly; the usual dose to relax the colon is 0.5 mg to 0.75 mg intravenously and 1 mg to 2 mg intramuscularly. After the end of the diagnostic procedure, give oral carbohydrates to patients who have been fasting, if this is compatible with the diagnostic procedure applied.

The sponsor's submission is based on a single BE study (study# 20090101) as outlined below:

STUDY TITLE: Bioequivalence of a Test Formulation of Glucagon for Injection, 1 mg (1 IU/mL) (manufactured by APP Pharmaceuticals) compared to GlucaGen® 1 mg (1 IU/mL) Manufactured by Bedford Laboratories Under Fasted Conditions.

OBJECTIVE: The primary objective of this study was to determine the pharmacokinetic bioequivalence of glucagon for injection, 1 mg (1 IU/mL) (manufactured by APP Pharmaceuticals) in comparison to the reference formulation, GlucaGen® 1 mg (1 IU/mL) (manufactured by Bedford Laboratories), via intramuscular route, in healthy adult subjects.

METHODOLOGY: This randomized, two-treatment, four-period, replicate-design crossover study was conducted to compare the relative bioavailability of two formulations of 1 mg (1 IU/mL) of glucagon for injection under fasted conditions. The study was conducted with 32 (25 completing) healthy adults in accordance with Protocol No. 20090101. In each study period, a single 1 mg (1 IU/mL) of glucagon was administered by intramuscular injection to subjects following an overnight fast. The test formulation was glucagon for injection 1 mg (1 IU/mL) (manufactured by APP Pharmaceuticals) and the reference formulation was GlucaGen® 1 mg (1 IU/mL) (rDNA origin) (manufactured by Bedford Laboratories). The subjects received the test product in two of the periods (once on the right and once on the left arm in each period) and received the reference product in the other two periods (once on the right and once on the left arm in each period). The order of administration was according to a two-treatment, two-sequence, four-period, replicate-design randomization schedule. There was a 7 day interval between treatments. Blood samples were collected pre-dose and at intervals over 4 hours after each dose in each period. The plasma concentration and actual time of the sample collection for each subject was used in the calculation of all the pharmacokinetic parameters.

Sponsor has claimed that their product is bioequivalent to the reference GlucaGen® based on the primary BE comparison of glucagon PK parameters. The secondary assessments based on glucose PD parameters are supportive. The key results as reported by the sponsor are mentioned below:

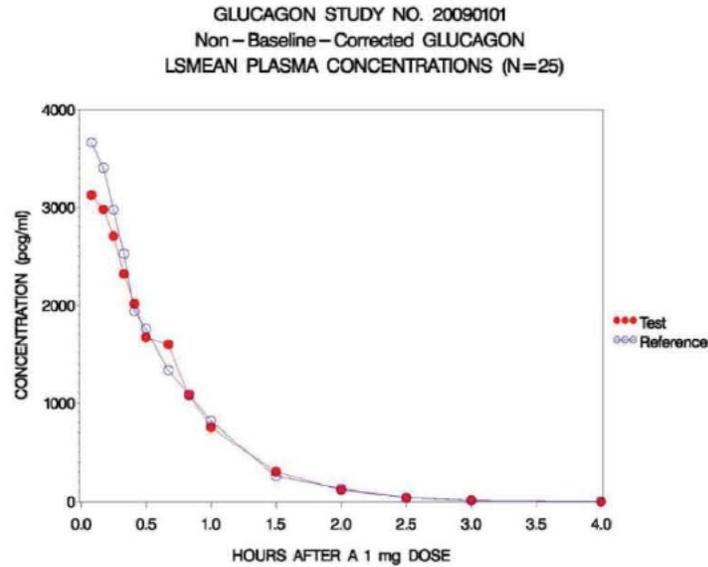
Primary Bioequivalence: GLUCAGON-Non-Baseline-Corrected:

According to the sponsor, the analytical method utilized for determining the concentration of glucagon in blood, did not detect any pre-dose concentrations at the lower limit of quantification and therefore all of the concentrations at baseline were zero. Thus, only uncorrected glucagon PK analysis is presented (Figure 1).

File name: 5_Clinical Pharmacology and Biopharmaceutics Filing Form/Checklist for NDA_BLA or Supplement 090808

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Figure 1 Non-baseline corrected glucagon plasma concentrations



Geometric Means, Ratio of Means, and 90% Confidence Intervals
Based on ANOVA of Ln-Transformed Data
Analyte: Non-Baseline-Corrected Glucagon
Test A vs Reference B

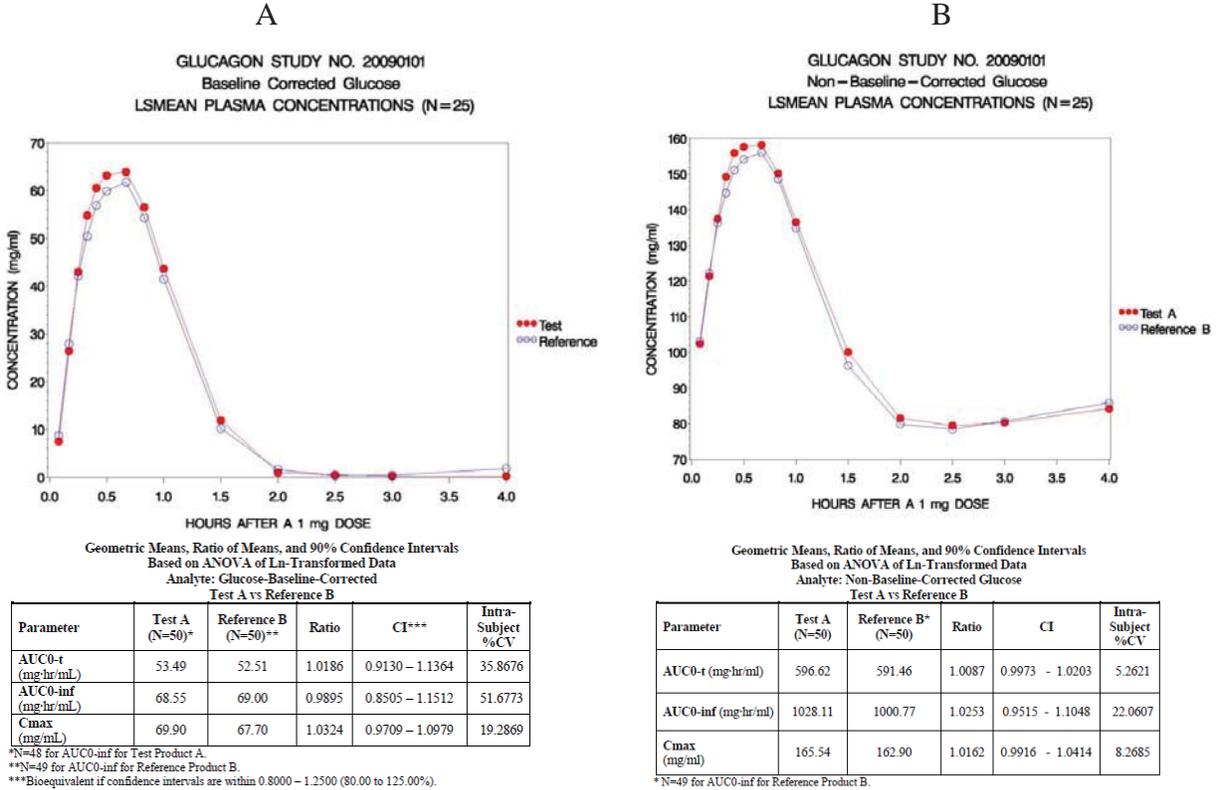
Parameter	Test A (N=50)	Reference B (N=50)	Ratio	CI*	Intra-Subject %CV
AUC _{0-t} (pg·hr/mL)	2829.16	2959.26	0.9560	0.9078 - 1.0069	21.9512
AUC _{0-inf} (pg·hr/mL)	2947.78	3074.50	0.9588	0.9094 - 1.0108	21.5108
C _{max} (pg/ml)	3391.01	3817.62	0.8883	0.8198 - 0.9625	28.5482

*Bioequivalent if confidence intervals are within 0.8000 - 1.2500 (80.00 to 125.00%).

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Supporting Evidence: Plasma Glucose Levels Non-Baseline and Baseline-Corrected (Figure 2):

Figure 2 Baseline corrected (A) and non-baseline corrected (B) plasma glucose concentrations



Clinical Pharmacology Review Question(s):

- Is the proposed to-be-marketed glucagon injection formulation bioequivalent to the reference GlucaGen® formulation?
- Are analytical methods adequate?

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

IMMO ZADEZENSKY
01/20/2012

JAYABHARATHI VAIDYANATHAN
01/20/2012

Office of Clinical Pharmacology
New Drug Application Filing and Review Form

General Information About the Submission

Information		Information	
NDA Number	201849	Brand Name	Glucagon for injection 1 mg (1 IU/mL)
OCP Division (I, II, III, IV, V)	DCP II	Generic Name	Glucagon
Medical Division	DMEP	Drug Class	Anti-hypoglycemic
OCP Reviewer	Manoj Khurana, Ph.D.	Indication(s)	(b) (4) DIAGNOSTIC USE TO INHIBIT GI MOTILITY FOR RADIOLOGIC EXAMS
OCP Pharmacometrics Reviewer		Dosage Form	Solution
OCPB Team Leader	Sally Choe, Ph.D.	Dosing Regimen	1 mg (1 IU/mL)
Date of Submission	September 30, 2010	Route of Administration	IM injection
Estimated Due Date of OCP Review	June 5, 2011	Sponsor	APP Pharmaceuticals, Melrose Park, IL 60160
PDUFA Due Date	August 5, 2011	Priority Classification	Standard
Division Due Date	July 5, 2011		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			Raw data sets not submitted
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X			
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:				
multiple dose:				
Patients-				
single dose:				
multiple dose:				
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				

Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:				
hepatic impairment:				
PD:				
Phase 1:				
Phase 2:				
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:	X	1		Study No. 20090101 (PK – Primary, PD-Secondary)
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		1		

Filability		
	“X” if yes	Comments
Is Application filable?	X	<p>Comments to the Sponsor:</p> <p>1. Please provide raw concentration data and PK parameter datasets for both glucagon (PK) and glucose (PD) (as SAS transport files) for the bioequivalence trial-Study No. 20090101.</p> <p>The concentration data-set should at least have the following columns: ID, Nominal Time, Actual Time, Concentration, Unit, Comments (if any), Treatment, Period, and Sequence.</p> <p>The PK and PD parameter data sets should at minimum have the following columns: ID, Parameter Name, Unit, Comments (if any), Treatment, Period, and Sequence. Please provide baseline uncorrected as well as baseline corrected PD data in separate files. Please include any other relevant information in these data sets that in your thinking could help us efficiently review your application.</p> <p>2. You have proposed in the Indication and Use for IM, ^{(b) (4)} and IV route. ^{(b) (4)}</p> <p>3. Please clarify the following discrepancies noted in your submission:</p> <ul style="list-style-type: none"> • Under Section 11.1 you indicate that “<i>For bioequivalence analysis (corrected glucagon data)</i>” was used. However, the primary PK comparison was based on non-baseline corrected glucagon data. • Section 9.7.1 in the Study Protocol (Study No. 20090101, 12/16/08) mentioned that “Primary determination of bioequivalence will be based on the baseline adjusted glucagon results. The uncorrected glucagon analysis and both sets of glucose analysis will be used as supporting evidence”. This is not concordant with the use of uncorrected glucagon PK parameters as the primary comparison in your study reports. No justification was provided for this deviation. • In Section 14.2 Efficacy Data you mentioned “<i>Mean concentration versus time plots (linear and In-linear) are presented below for both baseline-corrected and baseline-uncorrected glucagon and glucose</i>” but only non-baseline corrected glucagon is presented
Submission in Brief: See the details below.	<p>Reviewer’s Comments to project manager:</p> <ul style="list-style-type: none"> • Please send a DSI consult for inspection of the clinical and bioanalytical site. • Address of Bioanalytical Site: ^{(b) (4)} • Principal Investigator and Clinical Facility Address: Oscar De Valle, M.D. West Houston Clinical Research Services, 2026 Wirt Road, Houston Texas 77055, Telephone: 281-738-2642, Fax:713-344-0634 • From clinical pharmacology we have information request to be sent to sponsor. 	

Submission in Brief:

APP Pharmaceuticals, LLC (APP), has submitted this 505(b)(2) New Drug Application (NDA) for Glucagon which relies on the Agency's previous finding of safety and effectiveness for the reference listed drug (RLD), GlucaGen® 1 mg (1 IU), held by NOVO NORDISK (NDA 020918), and distributed by Bedford Laboratories. Thus, APP has not performed any duplicative clinical pharmacology, efficacy, or safety studies. The sponsor mentioned that the Glucagon API of this application is [REDACTED] (b) (4) and claim that it meets the requirements of the current USP and API manufacturer/supplier specifications.

Indication: Glucagon is indicated [REDACTED] (b) (4) as a diagnostic aid during radiologic examinations to temporarily inhibit movement of the GI tract.

Mode of Action: Glucagon induces liver glycogen breakdown, releasing glucose from the liver. GI motility inhibition is thought to be mediated via extra hepatic effects of glucagon include relaxation of smooth muscle of stomach, [REDACTED] (b) (4) small bowel, and colon.

Dosage Regimen (From the Proposed Label):

“Glucagon for Injection should be reconstituted with 1 mL of Sterile Water for Injection, USP (approximately 1 mg/mL glucagon).

[REDACTED] (b) (4)

For use as a diagnostic aid: The reconstituted Glucagon for Injection should be used immediately after reconstitution. When the diagnostic procedure is over, give oral carbohydrate to restore the liver glycogen and prevent occurrence of secondary hypoglycemia.”

This application is based on CMC and one bioequivalence (BE) study.

The BE study design and results are briefly discussed below:

STUDY TITLE: Bioequivalence of a Test Formulation of Glucagon for Injection, 1 mg (1 IU/mL) (manufactured by APP Pharmaceuticals) compared to GlucaGen® 1 mg (1 IU/mL) Manufactured by Bedford Laboratories Under Fasted Conditions.

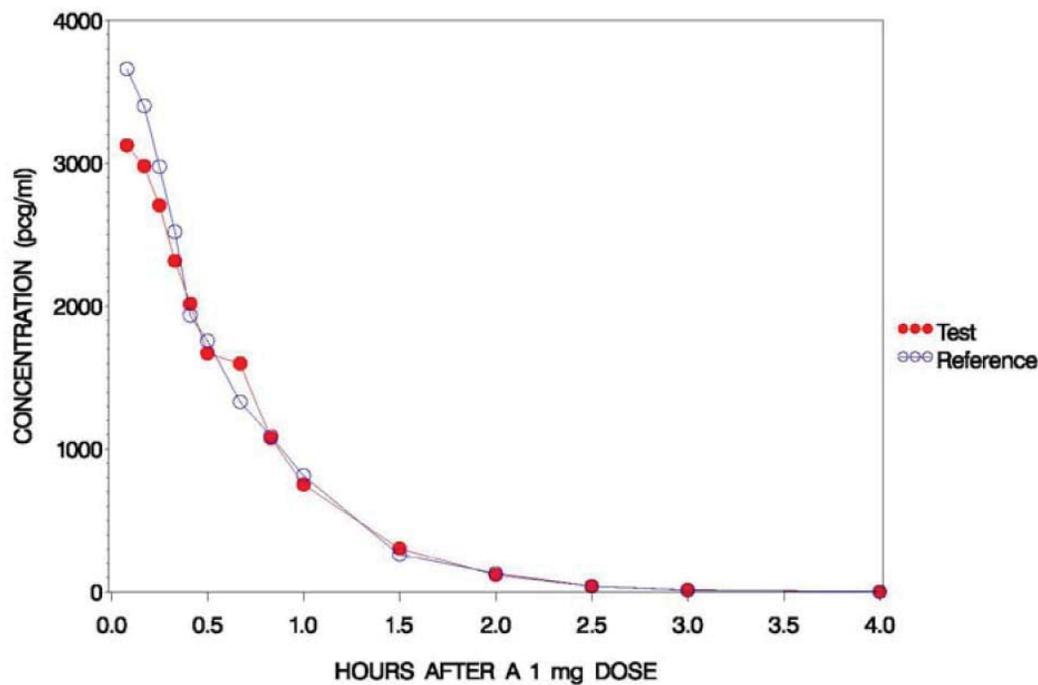
OBJECTIVE: The primary objective of this study was to determine the pharmacokinetic bioequivalence of glucagon for injection, 1 mg (1 IU/mL) (manufactured by APP Pharmaceuticals) in comparison to the reference formulation, GlucaGen® 1 mg (1 IU/mL) (manufactured by Bedford Laboratories), via intramuscular route, in healthy adult subjects.

METHODOLOGY: This randomized, two-treatment, four-period, replicate-design crossover study was conducted to compare the relative bioavailability of two formulations of 1 mg (1 IU/mL) of glucagon for injection under fasted conditions. The study was conducted with 32 (25 completing) healthy adults in accordance with Protocol No. 20090101. In each study period, a single 1 mg (1 IU/mL) of glucagon was administered by intramuscular injection to subjects following an overnight fast. The test formulation was glucagon for injection 1 mg (1 IU/mL) (manufactured by APP Pharmaceuticals) and the reference formulation was GlucaGen® 1 mg (1 IU/mL) (rDNA origin) (manufactured by Bedford Laboratories). The subjects received the test product in two of the periods (once on the right and once on the left arm in each period) and received the reference product in the other two periods (once on the right and once on the left arm in each period). The order of administration was according to a two-treatment, two-sequence, four-period, replicate-design randomization schedule. There was a 7 day interval between treatments. Blood samples were collected pre-dose and at intervals over 4 hours after each dose in each period. The plasma concentration and actual time of the sample collection for each subject was used in the calculation of all the pharmacokinetic parameters.

Sponsor has claimed that their product is bioequivalent to the reference GlucaGen® based on the primary BE comparison of glucagon PK parameters. The secondary assessments based on glucose PD parameters are supportive. The key results as reported by the sponsor are mentioned below:

Primary Bioequivalence: GLUCAGON-Non-Baseline-Corrected:

GLUCAGON STUDY NO. 20090101
Non – Baseline – Corrected GLUCAGON
LSMEAN PLASMA CONCENTRATIONS (N=25)



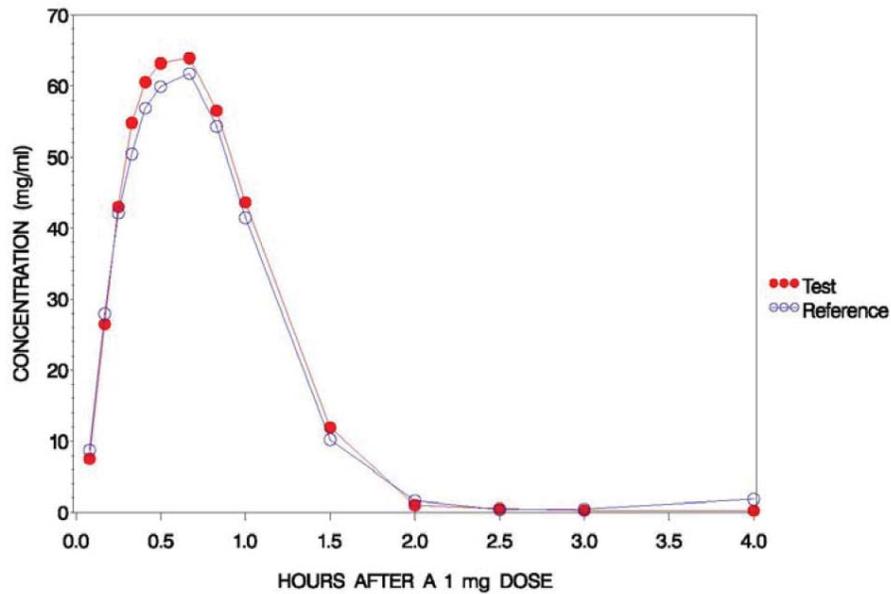
**Geometric Means, Ratio of Means, and 90% Confidence Intervals
Based on ANOVA of Ln-Transformed Data
Analyte: Non-Baseline-Corrected Glucagon
Test A vs Reference B**

Parameter	Test A (N=50)	Reference B (N=50)	Ratio	CI*	Intra-Subject %CV
AUC0-t (pg·hr/ml)	2829.16	2959.26	0.9560	0.9078 - 1.0069	21.9512
AUC0-inf (pg·hr/ml)	2947.78	3074.50	0.9588	0.9094 - 1.0108	21.5108
Cmax (pg/ml)	3391.01	3817.62	0.8883	0.8198 - 0.9625	28.5482

*Bioequivalent if confidence intervals are within 0.8000 – 1.2500 (80.00 to 125.00%).

Supporting Evidence: GLUCOSE-Baseline-Corrected

**GLUCAGON STUDY NO. 20090101
Baseline Corrected Glucose
LSMEAN PLASMA CONCENTRATIONS (N=25)**



**Geometric Means, Ratio of Means, and 90% Confidence Intervals
Based on ANOVA of Ln-Transformed Data
Analyte: Glucose-Baseline-Corrected
Test A vs Reference B**

Parameter	Test A (N=50)*	Reference B (N=50)**	Ratio	CI***	Intra-Subject %CV
AUC0-t (mg·hr/mL)	53.49	52.51	1.0186	0.9130 – 1.1364	35.8676
AUC0-inf (mg·hr/mL)	68.55	69.00	0.9895	0.8505 – 1.1512	51.6773
Cmax (mg/mL)	69.90	67.70	1.0324	0.9709 – 1.0979	19.2869

*N=48 for AUC0-inf for Test Product A.

**N=49 for AUC0-inf for Reference Product B.

***Bioequivalent if confidence intervals are within 0.8000 – 1.2500 (80.00 to 125.00%).

Clinical Pharmacology Review Question(s):

- Is the proposed to-be-marketed glucagon injection formulation bioequivalent to the reference GlucaGen® formulation?
 - Are analytical methods adequate?

Reviewer’s Comments:

1. Sponsor has not provided raw concentration data and PK parameter datasets for both glucagon (PK) and glucose (PD) for the bioequivalence trial-Study No. 20090101. The data listing was provided as PDF document under statistical report. However, the raw data cannot be extracted and reviewed reliably due to potential of human errors in this method.

2. Sponsor has proposed (Indication and Use section of proposed label) for IM, (b) (4) and IV route use of their product. (b) (4)

3. Sponsor need to clarify the following discrepancies noted in their submission:

- Under Section 11.1 you indicate that “*For bioequivalence analysis (corrected glucagon data)*” was used. However, the primary PK comparison was based on non-baseline corrected glucagon data.
- Section 9.7.1 in the Study Protocol (Study No. 20090101, 12/16/08) also mentioned that “Primary determination of bioequivalence will be based on the baseline adjusted glucagon results. The uncorrected glucagon analysis and both sets of glucose analysis will be used as supporting evidence”. This is not concordant with the use of uncorrected glucagon PK parameters as the primary comparison in the study reports.
- Section 14.2 Efficacy Data, mentioned “*Mean concentration versus time plot (linear and ln-linear) is presented below for both baseline-corrected and baseline-uncorrected glucagon and glucose*” but only non-baseline corrected glucagon is presented.

4. (b) (4)
IM bioequivalence data does not automatically waive the requirement (b) (4).

**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
FILING CHECKLIST FOR A NEW NDA/BLA**

		filing letter	
General			
13	On its face, is the clinical pharmacology and biopharmaceutical section of the NDA organized in a manner to allow substantive review to begin?	X	
14	Is the clinical pharmacology and biopharmaceutical section of the NDA indexed and paginated in a manner to allow substantive review to begin?	X	
15	On its face, is the clinical pharmacology and biopharmaceutical section of the NDA legible so that a substantive review can begin?	X	
16	Are the clinical pharmacology and biopharmaceutical studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	X	
17	Was the translation from another language important or needed for publication?		X

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?

YES

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

MANOJ KHURANA	11/23/10
Reviewing Pharmacologist	Date

SALLY CHOE	11/23/10
Team Leader/Supervisor	Date

File name: 5_Clinical Pharmacology and Biopharmaceutics Filing Checklist for a New NDA_BLA 110307

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/s/

MANOJ KHURANA
05/02/2011

SALLY Y CHOE
05/03/2011