Study Protocol

Protocol No.: API-I004-CL-B

Title: Comparison of the Pharmacokinetics (PK) and Pharmacodynamics (PD) Biosimilarity of Proposed Biosimilar Rapid-Acting Insulin Aspart (I004) and NovoLog® after Single-Dose Subcutaneous Administration to Healthy Volunteers

Version: 1.1

Date: 05 August 2021

NCT05539872

Comparison of the Pharmacokinetics (PK) and Pharmacodynamics (PD) Biosimilarity of Proposed Biosimilar Rapid-Acting Insulin Aspart (I004) and NovoLog® after Single-Dose Subcutaneous Administration to Healthy Volunteers

(A Single-Center, Randomized, Double-Blinded, Two-Treatment, Two-Period, Two-Sequence, Crossover, Hyperinsulinemia-Euglycemic Clamp Study)

Protocol ID: API-I004-CL-B, Ver. 1.1

Date: August/05/2021

Prepared by:
(Signature) [redacted] Manager of Clinical Dept.-B

Reviewed by:
(Signature) [redacted] Senior Director of Biostatistics

Reviewed by
(Signature) [redacted] Manager of Clinical Dept.-A

Approved by:
(Signature) [redacted] Sr. VP of Clinical Operations

Approved by:
(Signature) [redacted] Associate VP of Quality Assurance

Approved by:
(Signature) [redacted] Associate Director of Regulatory Affairs

Approved by:
(Signature) [redacted] Ph.D., Chief Scientist

Amphastar Pharmaceuticals, Inc.
(909-980-9484 ×2016)
11570 6th Street, Rancho Cucamonga, CA 91730

SYNOPSIS

General information

(1) Protocol No. API-I004-CL-B

(2) Title: Comparison of the Pharmacokinetics (PK) and Pharmacodynamics (PD) Biosimilarity of Proposed Biosimilar Rapid-Acting Insulin Aspart (I004) and NovoLog® after Single-Dose Subcutaneous Administration to Healthy Volunteers

(3) Subtitle: A Single-Center Randomized, Double-blinded, Two-treatment, Two-period, Two-sequence Crossover, Hyperinsulinemia-Euglycemic Clamp Study

(4)Proposed Drug: Biosimilar Insulin Aspart Solution (I004, to-be-marketed formulation) 100U/mL ([redacted])

(5) Comparator: NovoLog®, 100U/mL ([redacted])

(6) Delivery Path: Subcutaneous (SC) injection

(7) Sponsor: Amphastar Pharmaceuticals, Inc.

(8) Objectives: To determine PK/PD biosimilarity between the I004 and NovoLog® through assessment of *in vivo* PK and PD in healthy adult volunteers.

(9) Phase: Phase II/III (Pivotal)

I. Study Design

This study is a randomized, double-blinded, two-treatment, two-period, two-sequence crossover pivotal Biosimilar study in sixty (60) healthy volunteers (male and female, age between 18 and 65 years old). The purpose of this study is to establish the PK and PD biosimilarity of proposed biosimilar I004 and the US-approved NovoLog®. The I004 will use the to-be-marketed formulation.

The study includes one (1) screening visit, two (2) study visits (separated by at least 7 days) and a follow-up visit. In each study visit, a single injection will be administered before undergoing an euglycemic clamp that lasts for 12 hours. Adverse events will be documented. Study participation is expected to last up to 10 weeks. However, Screening visit window, Treatment visit window, and Follow-up visit window could be extended to accommodate state-issued Stay at Home orders due to the COVID-19 pandemic.

1) Screening Visit: Subject will sign informed consent and be assigned a screening ID, as **SI4-B-mm**, where S**I4-B** represents "Screening for I004 Study B", and **mm** is screening sequential number. Each subject will be evaluated per inclusion/exclusion criteria. Measurements of:

(1) physical examination (PE);

(2) vital signs;
(3) ECG;
(4) urinalysis (UA);
(5) urine drug screen;
(6) alcohol (urine or breathalyzer);
(7) pregnancy test (serum for screening and urine test on day 1; females of childbearing potential);
(8) fasting blood tests (hematology, clinical chemistry including fasting serum glucose, TSH, HIV, Hepatitis B-Antigen and C-Antibody tests)

shall be conducted. Qualified subjects will be scheduled for study visits.

2) Two (2) Study Visits:

After satisfying the enrollment criteria, each subject will be assigned a unique study subject ID as ***I4-B-nn***, **I4-B** represents study for "I004 Study B", and ***nn*** is the sequential number. The subject will be randomized into one of the two treatment sequences, T-R (I004 in the 1st visit and NovoLog® in the 2nd visit) or R-T (NovoLog® in the 1st visit, and I004 in the 2nd visit), respectively. A washout period of at least 7 days will be scheduled between the two study visits. At each study visit, the subject is given a single dose SC injection of either I004 or NovoLog® which is prepared per the randomization code.

Participants will be asked to be restrained from alcohol or caffeine-containing food or drinks for 24h and strenuous activity for the 72h period prior to coming in for study visits. Due to COVID-19 pandemic, subjects will be admitted to the clinical research unit on the day (day 1) of each treatment. A standard dinner will be given and finished before 8pm. The participants will take at least 10 hour fast before dosing.

Twenty-eight (28) serial PK blood samples after treatment and during the clamp period (12 hours) will be collected for serum insulin aspart, C-peptide, and endogenous human insulin measurements. No food will be allowed during the PK/PD sampling period (12 hours). Water intake and infusion will be preferably controlled at the minimal level during the clamp study.

Baseline vital signs and ECG will be performed at each study visit. On clamp days, both vital signs and ECG will be measured at baseline which is 15 min (± 5 min) prior to dosing, at 5 (± 3 min) minutes, 60 min (± 10 min), 180 min (± 20 min) and 720 min (±30 min) post-dose.

(3) Follow-up Evaluation

All treated subjects will be scheduled for a follow up visit after the study visit-2 (or ET visit).

Subjects will be asked if they have experienced any adverse event since the last study visit. An F/U evaluation will be performed, which includes: reviews of vital signs, ECG, fasting clinical lab tests (hematology, clinical chemistry, and Urinalysis), and physical examination (PE).

II. Subject

A sufficient number of healthy volunteers will be enrolled to obtain sixty (n=60) evaluable subjects. All subjects will be included in or excluded from the study based on the following inclusion/exclusion criteria.

Inclusion Criteria:

Candidates will be qualified only if they meet **all** of the following criteria:

(1) Upon review, agree to participate and sign informed consent.

(2) Healthy male and female subjects ≥ 18 to ≤ 65 years of age.

(3) Body mass index (BMI) ≥ 18.5 to ≤ 29.9 kg/m^2.

(4) Weight ≥ 50 kg.

(5) Fasting plasma glucose of < 100 mg/dL (5.5 mmol/L) measured with YSI at site; one repeat test is allowed.

(6) HbA1c < 5.7 %.

(7) Non-smoker for ≥ 3 months prior to Screening.

(8) Female candidates must be >1 year post-menopausal, surgically sterile, or practicing a clinically acceptable form of birth control and confirmed by negative serum pregnancy test at Screening.

Exclusion Criteria: Subjects will be excluded for any of the following reasons:

(1) History of diabetes mellitus.

(2) Resting blood pressure (BP) > 140/90 mmHg or < 90/60 mmHg. Subjects BP may be re-checked.

(3) Participation in an investigational drug/device study within 30 days or 5 half-lives within the last dose of any study drug, whichever is longer.

(4) History of any serious adverse reaction or hypersensitivity to any of the investigational product components.

(5) Have significant history of or current cardiovascular, respiratory, hepatic, renal, gastrointestinal, endocrine, hematological, or neurological disorders or abnormalities, or other major systemic disease that, according to the investigator, would unduly risk the subject's safety or may impact the conduct of the study.

(6) Subject shows evidence of significant active neuropsychiatric disease, including taking prescription medication for such diseases (including anti-depressant /anti-anxiety medication).

(7) Presence of clinically significant physical, laboratory, or ECG findings at Screening that, in the opinion of the Investigator, may interfere with any aspect of study conduct or interpretation of results, or may present a safety issue to that particular subject (laboratory results may be re-checked once on a separate day per Investigator discretion).

(8) Long QT syndrome or family history of long QT syndrome or corrected QT interval (QTcF) > 450 ms in men, > 470 ms in women at Screening.

(9) Liver function test results of AST and/or ALT ≥ 2.5 upper normal limit (ULN).

(10) Subject has a history of syncope.

(11) History of any major surgery within 6 months.

(12) History of any active infection, other than mild viral illness within 30 days prior to dosing.

(13) History of blood clots (e.g., deep vein thrombosis or embolism) or a frequent appearance in 1st degree relatives as judged by the Investigator.

(14) Known history or positive test of hepatitis B surface antigen (HBsAg), hepatitis C antibody (HCV Ab), or human immunodeficiency virus type 1 (HIV-1) or 2 (HIV-2) antibody.

(15) History of alcohol abuse as judged by the Investigator within approximately 1 year. Average weekly alcohol intake > 21 units/week (males) and > 14 units/week (females) or are unwilling to stop alcohol consumption from 24 hours prior to each dosing until discharged from the clinical research unit (CRU). Positive alcohol test at Screening. (One unit of alcohol equals about 250 mL of beer or lager, one glass of wine, or 20 mL of spirits).

(16) History of illicit drug abuse, including marijuana, within approximately 1 year or evidence of current use as judged by the Investigator. Positive drug test at Screening.

(17) Donation or loss of > 500 mL of blood within 56 days.

(18) Chronic use of or over-the-counter or prescription medication within 7 or 14 days prior to dosing (apart from vitamin/mineral supplements, occasional paracetamol, or birth control methods [Desogestrel is not allowed]).

(19) Unable to comply with the safety monitoring requirements of this clinical study or is considered by the investigator to be an unsuitable candidate for the study.

III.Dosing Regimens

Qualified subjects will participate in two (2) study visits separated by at least 7 days. These subjects will be treated at each study visit as described in **Table A** below by a qualified healthcare professional per the randomized sequences.

Table A. Treatments and Dose for I004-CL-B Study

Treatment	Arm-T	Arm-R
Study Drug Name	I004	NovoLog
Manufacturer	[redacted]	[redacted]
API and Scource	Insulin Aspart	Insulin Aspart
Dosage & Strength	Sterile Solution 100 U/mL	Sterile Solution 100 U/mL
No. of Subjects	60 healthy volunteers participate in two (2) crossover treatment sequences, T-R or R-T.	
Dose Regimen	0.2 IU/kg	0.2 IU/kg
Delivery Path	Subcutaneous injection	Subcutaneous injection

Double-blinded study medications will be dispensed in identical appearing syringes using aseptic technique by the designated site personnel. I004 or NovoLog will be drawn with 0.3mL insulin syringes that have a 0.01 mL (one unit on insulin syringe) graduation.

A Biostator, a 510(k)-cleared device that provides continuous assay of blood glucose measurements and minute-by-minute adaptations of glucose infusion rates (GIR), will be set up with infusion lines for blood glucose management and then the insulin aspart will be administered while subjects are under observation in the clinical research by qualified staff. The investigational product will be administered (at time-point zero) via subcutaneous injection into the abdominal wall into the peri-umbilical area using a standardized skin-fold technique. An area of skin approximately 5 cm lateral to the umbilicus on either side of the abdomen will be cleaned and used for the insertion of the needle. The needle will be promptly inserted at an approximately 45°degree angle into a lifted skin fold. The insulin aspart will be administered, and the needle should remain in the skin for at least 20 seconds after administration to reduce the possibility of leakage. The

needle will be very slowly withdrawn or may be held for a few seconds before it is fully withdrawn to minimize leakage of insulin. The injection site will be marked with a pen.

IV. PK/PD Blood Sampling

(1) PK Sampling

PK Sampling Procedure

A catheter will be inserted into one of the antecubital region veins for blood sampling. Alternatively, collection from a vein from the hand or forearm for easier access or via venipuncture is also acceptable. PK blood draws should use the opposite arm of the glucose infusion/clamp. Preferably, the arm with better venous access will be used for PK samplings, and the opposite arm for the glucose infusion/clamp. In case of extraordinary situations, such as IV infiltration etc., the PK samples could be collected from the arm of the clamp, for safety reason.

After each blood sample collection, a catheter flush will be performed to avoid catheter clot formation and prevent interference between samples.

At each PK sampling point, the first 1 mL of blood will be discarded for waste (utilizing a disposable tube), and blood samples (~6 mL per sample), will be collected in a serum separation tube and left to stand at room temperature for 30 - 60 minutes. Each sample will be centrifuged at 2-8°C, 1,000-1,300 g for 20 minutes.

Isolated serum will be distributed into two (2) 2.0 mL cryo vials, approximately 1.5 mL in the first aliquot and the remainder in the second aliquot. The serum samples will be frozen on dry ice and then immediately (within 60 minutes following serum isolation) stored in a freezer at -20°C or lower until analysis.

PK Sampling Schedules and Time Window

PK sampling: a total of twenty-eight (28) blood samples for each study visit will be collected. The blood sampling schedule and allowed time window during each study visit are listed in **Table B**.

Sample ID
The sample ID for PK samples (before centrifugation) is defined as

"*I4-B-nn-V-XX:*"

where, *I4-B-nn* is the subject ID; *V* is the study visit code, V=1 or 2; and *XX* is the two-digit time-

point sequential number of the sample: 01, 02, 03, …28 insulin as listed in **Table B**.

Table B PK Blood Sampling Schedule (6 mL per sample)

Seq. #	Scheduling	Time window	Sample No.,"XX"	#	Scheduling	Time window	Sample No.,"XX"
1	-60 min	± 5 min	01	15	75 min	±3 min	15
2	-30 min		02	16	80 min	±5 min	16
3	0 min	±1 min	03	17	90 min		17
4	5 min		04	18	105 min		18
5	10 min		05	19	120 min		19
6	20 min	±2 min	06	20	150 min		20
7	25 min		07	21	180 min	±10 min	21
8	30 min		08	22	210 min		22
9	40 min	±3 min	09	23	4 h		23
10	50 min		10	24	5 h		24
11	55 min		11	25	6 h		25
12	60 min		12	26	8 h	±20 min	26
13	65 min		13	27	10 h		27
14	70 min		14	28	12 h		28
Total							28 (168mL)

The sample ID for PK samples (after centrifugation) is defined as

"*I4-B-nn-V-XX-k*"

where *k* is the aliquot number (A or B). The sample ID labels will be printed in blue colored fonts with label size for the tube is 1/2"×1¾" (Avery label 5667), as demonstrated below:

I4-B-nn-V-XX-k

Sample Transportation and Analysis

Prior to sample transportation, all samples should be stored in a box and must be organized in sequential order by subject and separated by aliquot. The chain of custody, temperature control and related documents for sample transportation must be recorded. During transportation of PK samples from the site to Amphastar, the temperature should be below -20˚C. The transportation between the study site and the test laboratory must be completed within 48 hrs. The chain of custody, elapsed time for transport, temperature control and related documents for sample transfer must be recorded and documented. All serum samples will be stored at -50 ºC or lower in the

laboratory. Serum samples will be analyzed for the concentrations of (i) insulin aspart, (ii) C-peptide, and (iii) endogenous human insulin with a validated method of ultra-high pressure liquid chromatography/high resolution mass spectrum (UPLC/HRMS) by R & D Department of Amphastar under GMP, please refer to Section 7. 15 for details.

(2) PD Glucose Clamp

On the study visit, following ≥ 10 hour overnight fast, a 12-hour euglycemic clamp will be initiated.

At least 1 hour prior to the clamp, three venous catheters will be inserted into peripheral veins to connect subjects to the Biostator, to allow for the sampling of glucose, insulin and or other analytes, and to continuously infuse glucose 20%.

The determination of blood glucose concentrations will start at least 60 minutes prior to dosing. The mean of three blood glucose (BG) readings from -30 to -10 minutes (i.e., -30, -20 and -10 minutes) will be used to determine the individual subject's fasting blood glucose level for each dosing visit. The individual blood glucose clamp target level will be determined for each subject by the mean fasting glucose level minus 5 mg/dL.

If differences between initial fasting blood glucose values (-30, -20, -10 min) exceed the limit of 10 mg/dL, subjects will need to be rescheduled. Additionally, subjects will not be dosed if mean fasting plasma glucose is < 75 mg/dL due to proximity to overt hypoglycemia or > 99 mg/dL. Subjects may be re-scheduled once to come back to the site on a later day and may be dosed if the mean fasting plasma glucose is ≥ 75 mg/dL or ≤ 99 mg/dL prior to dosing.

The study drug will be administered at approximately 08:00 (t=0) by qualified staff via SC injection as described in section 6.4.

Following the study drug administration, the clamp procedure will start. Onset of insulin action will be identified by a ≥ 5mg/dL decline of BASELINE level and start of the glucose infusion rate (GIR). After the onset of insulin action is identified, an infusion of glucose 20% will then be utilized as needed to maintain target level of blood glucose concentration.

The GIR will be based on the rates suggested by a published clamp algorithm. The clamp operator can adapt the glucose infusion rate manually as deemed appropriate. The GIR required to keep blood glucose at target level (±10%) will be recorded every one minute over 12 hours and data will be used to calculate PD parameters.

The subjects will remain fasting in a supine or semi-supine position during the entire glucose

clamp. The subjects will be allowed to sip water throughout the clamp but will otherwise remain fasting.

V. Study Site and Investigators

The study will be performed at

[redacted]
Chula Vista, CA, 91911
USA

The detailed information about the study site can be found on FDA Form 1572, including: Name, address, and statement of qualifications of each principal investigator; name of each sub-investigator working under the supervision of the investigator; name and address of the research facilities to be used; name and address of each reviewing IRB per US 21 CFR 312.23 section 6(iii)b.

Principal Investigators (PI) responsible for the conduct of this study will be board certified physicians who are familiar with the study medications and trained to handle any untoward events related to medications.
The PI is responsible for the global aspects of the research study. For safety monitoring, a study physician or healthcare professional, who is certified for Advanced Cardiac Life Support, will be available at the study site at the time of study drug administration and during the entire time of PK blood sampling period. The Institutional Review Board (IRB) acknowledges one PI per study site. Qualifications for the PI and/or Co-Investigator(s) are listed on the FDA Form 1572.

VI. Study Evaluation

1) Pharmacokinetics (PK) Endpoints for Insulin Aspart (IA)

(1) Primary PK Endpoints for IA
   (i) C_{IAmax}, defined as the maximum serum IA concentration;
   (ii) $AUC_{IA(0\text{-}12h)}$, defined as area under the curve (AUC) of serum IA concentrations for time 0 to 12 hours; and

(2) Secondary PK Endpoints for IA

   (i) $AUC_{IA(0\text{-}\infty)}$ of Insulin Aspart,
   (ii) $AUC_{IA(0\text{-}2h)}$,
   (iii) t_{max} for IA (t_{IAmax}),
   (iv) Apparent clearance (CL/F),

(v) Apparent volume of distribution (Vz/F), and
(vi) Half-life ($t_{1/2}$).

2) Pharmacokinetics (PK) Endpoints for Human Insulin (HI)

(1) Primary PK Endpoints for HI
(i) C_{HImax}, defined as the maximum serum HI concentration;
(ii) $AUC_{HI(0-12h)}$, defined as area under the curve (AUC) of HI concentrations for time 0 to 12 hours; and

(2) Secondary PK Endpoints for HI: t_{max} for HI (t_{HImax})

3) Pharmacodynamics (PD) endpoints

The PD parameters are used to measure the study drug action over time as measured by the hyperglycemic-euglycaemic clamp procedure. During the clamp procedure, blood glucose concentrations are held constant after the administration of NovoLog® by adjusting the exogenous glucose injection rate (GIR). GIR, denoted as *G*, is defined as infusion rate of glucose administered intravenously needed to maintain target blood glucose level. GIR data will be reported as mg/kg/min.

(1) Primary PD Endpoints

(i) G_{max}: Maximum GIR, defined as maximum infusion rate of glucose administered intravenously needed to maintain target blood glucose level.
(ii) $AUC_{G(0-12h)}$ for GIR: defined as area under the curve (AUC) of GIR, i.e. the total amount of glucose infused over the duration of the clamp procedure.

Both G_{max} and AUC_G of GIR are calculated based on smoothed GIR curves.

(2) Secondary PD Endpoints

(i) $AUC_{GA(0-12h)}$ for GA (or G_A), where GA stands for "GIR for IA" to reflect the PD effect of only IA.
(ii) G_{Amax}
(iii) $AUC_{G(0-last)}$,
(iv) $AUC_{G(0-2h)}$,
(v) Value of last measurable GIR, G_{last}
(vi) Time until maximum glucose infusion rate is reached, t_{Gmax}
(vii) Time of start of GIR post-dose, t_{Gonset}

(viii) Time of last measurable GIR, t_{Glast}

(ix) $t\text{-}_{G50\%early}$, defined as the time to 50% maximal GIR before t_{Gmax}

(x) $t\text{-}_{G50\%late}$, defined as the time to 50% maximal GIR after t_{Gmax}

4) Statistical Analysis

The ratio (T to R) of geometric means and 90% confidence intervals of the ratio of geometric means for primary and secondary endpoints are to be estimated and evaluated.

VII. Safety Evaluation

(1) Changes in vital signs and ECG from baselines will be analyzed at designated post-dosing time points for two treatments, respectively.

(2) The results of laboratory tests, including hematology, clinical chemistry and UA, will be assessed. Comparisons between data at Screening and that at the follow-up visit (F/U) will be performed. Unscheduled visit for safety laboratory testing is allowed and at the discretion of PI during the COVID-19 pandemic.

(3) Adverse events (AEs): The occurrence of AEs will be compared for the two treatments. Two AE of special interests: hypoglycemia and injection site reaction will be closed monitored and elevated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AE	Adverse event
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
AUCGIR	Area under the glucose infusion rate-time curve
BG	Blood glucose
BMI	Body mass index
BW	Body weight
CDM	Clinical data management
CI	Confidence interval
CL/F	Apparent clearance
Cmax	Maximum concentration
CRF	Case report form
CRO	Contract Research Organization
CSR	Clinical Study Report
CV	Coefficient of variation
DMP	Data management plan
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
Ecrf	Electronic CRF
ELISA	Enzyme linked immunosorbent assay
FDA	Food and Drug Administration
FPG	Fasting plasma glucose
FSFV	First subject first visit
GA	Glucose infusion rate (GIR) for Insulin Aspart (IA)
GCP	Good Clinical Practice
GIR	Glucose infusion rate
HbA1C	Glycosylated hemoglobin
HbsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HI	Human Insulin
HIV	Human immunodeficiency virus
IA	Insulin Aspart
IP	Investigational Product
IB	Investigator's brochure
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
INR	International normalized ratio
IRB	Institutional Review Board
IV	Intravenous
Kg	Kilogram
Lb	Pound
LC/MS/MS	Liquid Chromatography with tandem mass

	spectrometry
LLOQ	Lower Limit of Quantification
LS mean	Least square mean
MSIA	Mass Spectrometric Immunoassay
MSIA-HR/MS	Mass spectrometric immunoassay with high resolution and accurate mass detection
Mcg	Microgram
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Milligram
m/z	mass to charge ratio
Nmol	Nanomol
OTC	Over the counter
PD	Pharmacodynamics
PE	Physical examination
PI	Principal Investigator
PK	Pharmacokinetics
PP	Per-Protocol
rDNA	Recombinant deoxyribonucleic acid
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous
SOE	Schedule of events
SUSAR	Suspected unexpected serious adverse reaction
t½	Terminal insulin half-life
TEAE	Treatment emergent adverse event
TG	Triglycerides
tGIRmax	Time to maximum glucose infusion rate
Tmax	Time to maximum serum insulin concentration (in concentration time curve)
TSH	Thyroid-stimulating hormone
ULN	Upper limit of normal
US	United States
USP	Unites States Pharmacopeia
Vz/F	Apparent volume of distribution at terminal phase
WHO	World Health Organization
YSI	YSI 2300 STAT Glucose Analyzer

1.0 INTRODUCTION

1.1 Background

In 2015, diabetes affected 30.3 million Americans or 9.4% of the total US population, including 7.2 million people who were undiagnosed.[1] Recently, there have been dramatic yearly increases in the incidence of diabetes, and should this upward trend continue, the total prevalence of diagnosed and undiagnosed diabetes in the US adult population is projected to increase to as much as 33% by the year 2050.[2]

The results of the Diabetes Control and Complication Trial (DCCT) demonstrated that tight glycemic control can prevent or decrease the microvascular complications of diabetes, such as retinopathy, nephropathy, neuropathy as well as reduce cardiovascular– related death.[3] To optimize blood glucose control in patients, intensive insulin regimens are used, although current replacement insulin formulations only approximate physiologic insulin secretion, resulting in high postprandial blood glucose peaks.[4]

Another disadvantage of intensive insulin regimens is the risk of hypoglycemia. The development of rapid-acting insulins with accelerated absorption has improved the postprandial glucose excursions and allowed for more precise dosing.[5,6,7]

The development of rapid-acting insulin analogs with fast absorption has reduced postprandial glucose excursions and has allowed greater flexibility in insulin therapy as well as a more physiological insulin replacement with more precise dosing regimens.[8]

Both I004 and NovoLog® are insulin Aspart [rDNA origin] injection, rapid-acting human insulin analogs used to lower blood glucose. I004 and NovoLog are homologous with regular human insulin, with the exception of a single substitution of the amino acid proline by aspartic acid in position B28, and is produced by recombinant DNA technology. The insulin formulation is available in a concentration corresponding to 100 units/mL (U-100).

Please refer to the Investigator's Brochure /Package Insert[9] for further information on NovoLog®.

1.2 Rationale for the Proposed Study

This study will assess PK and PD data that will be necessary for a future biosimilar approval. Therefore, the comparison of rapid-acting insulin I004 and Novolog with regard to the AUC_{IA} (0-12h), area under the serum insulin profile for the drug, and AUC_{GIR} (0-12h), area under the glucose infusion rate as primary endpoints, will be performed.

Safety, cardiac safety (ECG), and tolerability after SC administration will also be determined.

1.3 Summary of Pre-Clinical /Clinical Studies

Information regarding characterization, structure and properties of Insulin aspart [rDNA origin] are provided in the Novolog Investigator's Brochure (IB).

For data of in vitro and in vivo pharmacologic and toxicologic studies and clinical studies, reference is made to [redacted] *(Novolog,* [redacted]*).* The sponsor refers to the information and FDA safety determinations in the approved product labeling for these products.

Novolog will be commercially procured from the US market and will be used as the investigational product and comparator in this study. It will be administered at the same dose and in the same fashion, consistent with the approved product information.

1.4 Rationale for Treatment and Dose

The dose of 0.2 units/kg body weight has been chosen for this study in order to provide a robust dose-response relationship relevant for healthy subjects. In addition, the dose used is representative of doses in healthy subjects and is within the dose range used in other clinical glucose clamp studies conducted to date, where no safety concerns have been observed at this dose level.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

To assess and compare the PK profile of I004 and Novolog by:

(i) Maximum insulin concentration, C_{IAmax}
(ii) Area under the insulin concentration-time curve, $AUC_{IA\ (0\text{-}12h)}$

To assess and compare the PD profile of I004 and Novolog by:

(i) Maximum glucose infusion rate, G_{max}
(ii) Area under the curve for the glucose infusion rate (GIR)-time curve from administration to end of clamp time, $AUC_{G\ (0\text{-}12h)}$

2.2 Secondary Objectives

To assess and compare the PK profile of I004 and Novolog by:

(i) Partial AUC_{IA}, eg, $AUC_{IA\ (0\text{-}2h)}$
(ii) Area under the insulin concentration curve extrapolated to infinite time, $AUC_{IA(0\text{-}\infty)}$
(iii) Time until C_{max} is reached, t_{IAmax}
(iv) Apparent clearance, CL/F
(v) Apparent volume of distribution, Vz/F
(vi) Apparent terminal half-life, $t_{1/2}$

To assess and compare the PD profile of I004 and Novolog by:

(i) Total and partial AUC of GIR, eg, $AUC_{G(0\text{-}last)}$, $AUC_{GIR(0\text{-}2h)}$
(ii) Value of last measurable GIR, G_{last}
(iii) Time until maximum glucose infusion rate is reached, t_{Gmax}
(iv) Time of start of GIR post-dose, t_{Gonset}
(v) Time of last measurable GIR, t_{Glast}
(vi) $t\text{-}_{G50\%early}$, defined as the time to 50% maximal GIR before t_{Gmax}
(vii) $t\text{-}_{G50\%late}$, defined as the time to 50% maximal GIR after t_{Gmax}

To assess the confounding effect of endogenous human insulin:

(i) PK profile of endogenous human insulin

2.3 Safety Objectives

To assess safety and tolerability of I004 and Novolog by:

(i) Incidence of adverse events (including hypoglycemia)
(ii) Clinical findings on physical examination
(iii) Clinical laboratory parameters (hematology, serum chemistry and urinalysis)
(iv) Vital signs (blood pressure, temperature, respiratory rate, and heart rate) measurements
(v) 12-lead ECG parameters
(vi) Injection site reaction

2.4 Study Endpoints

1) Pharmacokinetic (PK) Endpoints for Insulin Aspart (IA)

(1) Primary PK Endpoints for IA
(i) C_{IAmax}, defined as the maximum serum IA concentration;
(ii) $AUC_{IA(0\text{-}12h)}$, defined as area under the curve (AUC) of serum IA concentrations for time 0 to 12 hours; and

(2) Secondary PK Endpoints for IA
(i) $AUC_{IA(0\text{-}\infty)}$ of Insulin Aspart,
(ii) $AUC_{IA(0\text{-}2h)}$,
(iii) t_{max} for IA (t_{IAmax}),
(iv) Apparent clearance (CL/F),
(v) Apparent volume of distribution (Vz/F), and
(vi) Half-life ($t_{1/2}$).

2) Pharmacokinetic (PK) Endpoints for Human Insulin (HI)

(1) Primary PK Endpoints for HI
(i) C_{HImax}, defined as the maximum serum HI concentration;
(ii) $AUC_{HI(0\text{-}12h)}$, defined as area under the curve (AUC) of HI concentrations for time 0 to 12 hours; and

(2) Secondary PK Endpoints for HI: t_{max} for HI (t_{HImax})

3) Pharmacodynamic (PD) endpoints

The PD parameters are used to measure the study drug action over time as measured by the

hyperglycemic-euglycaemic clamp procedure. During the clamp procedure, blood glucose concentrations are held constant after the administration of NovoLog® by adjusting the exogenous glucose injection rate (GIR). GIR, denoted as *G*, is defined as infusion rate of glucose administered intravenously needed to maintain target blood glucose level. GIR data will be reported as mg/kg/min.

(1) Primary PD Endpoints

(i) G_{max}: Maximum GIR, defined as maximum infusion rate of glucose administered intravenously needed to maintain target blood glucose level.
(ii) $AUC_{G(0\text{-}12h)}$ for GIR: defined as area under the curve (AUC) of GIR, i.e. the total amount of glucose infused over the duration of the clamp procedure.

Both G_{max} and AUC_G of GIR are calculated based on smoothed GIR curves.

(3) Secondary PD Endpoints

(i) $AUC_{GA(0\text{-}12h)}$ for GA (or G_A), where GA stands for "GIR for IA" to reflect the PD effect of only IA.
(ii) G_{Amax}
(iii) $AUC_{G(0\text{-}last),}$
(iv) $AUC_{G(0\text{-}2h)}$,
(v) Value of last measurable GIR, G_{last}
(vi) Time until maximum glucose infusion rate is reached, t_{Gmax}
(vii) Time of start of GIR post-dose, t_{Gonset}
(viii) Time of last measurable GIR, t_{Glast}
(ix) $t\text{-}_{G50\%early}$, defined as the time to 50% maximal GIR before t_{Gmax}
(x) $t\text{-}_{G50\%late}$, defined as the time to 50% maximal GIR after t_{Gmax}

3.0 INVESTIGATORS AND STUDY SITES

3.1 Investigational Site

The study will be performed at

[redacted]
Chula Vista, CA, 91911
USA

The detailed information about the study site can be found on FDA Form 1572, including: Name, address, and statement of qualifications of each principal investigator; name of each sub-investigator working under the supervision of the investigator; name and address of the research facilities to be used; name and address of each reviewing IRB per US 21 CFR 312.23 section 6(iii)b.

3.2 Principal Investigator(s)

Principal Investigators (PI) responsible for the conduct of this study will be board certified physicians who are familiar with the study medications and trained to handle any untoward events related to medications.

The PI is responsible for the global aspects of the research study. For safety monitoring, a study physician or healthcare professional, who is certified for Advanced Cardiac Life Support, will be available at the study site at the time of study drug administration and during the entire time of PK blood sampling period. The Institutional Review Board (IRB) acknowledges one PI per study site. Qualifications for the PI and Co-Investigator(s) are listed on the FDA Form 1572.

3.3 Laboratory

Samples of blood for evaluation of hematology and chemistry (metabolic panels) will be analyzed by the clinical laboratory with certification from a recognized accreditation agency (e.g., College of American Pathology or Clinical Laboratory Improvement Amendments certification). Refer to **Table 7-1** for a list of parameters analyzed, and the laboratory manual for details regarding specimen sample collection, processing, and shipment procedures. PK samples will be processed and analyzed by R&D Department, Amphastar Pharmaceuticals, Inc.

4.0 STUDY DESIGN AND DESCRIPTION

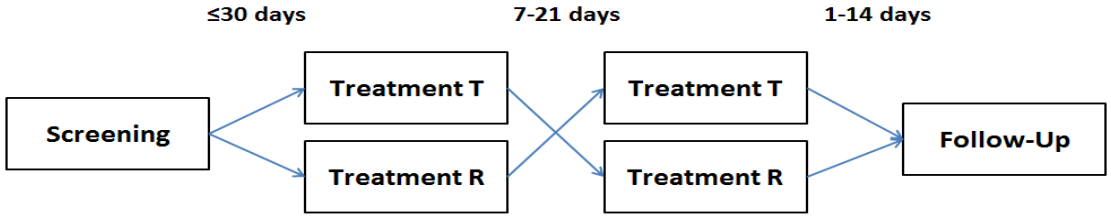
4.1 Study Design

This is a pivotal, phase II/III, single dose, double-blind, randomized, two-treatment, two-period, crossover, euglycemic glucose clamp study to assess the safety, pharmacokinetic and pharmacodynamic profiles of insulin I004 and Novolog in healthy subjects. Each period will include a 12-hour clamp and blood sampling procedure.

At least sixty (60) eligible subjects will be randomized to receive a total of two administrations of study drug on two separate dosing visits. Each subject will receive one administration of either 0.2 units/kg I004 or 0.2 units/kg Novolog. Replacement of dropouts and withdrawals will take place in order to ensure at least 60 subjects complete the study.

Each subject will undergo a Screening Visit, followed by two treatment periods, each consisting of a one-day in-house treatment period, with one euglycemic clamp procedure, for a total of two glucose clamp procedures per subject. The Wash-out Period between treatment periods will be at least 7 days. A Follow-up Visit will take place after the last dose of study drug in treatment period 2. The original duration of participation in this study, including Screening, Treatment and Follow-up will approximately be up to 10 weeks, as shown in **Figure 4-1**. However, Screening visit window, Treatment visit window, and Follow-up visit window could be extended to accommodate state-issued Stay at Home orders due to the COVID-19 pandemic.

Figure 4-1 Study Design Schematic



Due to the COVID-19 pandemic, original Screening visit window (≤30 days), Treatment visit window (7-21 days), and Follow-up visit window (1-14 days) could be extended to accommodate state-issued Stay at Home orders.

4.2 Study Description

Screening Visit:

Before the Screening takes place, potential subjects for the study will be provided with written and oral information about the study and the procedures involved. Subjects have to sign the IRB/IEC informed consent form (ICF) prior to entering the study.

The Screening Visit will be performed prior to the first dosing, in order to identify eligible subjects for the study. All assessments performed at the Screening visit are stated in the SOE (Appendix A) and will be recorded in the electronic case report form (eCRF). See section 7.1.2 and 9.2 for further details.

Treatment Period 1:

All eligible subjects will check in to the clinic in the morning of Day 1 for a one-day in-house period, to standardize meals and ensure adequate fasting time prior to the clamp procedure. Check–in assessments will be performed to ensure subject meets the check-in requirements.

Subjects will be randomized to one of two treatment sequences (TR or RT), where treatments will consist of single SC study drug administrations. Please see section 6.6 for further details.

On Day 1, following ≥ 10 hour overnight fast, a 12-hour euglycemic clamp will be initiated. EMEA (European Medicines Agency) recommends typical clamp duration to be 8 to 10 hours for rapid-acting insulins.[16] The 12-hour duration of the clamp study follows the recommendations from the EMEA and takes into account the known duration of action of the US-approved Novolog, with a half-life 81 minutes.[9]

At least 1 hour prior to the clamp, three venous catheters will be inserted into peripheral veins to connect subjects to the Biostator, to allow for the sampling of glucose, insulin and or other analytes, and to continuously infuse glucose 20%.

The determination of blood glucose concentrations will start at least 60 minutes prior to dosing. The mean of three blood glucose (BG) readings from -30 to -10 minutes (i.e., -30, -20 and -10 minutes) will be used to determine the individual subject's fasting blood glucose level for each dosing visit. The individual blood glucose clamp target level will be determined for each subject by the mean fasting glucose level minus 5 mg/dL.

If differences between initial fasting blood glucose values (-30, -20, -10 min) exceed the limit of 10 mg/dL, subjects will need to be rescheduled. Additionally, subjects will not be dosed if mean fasting plasma glucose is < 75 mg/dL due to proximity to overt hypoglycemia or > 99 mg/dL. Subjects may be re-scheduled once to come back to the site on a later day and may be dosed if the mean fasting plasma glucose is ≥ 75 mg/dL or ≤ 99 mg/dL prior to dosing.

The study drug will be administered at approximately 08:00 (t=0) by qualified staff via SC injection as described in section 6.4.

Following the study drug administration, the clamp procedure will start. Onset of insulin action will be identified by a ≥ 5mg/dL decline of BASELINE level and start of the GIR. After the onset of insulin action is identified, an infusion of glucose 20% will then be utilized as needed to maintain

target level glucose concentration.

The GIR will be based on the rates suggested by a published clamp algorithm. The clamp operator can adapt the glucose infusion rate manually as deemed appropriate. The GIR required to keep blood glucose at target level (±10%) will be recorded over 12 hours and data will be used to calculate PD parameters. For details on the clamp procedure please see section 7. 14.

The subjects will remain fasting in a supine or semi-supine position during the entire glucose clamp. The subjects will be allowed to sip water throughout the clamp but will otherwise remain fasting.

After the end of the clamp procedure, subjects will be disconnected from the Biostator.

Post-dose procedures, such as vital signs, ECG and injections site assessment will be performed during/after the clamp procedure as stated in the SOE (Appendix A).

Blood glucose will be measured to ensure that plasma glucose levels are stable and thereafter the subjects will receive a meal. Subjects will stay in the clinic throughout the treatment period until the glucose clamp has been completed and after deemed safe by the Investigator.

Wash-out period:

Subjects will be scheduled to come in for the next treatment period after a wash-out period of at least 7 days.

Treatment Periods 2:

All subjects will check in to the clinic in the morning of Day 1 for the second of the in-house treatment period. All procedures and the clamp will follow the same procedures as stated for the first treatment period, except for the change of the study drug.

Body weight from Day 1 of Treatment Period 1 under fasting condition will be used to calculate the drug dose for Treatment Period 2.

Follow-up Visit:

A Follow-up Visit will be performed after the last dosing to perform final safety procedures. The End of Study Form must be completed. Please see SOE (Appendix A) for details.

Sampling and Assessment:

Time points for study procedures and sample collection are specified in section 7.1.

Safety assessments will occur throughout the duration of the study, including monitoring of adverse events (AEs), clinical laboratory tests (chemistry, hematology and urinalysis), vital signs measurements (blood pressure, heart rate, respiration rate, and aural temperature), 12-lead electrocardiograms (ECGs) and physical examinations.
Sampling collections for PK, PD and glucose measurements will be performed as specified in Appendix B. Sample collection for PK and PD parameter will be collected under fasting condition for 12 hours.

Blood samples for the analysis of C-peptide and endogenous human insulin will be collected with every PK sample. The standardized measurements of the Biostator will be verified on a regular basis using the YSI reference device.

4.3 Rationale for Study Design and Endpoints

A single dose cross-over design is chosen in order to allow each subject to act as his/her own control in order to reduce variability.

A minimum 7-day wash-out period before and between the two treatment periods is introduced to ensure a sufficient wash-out from previous dosing, in order to avoid any carry-over effects.

Randomization is used to avoid bias introduced through an association between trial drug allocation order and subject characteristics.

Blinding of participants and personnel is introduced in order to avoid performance bias.

Due to the variability in fasting blood glucose concentrations in subjects with type 1 diabetes, which might affect the metabolic response to the study drug, healthy subjects have been chosen. A study in healthy subjects is likely to produce less PK variability compared with that in patients with potentially confounding factors such as underlying and/or concomitant disease and concomitant medications. C-peptide and endogenous human insulin in parallel to the insulin aspart concentration is measured during the study, to detect potential changes in endogenous insulin secretion and enable a correction for endogenous insulin secretion, if necessary.

Enrollment criteria will favor a generally insulin-sensitive population and diseases/conditions and medications associated with insulin resistance have been excluded.

The sample size of 60 subjects is chosen as it represents a typical number for a biosimilar study.
The use of the euglycemic glucose clamp method is chosen to assess PD in a standardized fashion and for safety reasons to avoid hypoglycemia.

Glucose levels below approximately 60 mg/dL must be avoided because they result in the

stimulation of counter regulatory hormones (epinephrine, glucagon, cortisol, growth hormone) to increase blood glucose concentrations and lead to a rapid and pronounced worsening of insulin sensitivity, thus influencing the estimated time-action profile of the investigated insulin preparation. Therefore, blood glucose (BG) inclusion criterion prior to the clamp is limited to a fasting plasma glucose value ≥ 75 mg/dL (for the mean of the -30 ≤ to -10 min glucose measurements), as the target clamp level is 5 mg/dL below the mean fasting BG values. The chosen target level of mean fasting glucose minus 5 mg/dL will ensure that endogenous insulin secretion is sufficiently suppressed and will not interfere with PK and PD assessments.

For the study design, the FDA and EU guidance on the development of similar biological medicinal products and prior studies, evaluating the pharmacology of rapid-acting insulins, have been taken into consideration.[10,11,12]

Safety is further emphasized by the design of the study: The clamp procedures in this study will be conducted at a specialized early phase research center, where the subjects will be under supervision of physicians, research nurses and additional medical staff experienced in investigating new insulins, incretins or other metabolic compounds. Following the glucose clamp procedures, subjects may be released from the investigational site or may be observed for longer, if deemed appropriate and needed by the Investigator to monitor for the risk of hypoglycemic episodes.

4.3.1 Criteria for Early Termination of the Study

The study will be completed as planned unless one or more of the following criteria are satisfied that require temporary suspension or early termination of the study.

- New information or other evaluation regarding the safety or efficacy of the study medication that indicates a change in the known risk/benefit profile for the compound, such that the risk/benefit is no longer acceptable for subjects participating in the study.

- Significant violation of Good Clinical Practice (GCP) that compromises the ability to achieve the primary study objectives or compromises subject safety.

- Failure to meet expected enrollment goals.

- Administrative Reasons.

In the event that the Sponsor, an institutional review board (IRB)/ independent ethics committee (IEC), or regulatory authority elects to terminate or suspend the study or the participation of an investigational site, a study-specific procedure for early termination or suspension will be provided by the Sponsor; the procedure will be followed by applicable investigational sites during the course of termination or study suspension.

4.3.2 Criteria for Early Termination of Individual Subjects

Subjects may withdraw their consent to participate in the study at any time.

If a subject withdraws consent, the date and reason for consent withdrawal should be documented. Subjects will be encouraged to remain in the clinic for safety assessments until the Investigator deems that it is safe for the subject to be discharged. Subject data will be included in the analysis up to the date of the consent withdrawal.

- AE or SAE that requires discontinuation at the discretion of the Investigator
- Protocol violation: If protocol violation or concurrent illness occurs, which, in the clinical judgment of the Investigator or after discussion with the Sponsor, may invalidate the study by interfering pharmacokinetically or pharmacodynamically with the investigational products, the subject will be withdrawn by the Investigator.
- Lost to follow-up. The subject did not return to the clinic and attempts to contact the subject were unsuccessful. Attempts to contact the subject must be documented.
- Voluntary withdrawal of consent (mandatory removal from study).
- Discretion of Investigator (document reason on CRF).
- Subject becomes pregnant or begins breastfeeding (mandatory).
- Study discontinuation by Sponsor.

Wherever possible, the tests and evaluations, including those listed for the Follow-up Visit should be performed for all subjects who discontinue prior to the completion of the study.

In the event the Investigator determines to terminate a subject participation in the Clinical Study, the Investigator must notify the Sponsor of such decision and rationale immediately in writing. In all cases, the appropriate IRB/IEC and other applicable regulatory authorities shall be informed.

5.0 STUDY POPULATION

5.1 Inclusion Criteria

Subjects must meet all of the following criteria at the Screening Visit to be considered eligible to participate in the study:

(1) Upon review, agree to participate and sign informed consent.

(2) Healthy male and female subjects ≥ 18 to ≤ 65 years of age.

(3) Body mass index (BMI) ≥ 18.5 to ≤ 29.9 kg/m^2.

(4) Weight ≥ 50 kg.

(5) Fasting plasma glucose of < 100 mg/dL (5.5 mmol/L) measured with YSI at site; one repeat test is allowed.

(6) HbA1c < 5.7 %.

(7) Non-smoker for ≥ 3 months prior to Screening.

(8) Female candidates must be >1 year post-menopausal, surgically sterile, or practicing a clinically acceptable form of birth control and confirmed by negative serum pregnancy test at Screening.

5.2 Exclusion Criteria

Subjects who meet any of the following criteria at the Screening Visit will be excluded from participating in the study:

(1) History of diabetes mellitus.

(2) Resting blood pressure (BP) > 140/90 mmHg or < 90/60 mmHg. Subjects BP may be re-checked.

(3) Participation in an investigational drug/device study within 30 days or 5 half-lives within the last dose of any study drug, whichever is longer.

(4) History of any serious adverse reaction or hypersensitivity to any of the investigational product components.

(5) Have significant history of or current cardiovascular, respiratory, hepatic, renal, gastrointestinal, endocrine, hematological, or neurological disorders or abnormalities, or other major systemic disease that, according to the investigator, would unduly risk the subject's safety or may impact the conduct of the study.

(6) Subject shows evidence of significant active neuropsychiatric disease, including taking prescription medication for such diseases (including anti-depressant /anti-

anxiety medication).

(7) Presence of clinically significant physical, laboratory, or ECG findings at Screening that, in the opinion of the Investigator, may interfere with any aspect of study conduct or interpretation of results, or may present a safety issue to that particular subject (laboratory results may be re-checked once on a separate day per Investigator discretion).

(8) Long QT syndrome or family history of long QT syndrome or corrected QT interval (QTcF) > 450 ms in men, > 470 ms in women at Screening.

(9) Liver function test results of AST and/or ALT ≥ 2.5 upper normal limit (ULN).

(10) Subject has a history of syncope.

(11) History of any major surgery within 6 months.

(12) History of any active infection, other than mild viral illness within 30 days prior to dosing.

(13) History of blood clots (eg, deep vein thrombosis or embolism) or a frequent appearance in 1st degree relatives as judged by the Investigator.

(14) Known history or positive test of hepatitis B surface antigen (HBsAg), hepatitis C antibody (HCV Ab), or human immunodeficiency virus type 1 (HIV-1) or 2 (HIV-2) antibody.

(15) History of alcohol abuse as judged by the Investigator within approximately 1 year. Average weekly alcohol intake > 21 units/week (males) and > 14 units/week (females) or are unwilling to stop alcohol consumption from 24 hours prior to each dosing until discharged from the clinical research unit (CRU). Positive alcohol test at Screening. (One unit of alcohol equals about 250 mL of beer or lager, one glass of wine, or 20 mL of spirits).

(16) History of illicit drug abuse, including marijuana, within approximately 1 year or evidence of current use as judged by the Investigator. Positive drug test at Screening.

(17) Donation or loss of > 500 mL of blood within 56 days.

(18) Chronic use of or over-the-counter or prescription medication within 7 or 14 days prior to dosing (apart from vitamin/mineral supplements, occasional paracetamol, or birth control methods [Desogestrel is not allowed]).

(19) Unable to comply with the safety monitoring requirements of this clinical study or is considered by the investigator to be an unsuitable candidate for the study.

5.3 Prohibited Medications

Use of prescription or nonprescription drugs is prohibited from 7-14 days prior to dosing.

Vitamins, mineral supplements, occasional paracetamol and /or birth control methods are allowed after discretion of the Investigator. Desogestrel is not allowed.

5.4 Check-in Criteria on Day 1

Subjects will be educated about the factors that may influence insulin sensitivity, such as refraining from strenuous exercise within 72 hours prior to the injection of the investigational product and during the in-house period, avoidance of alcohol and herbal supplements within 24 hours prior to dosing, caffeinated drinks (caffeine containing food and beverages within 24 hours prior to dosing), smoking or medication and illness/infection.

Treatment Period 1

At check-in for Treatment Period 1, subjects will not be allowed to check in if they meet any of the following criteria and the treatment period may be rescheduled one time with a time window of 1-14 days.

(1) Positive alcohol breath test.

(2) Positive urine drug screen test.

(3) Positive urine pregnancy test in female subjects. If sample is positive, blood sample will be sent to local lab for confirmation. A negative test result is mandatory prior to dosing.

(4) Any medical condition that could interfere with glucose metabolism, as judged by the Investigator.

(5) Any use of medicine (prescribed or over-the-counter) other than any allowed concomitant medications within the last 7-14 days prior to dosing.

(6) Strenuous exercise within the last 72 hours prior to injection of the investigational product.

(7) Consumption of caffeine containing food and beverages within 24 hours prior to dosing.

On Day 1, subjects will not be allowed to be dosed if they meet the following criterion and the treatment period may be rescheduled one time with a time window of 1-14 days.

(1) Subjects whose differences between initial fasting blood glucose values (-30, -20, -

10 min) determined by the YSI exceed the limit of 10 mg/dL or whose mean FPG is < 75 mg/dl or > 99 mg/dL will be rescheduled.

Treatment Period 2

At check-in for Treatment Period 2, subjects will not be allowed to check in if they meet any of the following criteria and the treatment period may be rescheduled one time (1-14 days later). The Sponsor will be notified about the rescheduling in a timely manner.

(1) Positive alcohol breath test.

(2) Positive urine drug screen test.

(3) Positive urine pregnancy test in female subjects. If sample is positive, blood sample will be sent to local lab for confirmation. A negative test result is mandatory prior to dosing.

(4) Any medical condition that could interfere with glucose metabolism, as judged by the Investigator.

(5) Any use of medicine (prescribed or over-the-counter) other than any allowed concomitant medications within the last 7-14 days prior to dosing.

(6) Strenuous exercise within the last 72 hours prior to injection of the investigational product.

(7) Consumption of caffeine containing food and beverages within 24 hours prior to dosing.

(8) Weight change of ≥ 5% compared to the weight for the first dosing.

On Day 1, subjects will not be allowed to be dosed if they meet the following criterion and the treatment period may be rescheduled one time with a time window of 1-14 days.

(1) Subjects whose differences between initial fasting blood glucose values (-30, -20, -10 min) determined by the YSI exceed the limit of 10 mg/dL or whose mean FPG is < 75 mg/dl or > 99 mg/dL will be rescheduled.

6.0 STUDY MATERIALS

6.1 Investigational Products

The investigational product is I004 (insulin aspart [rDNA origin] injection), a rapid-acting human insulin analogue. [redacted]

[redacted]

I004 is manufactured by [redacted].

For this study, I004 and Novolog, [redacted] will be provided by the Sponsor. The IP will be used as multi-dose vial (MDV). The Novolog product used in the study will be the U.S.-approved product ([redacted]).

For more detailed information please see the Novolog Product Insert.[9]

6.2 Packaging, and Labeling of Investigational Products

The Sponsor will provide the Investigator with the labeled drug in accordance with specific regulatory requirements.

The drug will be packaged and shipped to the site in an open label manner.

6.3 Storage and Drug Accountability of Investigational Products

All clinical material must be kept in an appropriate, limited-access, secure location. The investigational products, storage and preparation instructions will be provided by the Sponsor.

Table 6-1: Storage Conditions

I004 or Novolog	Unopened Refrigerated (2°C -8°C)	Unopened Room Temperature (below 30°C)	Opened Room Temperature (below 30°C)
[redacted] vial	Until expiration date	28 days	28 days (refrigerated/room temperature)

I004 or Novolog must be stored securely in a refrigerator at 2 ℃ to 8 ℃ (36 °F to 46 °F), and should not be exposed to excessive heat, direct sunlight and never be frozen. It must be allowed to come to room temperature on the morning of dosing.

The study staff is required to document the receipt, dispensing, and return/destruction of IP and supplies provided by or on behalf of the Sponsor. The Study Site must return all used and unused vials of I004 or Novolog to the Sponsor or designee or destroy them upon request. Drug supplies will be counted and reconciled at the site before being returned to the Sponsor or designee or being destroyed.

The quantity of reserve samples of study drugs under test will be sufficient to meet the retention sample requirements per 21 CFR 320.38 (b) and (c), and 320.63 [19, 20, 21]. The investigator and designated dispenser are responsible to carry out the on-site drug retained activities in accordance to this protocol, 21 CFR 320.38 and 320.63.

All samples will be stored in an area with controlled access. The retention samples will be stored for five (5) years or longer following product approval.

The Investigator or Investigator's authorized staff must ensure the availability of proper storage conditions. The temperature of all study drugs will be monitored over 24 hours a day, 7 days a week (24/7). In case of incorrect storage, the Sponsor and monitor must be contacted without delay.

No study drugs may be dispensed to any person not enrolled in the study.

6.4 Dose Regimen

I004 or Novolog will be drawn from *vials* by an unblinded staff with commercially available, appropriate insulin syringes (eg, Becton and Dickinson products) using standard sterile methods. The purpose of using insulin syringe dosing is to maintain blinding and consistency of dosing methodology across treatments.
The dose will be 0.2 units/kg at each dosing (Day 1/ Treatment Period 1 and Day 1/ Treatment Period 2), based on the body weight measured at Day 1 of Treatment Period 1 under fasting condition.

Dose calculation:

To calculate the amount of insulin for each subject, the body weight in kilograms (kg) measured on Day 1 of Treatment Period 1 under fasting condition will be determined (rounded to two decimal places) and the amount of insulin calculated will be rounded up or down to the nearest whole unit.

I004 or Novolog dose = 0.2 units/kg x _ _. _ _ kg = _ _ units. Doses will be rounded to nearest 1 unit (0.01ml).

Example: A subject with a body weight of 74.90 kg will receive 15 U insulin (74.9 × 0.2 = 14.98, which is rounded up to 15)

Standard rounding rules (0.49 unit round down, 0.50 unit round up) will be used. IP will be drawn with insulin syringes that have a 0.01 mL (one unit on insulin syringe) gradation.

The IP will be administered while subjects are under observation in the clinical research by qualified staff. The investigational product will be administered (at time-point zero) via subcutaneous injection into the abdominal wall into the peri-umbilical area using a standardized skin-fold technique. An area of skin approximately 5 cm lateral to the umbilicus on either side of the abdomen will be cleaned and used for the insertion of the needle. The needle will be promptly inserted at an approximately 45°degree angle into a lifted skin fold. The IP will be administered, and the needle should remain in the skin for at least 20 seconds after administration to reduce the possibility of leakage. The needle will be very slowly withdrawn or may be held for a few seconds before it is fully withdrawn to minimize leakage of IP. The injection site will be marked with a pen.

Every new injection should be administered in a new location, rotating around the umbilicus of the abdomen. The time of each dose will be recorded in the source documents and on the eCRFs.

For detailed instructions please see Novolog Product Insert.[9]

6.5 Overdose

If a study medication error occurs, it should be documented as Protocol Deviation. A brief description should be provided in the deviation, including whether the subject was symptomatic (list symptoms) or asymptomatic, and the event accidental or intentional.

Dosing details should be captured on the Electronic Case Report Form. If the subject takes a dose of Study Drug that exceeds protocol specifications and the subject is symptomatic, then the symptom(s) should be documented as an AE and be reported.

Should an overdose occur, the Investigator or designee should contact the Sponsor within 24 hours.

6.6 Randomization and Blinding

Subjects will be randomly assigned to one of two treatment sequences (TR or RT), where treatments will consist of single SC IP administrations:

- Treatment T: 0.2 units/kg I004; Solution for subcutaneous injection, 100 units/mL
- Treatment R: 0.2 units/kg Novolog; Solution for subcutaneous injection, 100 units/mL

Table 6-2 Sample Treatment Allocation Scheme

Number	Sequence	Treatment Period 1	Treatment Period 2
Sequence 1 (N=30)	**TR**	**0.2 units/kg I004**	**0.2 units/kg Novolog**
Sequence 2 (N=30)	**RT**	**0.2 units/kg Novolog**	**0.2 units/kg I004**

Subjects who met all eligibility requirements were invited to the in-house period. After check-in on Day 1 of Treatment Period 1, subjects will be randomized on the basis of their eligibility at check-in, according to a randomization list, generated by the Sponsor or designee.

They will be assigned a unique Randomization ID, as **I4-B-nn**, **I4-B** represents study B for "I004", and **nn** is the sequential number (the randomization subject will therefore have two individual ID numbers, a Screening number and a randomization number).

Two sets of sealed codes/randomization list with the subject randomization numbers, containing information about the treatment at each Treatment Period will be prepared. One set will be kept strictly confidential, in a secured place with limited access at [redacted] (during the entire study), the second set will be kept under the responsibility of the Sponsor's and both are to be used in case of code break need. While the set at the clinical study site is used for emergency unblinding, the sets with the Sponsor will be used for unblinding for regulatory reporting purposes.

In case subjects drop-out of the study, these subjects will be replaced. The replacement subject will receive a new, unique Randomization ID, as **I4-B-nn**, **I4-B** represents study B for "I004", and **nn** is the sequential number. In case of subject replacement, the subjects must always be assigned to the lowest available randomization number from the randomization list. Replacement of dropouts and withdrawals will ensure that a total of 60 subjects complete the study.

To maintain the double-blind aspect of the study, the insulin syringes for IP administration will be prepared by unblinded pharmacy staff, not participating in IP administration, or interacting with study subjects in any other way. The study staff, including the laboratory analysts, participating in other aspects of the study will be blinded to the treatment.

6.7 Breaking of Blinded Code

In case of an emergency, the drug identification information can be unblinded by contacting

[redacted] after consulting with the PI and, if possible, with the Sponsor. Drug identification information is to be unblinded only if this is required for subjects' safety.

In the event that an unblinding has occurred, the circumstances around the unblinding (e.g., date, time and reason) must be documented and the clinical monitor notified as soon as possible. Only the Principal Investigator or delegate and the respective subject's code will be unblinded. Study site personnel and Sponsor personnel directly associated with the conduct of the study will not be unblinded.

If the study site needs to unblind a subject, the sponsor will, if possible, be contacted prior to breaking the blinding. In all cases, the Study Monitor must be notified within 24 hours after emergency unblinding.

6.8 Auxiliary Supply

[redacted] will supply laboratory material necessary for PK sampling, all the safety hematology, biochemistry, and urinalyses (incl. pregnancy test) in collaboration with the specific laboratory.

[redacted] will also provide all necessary material needed for the glucose clamp procedure. Glucose, sodium chloride solutions, heparin, syringes, and needles used in the clamp will be provided by [redacted].

7.0 STUDY PROCEDURE

7.1 Informed Consent

Written informed consent will be obtained from each subject prior to performing any study-specific evaluations. The informed consent document is subject to review and approval by the Sponsor and will be approved by a qualified IRB. Only the most recently IRB-approved informed consent document must be used to consent prospective study subjects. The Investigator or a person designated by the Investigator and under the Investigator's responsibility, will fully inform the potential study subject of all pertinent aspects of the clinical study, including written information given approval/favorable opinion by the IRB/IEC. They will have the opportunity to ask questions. If the subjects wish to participate in the study, they must sign and date an ICF.

7.2 Screening

Subjects who have signed the informed consent, will be assigned a Screening ID, as

"SI4-B-mm", where **S** stands for Screening, **I4-B** represents "I004 Study B", and **mm** is the Screening sequential number.

If subjects are fasting (only water for ≥10 hours), all Screening assessments may be done on the same day. If subjects are not fasting, they will be invited to return for a second Screening visit to complete any missing Screening procedures (e.g. laboratory assessments).

Investigators must account for all subjects who sign informed consent forms. The Investigator will keep a Subject Screening Log at the site.

Subjects who have screen failed may be allowed to re-screen once at the discretion of the Investigator. A new Screening number will be assigned.

Subjects who meet all inclusion criteria and none of the exclusion criteria are eligible for this study. Subjects will be invited for the in-house treatment periods. They will be instructed on factors influencing insulin sensitivity such as refraining from strenuous exercise for three days prior to dosing, avoidance of alcohol and herbal supplements, caffeinated meals and drinks, smoking or medication and illness/infection as stated in section 5.4.

7.3 Demographics and Medical History

Demographic information and medical history, including smoking status, and medication history will be obtained at Screening.
The history of prior insulin exposure or use will be documented.

7.4 Physical Examination

The baseline physical examination will consist of the following body systems: (1) eyes; (2) ears, nose, throat, neck, thyroid; (3) cardiovascular system; (4) respiratory system; (5) gastrointestinal system, mouth; (6) dermatologic system; (7) extremities; (8) musculoskeletal system; (9) central and peripheral nervous system and (10) lymph nodes.

A physical exam will be performed by an Investigator (or a qualified physician at the investigational site) at time points indicated in the SOE (Appendix A). An additional physical examination may be performed in case the subjects have symptoms or at the discretion of the Investigator.

7.5 Height, Weight, and BMI

Height (without shoes) will be measured once, during the Screening Visit.

Weight (without shoes) will be measured fasting, in the morning, with light clothing and post void at time points indicated in the SOE (Appendix A).

BMI (kg/m^2) will be calculated from height and weight.

The body weight at Day 1, Treatment Period 1 under fasting condition will be used to calculate all further drug dose for the following clamp experiment. Therefore, the body weight will be recorded as stated in the SOE (Appendix A) and should stay within the range of 5 % throughout the study. If body weight is outside the range, subject will be rescheduled.

7.6 Vital Signs

Vital signs will include body temperature (*aural*), supine blood pressure (after 5 minutes resting), respiration rate and pulse rate (after 5 minutes resting). Vital sign measurements will be performed at days indicated in the SOE (Appendix A).

On clamp days, vital signs will be measured at baseline which is 15 min (± 5 min) prior to dosing, at 5 (± 3 min) minutes, 60 min (± 10 min), 180 min (± 20 min) and 720 min (±30 min) post-dose.

Additional measures will be performed as deemed necessary by the Investigator.

7.7 Concomitant Illness and Therapy

Concomitant therapy is any medication given in addition to the investigational product (including over-the-counter medications, herbal medications, and vitamin supplements) administered between Screening and follow-up.

Concomitant illness is any significant medical condition or disease that is present at study start (signing of informed consent). This includes clinically significant laboratory, electrocardiogram (ECG), or physical examination abnormalities noted at Screening examination.

Details of all concomitant illnesses and therapies must be recorded at study entry/Screening and must be recorded on the subject's CRF. Any changes in concomitant medication must be recorded at each visit. If the change influences the subject's eligibility to continue in the study, the Sponsor must be informed. The information collected for each concomitant medication includes, at a minimum, start date, stop date or continuing, and indication.

AEs related to administration of these therapies or procedures must also be documented on the appropriate eCRF.

7.8 Procedures for Clinical Laboratory Samples

All samples will be collected in accordance with acceptable laboratory procedures.
Laboratory samples will be taken as described in the SOE (Appendix A).

Table 7-1 Clinical Laboratory Assessments

Hematology	Serum Chemistry	Urinalysis
CBC with differential:	**Hepatic function panel:**	**Routine urinalysis with microscopic examination on positives**(a):
Hematocrit	Alanine aminotransferase (ALT/SGPT)	Color
Hemoglobin	albumin, serum	appearance,
mean corpuscular volume (MCV)	alkaline phosphatase, serum	specific gravity
mean corpuscular hemoglobin (MCH)	aspartate aminotransferase (AST/SGOT)	pH
mean corpuscular hemoglobin concentration (MCHC)	bilirubin, direct	protein
red cell distribution width (RDW)	bilirubin, total	glucose
percentage and absolute differential counts	protein, total, serum	ketones
platelet count	**Renal function panel:**	occult blood
red cell count (RBC)	Albumin, serum	leukocyte esterase
white blood cell count (WBC)	BUN	Nitrite
	BUN: creatinine ratio	bilirubin
	calcium, serum	urobilinogen
	carbon dioxide, total	
	chloride, serum	
	creatinine, serum	
	glucose, plasma (c)	
	phosphorus, serum	
	potassium, serum	
	sodium, serum	
	Additional parameters:	

	Lactic acid dehydrogenase (LDH) Gamma-Glutamyl Transferase Magnesium Uric Acid Free fatty acid (FFA)	

Diagnostic Screening		
Serum/Plasma/Whole Blood	**Urine**	**Breath**
HBsAg Anti-HCV TSH (b) PTT INR HbA1c Anti-HIV-1 Anti-HIV-2	**Drug Screen Profile** Urine drug screen, including marijuana, at timepoints stated in the SOE via commercial kit at the investigational site.	Alcohol breath test at timepoints stated in the SOE at the investigational site
Female Subjects Only human chorionic gonadotropin (hCG) as stated in SOE follicle-stimulating hormone (FSH) test for postmenopausal women (defined as amenorrheic female subjects < 60 years of age and not surgically sterile) at Screening.	Urine pregnancy testing via commercial kit at site.	

(a) These tests are done on all routine urinalysis and if protein, leukocyte, occult blood, and nitrites are all negative, microscopic examination is not performed; just the above parameters are reported.
(b) In the event of abnormal TSH, Free T3/T4 may be collected at Investigator discretion.

(c) Fasting Plasma Glucose at Screening in-house, test via YSI

The designated laboratory will perform all necessary laboratory tests listed above. The results of laboratory tests will be sent to the Investigator or designee, who is responsible for reviewing these results. All laboratory safety data will be faxed or transferred electronically.

Laboratory reports must be signed and dated by the Investigator or designee indicating that the report has been reviewed and any abnormalities have been assessed for clinical significance.

All clinically significant laboratory abnormalities detected after Screening must be recorded as an

AE. A clinically significant laboratory abnormality may be verified by retesting and may be followed upon discretion of the Investigator.

Unscheduled visit for safety laboratory testing is allowed and at the discretion of PI during the COVID-19 pandemic.

7.9 Contraception

Females must be non-pregnant and non-lactating, or either surgically sterile (e.g., bilateral tubal ligation, hysterectomy, bilateral salpingectomy, bilateral oophorectomy) or post-menopausal for >12 months. The site will make an effort to retrieve medical records to document the sterility, however, the absence of records will not exclude the subject. In the event that medical records cannot be obtained, serum pregnancy testing will be conducted at Screening, and serum and urine pregnancy testing will be conducted throughout the study. Postmenopausal status will be confirmed through testing of FSH levels outside the normal range (as specified by responsible lab) at Screening for amenorrheic female subjects <60 years of age.

Female subjects must use an adequate method of contraception, defined as adequate hormonal contraception to have started at least 7 days prior to the Screening visit, or an intra-uterine device to have been in place for at least 2 months prior to the Screening visit, and throughout the study (including washout intervals between treatments periods).

During the course of the study, regular human chorionic gonadotropin (hCG) pregnancy tests will be performed for sterilized women. In addition to a negative serum hCG pregnancy test at Screening, subjects also must have a negative urine hCG pregnancy test at check-in to in-house periods and at the follow-up visit.

7.10 Pregnancy

Women known to be of childbearing potential are allowed to participate in the study. In the event a subject becomes pregnant during the study, she should be withdrawn, and the study drug should be immediately discontinued. However, the subject will be encouraged to complete the post-treatment follow-up portion of the study to the extent that study procedures do not interfere with the pregnancy.

If the pregnancy occurs at any time during the study and the 30 days of last dose of active study medication, the pregnancy should be reported immediately to the Sponsor, using a pregnancy notification form.

Study subjects will give consent on enrollment that the Investigator will report any pregnancy during the study to the Sponsor and that they will be asked to provide information about her pregnancy, delivery, and the health of her infant until age one month. Payment for all aspects of obstetrical care, child, or related care will be the subject's responsibility.

All reported pregnancies will be followed up to final outcome, using the pregnancy and pregnancy follow-up forms. The outcome, including any premature termination, will be reported to the Sponsor. An evaluation after the birth of the child may also be conducted.

Pregnancy complications must be recorded as adverse event(s). If the infant has a congenital anomaly/birth defect this must be reported and followed up as a serious adverse event.

7.11 ECG Procedure

A standard 12-lead ECG will be recorded after 5 minutes in a supine position. The Investigator (or designee) will interpret the ECG's by use of an electronic measurement using the following categories: within normal limits, abnormal but not clinically significant, or abnormal with clinical significance. ECG's are performed according to the SOE (Appendix A). The following parameters will be recorded from the subject's ECG trace: heart rate, QT interval, PR interval, QRS interval, RR interval, and QTc (corrected) using the Fridericia correction (QTcF = QT ÷ cube root of the R-R interval [where R-R is the duration of the entire cardiac cycle]).

When ECGs are to be collected at the same time point as a blood collection, ECGs should be collected first to avoid any artificially increased heart rates due to the blood collection.

On clamp days, ECGs will be performed at baseline which is 15 min (±5 min) prior to dosing, at 5 min (±3 min), 60 min (±10 min), 180 min (± 20 min) and 720 min (±30 min) post-dose.

ECGs will be performed at days indicated in the SOE (Appendix A).

In some cases, it may be appropriate to repeat abnormal ECGs. If a machine-read QTc value is prolonged, repeat measurements may not be necessary if the Investigator's interpretation determines that the QTc value is in the acceptable range.

7.12 Fasting Plasma Glucose

Fasting plasma glucose will be measured during the in-clinic period with an YSI 2300 STAT glucose analyzer at time points stated in the SOE (Appendix A).

7.13 Standardized Meals

At each period, the importance of maintaining the body weight within a 5% range throughout the study will be explained. The counseling for each subject will be individualized based on their actual measured body weight. Additionally, subjects will be counseled to maintain their normal diet and exercise regimen. They will be instructed not to start any new diets, supplements, or exercise programs during the study.

During in-house period(s), subjects will receive standardized meals. Meal will be provided after the clamp procedure has been terminated.

7.14 Euglycemic Glucose Clamp

7.14.1 Biostator® Overview

The Biostator Automated Glucose Analyzer (MTB Medizintechnik, Amstetten, Germany) is a 510(k)-cleared device that automates the glucose clamp technique providing continuous assay of blood glucose measurements and minute-by-minute adaptations of glucose infusion rates (GIR).

In the clamp scenario, the Biostator automatically calculates and infuses the appropriate amount of Dextrose 20%, based on the actual measured blood glucose and its deviation from target level. The Biostator analyzer, using an internal algorithm, makes minute by minute adjustments to GIR, as necessary, to keep the blood glucose concentration at the target level. Adjustments are done per current blood glucose measurement and the variability observed during the last five readings.

7.14.2 Glucose Clamp Set-Up

The Biostator is set up with infusion lines for blood glucose management. One 0.9% sodium chloride (NaCl) and two Dextrose 20% (D20) IV infusion lines are connected via an infusion module. All three lines are linked together using three-way valves which are then connected to the subject through an IV catheter on the contralateral arm (contralateral and ipsilateral are defined as relative to Biostator). A second IV line is inserted (generally the dorsal hand vein, the lateral wrist vein of ipsilateral arm or any peripheral arm vein) and setup through a different Biostator module using a Double Lumen Catheter (DLC). One lumen is used for a continuous blood sample draw at an estimated rate of 2 mL/hr; while the other lumen, is used to infuse a low concentration of heparinized saline solution to the catheter tip where it is then mixed with the blood drawn. This mix avoids sample clotting within the tubing. The DLC vein site is covered by a heating pad for arterialization of venous blood flow. The Biostator system programming and operation is done per [redacted] standard operating procedures. An external D20% IV line is connected to the infusion lines. This infusion is manually operated by the technicians running the clamp in coordination with the medical staff and is used (e.g., in case the GIR delivered by the Biostator is insufficient).

As a background validation with a second methodology for safety, the standardized measurements of the Biostator will be verified on a regular basis using the YSI reference (YSI 2300 STAT or equivalent YSI Glucose Analyzer; Yellow Springs Instruments, Ohio, USA) device at approximately every 30 minutes.

Please see Appendix B for a detailed Clamp Sampling Schedule.

7.14.3 Glucose Clamp Procedure

During the study, subjects will undergo two euglycemic clamp procedures, one per treatment period. All clamp procedures will be performed identically.

Subjects will be connected to the Biostator through peripheral arm veins as described in section 7.14.2. The clamp procedure will follow instructions described in section 4.2.

The Biostator will be turned on and programmed. The body weight in kilograms used for Biostator programming on both clamps, corresponds to the weight measurement obtained on Day 1 of Treatment Period 1. At least one hour before dosing (t= - 60 min), the Biostator will start recording data. During this pre-dose monitoring period, glucose readings are obtained and analyzed with a YSI 2300 STAT or equivalent YSI Glucose Analyzer (Yellow Springs Instruments, Yellow Springs, Ohio, USA) at the following time points relative to the start of the trial drug infusion: -60, -30, -20 and -10 minutes. The average of blood glucose values prior to dosing (-30 min, -20 min, -10 min) correspond to each subject's individual BASELINE glycemic level. The glucose CLAMP TARGET level is determined by subtracting 5 mg/dL from the BASELINE glycemic level (Target level = BASELINE - 5 mg/dL). Subjects whose differences between initial fasting BG values (-30, -20, -10 min) exceed the limit of 10 mg/dL will be rescheduled to another day. Additionally, subjects will not be dosed if mean fasting plasma glucose is < 75 mg/dL due to proximity to overt hypoglycemia or > 99 mg/dL. Subjects may be re-scheduled once to come back to the site on a later day and may be dosed if the mean fasting plasma glucose is ≥ 75 mg/dL or ≤ 99 mg/dL prior to dosing.

At t= 0-min, the investigational product will be administered per randomization schedule.

After dosing, the onset of insulin action will be identified by a ≥ 5mg/dL decline of glucose concentration from the baseline level. After the onset of insulin action is identified, glucose infusion will then be utilized as needed to maintain the target level (± 10%) using a variable infusion of D20%. This GIR is calculated and infused by the Biostator. The glucose infusion is continuously diluted with saline solution at an approximate rate of 30 ml/hour to avoid the remote possibility of localized pain or venous thrombosis as complications. In case a high GIR is necessary, a part of the glucose infusion will be given by an external infusion pump. The infusion rate provided by the external pump will then be added to the infusion rate of the Biostator.

The subjects will remain fasting and in a supine or semi supine position during the entire glucose clamp. The subjects are allowed to sip water throughout the trial and will otherwise remain fasting.

7.14.4 Glucose Clamp Quality Parameter

The quality of the clamp data will be reviewed on a regular basis by the PI and the clamp supervisors at regularly scheduled Data Review Meetings. The following parameters are included in the review:

- Clamp coefficient of variation (CV): Coefficient of variation of device BG measurements calculated by the following formula:

  (SD of Device BG / Mean Device BG) * 100

- Clamp deviation from the target (DFT): Mean difference (mg/dL) between device and target BG level represented as a percentage, calculated as follows:

  [Mean (device BG – target level) / target level] * 100

- Accuracy: Mean difference of all paired BG measurements of device vs reference method represented as a percentage.

Calculations for CV and DFT will be based on each clamp from the minute that the glucose infusion rate is initiated (Start of GIR) to the minute that the glucose infusion rate is no longer required (End of GIR) or at the end of the clamp at the 12-hour mark, whichever appears first. Note that an End of GIR is not necessarily an end of the clamp.

In case a subject's GIR requirement is intermittent or has interrupted periods during the clamp, the PI will review the clamp data and determine how the clamp quality will be calculated.

Generally, overall mean clamp CV and DFT for the clamps should be less than 10%. If the calculated CV or DFT for an individual clamp is ≥ 12%, the clamp data for that clamp should be considered for exclusion from the final data analysis and the clamp should be repeated (if possible), or the subject should be replaced, following discussion between the Investigator and the Sponsor. If the accuracy calculation is > 12% but the CV and DFT are < 12%, it will be at the discretion of the Principal Investigator to determine if the clamp data quality is acceptable. [redacted] Clinical Data Management Team and the Sponsor will also be consulted.

7.15 Pharmacokinetic Assessments and Schedule

Blood for PK analysis of the IP, the human insulin analog, will be collected at the time points indicated in the SOE (Appendix A) and follow the PK blood sampling schedule below.

At each sampling time point, 1.0 mL of blood will be discarded for waste (utilizing a throw away tube). Thereafter, one 6.0 mL whole blood sample per scheduled time point will be collected to provide a minimum of 1.5 mL of serum for PK measurements and 1.5 mL of serum as a secondary back-up sample. Instructions for sample processing and shipment are provided below.

Table 7-2 PK Blood Sampling Schedule (6 mL per sample)

Seq. #	Scheduling	Time window	Sample No.,"XX"	#	Scheduling	Time window	Sample No.,"XX"
1	-60 min	± 5 min	01	15	75 min	±3 min	15
2	-30 min		02	16	80 min	±5 min	16
3	0 min	±1 min	03	17	90 min		17
4	5 min		04	18	105 min		18
5	10 min		05	19	120 min		19
6	20 min	±2 min	06	20	150 min		20
7	25 min		07	21	180 min	±10 min	21
8	30 min		08	22	210 min		22
9	40 min	±3 min	09	23	4 h		23
10	50 min		10	24	5 h		24
11	55 min		11	25	6 h		25
12	60 min		12	26	8 h	±20 min	26
13	65 min		13	27	10 h		27
14	70 min		14	28	12 h		28
Total							28 (168mL)

PK Sample Processing and Shipment Instructions are described below:

(1) Sampling Procedure

A catheter will be inserted into one of the antecubital region veins for blood sampling. Alternatively, collection from a vein from the hand or forearm for easier access or via venipuncture is also acceptable. PK blood draws should use the opposite arm of the glucose infusion/clamp. Preferably, the arm with better venous access will be used for PK samplings, and the opposite arm for the glucose infusion/clamp. In case of extraordinary situations, such as IV infiltration etc, the PK samples could be collected from the arm of the clamp, for safety reason.

After each blood sample collection, a catheter flush will be performed to avoid catheter clot formation and prevent interference between samples.

At each PK sampling point, the first 1 mL of blood will be discarded for waste (utilizing a disposable tube), and blood samples (~6 mL per sample), will be collected in a serum separation tube and left to stand at room temperature for 30 - 60 minutes. Each sample will be centrifuged at 2-8°C, 1,000-1,300 g for 20 minutes.

Isolated serum will be distributed into two (2) 2.0 mL cryo vials, approximately 1.5 mL in the first aliquot and the remainder in the second aliquot. The serum samples will be frozen on dry ice and then immediately (within 60 minutes following serum isolation) stored in a freezer at -20°C or lower

until analysis.

(2) PK Sample ID

The sample ID for PK blood samples (before centrifugation) is defined as:

"I4-B-nn-v-XX"

where **I4-B** represents study for "I004 Study B", **nn** is the subject ID, **v** is the treatment period/visit number and **XX** is the two-digit time-point sequential number of the sample: 01, 02, 03, …28 as listed in Table 7-2.

The sample ID for PK samples (after centrifugation) is defined as **"I4-B-nn-v-XX-k"** and **k** is the aliquot number (A or B). The PK sample ID labels, with the size of "1/2" × 1¾" (Avery label 5667), should be printed in black font as demonstrated below:

"I4-B-nn-XX-k"

(3) Sample Transportation

Prior to sample transportation, all samples should be stored in a box and must be organized in sequential order by subject and separated by aliquot. The chain of custody, temperature control and related documents for sample transportation must be recorded. During transportation of PK samples from the site to Amphastar, the temperature should be below -20ºC.

(4) Bioanalytical Methods of Insulin Aspart

An automated, high-throughput method for targeted quantification of Insulin Aspart (I004) in human serum includes (i) mass spectrometric immunoassay (MSIA) for immune-enrichment which is based on the Thermo Scientific™ MSIA™ platform and (ii) analysis by UPLC with high resolution mass spectrometer (UPLC/HRMS) will be performed.

MSIA™ platform approaches based on immune-enrichment prior to the mass spectrometric determination, using the insulin antibodies immobilized on a solid support first to capture the molecule of interest from the matrix, then subsequent washing cycles are used to remove contaminants and finally the analyte is eluted prior to its analysis by UPLC/HRMS.

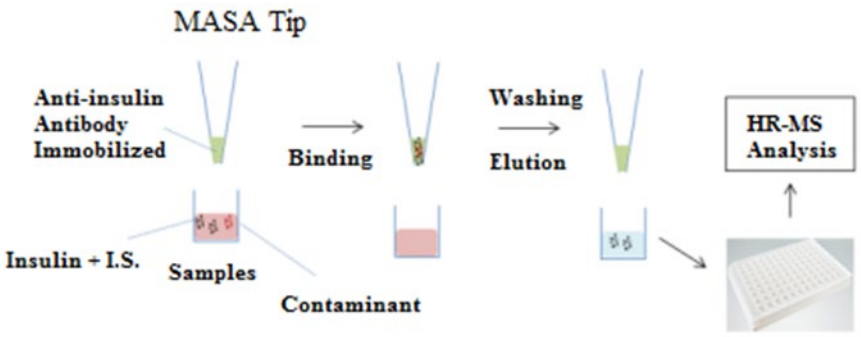
The HR-MS detection provides significant advantages over enzyme linked immunosorbent assay (ELISA) methods because the selectivity of MS allows detection of co-eluting analogs. Full-scan MS data could be analyzed due to the low noise and selectivity of the MSIA extraction technology. Co-eluting insulin analogs were easily separated and identified based on the accurate m/z values of each precursor's charge state and corresponding isotopes.

The results of the method feasibility study indicated the method is accurate with the LLOQ of 50 pg/mL or 0.05 ng/mL with a good linearity over the quantitation range.

(5) Bioanalytical Methods of Endogenous Insulin

Amphastar developed sensitive and specific UPLC/HR-MS method to detect the endogenous human insulin.

Figure 7-1 MSIA Technology Workflow



7.16 Pharmacodynamic Assessments and Schedule

The pharmacodynamic parameter GIR will be determined from the concentration-time data for all evaluable subjects.

Serum C-peptide and endogenous human insulin will be analyzed out of the PK sample at all PK sampling timepoints as stated in the PK sampling schedule. Instructions for PK sample processing and shipment are provided in section 7.15.

Please see Table 7-2 for PK Blood Sampling Schedule.

7.17 Tolerability Assessments

After injection of the study drug, the injection site will be marked with a pen. Assessment of study drug injection site will be performed at 15 (±3) min, at 60 (±10) min and at 720 (±30) min after dosing.

The local reaction from the injection site, the insertion site, and the adhesive will be evaluated quantitatively using a Draize scale or similar scale by qualified study staff. If an injection site reaction like pain on palpation, itching, erythema, edema, induration is observed, it must be recorded as an AE and then will be evaluated using the following scale:

Erythema will be evaluated as follows:

- 0 – No erythema
- 1 – Very slight erythema (barely perceptible)
- 2 – Well-defined erythema
- 3 – Moderate to severe erythema
- 4 – Severe erythema (beet redness) to slight eschar formations (injuries in depth)

Edema will be evaluated as follows:

- 0 – No edema
- 1 – Very slight edema (barely perceptible)
- 2 – Slight edema (edges of area well defined by definite raising)
- 3 – Moderate edema (raised approximately 1 mm)
- 4 – Severe edema (raised more than 1 mm and extending beyond the area of exposure)

The diameter of the affected area will be measured with a paper measurement in cm and the condition of the injection site will be recorded. Digital photography will be used to document all positive injection site reactions. In case of clinically significant injection site reactions, subjects may undergo a dermatological consultation.

7.18 Blood Volume

Total blood sampling volume for subjects will approximately be 390 mL.

Even in case of further unexpected blood sampling, extension of blood sampling period or necessary retesting, sampling volume will not exceed 420 mL.

8.0 ADVERSE EVENTS

8.1 Definitions

8.1.1 Adverse Event (AE)

An AE is any undesirable and unintended medical event occurring to a subject in a clinical study, whether or not related to the study products. This includes events from the first study related activity after the subject has signed the informed consent and until post treatment follow-up period as defined in the protocol. The following should not be recorded as AEs, if recorded as medical history/concomitant illness on the eCRF at Screening:

- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first study related activity after the subject has signed the informed consent

- Pre-existing conditions found as a result of Screening procedures

- Pre-existing events that has not worsened in intensity or frequency from baseline

8.1.2 Treatment Emergent Adverse Event (TEAE)

A treatment-emergent AE (TEAE) is defined as any clinically significant event not present before exposure to study drug or any event already present that worsens in either intensity or frequency after exposure to study drug.

8.1.3 Clinical Laboratory Event

A clinical laboratory AE is any clinically significant laboratory abnormality that suggests a disease and/or organ toxicity and is of a severity, which requires active management, (i.e. change of dose, discontinuation of study product, more frequent follow-up or diagnostic investigation).

A laboratory re-test and/or continued monitoring of an abnormal value is not considered an intervention. In addition, repeated or additional noninvasive testing for verification, evaluation or monitoring of an abnormality is not considered an intervention.

8.1.4 Unexpected Adverse Event/ Unexpected Suspected Adverse Reaction

An unexpected AE/unexpected suspected adverse reaction is an AE or suspected adverse reaction that is not listed in the Investigator Brochure or Protocol, as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation, or is not listed at the specificity or severity

that has been observed; or, if an Investigator Brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

8.1.5 Serious Adverse Event (SAE)/ Serious Suspected Adverse Reaction

An SAE is defined as any untoward medical occurrence that at any dose:

(1) Results in death.
(2) Is life threatening.
- The term "life threatening" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

(3) Requires inpatient hospitalization or prolongation of existing hospitalization. Hospitalization is defined as an admission of greater than 24 hours to a medical facility and does not always qualify as an AE.
(4) Results in persistent or significant disability/incapacity.
(5) Leads to a congenital anomaly/birth defect.

(6) Is an important medical event that satisfies any of the following:

- May require intervention to prevent items 1 through 5 above.

- May expose the subject to danger, even though the event is not immediately life threatening or fatal or does not result in hospitalization.

8.1.6 Life-Threatening Adverse Event/Life-Threatening Suspected Adverse Reaction

A life-threatening AE/life-threatening suspected adverse reaction, in the view of either the Investigator or Sponsor, places the patient or suspect at immediate risk of death. It does not include an adverse reaction that, had it occurred in a more severe form, might have caused death.

The determination of whether an AE is life threatening can be based on the opinion of the Investigator or Sponsor. If either, the Sponsor or investigator believes that the event is serious or life threatening, the event must be considered serious and evaluated by the Sponsor for expedited reporting (21 CFR 312.32(a) and 312.32(c)(1)).

8.1.7 Severity of AEs

The different categories of intensity (severity) are characterized as follows:

Mild:	The event is transient, easily tolerated by the subject and does not affect the subject's daily activities.
Moderate:	The event causes the subject discomfort and interrupts the subject's usual daily activities.
Severe:	The event is incapacitating and causes considerable interference with the subject's usual activities.

8.1.8 Relationship to Study Treatment

Very Likely Related:	There is clear evidence that the event is related to the use of the IP. The AE follows a reasonable temporal sequence from administration of a drug (including the course after withdrawal of the drug), or for which possible involvement of the drug can be argued.
Probably Related:	The AE follows a reasonable temporal sequence from the IP administration, and there is a high degree of certainty that the AE is related to study drug administration (eg, it represents a known reaction to the IP or other drugs in its class or is predicted by the known pharmacological properties of the drug).
Unknown:	The event follows a reasonable temporal sequence from the IP administration, and cannot be reasonably explained by the subject's clinical state or other causes (eg, disease under study, concurrent diseases, or concomitant medications).
Unlikely Related:	An event for which an alternative explanation is more likely (eg, concomitant medications or ongoing medical conditions) or the temporal relationship to IP administration and/or exposure suggests that a causal relationship is unlikely (for reporting purposes, Unlikely will be grouped together with Not Related)
Definitely Not Related:	An AE that does not follow a reasonable temporal sequence from administration of IP and/or that can reasonably be explained by other factors, such as underlying diseases, complications, concomitant drugs and concurrent treatments.

8.2 Procedures

8.2.1 Collection and Recording of AEs

Collection of all AEs (serious AEs and non-serious AEs) will commence from the time the subject signs the informed consent to participate in the study until the post-treatment follow-up visit. At each study visit, the Investigator will assess whether any subjective AEs have occurred. In order to avoid bias in eliciting AEs, a non-specific question, such as "How have you been feeling since your last visit?" may be asked. Subjects may report AEs occurring at any other time during the study.

All subjects experiencing AEs, whether considered associated with the use of the study medication or not, must be monitored and given appropriate medical treatment at the discretion and judgement of the Investigator until the symptoms subside and any clinically relevant changes in laboratory values have returned to baseline or until there is a satisfactory explanation for the changes observed. All AEs will be documented in the AE page of the eCRF, whether or not the Investigator concludes that the event is related to the drug treatment. The event term, start and stop dates, severity, action taken with study drug and outcome, will be documented, along with the Investigator's opinion of the causal relationship between the event and the IP.

8.2.2 Collection and Reporting of SAEs

When an SAE occur, it should be reported according to the following procedure:

An SAE form must be completed immediately or within 24 hours of first onset or notification of the event. The information should be completed as fully as possible but contain, at a minimum a short description of the event and the reason why the event is categorized as serious, subject identification number, Investigator's name, name of the study medication and a causality assessment.

In the interest of subject safety, and in order to fulfill regulatory requirements**,** all SAEs (regardless of their relationship to study drug) should be reported to the Sponsor or a designated qualified vendor within 24 hours of the study site's first knowledge of the event.

The collection of SAEs will begin after the subject signs the informed consent form and stop at the end of the subject's follow-up period. An Initial Serious Adverse Event Form should be completed, and a copy should be provided to the Sponsor or designated qualified vendor.

All serious and unexpected adverse event reporting will adhere to 21 CFR 312.32 for IND drugs and 21 CFR 314.80 for marketed drugs (15-day alerts). The Institutional Review Board(IRB) and all Investigators will be notified of the alert reports per FDA regulations.

8.3 Anticipated AEs

An anticipated AE is defined as any adverse events described in the Product Information or this protocol.

Normal precautions taken for a human study, including the provision of emergency equipment, will be taken during this study. Qualified and well-trained physicians and medical staff will instruct the subjects. Insulin or the preservatives in the insulin products may give rise to allergic reactions.

8.3.1 Drug Related AEs

8.3.1.1 Hypoglycemia

There is a small risk of severe hypoglycemia (requiring assistance of the study staff) when an exogenous insulin analog is administered. The glucose clamp will be performed under continuous surveillance and frequent monitoring of blood glucose levels as specified. Since there is simultaneous infusion of glucose, the occurrence of severe hypoglycemia is unlikely. If a blood glucose value ≤ 50 mg/dL is recorded and confirmed by YSI, or the subject has neurogenic symptoms (palpitations, tremor, hunger, sweats) or symptoms of neuroglycopenia (confusion, lethargy, agitation, reduced level of consciousness) at a BG ≤ 56 mg/dL, the subject must be treated at the discretion of the Investigator to prevent or alleviate neuroglycopenic symptoms. Treatment may include ingestion of carbohydrates (e.g. apple juice), iv glucose bolus injections, iv glucose infusions or other methods as required per Investigator. Subjects will be closely monitored, and it will be a clinical decision made by the Investigator to move from oral to iv glucose bolus, to iv glucose infusion. Severe hypoglycemia (as defined by the ADA Workgroup[13]) should be treated at the Investigator's discretion according to best available medical practice. As a safety precaution, a glucose solution will be available near the subject. An intravenous cannula will be placed, flushed with 2.0 mL sterile saline and maintained in a prominent forearm vein throughout the post-dose PK sampling period to enable prompt reliable venous access should iv glucose be required to treat hypoglycemia.

Hypoglycemic events will be recorded as adverse events, if symptoms develop and BG is ≤56 mg/dl, or if blood glucose measured is ≤ 50 mg/dL, even if no symptoms are present. The subjects will only be discharged from the clinic, if deemed safe by the Investigator.

8.3.1.2 Hypokalemia

Insulin products can cause a shift in potassium from extracellular to intracellular space, possibly leading to hypokalemia that, if left untreated, may cause respiratory paralysis, ventricular arrhythmia, and death.

8.3.1.3 Hypersensitivity and Allergic Reactions

- Local redness, lipodystrophy, swelling and itching at the injection site after I004 or Novolog injection.

- Local reaction and generalized myalgia due to metacresol, an excipient in I004 or

Novolog.

- Severe, life-threatening, generalized allergy, including anaphylaxis due to the insulin product, including whole body rash, dyspnea, wheezing, hypotension, tachycardia or diaphoresis.

- Vasovagal response, bruising, tenderness and rarely infection due to venous line.

8.3.2 Procedure Related AEs

8.3.2.1 Catheter Placement

Study procedures involve the placement of a catheter which may lead to allergic reaction, redness, swelling, bruising, pain, bleeding or infection at catheter insertion site.

8.3.2.2 Adhesive tape

The study procedures also involved the use of adhesive to secure the placement of medical equipment. The adhesive may cause an allergic reaction, redness, swelling or itching when in contact with the skin.

8.3.2.3 Blood draw

Subjects will participate in several blood draws throughout the course of the study which have the potential to cause a venous line-vasovagal response, bruising, tenderness, and rarely infection.

8.4 Follow-up of AEs and SAEs

All AEs should be followed up and subjects will be rendered appropriate medical care and treatment at the discretion of the Investigator until resolution or until the Investigator and Sponsor concludes that “further follow-up is not necessary”. If the AE has not resolved by the post-treatment follow-up visit, the stop date will be recorded as “ongoing.”

All SAEs should be followed up until resolution or permanent outcome of the event or until the Investigator and Sponsor judge that further follow-up is not necessary.
If information is not available at the time of the first report and becomes available at a later date, the Investigator should complete a follow-up SAE form at the earliest possible or provide other written documentation and fax it immediately within 24 hours of receipt of information to the Sponsor or designee. Copies of any relevant data from the hospital notes (e.g., ECGs, laboratory tests, discharge summary, postmortem results) should be sent accordingly.

All other non-serious AEs must be followed until the outcome of the event is “recovering” (for

chronic conditions), or "recovered", or until the end of the post-treatment follow-up stated in the protocol, whichever comes first, and until all queries related to these AE's have been resolved.

Safety Reporting to IRBs or IECs, and Regulatory Authorities is described below.

The Sponsor or designated qualified vendor will be responsible for reporting all suspected unexpected serious adverse reactions (SUSARs) and any other applicable SAEs to regulatory authorities, Investigators and IRBs or IECs, as applicable, in accordance with national regulations in the countries where the study is conducted.

Relative to the first awareness of the event by/or further provision to the Sponsor or Sponsor's designee, SUSARs will be submitted within 7 days for fatal and life-threatening events and 15 days for other SUSARs, unless otherwise required by national regulations.

The Sponsor or designated qualified vendor will also prepare an expedited report for other safety issues where these might materially alter the current benefit-risk assessment of an investigational medicinal product or that would be sufficient to consider changes in the investigational medicinal products administration or in the overall conduct of the study. The investigational site will forward a copy of all expedited reports to his or her IRB or IEC in accordance with national regulations.

9.0 DATA HANDLING AND MANAGEMENT

9.1. Data Quality Assurance and Monitoring

The trial will be monitored according to current Amphastar SOP.

The site monitor will conduct monitoring of the investigational activities for the purpose of ensuring subject safety and study compliance to applicable regulatory guidelines. The Investigator will permit Amphastar authorized monitors to access the subject source documents, eCRFs, clinical supplies dispensing and storage area and study documentation as frequently as necessary and agrees to assist the site monitors with their activities. The study site will make the eCRFs available, provide missing or corrected data and PI will sign the eCRFs. Personal or subject identifying information will be treated as confidential and will NOT be publicly accessible.
The trial information may be reviewed by regulatory authorities or independent QA auditors. The study site may be inspected during or after completing the study. The Investigators agree to allow inspectors from regulatory agencies to have access to all trial records, including subject source documents. By participating in this study, the Investigator agrees to these requirements and will assist the inspectors in their duties.

9.2. Electronic Case Report Forms (eCRFs)

Electronic Data Capture (EDC) system will be used for recording of eCRFs for the conduct of the study by Amphastar, eCRF will be used for the purpose of capturing data for all consented participants in the study. The 24 hour clock will be used for all time entries.

Subject source documents are the study subject records maintained at the study site. In some cases, the source documents may be the hospital's or the physician's clinical records. Therefore, the information collected on the eCRFs must match those records. Amphastar personnel will review all eCRFs to ensure the data is verifiable and accurate. At all times, the PI has final responsibility for the accuracy and authenticity of all clinical and laboratory data entered on the eCRFs. The eCRFs will be completed as soon as possible after the data become available.

9.3. Clinical Trial Drug Accountability Data

The Study Site Research Pharmacist or designated dispenser acknowledges that the drug supplies are investigational and, as such, must be handled in accordance with the protocol and labeling. The Research Pharmacist or designated personnel will maintain adequate records showing the receipt, dispensing, return, or other disposition of the investigational study material, including the date, quantity, lot or code number, and identification of subjects (number, initials) who received or returned study drug.

The Research Pharmacist or designated dispenser and Amphastar's unblinded site monitor will

review study supply records, including dispensing and accountability records, at monitoring visits.

It is the responsibility of the investigator and research pharmacist/designated dispenser to ensure that, when the study is completed or discontinued, unused supplies of study materials will be returned or disposed of, as directed by Amphastar's site monitors or CRA. Unused study medications must be available for verification by Amphastar's site monitors or CRA.

9.4. Trial Documents

The trial documents, including the protocol signature page signed by the investigators, Investigators' curriculum vitae, IRB approval notice and IRB member list, approved Informed Consent Form, laboratory verification and institutional accreditation, will be submitted to Amphastar Pharmaceuticals, Inc.

9.5. Reporting and Publication

The clinical trial report will be prepared according to the Amphastar Standard Operating Procedure for Clinical Trial Reporting.

9.6. Archive

The Investigator will retain a copy of all study documents, including reports to the IRB/Ethics Committee and to the sponsor, in accordance with the FDA and local regulations. The FDA regulations and/or ICH guidelines state that the Investigator must maintain study documents for a set period of time. The Investigator will arrange for the retention of the Subject Identification for at least 15 years after the Final Study Report has been signed. All other documents should be kept for the maximum period of time permitted by the institution.

10.0 STATISTICAL METHODS

10.1 Analysis Plan

A statistical analysis plan (SAP) will be prepared.

10.1.1 Populations

(1) Population for Safety Evaluation – Treated Population

The safety evaluation will be conducted for the treated population, namely all subjects who received at least one dose of study drug

(2) Population for Pharmacokinetic (PK) Evaluation

The PK evaluation population will include all subjects with sufficient evaluable PK data appropriate for the evaluation of interest (with no major protocol deviations or violations thought to significantly affect the PK of the drug).

(3) Population for Pharmacodynamic (PD) Evaluation

The PD evaluation population will include all subjects with sufficient evaluable PD data appropriate for the evaluation of interest (with no major protocol deviations or violations thought to significantly affect the PD of the drug).

10.1.2 Analysis of Demographics and Other Baseline Characteristics

Demographic and baseline characteristics will be summarized for all subjects overall and by treatment. Summary statistics (e.g., number of subjects, mean, median, standard deviation, and range) will be generated for continuous variables (e.g., age and weight) and the number and percentage of subjects within each category will be presented for categorical variables.

10.1.3 Analysis of the PK Endpoints

The analyses of the PK endpoints will assess and characterize the difference of the insulin I004 and Novolog in regard to the primary and secondary objective and endpoints.

The PK endpoints will be derived from the individual serum insulin I004 and Novolog profiles.

Insulin $AUC_{IA(0\text{-}12h)}$ and C_{IAmax} are the primary PK endpoints of interest. Partial AUCs, t_{IAmax}, etc, are of secondary interest as listed in Section 2.4.

PK assessments will be analyzed using descriptive and comparative statistical methods.

10.1.4 Analysis of the PD Endpoints

The analyses of the PD endpoints will assess and characterize the difference of the insulin I004 and Novolog in regard to the primary and secondary objective and endpoints.

The PD endpoints will be derived from the GIR parameter.

GIR parameter $AUC_{G(0\text{-}12h)}$ and G_{max} are the primary PK endpoints of interest. Other GIR profiles, such as t_{Gmax}, are of secondary interest as listed in Section 2.4.

PD assessments will be analyzed using descriptive and comparative statistical methods.

10.1.5 Safety Analysis and Endpoints

Safety and tolerability of the study drugs will be assessed by collection and review of adverse events, tolerability, laboratory parameters, physical examination, vital signs, and ECG parameters throughout the duration of the study. Safety analysis will involve examination of the descriptive statistics and individual subject listings for any effects of study treatment on clinical tolerability and safety.

AEs will be summarized using the treated population.

All AEs will be coded using MedDRA. Data will be summarized using preferred term and primary system organ class.

Adverse event summaries will include the overall incidence (by system organ class and preferred term), events by maximum intensity, event by relationship to study treatment, events leading to discontinuation of study drug, and serious adverse events. Physical exam, vital signs, ECG, and laboratory parameters (hematology, chemistry, and urinalysis) will be summarized descriptively by treatment.

10.2 Interim Analysis

No interim analysis is planned.

10.3 Determination of Sample Size

[redacted] the minimal sample size is estimated as 60

[redacted]

11.0 QUALITY CONTROL AND QUALITY ASSURANCE

11.1 Monitoring

The study will be monitored by the sponsor's monitor or a qualified designee.

Monitoring visits to the study site will be made periodically during the study to ensure that all aspects of the protocol and GCP are followed, CRFs are completed correctly, and drug accountability is monitored. The Monitor will visit the study site at least once before First Subject First Visit (FSFV) (Initiation Visit), at least once during the clinical part of the study, and at least once after Last Subject Last Visit (LSLV). Furthermore, the Monitor must be available for discussions by telephone.

The Monitor must be given direct access to source documents, such as original documents, data, and records. Direct access includes permission to examine, analyze, verify any record(s) and report(s) that are important to evaluation of the clinical study. The study will be monitored to verify integrity and validity of the data. Monitoring will follow a Monitoring Plan.

Additionally, QC monitoring of the clinical study for protocol and GCP compliance will be conducted periodically by qualified staff of [redacted]

11.2 Protocol Deviations

The Investigator should not deviate from the protocol, except where necessary to eliminate an immediate hazard to study subjects. Should other circumstances arise that will require deviation from protocol-specified procedures, unless there is an emergency or immediate need, the Investigator should contact the medical monitor and Sponsor to review and discuss the implications of the deviation and determine the appropriate course of action. Any deviation must be documented, stating the reason and date, the action taken, and the impact for the subject and/or the study. The documentation must be kept in the Investigator's Study File and the Sponsor's Study Master File.

12.0 ETHICAL ASPECTS OF THE STUDY

This study will be conducted in accordance with the Protocol, the International Conference on Harmonization (ICH), Guideline for Good Clinical Practice: Consolidated Guidance (E6) and applicable regulatory requirements including clinical research guidelines established by the Basic Principles defined in the U.S. 21 CFR Parts 50, 56, and 312 and the principles enunciated in the Declaration of Helsinki (revised version Fortaleza 2013).[14,15]

12.1 Institutional Review Board and/or Independent Ethics Committee

Prior to commencement of the study, the protocol, any amendments, subject information/informed consent form, any other written information to be provided to the subject, subject recruitment procedures, information about payments and compensation available to subjects if not mentioned in the subject information, the Investigator's current CV and/or other documentation evidencing qualifications, and other documents as required by the local Independent Ethics Committee (IEC)/Institutional Review Board (IRB) should be submitted. Written approval/favorable opinion must be obtained from IEC/IRB prior to commencement of the clinical study start.

The Investigator shall provide to Sponsor or its designee a copy of the written and dated approval/favorable opinion by the applicable IRB/IEC.

During the study, the Investigator must promptly report the following to the IEC/IRB: Updates to IB, unexpected SAEs where a causal relationship cannot be ruled out, substantial amendments to the protocol, non-substantial amendments, deviations to the protocol implemented to eliminate immediate hazards to the subjects, new information that may affect adversely the safety of the subjects or the conduct of the study (including new risk/benefit analysis in case it will have an impact on the planned follow-up of the subjects), annually written summaries of the study status and other documents as required by the local IEC/IRB.

Substantial amendments must not be implemented before approval/favorable opinion, unless necessary to eliminate hazards to the subjects.

The Investigator must maintain an accurate and complete record of all submissions made to the IEC/IRB. The records should be filed in the Investigator's Study File and copies must be provided to the Sponsor.

12.2 Regulatory Authorities

Regulatory Authorities will receive the Protocol, Amendments to the Protocol, and reports on SAEs and the Integrated Clinical Study Report (CSR) according to FDA regulations.

12.3 Responsibilities of the Investigator

The Investigator will conduct this clinical study in compliance with all applicable national, state, local or regional laws and regulatory requirements of the countries in which the clinical study is performed. The Investigator will align his or her conduct in accordance with the "Responsibilities of the Investigator". All study procedures and assessments may be performed by qualified designees per Investigator's discretion, such as medical doctors (MD), physician assistants (PA) and /or nurse practitioners (NP).

In compliance with the Clinical Study Protocol, Good Clinical Practice and applicable regulatory requirements, the Investigator must permit auditing by or on the behalf of the Sponsor and inspection by applicable regulatory authorities. The Investigator agrees to allow the auditors/inspectors to have direct access to his/her study records for review, being understood that these personnel are bound by professional secrecy and as such will not disclose any personal identity or personal medical information. The Investigator will make every effort to help with the performance of the audits and inspections, giving access to all necessary facilities, data, and documents. As soon as the Investigator is notified of a future inspection by the authorities, he or she will inform the Sponsor or its designee and authorize the Sponsor or its designee to participate in this inspection. Any results and information arising from the inspections by the regulatory authorities will be immediately communicated to the Sponsor. The Investigator shall take appropriate measures required by the Sponsor to take corrective actions for all problems found during the audit or inspections.

Clinical research studies sponsored by the Sponsor are subject to ICH GCP and all the applicable local laws and regulations. The responsibilities imposed on Investigators by the FDA are summarized in the "Statement of Investigators" (Form FDA 1572), which must be completed and signed before the Investigator may participate in this study.

12.4 Informed Consent

Once signed, the original informed consent form, subject authorization form (if applicable), and subject information sheet (if applicable) will be stored in the Investigator's site file. The Investigator must document the date the subject signs the informed consent in the subject's medical record. Copies of the signed informed consent form, the signed subject authorization form (if applicable), and subject information sheet (if applicable) shall be given to the subject.

All revised informed consent forms must be reviewed and signed by relevant subjects or the relevant subject's legally acceptable representative in the same manner as the original informed consent. The date the revised consent was obtained should be recorded in the subject's medical record, and the subject should receive a copy of the revised informed consent form.

12.5 Subject Confidentiality

The Sponsor and designees affirm and uphold the principle of the subject's right to protection against invasion of privacy. Subject records will be kept private except when ordered by law. The following individuals will have access to study subject records: Principal Investigator and designees, study Sponsor, monitors and auditors, the FDA, other government offices and the IRB.

Throughout this study, a subject's source data will only be linked to the Sponsor's clinical study database or documentation via a unique identification number. As permitted by all applicable laws and regulations, limited subject attributes, such as sex, age, or date of birth, and subject initials may be used to verify the subject and accuracy of the subject's unique identification number.

The Investigator must agree to permit the Sponsor's monitor or designee's monitor, representatives from any regulatory authority, the Sponsor's designated auditors, and the appropriate IRBs and IECs to review the subject's source data or documents, including, but not limited to, laboratory test result reports, ECG reports, admission and discharge summaries for hospital admissions occurring during a subject's study participation, and autopsy reports. Access to a subject's original medical records requires the specific authorization of the subject as part of the informed consent process. The confidentiality of the verified data and the protection of the subjects must be respected during these inspections.

Copies of any subject source documents that are provided to the Sponsor must have certain personally identifiable information removed.

12.6 Publication, Disclosure, and Clinical Study Registration Policy

The Investigator will provide the Sponsor with truthful, accurate and complete test results and all data derived from the study. During the study, only the Sponsor may make study information available to other study Investigators or to regulatory agencies, except as required by law or regulation. Except as otherwise allowable in the clinical study site agreement, any public disclosure (including publicly accessible websites) related to the protocol or study results, other than study recruitment materials and/or advertisements, is the sole responsibility of the Sponsor.

[redacted] or its designee will be responsible for preparing the Clinical Study Report. When all data has been fully analyzed, the Sponsor will communicate the results of the Clinical Study to the Investigator(s).

The Investigator or qualified designee agrees to use this information only and strictly in connection with this Clinical Study and must not use it for other purposes without the prior written permission from the Sponsor. Prior to any publication, the Sponsor must be given the opportunity to review and comment upon any manuscript, poster, or paper that contains data derived or generated from this study in order to be aware of all written and oral presentations of the data and does not imply any

editorial review or restriction of the contents of the presentation or use.

12.7 Insurance and Compensation for Injury

The Sponsor shall carry applicable insurance in the types and amounts necessary to cover its obligations herein in accordance with local laws and requirements and/or guidelines for conducting clinical studies in any country, unless others have shown negligence. The Sponsor renounces liability in the event of negligence or any other liability by the clinics or doctors conducting experiments or by persons for whom the said clinic or doctors are responsible. The Sponsor accepts liability in accordance with all applicable regulations per the Code of Federal Regulations (CFR) and all other applicable federal or state regulations.

Each subject in the study must be insured in accordance with the regulations applicable to the site where the subject is participating.

If a subject becomes ill or injured due to an adverse event directly resulting from use of the study drug or a study procedure in the course of their participation in this Clinical Study, medical treatment will be provided. Sponsor will pay the costs of such treatment.

13.0 REFERENCES

1. Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2017. http://www.diabetes.org/assets/pdfs/basics/cdc-statistics-report-2017.pdf

2. Boyle JP, Thompson TJ, Gregg EW, et al. Projection of the year 2050 burden of diabetes in the US adult population: dynamic modeling of incidence, mortality, and prediabetes prevalence. Popul Health Metr. 2010;8(1):29.

3. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329:977-86.

4. Pieber TR et al. Evaluation of a structured outpatient education program for intensive insulin therapy. Diabetes Care 1995;18:625-30.

5. Lindholm A et al. Improved postprandial glycemic control with insulin aspart: a randomized double-blind cross-over trial in type I diabetes. Diabetes Care 1999;22:801-5.

6. Gale EAM, the UK Trial Group: A randomized controlled trial comparing insulin lispro with human soluble insulin in patients with type I diabetes on intensified insulin therapy. Diabetic Med 2000;17:209-14.

7. Kotsanos JG et al. Health-related quality-of life results from multinational clinical trials of insulin lispro: assessing benefits for a new diabetes therapy. Diabetes Care 1997;20:948-58.

8. Kang S et al. Subcutaneous absorption explained by insulin's physiochemical properties: evidence from absorption studies of soluble human insulin and insulin analogues in humans. Diabetes Care 1980;14:942-48

9. Product information NovoLog®, revised 03/2021: https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/020986s093lbl.pdf

10. EMA: Annex to guideline on similar biological medicinal products containing biotechnology- derived proteins as an active substance. Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues The European Agency for the evaluation of Medicinal Products. EMA/CHMP/BMWP/32775/2005_Rev. 1

11. EMA: Guideline on similar biological products containing biotechnology-derived proteins as active substance: Quality issues. EMA /CHMP/BWP/49348/2005

12. US Food and Drug Administration. United States Code of Federal Regulation (CFR), Part 320: Bioavailability and Bioequivalence Requirements. Federal Regulations] [Title 21, Volume 5] [Revised as of April 1, 2019] [CITE: 21CFR320].

13. ADA Workgroup Report on Hypoglycemia. Diabetes Care 2013; 36:1384-95.

14. World Medical Association. Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. 52nd WMA General Assembly, Edinburgh, Scotland, October 2000. Last amended with Note of Clarification on Paragraph 29 by the WMA General Assembly, Washington 2002.and Note of clarification on paragraph 30 by the WMA General assembly, Tokyo 2004

15. International Conference on Harmonization. ICH Harmonized Tripartite Guideline. Good Clinical Practice. 01-May-1996

16. EMA: Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues. EMEA/CHMP/BMWP/32775/2005_Rev. 1

17. Shankar G, Arkin S, Cocea L, et al.: Assessment and reporting of the clinical immunogenicity of therapeutic proteins and peptides—harmonized terminology and tactical recommendations. AAPS J 2014;16: 658–673.

18. FDA Guidance for Industry: Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products. April 2016.

19. Guidance for Industry: Handling and Retention of BA and BE Testing Samples and Retention (May 2004)

20. Guidance for Industry: Compliance Policy for the Quantity of Bioavailability and Bioequivalence Samples Retained Under 21 CFR 320.38 (c) (August 2020)

21. Questions and Answers on Biosimilar Development and the BPCI Act Guidance for Industry: Guidance for Industry (December 2018)

Protocol Signature Page

I confirm that I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the ICH Good Clinical Practice guideline and adhere to the core principles of the Declaration of Helsinki, or the applicable local laws and regulations of the study site for which I am responsible, whichever provides the greater protection of the individual. I will abide by the publication rules set forth in my agreement with Amphastar Pharmaceuticals, Inc. I will accept the monitor's inspection and overseeing of the study. I will promptly submit the protocol to the applicable Institutional Review Board/Ethical Committee for approval.

_____ _____

Signature of Investigator Date

_____

Investigator Name (Print)

_____

Title

_____

Name of Facility

_____

Street Address

_____

City, State

_____ _____

Signature of Representative of Amphastar Pharmaceuticals, Inc. Date

APPENDIX A
Schedule of Events (SOE)

ASSESSMENT	SCREEN	IN-HOUSE PERIOD 1	WASH-OUT	IN-HOUSE PERIOD 2	FOLLOW-UP[1]
IP administration		X		X	
Informed consent	X				
Assess eligibility criteria	X				
Demography	X				
Medical history	X				
History of prior Insulin exposure/use	X				
Sequestered in clinic		X		X	
Physical examination (PE)	X				X
Hematology, serum chemistry & urinalysis[5]	X				X
HbA1c	X				
Coagulation	X				
TSH	X				
Hep B, Hep C, HIV	X				
FSH if postmenopausal	X				
Pregnancy test(serum)	X				
Pregnancy test(urine)		X		X	X
Urine drug screen & alcohol breath test	X	X		X	X
Weight	X	X		X	
Height, BMI	X				
Vital signs	X	X[2]		X[2]	X
12-lead ECG	X	X[3]		X[3]	X
Standardized meals		X		X	
Record concomitant medications	X	X		X	X
Record AEs	X	X		X	X
Assess check-in criteria		X		X	
Randomization		X			
Injection site assessment		X[4]		X[4]	
Clamp procedure		X		X	
PK/PD assessments		X		X	
Blood glucose (YSI)	X	X		X	

[1]To be performed after second dosing or at early termination

[2]On each Day 1, vital signs to be measured 15 (± 5) min pre-clamp/pre-dose and at 5(± 3) min, 60 (± 10) min, 180 (± 20) min and 720 (± 30) min post-dose.

[3]On each day 1, ECG 15 (± 5) min pre-clamp/pre-dose and at 5 (± 3) min, 60 (± 10) min, 180 (± 20) min and 720 (± 30) min post-dose.

[4]Assessment of reaction at 15 min, 60 min and 720 min post-dose.

[5] Unscheduled visits for safety lab testing are allowed during COVID-19 pandemic.

APPENDIX B
Clamp Sampling Schedule

Approx. hour[1]	Nominal timing	Activity	Blood Glucose (via YSI)	Blood Sampling[2] for PK/PD		Sampling window for PK/PD
				I004 or Novolog	C-peptide; endogenous human insulin	
06:00	120 to –60 min	Connection to Biostator.	Sampling at least every 30 min	--	--	
07:00	-60 min			X	X	± *5 min*
	-30 min	The average of BG values at -30 min, -20 min and -10 min time points, correspond to subject's individual BASELINE glycemic level.	X	X	X	
	-20 min		X	--	--	
	-10 min		X	--	--	
08:00	0 min	**IP administration** Start of clamp period/IV variable glucose infusion (GIR) to keep BG at clamp target (±10%). Clamp target level is determined by BASELINE level minus 5 mg/dL.	Sampling pre-dose and thereafter at approximately every 30 min	X	X	-5 to 0 min
	5 min			X	X	± *1 min*
	10 min			X	X	
	20 min			X	X	± *2 min*
	25 min			X	X	
	30 min			X	X	
	40 min			X	X	± *3 min*
	50 min			X	X	
	55 min			X	X	
09:00	60 min (1h)			X	X	
	65 min			X	X	
	70 min			X	X	
	75 min			X	X	
	80 min			X	X	± *5min*
	90 min			X	X	
	105 min			X	X	
10:00	120 min (2h)			X	X	
	150 min			X	X	
11:00	180 min (3h)			X	X	± *10 min*
	210 min			X	X	
12:00	240 min (4 h)			X	X	
13:00	300 min (5 h)			X	X	
14:00	360 min (6 h)			X	X	
16:00	480 min (8 h)			X	X	± *20 min*
18:00	600 min (10 h)			X	X	
20:00	720 min (12 h)	End of clamp		X	X	

		Disconnect from Biostator				

[1]Actual starting time is approximate but nominal timing should be followed in any case.
[2]Collection of blood samples for pharmacokinetic assessment at the correct time point relative to dosing is of primary importance.