Protocol Title: A randomized, examiner-blind, comparator-controlled, cross-over bioequivalence study on vitamin C in healthy adults

NCT03562988

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CRO: KGK Synergize Inc.
[redacted]

Protocol approval date – June 13, 2017

OUTLINE OF RESPONSIBILITIES REGARDING CLINICAL SAMPLES

Responsibility	Laboratory	Address
Blood & urine collection	KGK Canadian Clinic	[redacted]
Sample processing	KGK Canadian Clinic	
Sample storage	KGK Canadian Clinic	
Sample shipment	KGK Canadian Clinic	
Blood safety parameters	LifeLabs	
Plasma ascorbic acid level	LifeLabs	
WBC ascorbic acid level	Mount Sinai Laboratory	
Urine ascorbic acid level	Mount Sinai Laboratory	

PROTOCOL SIGNATURE SHEET

KGK Synergize Inc. agrees to conduct the title study as provided in this protocol, in accordance with all government regulations and to make no changes without prior notification to the sponsor except for a modification that is deemed necessary to eliminate or reduce risk to human volunteers.

Name	Signature	Date
Sponsor: [redacted]	CONFIDENTIAL	
Scientific Director: [redacted]	CONFIDENTIAL	

LIST OF ABBREVIATIONS AND SYMBOLS

ADE	adverse device effect
AE	adverse event
ALT	alanine transaminase
AST	aspartate aminotransferase
BMI	body mass index
°C	degree Celsius
Ca	calcium
CBC	complete blood count
Cl	chloride
CIP	Clinical Investigation Plan
cm	centimetre
CPT	cell preparation tube
CRF	case report form
EC	ethics committee
EDTA	diaminoethanetetraacetic acid
e.g.	for example
et al	and others
g	Gram
GCP	Good Clinical Practice
HPLC	High Performance Liquid Chromatography
Ibs	pounds
ICH	International Conference of Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
K	potassium
kg	kilogram
L	Litre
m	metre
mg	milligram
Mg	magnesium
mL	millilitre
MVM	multivitamin or mineral
Na	Sodium
NHANES	National Health and Nutrition Examination Survey
PBS	phosphate buffer saline
RBC	red blood cells
RCF	relative centrifugal force
RPM	revolutions per minute
SAE	serious adverse event
SOP	standard operating procedure
SST	serum separating tube

TPD	Therapeutic Products Directorate
WBC	white blood cell

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1 INTRODUCTION

According to the National Health and Nutrition Examination Survey (NHANES), approximately 40% of all adults and 31% of children have been found to take multivitamin or mineral supplements (MVM) (1;2). Moreover, 71% of dietary supplement consumers have reported routinely taking a MVM in a recent panel of nationally representative surveys of adults in the United States from 2007 to 2011 (3). Aside from MVM, vitamin C (32%) is also commonly consumed by supplement users who eat a balanced diet and follow a lifestyle routine that is associated with good health (3).

Vitamin C is frequently reported to be below the estimated average requirements for vitamins obtained through dietary sources (2). Moreover, adequate levels of vitamin C are vital for the maintenance of numerous biological functions within the body.

Vitamin C (Ascorbic acid) is an essential water-soluble vitamin in humans, which can be obtained through the diet mainly through fruits and vegetables (4). This nutrient plays an important role as a cofactor for various biological processes, an anti-oxidant, and it provides support for the immune system (5). Pharmacokinetic studies have found that vitamin C has a relatively short plasma half-life that can range from 30 minutes to 8 hours (6). Moyad et al (2008) found that vitamin C accumulates in leukocytes following an acute oral dose of either calcium ascorbate with calcium threonate, or with ascorbic acid alone (7).

While people perceive taking MVM supplements as a healthy lifestyle choice, bioequivalence studies centered on gummy vitamin C formulations are lacking. This may be due to the difficulty in having two competing vitamin products with similar micro-nutrient profiles. This current randomized, examiner-blind, cross-over study will evaluate the bioequivalence of a gummy containing vitamin C relative to a caplet comparator product in healthy adults.

2 STUDY OBJECTIVES

The objective of this exploratory pharmacokinetic research study is to demonstrate that both caplets and gummies provide an effective dose of ascorbic acid on healthy adults.

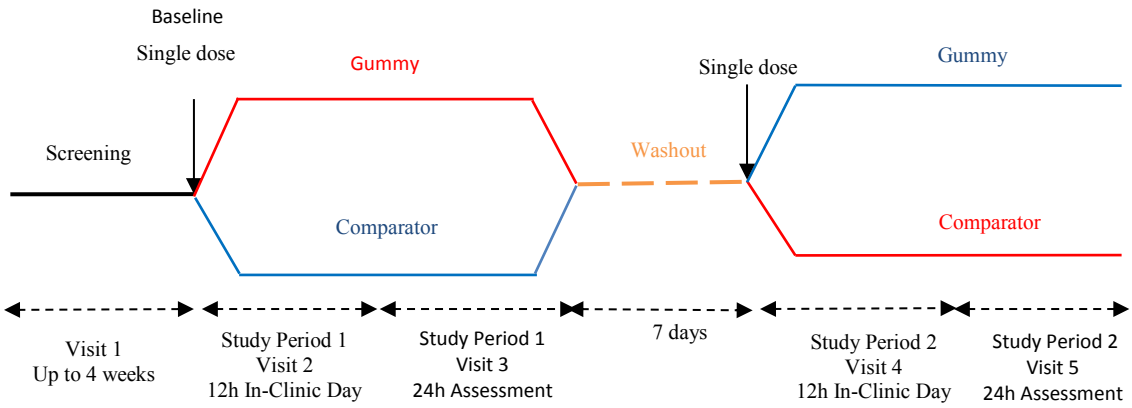
Outcomes:

1. The vitamin C $AUC_{0\text{-}24h}$ of the gummy formulation and that of a caplet comparator product
2. The vitamin C C_{max} of the gummy formulation and that of a caplet comparator product
3. The vitamin C T_{max} of the gummy formulation and that of a caplet comparator product
4. The vitamin C AUC to infinity (AUC_I) of the gummy formulation and that of a caplet comparator product
5. The vitamin C $AUC_{0\text{-}24h}$ /AUC_I of the gummy formulation and that of a caplet comparator product
6. The vitamin C terminal disposition rate constant (λ) of the gummy formulation and that of a caplet comparator product
7. The vitamin C terminal half-life ($t_{½}$) of the gummy formulation and that of a caplet comparator product

Safety outcomes:

1. The difference in vital signs, hematology and clinical chemistry parameters between the gummy formulation vs. that of a comparator product
2. The difference in the incidence of adverse events between the gummy formulation vs. that of a caplet comparator product

3 STUDY DESIGN



This will be a randomized, examiner-blind, cross-over, comparator-controlled bioequivalence study.

The planned sample size for this study is 30 participants to complete (equal number of males and females). Assuming 20% drop-out rate from enrollment to completion, 36 participants will be randomized equally to each of the two study arms in an examiner-blind manner at a ratio of 1:1.

Study Arm	**Participant Number***
Gummy → Comparator	N = 18
Comparator → Gummy	N = 18
Total	**N = 36**

*Assumed 20% drop-out rate from enrollment to completion

To evaluate study outcomes, assessments will be conducted at screening, baseline and, all study visits.

The study will be conducted at a single site in KGK Synergize, [redacted].

4 SELECTION OF STUDY POPULATION

This exploratory research study will include 30 healthy male and female participants. Each participant must fulfill the inclusion criteria and not meet any of the exclusion criteria as described in sections 4.1 and 4.2 respectively.

4.1 Inclusion Criteria

1. Healthy male or female from 18 and 55 years of age inclusive.
2. If female, participant is not of child bearing potential, which is defined as females who have had a hysterectomy or oophorectomy, bilateral tubal ligation or are post-menopausal (natural or surgically with > 1 year since last menstruation)
   OR
   Females of childbearing potential must agree to use a medically approved method of birth control and have a negative urine pregnancy test result. A minimum of 3-months stable dose is required for females on a hormonal birth control. Acceptable methods of birth control include:
   - Hormonal contraceptives including oral contraceptives, hormone birth control patch ([redacted]), vaginal contraceptive ring ([redacted]), injectable contraceptives ([redacted]), or hormone implant ([redacted])
   - Double-barrier method
   - Intrauterine devices
   - Non-heterosexual lifestyle or agrees to use contraception if planning on changing to heterosexual partner(s)
   - Vasectomy of partner (shown successful as per appropriate follow-up)
3. BMI 18.5 to 29.9 kg/m^2 (±1 kg/m^2)
4. Healthy as determined by laboratory results and medical history
5. Agrees to maintain current level of physical activity throughout the study
6. Agrees not to donate blood for the next 3 months after completing the study
7. Agrees to avoid foods and beverages fortified with vitamin C for at least 7 days prior to enrolment and during the study
8. Agrees to avoid citrus foods, citrus juices (e.g. orange, grapefruit, lemon, lime, orange juice, grapefruit juice and lemonade), and tomato juice for at least 7 days prior to enrolment and during the study
9. Non-smoker or ex-smoker >1 year
10. Has given voluntary, written, informed consent to participate in the study
11. Agrees to avoid high caffeine and alcohol intake 72 hours prior to in-clinic test days and during the 24-hour in-clinic test days

4.2 Exclusion Criteria

1. Women who are pregnant, breast feeding, or planning to become pregnant during the trial

2. Duodenal or gastric ulcer, gastritis, hiatus hernia, or GERD within past 3 months
3. History irritable bowel syndrome and related disorders
4. Significant gastrointestinal disease (examples include but are not limited to Celiac disease)
5. History of malabsorption
6. Unstable medical conditions as determined by the Qualified Investigator
7. Blood pressure greater than 150/90 mmHg
8. Cancer except skin cancers completely excised with no chemotherapy or radiation following and with a negative follow up. Volunteers with cancer in full remission for more than 5 years after diagnosis are acceptable.
9. Clinically significant abnormal laboratory results at screening
10. Metabolic disease or chronic diseases (examples include and not limited to hyperlipidemia, hypertension, and hypercholesterolemia)
11. Type I or Type II diabetes
12. History of kidney stones
13. Use of prescription or over the counter products known to interact with vitamin C within 72 hours of enrolment and during the trial such as aspirin and NSAIDs (including acetaminophen containing medications), aluminum (found in most antacids),, iron, and proton pump inhibitors
14. Use of acute over the counter medication within 72 hours of test product dosing
15. Use of tobacco products within the last year
16. More than 2 alcoholic drinks per day
17. Drug abuse within 1 year of enrolment
18. Use of medicinal marijuana
19. Immunocompromised individuals such as individuals that have undergone organ transplantation or individuals diagnosed with human immunodeficiency virus (HIV)
20. Individuals who have planned surgery during the course of the trial
21. St. John's wort in the last 30 days prior to enrolment and during the study
22. Use of vitamin C, multivitamins containing vitamin C, or foods or beverages fortified with vitamin C and other natural health products containing vitamin C within 7 days of enrolment and during the study
23. Consumption of citrus foods, citrus juices (e.g. orange, grapefruit, lemon, lime, orange juice, grapefruit juice and lemonade), and tomato juice within 7 days of enrolment and during the study
24. Use of anticoagulants (warfarin), barbiturates, tetracycline antibiotics, beta-blockers, cyclosporine, prednisone, tricyclic antidepressants, diuretics and nitrate medications (including daily use of low-dose Aspirin)
25. Use of natural health products/dietary supplements within 7 days of enrolment or during the study
26. Current diagnosis and history of blood/bleeding disorders
27. Current diagnosis and history of anemia of any etiology defined as hemoglobin < 145 g/L for males and < 123 g/L for females
28. History of hemoglobinopathies such as sickle cell anemia or thalassemia, sideroblastic anemia

29. History of hemochromatosis
30. Blood donation in the past 3 months
31. Individuals who plan to donate blood during the study or within 30 days of completing the study
32. Participation in a clinical research trial within 30 days prior to enrolment
33. Allergy or sensitivity to supplement ingredients or to any food or beverage provided during the study
34. Individuals who are cognitively impaired and/or who are unable to give informed consent
35. Any other condition which in the Qualified Investigator's opinion may adversely affect an individual 's ability to complete the study or its measures or which may pose significant risk to the individual

4.3 Concomitant Medications

Participants who are currently taking any prescribed medications except for birth control will be excluded from the study.

- Use of natural health products/dietary supplements within 7 days of enrolment or during the study is not permitted.
- Use of acute or over the counter medication within 72 hours of test days is not permitted.
- Consumption of tomato juice, citrus foods, or citrus juice (e.g. orange, orange juice, grapefruit, grapefruit juice, lime, lemon, and lemonade)
- Food and beverages fortified with vitamin C within 7 days of enrolment and during the trial.
- St. John's wort is prohibited 30 days prior to enrolment and during the trial.
- Use of prescription or over the counter product know to interact with vitamin C such as aspirin (including daily use of low-dose Aspirin), NSAIDs (including medications containing acetaminophen), aluminum, iron and proton pump inhibitors are prohibited 72 hours prior to enrolment and during the study.
- Vitamin C or multivitamins containing vitamin C are not allowed within 7 days of enrolment and during the duration of the study.
- The following are prohibited in this study: anticoagulants (warfarin), barbiturates, tetracycline antibiotics, beta-blockers, cyclosporine, prednisone, tricyclic antidepressants, diuretics, nitrate and marijuana.
- Tobacco products are prohibited within 1 year of enrolment and during the study.

4.4 Early Withdrawal

Personal reasons
As stated in the Informed Consent Form, a participant may withdraw from the study for any reason at any time.

Removal by Qualified Investigator:

Participant discontinuation should be considered at the discretion of the Qualified Investigator. The circumstances of any discontinuation must be documented in detail in the participant file and final report. If possible, the evaluations planned for the end of study will be carried out at the time when the participant is withdrawn from the study. A participant leaving the study prematurely will be replaced by another if required to ensure 30 participants complete the study. It is understood by all concerned that an excessive rate of withdrawals can render the study un-interpretable; therefore, unnecessary withdrawal of participants should be avoided.

Criteria for removal of participants from the study will include:

Clinical reasons

A participant may be withdrawn from the study if, in the opinion of the Qualified Investigator, it is not in the participant's best interest to continue. Any participant who experiences a serious adverse event (SAE) may be withdrawn from the trial at the discretion of the Qualified Investigator. A participant will also be withdrawn due to adverse events causing clinically significant illness or the need for prohibited medication(s) during the trial. Any female participant who becomes pregnant during the course of the trial will be withdrawn.

Protocol violation

Any participant found to have entered this study in violation of the protocol will be discontinued from the study at the discretion of the Qualified Investigator. This will include any participant found to have been inappropriately enrolled (did not meet eligibility criteria). Participant non-compliance includes not showing up for study visits or refusing to undergo study visit procedures. Participants who are found to be taking prohibited medications or supplements without the knowledge of the Qualified Investigator will also be withdrawn. Any major protocol deviations (i.e., those that increase the risk to participants and/or compromise the integrity of the study or its results) will result in participant discontinuation.

5 INVESTIGATIONAL PRODUCT

5.1 Manufacturing and Storage

The investigational product will be provided to KGK by the Sponsor. The investigational product will be carefully stored at the study site in a lockable, limited access area, accessible only to study team personnel in compliance with pertinent regulations. Only authorized persons will have access to the investigational product. The products will be stored at room temperature and will not be exposed to direct sunlight or heat. The investigational products will be kept in a locked investigational product storage room at KGK Synergize Inc. on receipt. An accountability log will be kept for the investigational products.
All unused investigational product will be returned to the study sponsor by KGK (at the sponsor's expense) or destroyed on receipt of written confirmation from the sponsor at study closeout (within one month of last participant visit).

5.2 Labeling and Coding

The investigational product will be labeled per the requirements of ICH-GCP guidelines and applicable local regulatory guidelines. The investigational product will be randomized and coded by an un-blinded person at KGK who is not involved in data collection or analysis.

5.3 Investigational Products

5.3.1 Gummy Product:

[redacted]

Sponsor Reference No.	Dietary Ingredient	Amount per serving per label claim	Use Instructions
4796-81A	Vitamin C	240 mg / two gummies	Chew thoroughly before swallowing

Other ingredients: *Sodium Citrate, Natural Favor, Glucose Syrup, Rose Hips, Sucrose, Gelatin, Citric Acid, Color (annatto extract), Fumaric Acid, Lactic Acid, Sodium Citrate.*

5.3.2 Comparator Product:

[redacted]

Sponsor Reference No.	Dietary Ingredient	Amount per serving per label claim	Use Instructions
4796-81B	Vitamin C	500 mg / one caplet	Take with water

Other ingredients: *cellulose gel, hydroxypropyl Methylcellulose, Rose Hips, Croscarmellose Sodium, Stearic Acid, Silicon Dioxide, Magnesium Stearate.*

5.5 Dosing Directions

A dose of 1000 mg vitamin C will be used in this study based on a recently published article in which a threshold of plasma ascorbic acid level of 37 µM was observed post dosing (8). Participants will consume the investigational products with 250 mL water in front of the clinical coordinator. The investigational products will be provided based on analytical results in the number of gummy or caplet in a quantity as close as possible between 975 and 1025 mg/dose (1000 mg ± 2.5%). To ensure examiner blinding clinic coordinators not involved in collecting study data will dispense investigational products to the participants. Participants will be instructed to chew the gummies thoroughly before swallow. A total of 250 mL of water is to be consumed together with the study product. The time of investigational product intake will be recorded. Water will be permitted ad libitum starting 1-hour after dosing and the clinic coordinator will encourage the participants to drink water throughout the day. Participants will be allowed to sit / rest yet will not be permitted to recline for 2 hours after dosing.

5.6 Randomization

A randomization schedule will be created and provided to the Investigator indicating the order of randomization (Appendix 5). Each participant will be assigned a randomization code according to the order of the randomization list generated using www.randomization.com. Enrolled participants will be randomized to the different study arms at the Baseline Visit.

5.7 Un-blinding and Allocation Concealment

This study is single blinded (examiner only) due to the obvious difference in product form (gummy vs. caplet). Un-blinding should not occur except in the case of emergency situations. If a serious adverse event occurs, for which the identity of the investigational product administered is necessary to manage the participant's condition, the supplement received by the participant will be un-blinded and the investigational product identified. Concealment of the allocation of supplement will be employed using opaque sealed envelopes, each labeled with a randomization number. Each envelope will contain information regarding the supplement associated with each randomization number. These envelopes will be readily available for the investigator to open if it becomes necessary to know which product a participant is taking for the sake of the participant health care. The sponsor must be notified of any un-blinding within 24 hours. Details of participants who are un-blinded during the study will be included in the Final Report.

6 STUDY ASSESSMENTS

See Appendix 1 for the schedule of assessments and procedures, and Appendix 1a for time points for blood and urine sampling:

- Table 1A: A total of twelve (12) time points for blood sampling including baseline prior to dosing at 0h, and 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h post dosing for ascorbic acid analysis.
- Table 1B: A total of four time points for blood safety parameters.
- Table 1C: A total of six time points for urine ascorbic acid.

6.1 Visit 1 – Screening (Day -28 to Day -8)

At screening, a volunteer Informed Consent Form will be given to the potential participant. The volunteer will read the information carefully and will be given the opportunity to seek more information if needed. The volunteer will also be provided with the option of taking the consent form home to review prior to making his or her decision. If agreeable, the volunteer will sign the consent form and receive a duplicate. Once consent has been obtained, the screening visit will proceed. After the volunteer has signed the informed consent, the screening number will be assigned sequentially and entered in the Screening and Enrolment Log. Screening numbers will be allocated in the chronological order of the volunteer signing the informed consent. Participants will be provided with a list of meal items to choose from during the 8-h clinic visits.

Visit 1 assessments include:

- Reviewing of medical history, concomitant therapies, and current health status
- Urine pregnancy test for females of childbearing potential and participants that are not post-menopausal
- Recording weight, height, and BMI
- Recording seated resting blood pressure and heart rate
- Assessing inclusion and exclusion criteria
- Blood collection for the analysis of CBC, electrolytes (Na, K, Cl), HbA1c, creatinine, estimated glomerular filtration rate (eGFR), aspartateaminotransferase (AST), alanine aminotransferase (ALT), and bilirubin
- Dispensing 7-day food record and instructing on completion
- Nutrition counselling
- Instructing volunteers to maintain their current dietary habits but to refrain from consuming St. John's Wort, health supplements, foods and beverages containing vitamin C (Refer to Appendix 3), tomato juice, citrus food, and juice (orange, grapefruit, lemon, lime, orange juice, grapefruit juice and lemonade) 7 days prior to their next visit.
- Volunteers will be instructed to fast (water is allowed) for 12 hours prior to their next visit and avoid alcohol for 24 hour prior to coming to the clinic

Individuals will be counseled on foods to avoid during the washout period and on determining vitamin C content of fortified foods by reading labels. Foods and fortified foods containing less than 2.4 mg or 4% of recommended dietary intake (RDI) of vitamin C will be permitted during the washout period. They will be provided with a list of low vitamin C foods to be followed during their washout period, as well as examples of low vitamin C meal plans (Appendix 3).

6.2 Washout period (Day -8 to Day -1)

Eligible volunteers will follow a recommended food plan and record their food/drink intake for the 7 days preceding the baseline visit. Volunteers will start fasting 12 hours before visit 2.
The next visit will be scheduled for eligible participants immediately following the 7-day washout period.

6.3 Visit 2 – Baseline: Clinic Day 1 (Test Period 1, 8h In-Clinic Day)

Eligible participants will return to the clinic after fasting for 12 hours (only water is allowed) for baseline assessments.

Baseline assessments include:

- Reviewing of concomitant therapies and current health status
- Physical exam
- Assessing of inclusion and exclusion criteria
- Collecting 7-day food record and reviewing dietary compliance
- Randomizing eligible participants

- Recording weight and BMI
- Recording seated resting blood pressure and heart rate
- Blood collection (plasma and WBC) for pre-dose baseline (0h) analysis of L-ascorbic acid
- Pre-dose urine collection (baseline 0h) and total urine volume will be recorded and a sample for L-ascorbic acid analysis will be retained
- Following randomization and collection of pre-dose samples, Investigational product administration with 250 mL of room temperature water and recording of consumption time. Participants who receive the gummies will be instructed to chew gummies thoroughly before swallowing. Water will be permitted ad libitum 1-hour after dosing.
- Participants will be instructed to avoid reclining for 2 hours after dosing.
- Blood collection (plasma and WBC) for post-dose analysis of L-ascorbic acid at 30min, 1h, 2h, 3h, 4h, 5h, 6h, 8h,10h and 12h post-dose
- Urine collection between 0-2h post-dose and total volume will be recorded.
- Sample of 0-2h post dose urine will be retained for L-ascorbic acid analysis
- Urine collection between 2-4h post-dose and total volume will be recorded.
- Sample of 2-4h post dose urine will be retained for L-ascorbic acid analysis
- Urine collection between 4-8h post-dose and total volume will be recorded
- Sample of 4-8h post dose urine will be retained for L-ascorbic acid analysis
- Urine collection between 8-12h post-dose and total volume will be recorded
- Sample of 8-12h post dose urine will be retained for L-ascorbic acid analysis
- Recording of adverse events

Standardized meals devoid of vitamin C containing products will be provided to participants in the clinic (Refer to appendix 4). The following meals will be provided at the clinic: Breakfast after the 2h blood sampling, lunch after the 5h blood sampling, and supper between the 6h and 12h sampling. All food items and quantities consumed will be recorded by clinic staff for each individual participant.

Participants will remain in the clinic during the study visit from pre-dose until the 12h post-dose blood sampling. They will be allowed to watch television, use computers/laptops, read, talk, play video or board games, or sleep.

Prior to leaving the clinic participants will be reminded to maintain compliance and refrain from consuming St. John's Wort, other health supplements, foods and beverages containing vitamin C and high caffeine, alcohol, grapefruit and grapefruit juice. Participants will be reminded to maintain similar levels of physical activity throughout the study.

Participants will be provided with containers for the 12-24h urine collection.

Participants will be provided with study diary to record concomitant therapies and adverse events between the 12 and 24h clinic visit. Participants will also be provided with a food record to record food or beverages consumed between the 12 and 24h clinic visit. Participants will be instructed to return the completed food record and study diary to their next visit.

Participants will return the following day for their 24h assessment (Visit 3).

6.4 Visit 3 – Clinic Day 2 (Test Period 1, 24h Assessment)

Participants will return to the clinic for the 24h post-dose sample collections.

- Study diary will be collected and a new diary dispensed
- Concomitant therapies and adverse events will be reviewed
- Food record will be collected and dietary compliance will be reviewed
- Weight, BMI, seated resting blood pressure and heart rate will be recorded
- Urine containers will be collected, total urine volume will be recorded and a sample for L-ascorbic acid analysis will be retained
- 24h post-dose blood sample (plasma and WBC) will be collected for the analysis of L-ascorbic acid
- Blood collection for the analysis of CBC, electrolytes (Na, K, Cl), creatinine, eGFR, AST, ALT, and bilirubin
- 7-day food records will be dispensed to record all intake of between now and their next visit

Prior to leaving the clinic participants will be reminded to maintain compliance and refrain from consuming St. John's Wort, other health supplements, foods and beverages containing vitamin C (Appendix 2) and high caffeine, alcohol, grapefruit and grapefruit juice. Participants will be reminded to maintain similar levels of physical activity throughout the study.

6.5 Washout period (Day 3 to Day 10)

Following test period 1, participants must follow a recommended food plan and record their food/drink intake for the all days preceding the next visit. Participants will start fasting 12 hours before visit 4.

The next visit will be scheduled after a 7-day washout where a two-day window (+2 days) will be allowed to accommodate scheduling. Participants will be advised on what foods to avoid during the washout period and to complete a 7-day food (+2 days if applicable) record during this time. Participants will be reminded not to consume alcohol 24h prior to their next clinic visit and that they will be required to provide a urine sample at the start of the visit. Participants will be reminded to maintain similar levels of physical activity throughout the study.

Participants will be provided with study diary to record concomitant therapies and adverse events between clinic visit 3 and visit 4. Participants will be instructed to return both the diary and 7-day food record at their next study visit.

6.6 Visit 4 – Clinic Day 3 (Test Period 2, 8h In-Clinic Day)

Participants will return to the clinic, after fasting for 12 hours (water is allowed), for the second test period assessments. Study diaries will be collected and new diaries dispensed.

Clinic Day 3 assessment includes:

- Reviewing of concomitant therapies, adverse events, and current health status
- Collecting 7-day food diary food record and reviewing dietary compliance
- Recording weight and BMI
- Recording seated resting blood pressure and heart rate
- Pre-dose blood will be collected for the analysis of CBC, electrolytes (Na, K, Cl), creatinine, eGFR, AST, ALT, and bilirubin
- Blood collection (plasma and WBC) for pre-dose (baseline, 0h) analysis of L-ascorbic acid
- Pre-dose urine collection (baseline, 0h) and total urine volume will be recorded and a sample for L-ascorbic acid analysis will be retained
  - Administration of the investigational product that the participant did not received in round 1 with 250 mL of room temperature water. Participants who receive the gummies will be instructed to chew gummies thoroughly before swallowing. Time of consumption will be recorded. Water will be permitted ad libitum 1 hour after dosing.
- Participants will be instructed to avoid reclining for 2 hours after dosing.
- Blood collection (plasma and WBC) for post-dose analysis of L-ascorbic acid at 30min 1h, 2h, 3h, 4h, 5h, 6h, 8h, 10h and 12h post-dose
- Urine collection between 0-2h post-dose and total volume will be recorded.
- Sample of 0-2h post dose urine will be retained for L-ascorbic acid analysis
- Urine collection between 2-4h post-dose and total volume will be recorded.
- Sample of 2-4h post dose urine will be retained for L-ascorbic acid analysis
- Urine collection between 4-8h post-dose and total volume will be recorded
- Sample of 4-8h post-dose urine will be retained for L-ascorbic acid analysis
- Urine collection between 8-12h post-dose and total volume will be recorded
- Sample of 8-12h post-dose urine will be retained for L-ascorbic acid analysis
- Recording of adverse events

Standardized meals devoid of vitamin C containing products will be provided to participants in the clinic (Refer to appendix 4). The following meals will be provided at the clinic: Breakfast after the 2h blood sampling, lunch after the 5h blood sampling, and supper between the 6h and 12h sampling. All food items and quantities consumed will be recorded by clinic staff for each individual participant.

Participants will remain in the clinic during the study visit from pre-dose until the 12h post-dose blood sampling. They will be allowed to watch television, use computers/laptops, read, talk, play video or board games, or sleep.

Prior to leaving the clinic participants will be reminded to maintain compliance and refrain from consuming St. John's Wort, other health supplements, foods and beverages containing vitamin C and high caffeine, alcohol, grapefruit and grapefruit juice. Participants will be reminded to maintain similar levels of physical activity throughout the study.

Participants will be provided with containers for the 12-24h urine collection.

Participants will be provided with study diary to record concomitant therapies and adverse events between the 12 and 24h clinic visit. Participants will also be provided with food record to record food or beverages consumed between the 12 and 24h clinic visit. Participants will be instructed to return the completed food record and study diary to their next visit.

Participants will return the following day for their 24h assessment (Visit 5).

6.7 Visit 5 - End of Study: Clinic Day 4 (Test Period 2, 24h Assessment)

Participants will return to the clinic for the 24h post-dose sample collections.

- Study diary will be collected
- Concomitant therapies and adverse events will be reviewed
- Food records will be collected and dietary compliance will be reviewed
- Weight, BMI, seated resting blood pressure and heart rate will be recorded
- Urine containers will be collected, total urine will be recorded and a sample for L-ascorbic acid analysis will be retained
- 24h post-dose blood sample (plasma and WBC) will be collected for the analysis of L-ascorbic acid
- Blood collection for the analysis of CBC, electrolytes (Na, K, Cl), creatinine, eGFR, AST, ALT, and bilirubin

At the end of the study participants will be advised to return to their normal diet.

6.8 Clinical Assessments and Procedures

Calculations or measurements of specific parameters are required as indicated in the schedule of assessments. Instructions for determining these parameters are provided in the following sections.

6.8.1 Height, Weight

Weight measurements will be performed with shoes removed, and bladder empty. Participants will be weighed on the same scale at all visits.

At least two separate measurements will be taken at each visit. If the two measurements are more than 0.5 kg (1.1 lbs) apart, a third measurement will be taken. Then the two closest values will be selected and entered in the database. However, if the 3 values are equidistant a fourth measurement will be taken and the two closest values will be selected and entered into the database.

Measurement of height will be performed with the participant's shoes removed. The participant's knees will be straightened, and head held upright.

6.8.2 Blood Pressure

In office, seated resting blood pressure and heart rate will be determined from 3 measurements obtained at least 1 minute apart. One arm will be chosen and used consistently throughout the study. Blood pressure will be checked in both arms at the first examination. The arm with the higher systolic blood pressure will be used throughout the study. The arm selected for use at the initial visit will be documented in the study file.

The participant should be seated comfortably with the back supported and the upper arm bared without restrictive clothing. Feet should be flat on the floor, legs will not be crossed. The participant will rest in this position for at least 5 minutes prior to the first reading.

The same recording method and the same equipment will be used for each participant throughout the study.

6.8.3 Compliance

Investigational product compliance calculations are not necessary in this study as the study supplements will be administered in the clinic. Participants will be asked to consume the supplements in front of the clinical coordinator. Compliance to the dietary restrictions of the protocol will be assessed using the food records completed by the participant. In the event that a participant consumes foods and/or beverages with high vitamin C content (see Appendix 2), the Qualified Investigator will determine the significance and if the participant should continue in the study.

6.9 Sample collection and Processing (Appendix 6 for Flow Chart)

Blood samples will be drawn from the participants at screening (Visit 1), baseline visit (Visit 2, Day 0), visit 3, visit 4 and at the end-of-study visit (visit 5) as indicated in the schedule of assessments.

Protection of volunteer confidentiality will extend to all data generated from the assaying of these samples. These samples will be alphanumerically coded and the persons (examiner blinded) performing the analysis will not be aware of the participant's identity or the allocated product they received.

Blood collected for the analysis of CBC, electrolytes (Na, K, Cl), creatinine, eGFR, AST, ALT, and bilirubin will be delivered to LifeLabs [redacted].

The collected whole blood will be processed to generate plasma and WBC for vitamin C analysis. Plasma and WBC will be separated and stored in light resistant cryogenic tube and stored at -80°C. Plasma samples will be delivered on dry ice to LifeLabs [redacted]. WBC samples will be delivered on dry ice to Mount Sinai laboratory in [redacted]. Stability of non-acidified vitamin C at -80°C is up to 21 days. Retainer samples will be stored at -80°C.

Urine collected for vitamin C analysis will be aliquoted into light resistant cryogenic tubes and stored at -80°C. Urine samples will be delivered on dry ice to Mount Sinai laboratory [redacted]. Stability of non-acidified vitamin C at -80°C is up to 21 days. Retainer samples will be stored at -80°C.

Blood Collection:

At screening (Visit 1), 13 mL of whole blood will be collected in:

1. Two 4 mL EDTA vacutainer tubes to generate plasma for:
    a. CBC analysis (1 tube)
    b. Hb1Ac analysis (1 tube)
2. One 5 mL SST vacutainer tube to generate serum for:
    a. electrolytes (Na, K, Cl), creatinine, eGFR, AST, ALT, and bilirubin analysis (1 tube)

At baseline (Visit 2), 176 mL of whole blood will be collected in:

1. Twenty-two 8 mL sodium-heparin CPT™ vacutainer tubes to generate plasma and WBC for:
    a. L-ascorbic acid analysis (total of 22 tubes – 2 tubes per time point: pre-dose (0h), 30min, 1h, 2h, 3h, 4h, 5h, 6h, 8h, 10h, and 12h post-dose)

Visit 3, 25 mL of whole-blood will be collected in:

1. One 4 mL EDTA vacutainer tube to generate plasma for:
    a. CBC analysis (1 tube)
2. One 5 mL SST vacutainer tube to generate serum for:
    a. electrolytes (Na, K, Cl), creatinine, eGFR, AST, ALT, and bilirubin analysis (1 tube)
3. Two 8 mL sodium-heparin CPT™ vacutainer tubes to generate plasma and WBC for:
    a. L-ascorbic acid analysis at 24h post-dose (2 tube)

Visit 4, 185 mL of whole-blood will be collected in:

1. One 4 mL EDTA vacutainer tube to generate plasma for:
    a. CBC analysis (1 tube)
2. One 5 mL SST vacutainer tube to generate serum for:
    a. electrolytes (Na, K, Cl), creatinine, eGFR, AST, ALT, and bilirubin analysis (1 tube)
3. Twenty-two 8 mL sodium-heparin CPT™ vacutainer tubes to generate plasma and WBC for:
    b. L-ascorbic acid analysis (total of 22 tubes – 2 tubes per time point: pre-dose (0h), 30min 1h, 2h, 3h, 4h, 5h, 6h, 8h, 10h, and 12h post-dose)

At the end of the study (Visit 5), 25 mL of whole-blood will be collected in:

4. One 4 mL EDTA vacutainer tube to generate plasma for:
    a. CBC analysis (1 tube)
5. One 5 mL SST vacutainer tube to generate serum for:
    a. electrolytes (Na, K, Cl), creatinine, eGFR, AST, ALT, and bilirubin analysis (1 tube)

6. Two 8 mL sodium-heparin CPT™ vacutainer tubes to generate plasma and WBC for:
    a. L-ascorbic acid analysis at 24h post-dose (2 tubes)

The total blood volume collection for the laboratory assessments listed above will be approximately 424 mL, over the period from screening to end-of-study (approximately 16 days). At any study visit, blood loss per volunteer is not expected to exceed 185 mL. Additional blood samples may be collected during the study to perform repeat laboratory tests outlined in the Schedule of Assessments if needed.

A Central Laboratory, LifeLabs ([redacted]), will be contracted to measure safety blood (CBC, Hb1Ac, electrolytes (Na, K, Cl), creatinine, eGFR, AST, ALT, and bilirubin) and ascorbic acid content in plasma. Mount Sinai Laboratory ([redacted]) will also be contracted to measure L-ascorbic acid content in WBC and urine.

Urine Collection:

At screening (Visit 1), a urine pregnancy test will be done at the KGK Synergize clinic on females of childbearing potential.

At Baseline (Visit 2), urine will be collected for L-ascorbic acid analysis prior to dosing and total volume recorded. A sample will be retained for L-ascorbic acid analysis. Urine will be collected during the following post-dose time intervals 0-2h, 2-4h, 4-8h, and 8-12h and the total volume of each post-dose time interval will be recorded. A sample will be retained from each post-dose time interval for L-ascorbic acid analysis.

At visit 3, urine will be collected from 12-24h (a collection container will be dispensed to participants and they will be instructed to collect their urine up until their 24h post-dose appointment) and total volume will be recorded. A sample will be retained for L-ascorbic acid analysis.

At visit 4, urine will be collected for L-ascorbic analysis prior to dosing and total volume recorded. A sample will be retained for L-ascorbic acid analysis. Urine will be collected during the following post-dose time intervals 0-2h, 2-4h, 4-8h, and 8-12h and total volume of each post-dose time interval will be recorded. A sample will be retained from each post-dose time interval for L-ascorbic acid analysis.

At visit 5, Urine will be collected from 12-24h (a collection container will be dispensed to participants and they will be instructed collect their urine up until their 24h post-dose appointment) and the total volume will be recorded. A sample will be retained for L-ascorbic acid analysis.

Sample Processing for L-Ascorbic Acid Analysis

Plasma and WBC preparation at KGK Canadian Clinic ([redacted]):

1. Whole blood will be collected in two 8 mL sodium-heparin CPT™ vacutainer tube for plasma and WBC L-ascorbic acid analysis.
2. After the vacutainer tube is completely filled the tube will be inverted 8-10 times to ensure a complete mixing of blood and anticoagulant.
3. Sodium-heparin CPT™ vacutainer tubes will be immediately centrifuged for 20 minutes at 1500 RCF at 18 – 25 °C
4. 2 mL of plasma will be aliquoted into two amber cryovials and frozen at -80°C
5. The cell layer (whitish layer under the plasma layer) will be collected with a Pasteur pipette and transferred to a conical centrifuge tube that is in an ice slurry
6. PBS (without Ca^{++} or Mg^{++}) will be added to the conical tube to bring the volume to 15 mL
7. The conical tube will caped and inverted 5 times to mix the cells
8. The conical tube will be centrifuged for 15 minutes at 300 CRF at 4°C
9. The supernatant will be aspirated as much as possible without disturbing the cell pellet
10. The cell pellet will be resuspended by gently vortexing the conical tube
11. PBS (without Ca^{++} or Mg^{++}) will be added to the conical tube to bring the volume to 10 mL
12. The conical tube will be caped and inverted 5 times to mix the cells
13. The conical tube will be centrifuged for 10 minutes at 300 CRF at 4°C
14. The supernatant will be aspirated as much as possible without disturbing the cell pellet
15. The cell pellet (WBC mononuclear cells) will be suspended in PBS (without Ca^{++} or Mg^{++}) and transferred to amber cryovial and frozen at -80°C
16. One cryovial containing plasma will be delivered to LifeLabs for analysis and the second plasma cryovial will be retained at KGK
17. One cryovial containing WBC will be delivered to Mount Sinai Laboratory for analysis and the second WBC cryovial will be retained at KGK
18. Throughout this process the sample will be protected from light.

Urine Processing at KGK Canadian Clinic ([redacted]):

1. Total urine will be collected at pre-dose (0 h) and at post-dose (0-4h, 4-8h and 8-24h).
2. Total volume will be recorded for each collection period.
3. Urine collected pre and post-dose will be kept at 4°C until the end of each collection period.
4. At the end of each collection period urine will be agitated to ensure homogeneity of the collected urine sample and 8 mL sample will be transferred into a opaque tube.
5. The opaque tube will be centrifuged at 1000 x g for 20 minutes at 4°C.
6. 2 mL aliquot of the supernatant will be transferred into two amber cryovials and stored at -80°C.
7. One cryovial will be delivered to Mount Sinai Laboratory for analysis and one vials will be retained at KGK.
8. Throughout this process the sample will be protected from light.

Analytical method for L-Ascorbic acid level

Plasma samples will be frozen and protected from light prior to being delivered to LifeLabs ([redacted]) for analysis of L-ascorbic acid by standard procedures using HPLC methodology. Sample will be processed as follows:

1. Sulfosalicylic acid will be added to the sample to precipitate proteins and to extract ascorbic acid.
2. The supernatant will then be separated and quantified by HPLC using a reverse phase column and electrochemical detector.

WBC and Urine samples will be frozen and protected from light prior to being delivered to Mount Sinai laboratory ([redacted]) for analysis of L-ascorbic acid by ELISA method. Sample will be processed at Mount Sinai Laboratory as follows:

1. 50 µl of Standard will be added to each Standard well, 50 µl of Sample will be added to each Sample well, and 50 µl of Sample Diluent will be added to each Blank/Control well. All Standards, samples and Sample Diluent are analyzed in duplicate.
2. 100 µl of HRP-conjugate reagent will be added to each well, and covered with a Closure Plate Membrane and incubate for 60 minutes at 37°C.
3. The plate will be washed 4 times.
4. To each well 50 µl of Chromogen Solution A will be added and 50 µl Chromogen Solution B will be added successively.
5. The plate will be protected from light and incubated for 15 min at 37°C.
6. After incubation 50 µl of Stop Solution will be added to each well.
7. Optical Density (O.D.) will be read at 450 nm using an ELISA reader within 15 minutes after adding Stop Solution (5 minutes is the optimal reading time).

All measured vitamin C concentrations in µM will be used in report for plasma, WBC and urine samples at each time point during the study.

6.10 Termination of the Trial

In the case of premature termination of the trial, participating investigators/participants, and the Institutional Review Board must be promptly informed of the termination.

6.11 Protocol Amendments

If amendments to the study protocol are required after approval such changes will be captured in writing the reasons for the change documented and signed and dated by the sponsor. Any such amendments may be subject to IRB and Health Canada review/approval prior to implementation. Exception: if it becomes necessary to alter the protocol to eliminate an immediate hazard to participants, an amendment may be implemented prior to IRB approval.

In this circumstance, the Investigator must notify IRB and Health Canada in writing within five (5) working days of the implementation.

7 Safety Instructions and Guidance

7.1 Adverse Events and Laboratory Abnormalities

7.1.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation participant who has been administered an investigational product and which does not necessarily have a causal relationship with the investigational product. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a product, whether or not it is considered related to that product. Pre-existing conditions which worsen during a study are to be reported as AEs.

During the study, participants should record any adverse effects in their diary. At each visit the participant will be asked "Have you experienced any difficulties or problems since I saw you last"? Any adverse events (AEs) will be documented and in the study record and will be classified according to the description, duration, intensity, frequency, and outcome. The Qualified Investigator will assess any AEs and decide causality.

Intensity of AEs will be graded on a three-point scale (mild, moderate, severe) and reported in detail in the study record.

Mild:	Awareness of event but easily tolerated
Moderate:	Discomfort enough to cause some interference with usual activity
Severe:	Inability to carry out usual activity

The causality relationship of investigational product to the adverse event will be assessed by the Qualified Investigator as either:

Most probable:	There is a reasonable relationship between the investigational product and AEs. The event responds to withdrawal of investigational product (dechallenge) and recurs with rechallenge when clinically feasible.
Probable:	There is a reasonable relationship between the investigational product and AEs. The event responds to dechallenge.
Possible:	There is a reasonable relationship between the investigational product and AEs. Dechallenge information is lacking or unclear.

Unlikely:	There is a temporal relationship to the investigational product administration but there is no reasonable causal relationship between the investigational product and the AEs.
Not related:	No temporal relationship to the investigational product administration or there is a reasonable causal relationship between non-investigational product, concurrent disease or circumstance and the AEs.

7.1.2 Serious Adverse Event

A serious adverse event (SAE) is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability or incapacity
- A congenital anomaly/birth defect in the offspring of a participant who received the study product
- Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or the development of drug dependency or drug abuse.

7.1.3 Unexpected Adverse Reaction

An unexpected adverse reaction is an adverse reaction, the nature and severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product).

7.1.4 Laboratory Test Abnormalities

The investigator must assess the clinical significance of all abnormal laboratory values as defined by the compendium of normal values for the reference laboratory.

Any investigational product emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the AEs form in the study record:

- Accompanied by clinical symptoms

- Leading to interruption or discontinuation of the investigational product
- Requiring a change in concomitant therapy

This applies to any protocol and non-protocol specified laboratory result from tests performed after the first dose of the investigational product, which falls outside the laboratory reference range and meets the clinical significance criteria for liver and kidney tests as well as for hematology and clinical chemistry, etc.This does not apply to any abnormal laboratory result which falls outside the laboratory reference range but which does not meet the clinical significance criteria or those which are a result of an AE which has already been reported.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event (SAE) should be reported as such, in addition to being reported as an AE in the study record.

7.2 Treatment and Follow-up of AEs and Laboratory Abnormalities

7.2.1 Treatment and Follow-up of AEs

AEs, especially those for which the relationship to the investigational product is suspected, should be followed up until they have returned to baseline status or stabilized.
If after follow-up, return to baseline status or stabilization cannot be established, an explanation should be recorded in the study record.

7.2.2 Treatment and Follow-up of Laboratory Abnormalities

In the event of participant-initiated withdrawal or clinically significant unexplained abnormal laboratory test values, the participant will be withdrawn from receiving study product. Following this, the participant will be encouraged to have a termination visit where blood will be withdrawn for safety and any other relevant information.

7.3 Reporting of SAEs and Unexpected Adverse Reactions

The Qualified Investigator will be responsible for classification of an AE as an SAE within 24h of notification. Causality should be signed off by the Qualified Investigator prior to reporting to ethics and regulatory bodies. Notification of any serious adverse events must be made in writing to the study sponsor. The IRB will be notified of all SAEs and unexpected adverse reactions. All SAEs will be reported to the Therapeutics Products Directorate (TPD) in an expedited manner.
The sponsor must notify the TPD of all serious adverse reactions as follows:

a) If it is neither fatal or life threatening, within 15 calendar days after the day on which the sponsor becomes aware of the information; and

b) If it is fatal or life threatening, must be reported as soon as possible, but not later than seven (7) days after the day on which the sponsor becomes aware of the information.

8 STATISTICAL EVALUATION

8.1 Determination of sample size

This is a pilot test and no calculation was conducted for sample size.

8.2 Study Population

- The **Safety Population** will consist of all participants who received any amount of either product, and on whom any post-randomization safety information is available.
- The **Per Protocol (PP) Population** consists of all participants who do not have any major protocol violations and complete all study visits and procedures connected with measurement of the primary variable.

8.3 Analysis Plan

The primary outcome variable will be the peak plasma ascorbic acid level (C_{MAX}). The responses from all time points will be compared to determine the time at which the plasma ascorbic acid level reaches its peak (T_{MAX}). For each product, a one sample t-test will be utilized to test whether the mean peak plasma ascorbic acid level is greater than 37 µM.

H_0: $C_{MAX} \leq 37$ µM

H_A: $C_{MAX} > 37$ µM

Because the plasma ascorbic acid level reaches a saturation point at approximately 80 µM (9;10), some of the participants may reach saturation at the peak time. Depending on the proportion of participants who peak at saturation level, a one sample t-test may be inappropriate and nonparametric techniques may be required to judge the typical peak plasma ascorbic acid level for each product.

The action standard for each product will be that the typical peak plasma ascorbic acid level is statistically significantly greater than 37 µM and each product which meets the action standard will be determined to have provided an effective dose.

Additional outcome variables which will be measured include the ascorbic acid level in white blood cells and the ascorbic acid level in urine. These data will be collected for informational purposes only.

Descriptive statistics will be provided for participant demographic and baseline characteristics. Line graphs showing the individual and mean concentrations in µM of plasma, WBC and urine ascorbic acid over the 24 h will be provided for the investigational products.

Pharmacokinetic parameters, including the incremental area under the curve ($iAUC_{0-24h}$ and $iAUC_{0-\infty}$), the maximum observed concentration (C_{max}) time, and maximum concentration (T_{max}) will be calculated for each participant. The area under the curve parameter will be calculated using the trapezoid approximation from 0h to 24h and by using the exponential decay approximation for 24h to infinity.

8.3.1 Premature Discontinuation Description

For each premature discontinuation, the following parameters will be listed: participant number, dates of start and end of supplementation, and the reason of premature discontinuation. If a participant is withdrawn or drops out of the pharmacokinetic study, KGK will replace the participant to ensure a total number of 30 participants complete the study.

8.3.2 Safety

For adverse events, a descriptive analysis will be given. Adverse events will be presented in a frequency table by category and investigational product. Furthermore, description, frequency, severity and causality will be reported for each adverse event.

Continuous safety parameters (e.g. hematology, clinical chemistry, heart rate and blood pressure) will be summarized using a table including mean, standard deviation, median, minimum value, and maximum value for each measurement point. The changes from baseline will also be summarized similarly.

8.4 Protocol Deviation Description

Protocol deviations will be listed in the final study report.

8.5 Protocol Amendments

Once the protocol has been approved by the IRB and Health Canada, any changes to the protocol will be documented in the form of an amendment. All amendments will be included in the final study report.

9 DATA COLLECTION AND STORAGE

All data collection and record storage will be done in compliance with ICH GCP Guidelines and applicable local regulatory guidelines and with KGK standard operating procedures (SOPs).

10 ETHICAL ASPECTS OF THE STUDY

This study will be conducted with the highest respect for the individual participants according to the protocol, the ethical principles that have their origin in the Declaration of Helsinki, and the ICH Harmonised Tripartite Guideline for GCP.

10.1 IRB Approval

KGK Synergize Inc. will supply relevant documents for submission to an IRB for the protocol's review and approval. The following must be submitted to the IRB: This protocol, a copy of the informed consent form, and, if applicable, volunteer recruitment materials and/or advertisements and other documents required by all applicable laws and regulations. The IRB's written approval of the protocol and volunteer informed consent must be obtained before commencement of the study. The IRB approval must refer to the study by exact protocol title, number, and version date; identify versions of other documents (e.g., informed consent form) reviewed; and state the approval date.

KGK must adhere to all requirements stipulated by the IRB. This may include notification to the IRB regarding protocol amendments, updates to the informed consent form, recruitment materials intended for viewing by volunteers, local safety reporting requirements and submission of the investigator's annual/final status report to the IRB.

10.2 Volunteer Information and Informed Consent

Written consent documents will embody the elements of informed consent as described in the declaration of Helsinki and the ICH Guidelines for GCP and will be in accordance with all applicable laws and regulations. The informed consent form describes the planned and permitted uses, transfers, and disclosures of the volunteer's personal and personal health information for purposes of conducting the study. The informed consent form further explains the nature of the study, its objectives, and potential risks and benefits, as well as the date informed consent is obtained. The informed consent form will detail the requirements of the volunteer and the fact that he or she is free to withdraw at any time without giving a reason and without prejudice to his or her further medical care. A properly executed informed consent document will be obtained from each subject prior to entering the study. The signed informed consent document will be maintained in the study file and a copy given to the subject.

10.3 Potential Risks and Procedures to Minimize Risk

All potential risks are disclosed to study participants prior to their participation. The potential risks associated with this study include venipuncture and the associated risks. Risks associated with venipuncture include pain, bruising, and infection at the site. Alcohol swabs and proper venipuncture procedure will be followed to minimize the risk of infection.

11 QUALITY ASSURANCE AND QUALITY CONTROL

11.1 Auditing

All material used in clinical studies are subjected to quality control. Quality assurance audits may be performed by the sponsor or any health authority during the course of the study or after its completion.

The Investigator agrees to comply with the sponsor and regulatory requirements in terms of auditing of the study. This includes access to the source documents for source data verification.

11.2 Monitoring

An initiation meeting in person or via conference call will be conducted by the sponsor. At this meeting, the protocol and logistical aspects of the study will be reviewed with the Investigator and all study staff.

The sponsor monitor may make site visits during the study and may inspect all case report forms, the clinical staff training and competency record and other documentation directly associated with the study. Source documents will be reviewed to ensure that all items have been completed and that the data provided are accurate and obtained in the manner specified in the protocol. The participant files will be reviewed to confirm that:

- Informed consent was obtained and documented;
- Enrolled participants fulfilled all inclusion criteria and did not meet any exclusion criteria;
- AE/SAE reporting has been performed as applicable;
- Study visits have been conducted as per protocol and information has been recorded in the appropriate place in the source document;
- The study product is being stored correctly and an accurate record of its dispensation to the study participants is being maintained (accountability).

Incorrect, inappropriate, or illegible entries in the participant files will be returned to the Investigator or designee for correction. No data disclosing the identity of participants will leave the study center. The Investigator and any designees will maintain confidentiality of all participant records.

The Investigator will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspections and will allow direct access to source data and documents for these purposes.

11.3 Data Management

Data required for the analysis will be acquired from source documentation (including laboratory reports) and entered into a Microsoft Office Access database designed specifically for this study. All data points entered into the study database are source data verified.

High safety standards for the transfer and storage of study data are guaranteed by the use of technologies such as password protection, firewalls and periodic backup to protect stored data. Writing access to the system will be limited to authorized personnel.

All data including originals or copies of all case report forms, correspondence, study reports, and all source data is archived for a period not less than 25 years from the date of completion of the study in accordance with Health Canada regulatory requirements.

Study Report

A draft study report is prepared by the test facility and written as per ICH E3 guidelines. The following information will be included, as appropriate, in the draft study report:

1. Introduction/objective
2. Investigational site location
3. Study reference number(s)
4. Test products
5. Study dates
6. Subject selection
7. Methods including any statistical analysis
8. Results
9. Discussion/conclusion
10. Authorized signatures

The test facility will finalize the study report upon receiving comments from Sponsor

12 Reference List

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13 APPENDICES

13.1 Appendix 1 Schedule of Assessments

Procedures/assessments	Visit 1 Screening		Visit 2 Baseline 0-12 hour 1st study period	Visit 3 24 hour 1st study period		Visit 4 0-12 hour 2nd study period	Visit 5 24 hour End of study 2nd study period
Informed Consent	X						
Review of Inclusion/Exclusion criteria	X		X				
Review of medical history/current health status	X		X			X	
Review of concomitant therapies	X		X	X		X	X
Review adverse events				X		X	X
Height*, weight, blood pressure and heart rate	X		X	X		X	X
Randomization			X				
Physical Exam			X				
Nutrition counselling	X						
Laboratory Tests Blood sample collection for CBC, electrolytes, HbA1c*, creatinine, eGFR, AST, ALT, and bilirubin analysis	X			X		X	X
Urine pregnancy test	X	Washout 7 days			Washout 7 days		
Blood samples: Pre-dose (t=0) and Post-dose (30 min, 1h, 2h, 3h, 4h, 5h, 6h, 8h, 10 h, 12 h) for L-Ascorbic Acid analysis			X			X	
Blood samples: 24 hours for L-Ascorbic Acid analysis				X			X
Pre-dose Urine sample for L-Ascorbic Acid analysis			X			X	
Post-dose urine samples: 0-2h, 2-4h, 4-8h, 8-12h post-dose L-Ascorbic Acid analysis			X			X	
Dispense urine collection kits for 8-24h urine collection: L-ascorbic acid			X			X	
Return post-dose urine sample collection: 12-24h post-dose L-Ascorbic Acid analysis				X			X
IP or Comparator Dispensed			X			X	
Breakfast, lunch, and dinner provided			X			X	
Study diary dispensed			X	X		X	
Study diary returned				X		X	X
24h-food record dispensed			X			X	
24h-food record returned				X			X
7-day food record dispensed	X			X			
7-day food record returned			X			X	

* Will only be measured at screening visit (Visit 1)

13.2 Appendix 1a Time Points for Sampling

Blood Draw for Ascorbic Acid Measurement (Table 1A), Safety Parameters (Table 1B) and Urine Collection (Table 1C). One of two vials will be retained.

Table 1A. Time Points for Blood Draw – Ascorbic Acid Measurement
Blue cells are vials that will be aliquoted from tubes in green cells

	Visits 2 & 4											**Visits 3 & 5**
Timing (Hour)	**0 h**	30 min	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**	6 h	**8 h**	**10 h**	**12 h**	**24 h**
Number of Vacutainer tubes (8 mL/tube)	2	2	2	2	2	2	2	2	2	2	2	2
Total blood volume (mL)	16	16	16	16	16	16	16	16	16	16	16	16
Number of vials for Plasma	2	2	2	2	2	2	2	2	2	2	2	2
Number of cryovials for WBC	2	2	2	2	2	2	2	2	2	2	2	2

Table 1B. Time Points for Blood Draw – Safety Parameters

Timing	**Screening Visit 1**	**Visit 4 0 h**	**Visits 3 & 5 24 h**
Number of vacutainer tubes	2 tubes (4mL/tube) 1 tube (5mL/tube)	1 tube (4mL/tube) 1 tube (5mL/tube)	1 tube (4mL/tube) 1 tube (5mL/tube)
Total volume (mL)	13 mL	9 mL	9 mL

Table 1C. Time Points for Urine Collection – Ascorbic Acid Measurement

	Visits 2 & 4													**Visits 3 & 5**
Hour	**0 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**	**6 h**	**7 h**	**8 h**	**9 h**	**10 h**	**11 h**	**12 h**	**12 h → 24 h**
Timing	Prior to Dosing	0-2 h Post-dose		2-4 h Post-dose		4-8 h Post-dose				8-12 h Post-dose				13-24 h post-dose
Number of vials	2 Vials	2 Vials		2 Vials		2 Vials				2 Vials				2 Vials

13.3 Appendix 2 Vitamin C Foods

For this study, we ask that you lower your consumption of foods containing high amounts of vitamin C during the study period.
Foods containing high amounts of vitamin C:

- Fruit Juices
- Veggie juices
- Fruits
- Fruit Based Snacks (Welch's Fruit Snacks, Fruit Roll-Ups, etc.)
- Kale
- Cauliflower
- Broccoli
- Cabbage
- Bell Peppers

13.4 Appendix 3 Meal Items low in Vitamin C for Meal Planning During the Study

Breakfast	Lunch	Dinner
Cereals ○ Raisin Bran ○ Shredded Wheat ○ Unprocessed bran ○ Hominy grits ○ Cream of Wheat **Entrees** ○ Scrambled eggs ○ Hard poached eggs ○ Cheddar cheese ○ French toast ○ Syrup ○ Plain yogurt ○ Strawberry yogurt **Breads and spreads** ○ Bran muffin ○ Glazed doughnut ○ Mini bagel ○ Cream cheese ○ White toast ○ Wheat toast ○ Margarine ○ Butter ○ Honey ○ Peanut butter **Other** ○ Sugar substitutes ○ Low-fat cream cheese **Beverages** ○ Coffee ○ Decaffeinated coffee ○ Tea ○ Decaffeinated tea ○ Cream	**Appetizers, salads, and dressings** ○ Chicken noodle soup ○ Cream of chicken soup ○ Croutons **Entrees** ○ Escalloped chicken ○ Pork chops ○ Brown gravy ○ Garlic herb pizza ○ Grilled cheese sandwich ○ Vanilla yogurt ○ Tuna chunks ○ Plain yogurt ○ Blueberry yogurt **Vegetables and starches** ○ Rice royale ○ Black beans ○ Rice ○ Pretzels **Desserts** ○ Diet Jello ○ Chocolate chip cookie ○ Vanilla ice cream ○ Chocolate ice cream ○ Sugar cookie **Breads and spreads** ○ White bread ○ Margarine, butter ○ Wheat bread ○ Saltines ○ Sour cream ○ Peanut butter	**Appetizers, salads, and dressings** ○ Chicken and rice soup ○ Cottage cheese ○ Croutons **Entrees** ○ Fried shrimp ○ Roast beef ○ Brown gravy ○ Macaroni and cheese ○ Cheeseburger ○ Chicken salad ○ Plain yogurt ○ Peach yogurt **Vegetables and starches** ○ Pinto beans ○ Rice **Desserts** ○ Diet Jello ○ Vanilla ice cream ○ Angelfood cake **Breads and spreads** ○ Margarine ○ Butter ○ Peanut butter ○ White bread ○ Wheat bread

MILK ○ WHOLE ○ 2% FAT ○ SKIM ○ CHOCOLATE ○ BUTTERMILK	**OTHER** ○ MUSTARD ○ MAYONNAISE ○ SUGAR SUBSTITUTE ○ RELISH **BEVERAGES** ○ COFFEE ○ DECAFFEINATED COFFEE ○ TEA ○ DECAFFEINATED ○ TEA ○ ICED TEA ○ CREAM	**BEVERAGES** ○ COFFEE ○ DECAFFEINATED COFFEE ○ CREAM ○ TEA ○ DECAFFEINATED TEA ○ ICED TEA **EVENING SNACKS** ○ PEANUT BUTTER CRACKERS ○ GRAHAM CRACKERS ○ CHOCOLATE CHIP COOKIE ○ POPCORN ○ GINGER ALE ○ DIET COLA

13.5 Appendix 4 Clinic Meal Choices

Please choose from the following items for each meal (√ or X). You may drink as much water as you like.

Breakfast:

White Toast (1 slice)	
Wheat Toast (1 slice)	
Peanut Butter (1 tbsp.)	
Honey Nut Cheerios (1 cup)	
Shredded Wheat (1 cup)	
Scrambled Egg (1 cup)	
Hash Browns (1/2 cup)	
Coffee	
Tea	
Cream (1 tbsp.)	
Sugar (1 tsp)	

Lunch:

Minestrone Soup (1 regular)	
Chicken Noodle Soup (1 regular)	
Chili (1 regular)	
Crackers	
Dinner Roll (1 small roll)	
BLT Sandwich	
Ham & Swiss Sandwich	
Granola Bar (1 bar)	
Chocolate Chip Cookie (1 cookie)	
Jell-O Cup (1 pack)	
Coffee	
Tea	
Cream (1 tbsp.)	
Sugar (1 tsp)	
Ginger Ale (1 can)	
Coca Cola (1 can)	

Dinner:

Minestrone Soup (1 regular)	
Chicken Noodle Soup (1 regular)	
Chili (1 regular)	
Crackers	
Dinner Roll (1 small roll)	
Beef Lasagna	
Vegetarian Lasagna	
Granola Bar (1 bar)	
Chocolate Chip Cookie (1 cookie)	
Jell-O Cup (1 pack)	
Coffee	
Tea	
Cream (1 tbsp.)	
Sugar (1 tsp)	
Ginger Ale (1 can)	
Coca Cola (1 can)	

13.6 Appendix 5 Randomization Schedule and Dispensing Log – 18 Participants Per Sex

17MBHC Randomization Schedule and Dispensing Log

Ensure you select the appropriate Male of Female Randomization Log

MALES							
Randomization Number	Participant ID (ex. ABC 123)	Week 1	Date Dispensed	Initial	Week 2	Date Dispensed	Initial
7508		B			A		
7511		A			B		
7517		A			B		
7520		B			A		
7518		A			B		
7505		B			A		
7514		B			A		
7501		A			B		
7510		B			A		
7502		A			B		
7513		B			A		
7506		A			B		
7516		A			B		
7504		A			B		
7503		B			A		
7509		B			A		
7519		A			B		
7512		B			A		
7515		B			A		
7507		A			B		

Note: The last two randomization numbers from this list (randomization number 7515 and 7507) will not be used.

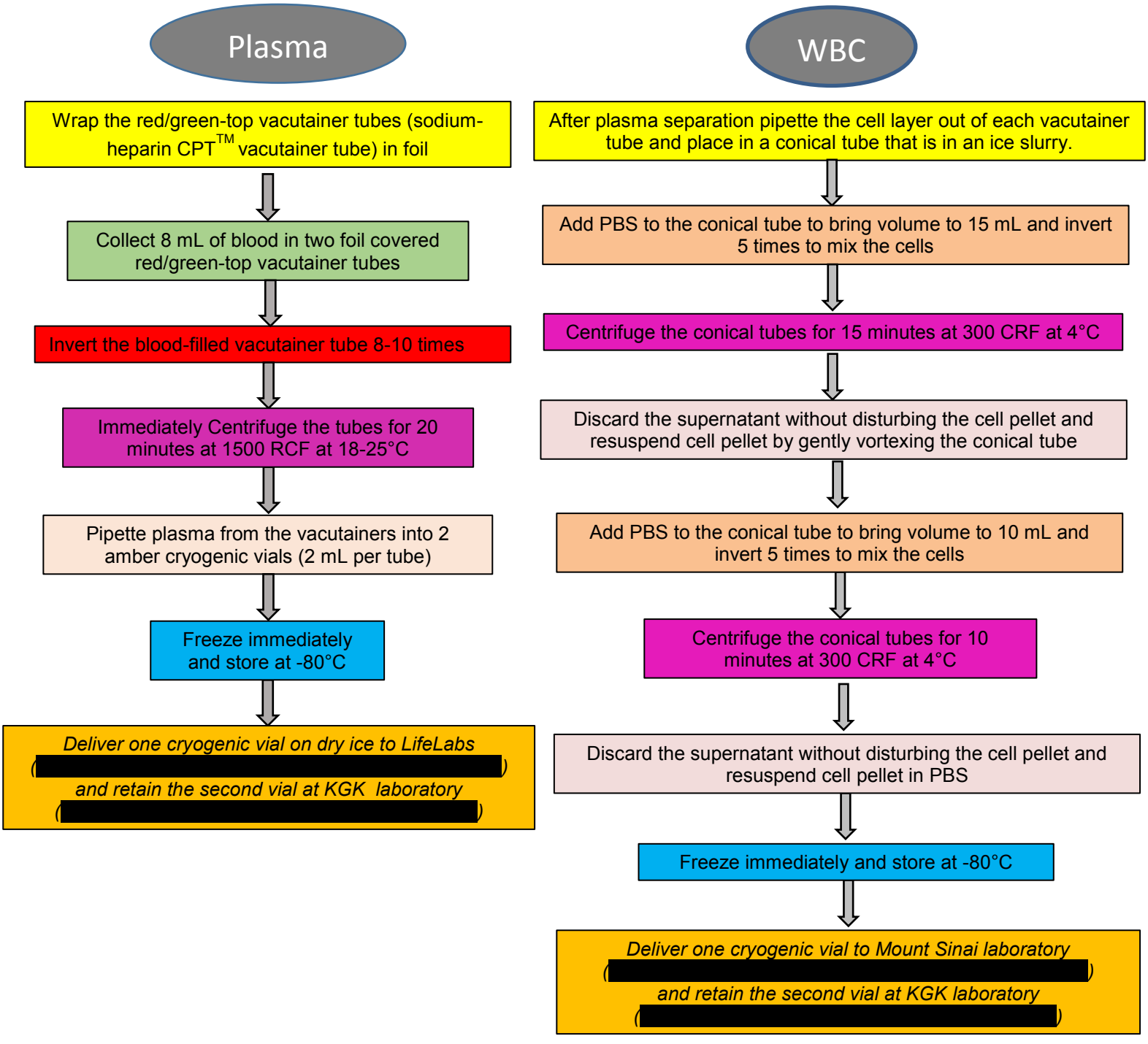
17MBHC Randomization Schedule and Dispensing Log
Ensure you select the appropriate Male of Female Randomization Log

FEMALES							
Randomization Number	Participant ID (ex. ABC 123)	Week 1	Date Dispensed	Initial	Week 2	Date Dispensed	Initial
7529		B			A		
7526		A			B		
7521		B			A		
7533		A			B		
7534		B			A		
7535		A			B		
7539		B			A		
7540		A			B		
7528		A			B		
7530		B			A		
7524		A			B		
7527		B			A		
7532		A			B		
7537		B			A		
7523		A			B		
7538		B			A		
7525		A			B		
7531		B			A		
7522		B			A		
7536		A			B		

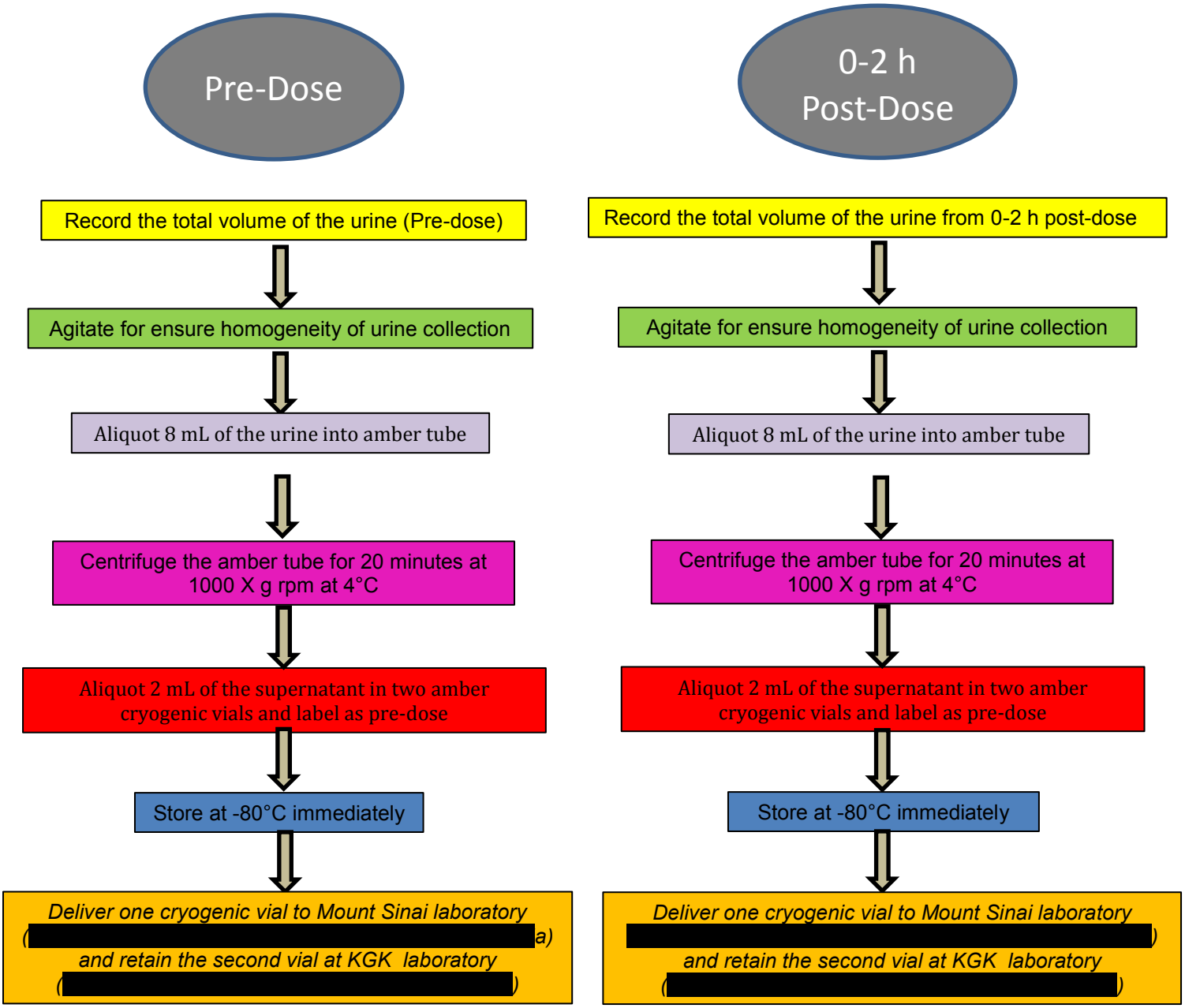
Note: The last two randomization numbers from this list (randomization number 7522 and 7536) will not be used.

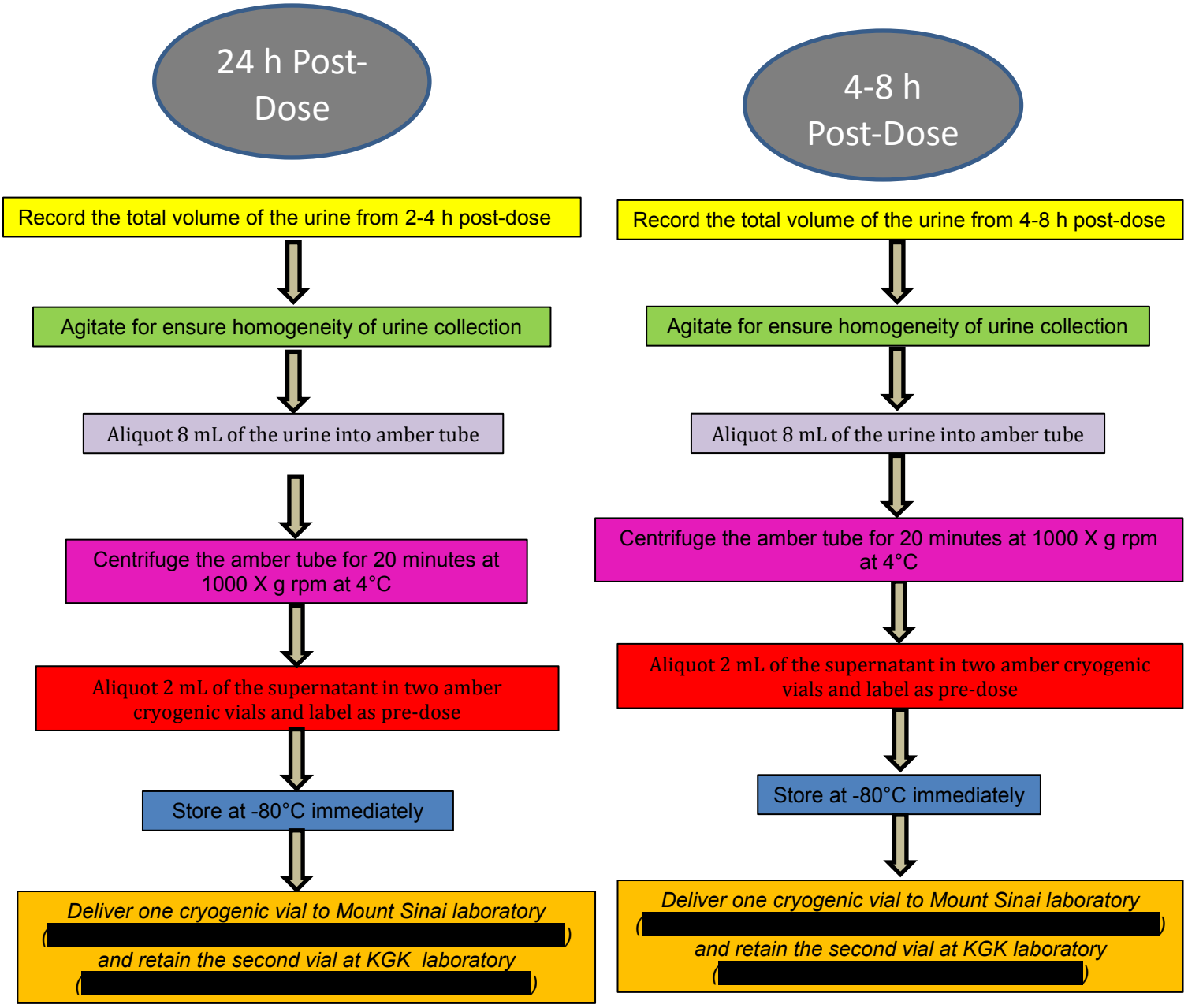
13.7 Appendix 6 Laboratory Processing Flow Chart

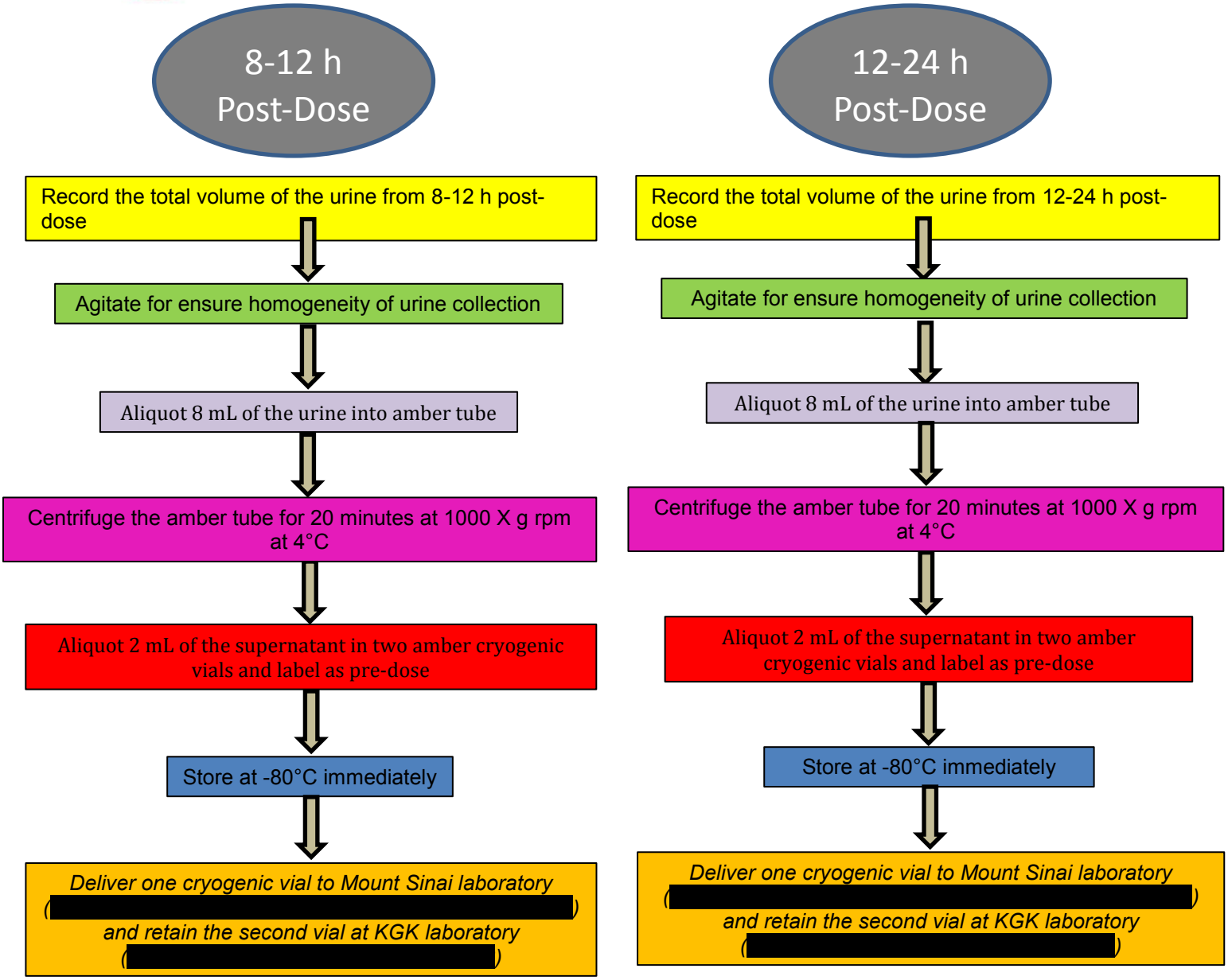
Blood Samples for Ascorbic Acid Analysis



13.8 Urine Samples for Ascorbic Acid Analysis




24 h Post-Dose
Record the total volume of the urine from 2-4 h post-dose
Agitate for ensure homogeneity of urine collection
Aliquot 8 mL of the urine into amber tube
Centrifuge the amber tube for 20 minutes at 1000 X g rpm at 4°C
Aliquot 2 mL of the supernatant in two amber cryogenic vials and label as pre-dose
Store at -80°C immediately
Deliver one cryogenic vial to Mount Sinai laboratory
[redacted]
and retain the second vial at KGK laboratory
[redacted]
4-8 h Post-Dose
Record the total volume of the urine from 4-8 h post-dose
Agitate for ensure homogeneity of urine collection
Aliquot 8 mL of the urine into amber tube
Centrifuge the amber tube for 20 minutes at 1000 X g rpm at 4°C
Aliquot 2 mL of the supernatant in two amber cryogenic vials and label as pre-dose
Store at -80°C immediately
Deliver one cryogenic vial to Mount Sinai laboratory
[redacted]
and retain the second vial at KGK laboratory
[redacted]


8-12 h Post-Dose
Record the total volume of the urine from 8-12 h post-dose
Agitate for ensure homogeneity of urine collection
Aliquot 8 mL of the urine into amber tube
Centrifuge the amber tube for 20 minutes at 1000 X g rpm at 4°C
Aliquot 2 mL of the supernatant in two amber cryogenic vials and label as pre-dose
Store at -80°C immediately
Deliver one cryogenic vial to Mount Sinai laboratory
([redacted])
and retain the second vial at KGK laboratory
([redacted])
12-24 h Post-Dose
Record the total volume of the urine from 12-24 h post-dose
Agitate for ensure homogeneity of urine collection
Aliquot 8 mL of the urine into amber tube
Centrifuge the amber tube for 20 minutes at 1000 X g rpm at 4°C
Aliquot 2 mL of the supernatant in two amber cryogenic vials and label as pre-dose
Store at -80°C immediately
Deliver one cryogenic vial to Mount Sinai laboratory
([redacted])
and retain the second vial at KGK laboratory
([redacted])

13.9 Safety Blood