Clinical evaluation of the accuracy of Vitastiq device for tracking vitamin and mineral trend in human body

(VITASTIQ)

Protocol no.: VITA-01, Ver. 1.0

Sponsor: Vitastiq d.o.o.

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10000 Zagreb, Croatia

May 3, 2017

TABLE OF CONTENTS

LIST OF ABBREVATIONS

AE Adverse Event

AEV Electro-Acupuncture according to Voll

BMI Body Mass Index

CRF Case Report Form

CRO Clinical Research Organisation

EC Ethical Committee

ICF Informed Consent Form

GCP Good Clinical Practice

SAE Serious Adverse Event

TMF Trial Master File

STATEMENT OF COMPLIANCE

Clinical evaluation of the accuracy of Vitastiq device for tracking vitamin and mineral trend in human body

I have read this protocol and agree to conduct the study as outlined. I also agree that prior to seeking approval from the Ethics Committee (EC) any changes to the protocol must be approved by Vitastiq.

This study will be conducted in accordance with Good Clinical Practice (GCP) guidelines, the Declaration of Helsinki, and Slovenian ethical and legal requirements.

I will supervise all testing of the device involving human subjects and ensure that the requirements for obtaining informed consent are met.

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PROTOCOL SUMMARY

Title:	Clinical evaluation of the accuracy of Vitastiq device for tracking vitamin and mineral trend in human body
Study acronym:	VITASTIQ
Protocol No.:	VITA-01
Protocol version:	1.0
Study design:	The single site evaluation study will include 45 subjects who will test Vitastiq device for 57 days. On day 1, 29 and 57, participants' blood sampling will be collected and analysed, and measurements using Vitastiq device will be performed. Data will be analysed retrospectively to evaluate Vitastiq performance compared to blood test results.
Study population:	Healthy women and men in ratio 1:1 (approximately), aged between 18 and 64 years will be included in the study.
Number of participants:	45
Study coordinator:	Vizera d.o.o., Slovenia
Study Sponsor:	Vitastiq d.o.o., Croatia
Study center:	Adria lab d.o.o., Ljubljana
Study period:	First patient in to last patient out (months): 6 Duration of the study (months): 2 Recruitment period (months): 4 FPFV: May 2017 LPLV: November 2017
Inclusion criteria:	• Informed consent form (ICF) is signed by a volunteer • Age between 18 and 64 years at the time of the signature of ICF • A body mass index (BMI) between 18 and 28 kg/m^2 • Healthy, meaning absence of any chronic or acute medical therapy for a month prior to the inclusion to the study • Willing to follow all study procedures, including attending all site visits, and keeping a food diary and diary of activities on a weekly basis
Exclusion criteria:	• Intake of any drugs to treat chronic disease within 1 month before the beginning and during the study • Intake of any drugs to treat serious acute disease within 1 month before the beginning of the study and within 5 days of each visit

- Any clinically significant medical history of serious digestive tract, liver, kidney, skin or haematological disease or hormone imbalance
- Pregnant and breastfeeding women
- Women who planning pregnancy during the study
- Inadequate veins (in the opinion of the investigator)
- Known drug or alcohol abuse
- Using any form of nicotine or tobacco
- Mental incapacity that precludes adequate understanding or cooperation
- Participation in another investigational study or blood donation within 3 months prior to or during this study

Study objectives: The main objective is to define the accuracy of Vitastiq device readings.

Study endpoints The **primary endpoint** of the study is to determine the Vitastiq device accuracy and performance. The goal is to achieve at least 70% Vitastiq accuracy performance meaning that at least 70% of Vitastiq readings for each mineral and vitamin fall within the range of matching blood test results.

The **secondary endpoint** of the study is to define repeatability of Vitastiq readings method. The goal is to achieve at least 80% Vitastiq readings method repeatability meaning that at least 80% of Vitastiq readings for each mineral and vitamin performed by less experienced investigator and participant fall within the range of matching Vitastiq readings performed by the most experienced investigator.

Statistical analysis Statistical analysis of primary study outcome will be based on calculation of 95% confidence interval (CI) of the estimated accuracy of Vitastiq readings for each mineral or vitamin. The expected accuracy level is 70%, therefore by using 135 readings the radius of 95% confidence interval would be estimated with 10% relative margin of error. The point estimate of the accuracy value together with 95% CI will be presented for each mineral or vitamin

Statistical analysis of secondary outcomes will include Kappa statistic for interrater reliability on Vitastiq readings or Friedman ANOVA for testing the possible change of each mineral/vitamin blood level during the study period. Kappa statistic for interrater reliability will be calculated separately for each analyte and for all 135 readings. Additionally, the results of the second and the third rater (second investigator and participant) will be separately compared to the results of the first rater (first investigator). In Friedman ANOVA test 3 consecutive blood test results, that is on day 1, 29, and 57, will be compared.

1. INTRODUCTION

Poor dietary choices not only manifest in obesity, which is currently the main public health focus in the world, but can also lead to inadequate micronutrient intakes, with implications for health. Recent dietary survey data and measurements of status biomarkers have highlighted folate, vitamin D, calcium, iron and iodine to be amongst the micronutrients of most concern [1]. in order to anticipate and prevent the health consequences of micronutrient deficiencies, an easy and cost effective way of determining and tracking vitamin and mineral status is of particular importance.

Vitamin and mineral status in our body can be obtained in several ways. The most common and well acceptable way is to assess vitamin and mineral concentration from blood serum or plasma. Results obtained by these well-established analytical methods require professional explanation. An alternative to blood testing is Electro-Acupuncture method according to Voll (EAV). EAV has been used for decades in pharmacies and specialist offices. It is based on the acupuncture energy meridians theory and Reinhold Voll's electro-acupuncture research.

1.1 BACKGROUND AND SCIENTIFIC BASIS FOR THE STUDY

In 1950's, a German medical doctor, Internal Medicine specialist, and engineer, Dr. Reinhold Voll, began researching and proving the diagnostic and therapeutic benefits of an innovative testing method now known as EAV (Electro-Acupuncture according to Voll). The EAV method [2] is used to assess the individual's energy with a painless and non-invasive procedure. These are acupoints and a system of meridians, a network of energy channels connected among each other and to parts of the body or organs. Electro-Acupuncture according to Voll is a big part of the Meridian Stress Assessment (MSA) which focuses on measuring organs in the body. They are either balanced, weakened or stressed.

EAV measurement device consists of the patient holding a negative electrode on his or her hand and the practitioner touching the body with a stylus. The EAV device generates a safe electric current. Dr. Voll saw that testing electrical conductance at specific acupoints had a higher electrical flow than other locations. Dr. Voll soon discovered that when an internal organ's function or structure changes, the performance of the related meridian and acupuncture points also changed, and that this change could be measured using a device.

In modern devices used in this type of assessment the voll machine comes with specialized software. This can lead to a support protocol for stressed or weakened organs along with reports about the final results.

This method was documented and tested in over a decade of hospital studies in Germany [3-5]. EAV is part of alternative and holistic medicine techniques to help determine the body's health imbalances and it is widely used throughout Europe and the world [6].

1.2 INVESTIGATIONAL DEVICE (ID)

An EAV device is a type of Electrical Conductivity Meter and is considered to be safe [7]. The newer generation devices have specialized software but, essentially, any EAV device is a Conductivity Meter.

In using an EAV device, each Meridian Point is tested with a Point Probe (a positive electrode [+]). Regardless of age, weight, sex or race of testing individuals, a reading of 50 with no change over time (no indicator drop) is an indication of an Energetically healthy or »Balanced« meridian. Readings at points that are significantly above 50 (65+) indicate »Irritation« of the Meridian. Readings above 75 exhibit »Inflammation« of the Meridian. When a reading is significantly lower than 50 (below40) then is believed that this meridian is displaying »low energy« properties.

One of EAV devices is Vitastiq device for personal use. It helps indicate the user's vitamin and mineral trend. The accompanying user-friendly Vitastiq app saves the history of previous readings to allow Vitastiq users to keep track of their progress.

Vitastiq is a single innovative hardware and software concept that provides a personalised way of measuring vitamins and minerals through a smartphone.

It measures the vitamin or mineral content in the skin at various acupuncture points that are outlined on the body and then displays the information on the smartphone screen as LOW, NORMAL or HIGH. Results are achieved by measuring the electrical status of the skin. The Vitastiq mobile app saves and evaluates the history of readings, which enables the user can keep track of the progress.

Today EAV is widely used throughout Europe by over 25,000 medical practitioners [8]. However, there is still a lack of reliable data obtained by clinical trials that would show the reliability and/or accuracy preferences of individual EAV device. It thus causes doubts and resistance to use EAV for personal or professional use. Therefore, we would like to define accuracy of Vitastiq device, a vitamin and mineral trend tracker.

1.3 STUDY PURPOSE

The purpose of the study is to test the accuracy of Vitastiq device as a personal tracker of vitamins and minerals in human body and to obtain and analyse data to enhance the accuracy of new version of Vitastiq device.

The main objective is to define the accuracy of Vitastiq device. Data (readings) obtained by Vitastiq will be compared to blood test results obtained by commonly used analytical methods. Both, Vitastiq readings and blood sampling will be taken at the same time. Vitastiq accuracy performance for each mineral and vitamin reading is considered acceptable if at least 70% of Vitastiq readings fall within the range of matching blood test results.

The study should provide answers to two main questions:

a) Do 70% of Vitastiq readings for each mineral and vitamin fall within the range of matching blood test results?
b) Is Vitastiq reading method at least 80% repeatable where the readings performed by the most experienced investigator are compared to the readings performed by less experienced investigator and participant.

2. STUDY DESIGN

2.1 DESCRIPTION OF THE STUDY DESIGN

This is a single site evaluation study of Vitastiq device accuracy in healthy men and women over the age 18. A total of 45 Vitastiq personal devices will be used by volunteers for two months. The Vitastiq device will be evaluated during three site visits: on day 1, 29 ± 4 days and 57 ± 4 days and 54 ± 4 home use days (every day between first and last day of the study except on visit days). During site visit days, blood sampling will be collected and analysed and readings using Vitastiq device will be performed. Data will be analysed retrospectively to evaluate Vitastiq performance compared to blood tests results.

Participant will complete a home diary which will include all readings for selected minerals and vitamins once per day – in the mornings, after waking up. Subject's will report about physical activities, and food intake during every week.

Some participants may not complete a full 57-day (± 4 days) study period for various reasons, such as the device failure. All device failures or problems will be documented.

2.2 STUDY ENDPOINTS

2.2.1 Primary endpoint

The primary endpoint of the study is to determine the Vitastiq device accuracy and performance. The goal is to achieve at least 70% Vitastiq accuracy performance meaning that at least 70% of Vitastiq readings for each mineral and vitamin fall within the range of matching blood test results. Vitastiq readings will be presented as low, normal and high, where:

- low means below the agreed normal level for selected mineral or vitamin determined by biochemical blood analyses tests (see Table 1)
- normal means within the range of agreed normal level and
- high means above the agreed normal level.

Data will be collected from Vitastiq device utilizing 45 volunteers. Volunteers will visit the site three times (day 1, 29 ± 4 days and 57 ± 4 days). Three complete Vitastiq readings by two investigators (who differs in years of experiences in performing EAV by Vitastiq device) and by the participant in the study will be performed at each visit (altogether, 405 Vitastiq readings will be collected on visit days (day 1, 29 ± 4 days and 57 ± 4 days) for 45 volunteers). To confirm the primary endpoint only Vitastiq readings performed by the most experienced investigator in acupuncture will be taken to evaluate the accuracy. Therefore, 135 Vitastiq readings taken on visit days (day 1, 29 ± 4 days and 57 ± 4 days) will be compared to blood test results. Analyses of Vitastiq data generated by matching minerals and vitamins levels to venous blood minerals and vitamins concentration by well-established analytical methods on day 1, 29 ± 4 days and 57 ± 4 days will determine Vitastiq device accuracy and performance.

Table 1: Agreed normal levels of vitamins and minerals in blood serum (fasting).

Mineral/Vitamin	Low	Normal	High
Sodium	< 135 mmol/L	135 – 145 mmol/L	> 147 mmol/L
Potassium	< 3.8 mmol/L	3.8 – 5.5 mmol/L	> 5.5 mmol/L
Calcium	< 2.15 mmol/L	2.15 – 2.55 mmol/L	> 2,55 mmol/L
Magnesium	0,66 mmol/L	0,66 – 1,07 mmol/L	> 1,07 mmol/L
Iron	Men: < 10,6 µmol/L Women: < 6,6 µmol/L	Men: 10,6 – 28,3 µmol/L Women: 6,6 – 26,0 µmol/L	Men: > 28,3 µmol/L Women: > 26,0 µmol/L
Zink	< 70 µg/dL	70 – 150 µg/dL	> 150 µg/dL
Selenium	< 50 µg/L	50 – 120 µg/L	> 120 µg/L
Folic acid	< 7,0 nmol/L	7,0 – 46,4 nmol/L	> 46,4 nmol/L
Vitamin A	< 250 µg/L	250 – 1100 µg/L	> 1100 µg/L
Vitamin B12	< 138 pmol/L	138 – 652 pmol/L	> 652 pmol/L
Vitamin D	< 30 µg/L	>30 µg/L	/
Vitamin E	Men: < 8,9 mg/L Women: < 9,4 mg/L	Men: 8,9 – 18,3 mg/L Women: 9,4 – 15,0 mg/L	Men: > 18,3 mg/L Women: > 15,0 mg/L

2.2.2 Secondary endpoints

Three Vitastiq readings by two investigators (who differs in years of experiences in performing EAV readings by Vitastiq device) and by the participant in the study will be performed and one blood sample will be collected per participant at each visit (on day 1, 29 ± 4 days and 57 ± 4 days). The purpose of the secondary endpoint is to determine repeatability of Vitastiq reading method. Therefore, the results of Vitastiq readings performed by less experienced investigator and by participant will be compared to the results of Vitastiq readings collected by the most experienced investigator. The goal is to achieve at least 80% Vitastiq reading method repeatability meaning that at least 80% of Vitastiq readings for each mineral and vitamin performed by less experienced investigator and participant fall within the range of matching Vitastiq readings performed by the most experienced investigator.

Using Vitastiq device the participants will measure their level of minerals and vitamins on daily basis. The results of each mineral and vitamin will be presented as low, normal or high (see Table 1 above). Participants will receive their numerical blood sample results of previous visit at next visit. The aim of

third study endpoint is to define which results/information (obtained by Vitastiq device or well-established blood test analyses) has bigger impact on people's life style changes (food intake, additional exercises).

2.2.3 Other data that will be collected

a) Gender impact on result matching
b) Age impact on result matching
c) BMI Impact on result matching
d) Impact of vascular body fat rate on results matching

2.3 STUDY POPULATION

45 healthy women and men in ratio 1:1 (approximately) between ages 18 and 64 years will be included in the study. Healthy women and men who don't use any chronic medical therapy will be eligible.

2.3.1 Rationale for the study population

Due to unbalanced eating pattern in modern society women and men are put at risk of nutrition deficiency. The nutrients found to be deficient the most often include iron, iodine and vitamins B2, B6 and D [9]. Most research studies are oriented to define the nutrition deficiency of pregnant women, children and geriatric population. Due to "healthy appearance" generally healthy people do not have any particular need to make blood assessment of their body nutrition status. Because of that, there is a lack of reliable and available evidence, if vitamin and mineral status of the generally healthy population is really balanced and sufficient.

2.4 STUDY DURATION AND TIMELINES

Study duration for individual volunteer will include 57-day (± 4) test period. On day 1, 29 ± 4 days and 57 ± 4 days the blood sampling will be collected and analysed, and at the same time the Vitastiq readings by two investigators and participant will be performed.

The study recruitment period is planned for 4 months, starting in May 2017. If needed, this period of the study can be prolonged to allow the inclusion of the planned number of volunteers.

The completion of the study is planned for November 2017. If the target number of participants will complete the study before the estimated time schedule, the study will be terminated accordingly.

2.5 NUMBER OF CENTRES AND PARTICIPATING COUNTRIES

Study will be conducted in Slovenia at private analytical laboratory where blood sampling will be collected and analysed, and Vitastiq readings will be performed.

3. STUDY ENROLLMENT AND WITHDRAWAL

3.1 SUBJECT SELECTION

The following criteria will determine the eligibility of the subjects. Each subject should meet all of the inclusion criteria. In case any of the exclusion criteria are confirmed for the subject, he is not eligible for the study and should not be included.

3.1.1 Inclusion criteria

- Informed consent form (ICF) is signed by a volunteer
- Age between 18 and 64 years at the time of the signature of ICF
- A body mass index (BMI) between 18 and 28 kg/m^2
- Healthy, meaning absence of any chronic or acute medical therapy for a month prior to the inclusion to the study
- Willing to follow all study procedures, including attending all site visits, and keeping a food diary and diary of activities on a weekly basis

3.1.2 Exclusion criteria

- Intake of any drugs to treat chronic disease within 1 month before the beginning and during the study
- Intake of any drugs to treat serious acute disease within 1 month before the beginning of the study and within 5 days of each visit
- Any clinically significant medical history of serious digestive tract, liver, kidney, skin or haematological disease or hormone imbalance
- Pregnant and breastfeeding women
- Women who planning pregnancy during the study
- Inadequate veins (in the opinion of the investigator)
- Known drug or alcohol abuse
- Using any form of nicotine or tobacco
- Mental incapacity that precludes adequate understanding or cooperation
- Participation in another investigational study or blood donation within 3 months prior to or during this study

3.2 RECRUITMENT AND RETENTION

Volunteers will be screened for the inclusion and exclusion criteria and up to 50 volunteers will be enrolled. At least 45 volunteers should complete the study. Volunteers will be invited to join the study by public announcement on the web page of the Vizera, d.o.o., Ljubljana, Slovenia (www.vizera.eu). The invitation will be also shared on social media (facebook, linkedin, twiter). Viewers of the

advertisement will be invited to inform their colleagues and friends about the possibility to collaborate in the study.

Considering that participants will need to perform EAV using Vitastiq device every day of the study and visit a site for 4 times (screening visit + 3 visits), their participation will be compensated with 40 € (bruto) and a Vitastiq device will be given to them for their permanent use. Additionally, participant will receive the results of screening medical examination and blood tests results of all visits.

3.3 SUBJECT WITHDRAWAL OR TERMINATION

3.3.1 Reason for withdraw or termination

In accordance to the Declaration of Helsinki and other applicable regulations, a subject has the right to withdraw from the study at any time and for any reason without prejudice to their future medical care by the physician or the institution.

Additionally, the subject may be removed from the study if any of the following occurs:

- Decision by the investigator or the sponsor that termination is in the subject's best medical interest

- Subject's noncompliance with study requirements

- Study discontinuation

- Subject's decision

If a subject requests or decides to withdraw from the study, all efforts will be made to complete and report study observations at the time of withdrawal. One last study visit should be performed and study diary and study device collected. If data are available, the reason for each dropout should be documented.

3.3.2 Handling of subject withdraw or termination

If a subject is withdrawn from the study, data collected will be analysed and results will be retained for accuracy assessments. Data will not be retained in analyses related to the primary objective of the study if there is a valid reason to believe that the data may be biased, incorrect, or confounded.

3.3.3 Premature termination or suspension of study

This study may be temporarily suspended or prematurely terminated, if there is sufficient reasonable cause. Written notification documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the appropriate persons and/or regulatory authorities. Study may resume once concerns about safety, protocol compliance, or data quality are addressed.

Unacceptable Number of Device Failures

If more than six devices fail, based on sponsors established failure rate, the study may be stopped and/or terminated.

Unacceptable Number of Adverse Events

The study will be stopped and/or terminated at the discretion of the investigator or sponsor if there is reason to believe that subjects are being exposed to an unacceptable number of adverse events or to any additional significant risk that was not originally anticipated. If more than 3 serious adverse events occur during the study, the EC will be informed and asked to provide input whether or not to continue the study.

4. STUDY PROCEDURES AND SCHEDULE

4.1 SCREENING PROCESS

Screening will take place within a week prior to a subject's first study visit. The screening visit could be also performed together with Visit 1.

All potential participants will visit the study site for a Screening Visit. Information will be provided volunteers about the clinical study and a consent shall be obtained prior to screening commencement. Participants will be screened for health conditions, their medical history will be taken, and laboratory testing will be performed to confirm if participant is healthy. The initial blood test (CRP) must be in normal range, while cholesterol level and blood glucose level could be slightly higher (in the range that it could be managed with food - medical treatment is not needed).

The site study staff will perform the following during the screening visit:

- A unique screening identification number will be assigned to each participant
- Medical history will be collected
- Measurements and tests will be performed, including:
  - Chemistry panel (CRP, blood glucose and cholesterol)
  - Height and weight (for calculation of BMI)
  - Waist circumference
  - Body fat percentage
  - Vital signs (temperature, respiratory rate, hart rate, blood pressure)

4.2 ENROLLMENT PROCESS

Once all results for a participant's screening evaluations are available, a decision will be made by the investigator whether or not the subject qualifies for the study. Inclusion / exclusion criteria will be met. Volunteers who do not meet these criteria will not be included in the study. Volunteers who meet the inclusion / exclusion criteria will be scheduled for the study Day 1 visit.

Participants will receive a training how to use Vitastiq device and will be given the following instruction prior to the Day 1, 29 ± 4 days and 57 ± 4 days visits:

- Do not eat or drink anything (other than water) after 10:00 pm the night before each visit.

4.3 BLOOD SAMPLES PREPARATION

Venus capillary blood samples are collected into appropriate tubes that are defined by a procedure for blood sample analyses for selected analyte (minerals and vitamins).

4.4 DAYS 1, 29 and 57

First, study stuff documents if participant took any medications to treat acute disease (participant will be withdrawn if he or she took any medicine within 5 days before visit). The following procedure will be performed:

- On Day 1 participants will receive participant's diary and instruction how to complete it
- On Day 29 ± 4 days and 57 ± 4 days the study stuff will review participant's diary and document any new adverse events reported by participant.
- On Day 29 ± 4 days and 57 ± 4 days the Study staff will document any new device problems encountered by participants during home use.
- On Day 29 ± 4 days and 57 ± 4 days the Study staff will inform participants about the blood test results of previous visit. Since blood test results are not available on the day of their sampling, the blood test results of day 57 with their explanation will be sent by mail to the participants.
- During all three visits the Study staff will collect venous blood sampling.
- During all three visits the 2 investigators and participant will perform AEV readings at selected acupuncture points using Vitastiq device.

If a volunteer does not use Vitastiq device for the full 57-day period (± 4 days), documentation will include the date and reason for not using. The date of these events will be documented by the study staff during site visits or from subject self-report or diary entries.

4.5 AT HOME DAYS

At screening visit, the study stuff will instruct the participants of the study tasks to be completed during 57-day study period (± 4 days). All subjects will complete these tasks at home every day during the study. The participants will perform Vitastiq readings for selected minerals and vitamins, and on weekly basis they will record diary of adverse events, exercise, food intake and device problems.

4.6 FINAL STUDY VISIT

Day 57 (± 4 days) is the final scheduled study visit. The investigator will submit a report summarizing subject disposition and other study details, as appropriate at that site, to the Sponsor designee and the reviewing EC. This report will be completed within 1 month of the study closure date. The Sponsor designee will send a letter to the site informing them that the study is closed. The study will be considered closed when:

- Enrolment is complete
- Last subject has completed study
- Close-out visit is complete

- All major outstanding, reconcilable queries are resolved

The Sponsor designee will report the completion of the study to the EC.

4.7 FOLLOW UP

Since all blood test results are not available on the day of their sampling, the blood test results of day 57 (± 4 days) could not be delivered to the participants during the study. Therefore, the participants will receive their last blood sample results with their explanation within 14 days after the last site visit.

4.8 SCHEDULE OF EVENTS TABLE

Study procedures	Screening visit	Day 1 visit	Day 29 visit	Day 57 visit	Follow up
Informed consent process	**X**				
Demographics, medical history, medications (Rx & OTC)	**X**				
Physical exam	**X**				
Blood drawn for initial laboratory testing: CRP, glucose, cholesterol	**X**				
Enrolled volunteers each given instructions for site visits	**X**				
Train participant on home testing and diary completion	**X**				
Weight, body fat and waist circumference measurement	**X**	**X**	**X**	**X**	
Study staff collects participant health status		**X**	**X**	**X**	
Blood sampling for selected minerals and vitamins		**X**	**X**	**X**	
Study staff and participant perform measurements using Vitastiq device		**X**	**X**	**X**	
Study staff collects new AEs and device problems			**X**	**X**	
Study staff sends blood test results of day 57 to participants					**X**

4.9 RESTRICITIONS IN FOOD CONSUMPTION AND MEDICATIONS

There are no restrictions in food consumption mandated by study.

Using any medications to treat chronic disease or using any medication to treat serious acute disease within 5 days of each visit is an exclusion criterion. Therefore, the subject who uses medications to treat acute illness or disease will be excluded from the study.

4.10 PROTOCOL DEVIATIONS

Protocol deviations will be appropriately documented in study files and will be included in final report.

Study deviations include, but are not limited to:

- failure to obtain ICF prior participation
- incorrect version of the ICF used
- enrolled patient did not meet inclusion/exclusion criteria
- inappropriate use of Study device
- Subject Diary not available

4.11 PARTICIPANT ACCES TO STUDY DEVICE AT STUDY CLOSURE

Vitastiq device is already on the market registered as a device for personal use (it is not a medical device) and as such will be available for use at study closure.

5. ADVERSE EVENTS

5.1 ADVERSE EVENT

Adverse Event (AE): *Any untoward medical occurrence in a patient or clinical investigation subject administered an Investigational Product (pharmaceutical product, medical device, food supplement) and which does not necessarily have to have a causal relationship with this treatment.*

No AEs linked to the Study device are expected. Nevertheless, subjects will be closely followed and will be asked to report any observations regarding their health and overall status. At the end of the study, all reported AEs will be analysed to see if there might be an important prevalence of individual event or symptom.

All observations and conclusions regarding the AEs will be included in the final report.

5.2 SERIOUS ADVERSE EVENT

Serious Adverse Event (SAE): *Any AE is serious if the outcome is any of the following:*

- *Death*
- *Life-threatening*
- *Hospitalization or prolonged hospitalization*
- *Disability or permanent damage*
- *Congenital anomaly/Birth defect*
- *Other Serious (Important Medical Events)*

Any SAE that might occur will be appropriately reported according to local regulation and included in the final report.

5.3 DEVICE ADVERSE EFFECT

Vitastiq device is already on the market for 2 years. There was no device adverse effect reported by users or recognized by designers. Nevertheless, subjects will be closely followed and will be asked to report any observations regarding device adverse effect. At the end of the study, all reported device AEs will be analysed to see if there might be an important prevalence of individual event.

6. STUDY DEVICE

For the purpose of this protocol the term “Study device” refers to Vitastiq device that will be given to the participants.

6.1 STUDY DEVICE DESCRIPTION

Study device is Vitastiq device that measures skin resistance. It has titanium body. Vitastiq device has one electrode in the body, and the other electrode is at the top of the "pen". Device uses wireless technology (bluetooth). The device has a rechargeable battery.

The Vitastiq device measures electrical DC (low frequency) resistance between the tip of the device and its metal enclosure. The device charges a small 2.2nF capacitor to 3.3V which is discharged through the unknown resistance being measured. Based on the rate of discharge, the time constant is determined and hence the unknown resistance value. The process is repeated a couple of times per second, with the measurement range being between 10kR and 120MR. The resistance measured at each measuring point is being interpreted in relation to the electrical resistance measured at the reference point (reference point is determined at the beginning, during calibration).

The readings that are obtained during the measurement are presented to the user as: LOW, NORMAL, and HIGH.

6.1.1 Study device supply and return

The Sponsor will be responsible for manufacturing, packaging and labelling of study devices. The devices will be provided to the site from Vitastiq facility in Zagreb, Croatia or hand carried by the sponsor.

The site will be responsible for appropriate safe storage and distribution to study participants.

Monitor will check the supplies packages at initiation visit and first monitoring visit.

At the end of the study the Study devices will be given to participants for permanent.

6.1.2 Device packaging and labelling

The Vitastiq device is device for personal use and is already available for sale. This study is not blinded and randomized, therefore, all Study devices will be packed and labelled in their original packaging.

6.1.3 Device storage and stability

The devices will be stored at ambient temperature and humidity and are stabile for 2 years under this condition.

6.1.4 Device assembly

A guide for study staff and study subjects will be provided prior at study start. The guide includes device component images, application download instructions and descriptions of Vitastiq reading procedure for both study staff and study subjects.

6.1.5 Accountability records

Accountability records will be kept for all study material on study, site, and participant level.

All documents regarding the distribution of the material to and from CRO will be filed in Study Master File (TMF). The same applies to all tracking documents on a site level. At the end of the study, all site and participant accountability records will be copied for the filing in TMF; original documents will be archived at the site.

During each monitoring visit, accountability records will be compared to the supplies inventory at the site to make sure the material is distributed according to protocol, and is appropriately recorded.

7. QUALITY CONTROL AND PROCESSES

This study data will not be used for regulatory purposes.

7.1 RESPONSIBILITIES

7.1.1 CRO Project Manager/CRA

CRO will prepare and provide all study documents, obtain regulatory approvals, and monitor the study. Project Manager will be responsible for the overall conduct of the study and will make sure that Monitoring Plan and Data Management Plan are appropriately executed.

CRO will also be responsible for the statistical analysis and final report.

7.1.2 Investigator

Principle investigator will be principally responsible for the conduct of the study at laboratory site, as well as actions listed below:

- Conduct the study in accordance with the Protocol and GCP requirements
- Organize and manage the study team at the site
- Obtain the written informed consent of each volunteer participating in the study
- Report all adverse events related to the study as required
- Permit study monitors from the appointed CRO to have access to all records, including source documentation, for monitoring purposes related to this Study
- Provide additional information or data in the event a governmental regulatory agency requires such information or data

Ensure that only authorized study personnel will be permitted to receive, distribute, and dispose of Study device, enter data and sign CRFs, and consent subjects. These tasks may be delegated by the Investigator if so desired.

7.2 DATA COLLECTION AND MANAGEMENT

Data collected for each participant will be recorded in the CRF provided by CRO. The Investigator will be responsible for completion and correction of all sections of the CRF and will ensure that entries can be verified against source data (i.e. participant files, study working sheets, etc.). Monitor will review and collect CRF pages during each Monitoring Visit, according to the Monitoring Plan.

The CRO will only consider CRFs to be complete when each CRF section has been reviewed and signed by the Investigator, indicating their assurance on the accuracy of all recorded data. Data Management Plan specifies the procedures for collection, quality check, and processing of the CRF data.

Statistical analysis will be performed by expert statistician and according to study specific statistical plan.

7.3 MONITORING

Site monitoring will include initiation visit, interim monitoring visits and a close-out visit. CRFs will be compared to source documents to ensure that the investigation is conducted according to the protocol design.

A record of all study visits to the site will be kept in the Monitoring Visit Log in the ISF. Each visit will be described in Monitoring Visit Report, summarizing all activities conducted during the visit and actions for follow-up.

7.3.1 Source Data Verification

At the study site, the monitor will check complete set of CRFs for 100% of volunteer pool.

Participant files (electronic or paper), completed worksheets, and lab reports will constitute source data.

Source data verification shall compare the CRF with the participant records to verify:

- the accuracy and completeness of the information
- that the adverse events reported in the medical records are reported in the CRF according to protocol requirements and definitions

Corrections can only be made by the investigator or delegated study staff as follows:

The correction will be made by use of a single line through the error in indelible ink, writing the corrected entry, dating and initialling the corrections.

7.3.2 Participant Diary

Participant diary will be provided in local language.

For the purpose of practicality, the Participant diary will be designed in the same way as the core CRF and will be also treated as such. Therefore, the additional transcription of data into CRF won't be needed. In this case, the diary will be considered as both, the Source data and the CRF. Investigator will confirm with his or her signature that they discussed the entries with the participant, and that the data is complete and correct.

8. STATISTICAL DESIGN AND METHODS

Statistical analysis of primary study outcome will be based on calculation of 95% confidence interval (CI) of the estimated accuracy of Vitastiq readings for each mineral or vitamin. The expected accuracy level is 70%, therefore by using 135 readings the radius of 95% confidence interval would be estimated with 10% relative margin of error. The point estimate of the accuracy value together with 95% CI will be presented for each mineral or vitamin.

Statistical analysis of secondary outcomes will include Kappa statistic for interrater reliability on Vitastiq readings or Friedman ANOVA for testing the possible change of each mineral/vitamin blood level during the study period. Kappa statistic for interrater reliability will be calculated separately for each analyte (vitamin and mineral) and for all 135 readings. Additionally, the results of the second and the third rater (second investigator and participant) will be separately compared to the results of the first rater (first investigator). In Friedman ANOVA test 3 consecutive blood test results, that is on day 1, 29 ± 4 days and 57 ± 4 days, will be compared.

9. DATA ANALYSIS AND REPORTING

9.1 STATISTICAL DATA ANALYSIS

The detailed statistical analysis will be outlined in a separate statistical analysis plan and executed by the statistician.

Participant demographics and baseline characteristics will be presented using appropriate summary statistics (mean, standard deviation, median, minimum and maximum for continuous variables; frequency counts and percentages for categorical variables). Number of enrolled volunteers and number of completed visits will be reported. An overview of study discontinuations will be provided.

No interim analysis will be performed in this study.

9.2 PUBLICATION POLICY

The Sponsor and CRO will be responsible for publication of the study results.

9.2.1 Study Report

The final report will be prepared and submitted to competent authorities as per the ICH-GCP. It will be also available to all participating Investigators.

9.2.2 Use of information and publications

Information concerning Study device, unpublished scientific data and other pertinent information are confidential and remain the property of Vitastiq. Details should be disclosed only to the persons involved in the approval or conduct of the study. The Investigators may use this information for the purpose of the study only. It is understood by the Investigators that the company will use the information obtained during the clinical study in connection with the marketing and therefore may disclose it as required to other clinical Investigators or to competent bodies. In order to allow the use of the information derived from this clinical study, the Investigators understand that they have an obligation to provide the sponsor with all data obtained during this study.

The results of the work performed under this protocol and collected per study CRFs and diaries might be considered for publication or presentation at symposia and congresses as long as it is in accordance with the agreed publication policy. The Investigators will be entitled to publish or disclose these results only after allowing Vitastiq d.o.o. to review all transcripts, texts of presentations, and abstracts related to the study, 6 weeks prior to the intended submission for publication or any other disclosure. The study manager will ensure that no participant will be identifiable either from the final report or the published results. Vitastiq shall limit its review to a determination of whether any confidential information is disclosed and shall not attempt to censor or in any way interfere with investigators presentation or conclusions beyond the extent necessary to protect Vitastiq confidential information and to allow a check of technical accuracy.

10. STUDY MANAGEMENT

10.1 STUDY MANAGEMENT

Overall, the CRO and Sponsor will undertake responsibility and management of the study.

The statistician will manage the data analysis.

10.2 INSURANCE

Study insurance will be arranged according to local requirements.

Each participating entity (i.e. the CRO, Institution) maintains appropriate liability insurance coverage for its line of work, as required under applicable local laws and regulations.

11. LITERATURE

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