User Performance of the T-PLUS Blood Glucose Monitoring Systems

Protocol GCA-PRO-2019-001-01

Sponsor: Ascensia Diabetes Care
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I have read this protocol and agree to conduct the study as outlined. I also agree that prior to seeking approval from an Institutional Review Board (IRB), the Ascensia Diabetes Care study manager must approve any changes to the protocol.

SITE: Diablo Clinical Research

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Signature Printed Name Date

Revision Status

Date	Revision History
04/18/2019	Initial Release
05/09/2019	1. Secondary Objectives Section 2.2.8 Fingerstick Test • 95% of glucose results shall fall within ±12.5mg/dL of laboratory method at glucose concentrations <100 mg/dL and within ±12.5% at glucose concentrations ≥100 mg/dL** **CHANGED TO** • Test the hypothesis that there is at least a 95% probability that meter results would be within ±12.5mg/dL of laboratory method at glucose concentrations <100 mg/dL and within ±12.5% at glucose concentrations ≥100 mg/dL** 2. Secondary Objectives Section 2.2.9 Study Staff Test • 95% of glucose results shall fall within ±15mg/dL of laboratory method at glucose concentrations <100 mg/dL and within ±15% at glucose concentrations ≥100 mg/dL** **CHANGED TO** • Test the hypothesis that there is at least a 95% probability that meter results would be within ±15mg/dL of laboratory method at glucose concentrations <100 mg/dL and within ±15% at glucose concentrations ≥100 mg/dL** 3. Section 4.2 Exclusion Criteria **Added the following exclusion criteria:** • Being in this trial during or soon after xylose absorption testing (Xylose in the blood is known to cause interference). However, subjects can be enrolled as soon as 2 days after the test is performed (25 gms of oral xylose administered during this test is cleared within 480 mins or 8 hours. 4. Removed Section 5.6 Subject Enrolment and merged all information regarding the study enrolment to Section 4.3 Subject Recruitment (the section was previously named as General Enrolment Details).

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List of Abbreviations

AE	Adverse Event
AST	Alternative site test
BG	Blood Glucose
BGM	Blood Glucose Meter
BGMS	Blood Glucose Monitoring System
CCRA	Certified Clinical Research Associate
CLSI	Clinical and Laboratory Standards Institute
CSR	Clinical Study Report
EDC	Electronic Data Capture
FDA SMBG: 2018	Self-Monitoring Blood Glucose Test Systems for Over-the-Counter Use, Draft Guidance for Industry and Food and Drug Administration Staff. Issued 30 Nov2018, Food and Drug Administration, USA.
ICF	Informed Consent Form
IRB	Institutional Review Board
ISO 15197:2013 (and EN ISO 15197:2015)	International Organization for Standardization, Switzerland. **Note:** All references to ISO:15197:2013 in this protocol also encompass EN ISO 15197:2015.
PI	Principal Investigator
QRG	Quick Reference Guide
SMBG	Self-Monitoring of Blood Glucose
T-PLUS	Thunder PLUS
UADE	Unanticipated Adverse Device Effect
UG	User Guide
YSI	YSI Glucose Analyzer™ (YSI Life Sciences, Yellow Springs, OH)

1.0 Background Information

T-PLUS (Thunder PLUS) is the code name of a blood glucose monitoring system (BGMS) consisting of a new meter and CONTOUR PLUS test strips. The proposed brand name for this BGMS is "Contour Plus BLUE". This system will be marketed in the US and globally. The meter can wirelessly transmit BG results using Bluetooth® Low Energy technology to the meter mobile application that will be available on specified versions of the iOS and Android mobile platforms.

This clinical trial will assess the performance (accuracy) of the T-PLUS meters by lay users enrolled as subjects in the study, and by health care professionals (also called study staff).

The trial will follow the requirements and procedures described in Draft FDA Guidance on Self-monitoring Blood Glucose Test Systems: 2018 (FDA SMBG:2018)[1] and ISO 15197:2013 International Standard, Section 8[2]. Results will be evaluated and reported for the T-PLUS system according to each of the two standards (FDA and ISO), for a total of two reports.

2.0 Study Objectives

Study objectives 2.1 is in accordance with **ISO 15197:2013, Section 8**, and apply to data collected ONLY from **subjects who have diabetes**.

2.1 Primary Objectives, **ISO 15197:2013**

2.1.1 Fingerstick Test: Obtain T-PLUS BGMS performance data in the hands of lay users with diabetes using fingerstick capillary blood obtained with Microlet NEXT® lancing device.

- **95% of glucose results shall fall within ±15mg/dL of laboratory method at glucose concentration <100 mg/dL and within ±15% at glucose concentrations ≥100 mg/dL**

2.2 Secondary Objectives: Failure of any sub objectives listed below does not constitute the failure of the secondary objective.

Study objectives 2.2.1 to 2.2.3 are in accordance with **ISO 15197:2013, Section 8**, and apply to data collected ONLY from **subjects who have diabetes**.

2.2.1 Alternative Site Test (AST): Obtain T-PLUS BGMS performance data in the hands of lay users with diabetes using capillary blood from the palm site obtained with Microlet NEXT lancing device with the AST endcap. Acceptance criteria will be the same as fingerstick 2.1.1.

2.2.2 Venous Blood Test: Obtain T-PLUS BGMS performance data using venous blood from lay users with diabetes. Acceptance criteria will be the same as fingerstick 2.1.1.

[1] Self-Monitoring Blood Glucose Test Systems for Over-the-Counter Use, Draft Guidance for Industry and Food and Drug Administration Staff. Issued 30Nov2018, Food and Drug Administration, USA. (abbreviated FDA SMBG:2018)

[2] In vitro diagnostic test systems – Requirements for blood-glucose monitoring systems for self-testing in managing diabetes mellitus. Issued 15 May2013, International Organization for Standardization, Switzerland. (abbreviated ISO 15197:2013)

2.2.3 Subject Questionnaire 1: The questionnaire will include statements about the T-PLUS BGMS instructions for use (product labeling) and ease of use of the basic blood testing operations of the meter system. These statements will be given a rating by the subjects with diabetes (1=strongly disagree, 2=disagree, 3=neutral, 4=agree, 5=strongly agree), and frequency distributions will be tabulated. Results will be compared to the following criteria:

- **Acceptance criteria: There must be at least a 90% probability that a user would respond with a score of 3 or higher to statements concerning instructions for use or ease of use.***

Study objectives 2.2.4 to 2.2.7 are in accordance with **FDA SMBG: 2018,** and apply to data collected from ALL subjects (**subjects who are diabetic and Naïve)**.

2.2.4 Fingerstick Test: Obtain T-PLUS BGMS performance data in the hands of **lay users with diabetes and naïve users** (without diabetes) from fingerstick capillary blood using Microlet NEXT lancing device.

- **95% of glucose results shall fall within ±15% of laboratory method across the entire tested range**
- **99% of glucose results shall fall within ±20% of laboratory method across the entire tested range**

2.2.5 Alternative Site Test (AST): Obtain T-PLUS BGMS performance data in the hands of **lay users with diabetes and naïve users** (without diabetes) from the palm site using the Microlet NEXT lancing device with the AST endcap. Acceptance criteria will be the same as fingerstick 2.2.4.

2.2.6 Samples will adequately span the claimed measuring range. At least ten unaltered samples with blood glucose concentration at the lower limits of the measuring range (less than 80 mg/dl) and at least ten unaltered samples at the upper limit measuring range (greater than 250 mg/dl) will be obtained for each anatomical site (i.e. fingerstick & palm).

2.2.7 Subject Questionnaire 1: The questionnaire will include statements about the T-PLUS BGMS instructions for use (product labeling) and ease of use of the basic blood testing operations of the meter system. These statements will be given a rating by all of the subjects (1=strongly disagree, 2=disagree, 3=neutral, 4=agree, 5=strongly agree), and frequency distributions will be tabulated. Results will be compared to the following criteria:

- **Acceptance criteria**: There will be no pass/fail criteria for FDA: SMBG 2018**

*This is a "total population" (hypothesis test) requirement for the pool of all subjects with diabetes.

**This is a "total population" (hypothesis test) requirement for the pool of all subjects (naïve and diabetic).

Study objectives 2.2.8 to 2.2.10 below are internal objectives and apply to data collected from all subjects OR subjects who have diabetes, as specified for each objective.

2.2.8 Fingerstick Test **(only subjects with diabetes)**: Obtain T-PLUS BGMS performance data in the hands of lay users with diabetes using fingerstick capillary blood obtained with Microlet NEXT lancing device.

- **Test the hypothesis that there is at least a 95% probability that meter results would be within ±12.5mg/dL of laboratory method at glucose concentrations <100 mg/dL and within ±12.5% at glucose concentrations ≥100 mg/dL**

2.2.9 Study staff test **(two analyses: all subjects and for subjects with diabetes)**: Obtain T-PLUS BGMS performance data in the hands of trained study staff testing subjects' fingerstick blood obtained with Microlet NEXT lancing device.

- **Test the hypothesis that there is at least a 95% probability that meter results would be within ±15mg/dL of laboratory method at glucose concentrations <100 mg/dL and within ±15% at glucose concentrations ≥100 mg/dL**

2.2.10 Subject Questionnaire 2 **(only subjects with diabetes)**: This questionnaire for subjects with diabetes will include statements about user preferences and feedback regarding the features of the T-PLUS BGMS in routine diabetes management. These statements will be given a rating by the subjects (1=strongly disagree, 2=disagree, 3=neutral, 4=agree, 5=strongly agree), and frequency distributions will be tabulated.

- **Acceptance criteria: There are no pass/fail criteria for Questionnaire 2.**

3.0 Study Design Summary

This study is designed to satisfy both the ***FDA SMBG:2018*** and the ***ISO 15197:2013, Section 8*** requirements. Besides different analysis criteria, the main difference between these regulations is that FDA SMBG:2018 requires that both naïve users (people without diabetes) and people with diabetes to be included in the study population. The ISO 15197:2013 standard states that the study population shall only include people with diabetes.

The study will be conducted at minimum of one clinical site and enroll a total of approximately 370 lay users. Ten to fifteen percent (10 - 15%) of the enrolled subjects will be people without diabetes and twenty percent (20%) of subjects enrolled with diabetes will have Type 1 diabetes. At least 70% of subjects will be younger than age 65.

For this study, there must be at least 350 total evaluable fingerstick self-test results and at least 350 total evaluable AST palm self-test results. ISO 15197:2013 criteria requires at least 100 evaluable results each for fingerstick self-test results, AST palm self-test results, study staff performed fingerstick blood and venous blood tests.

In addition, per FDA SMBG:2018, at least 10 unaltered evaluable subject fingerstick and AST palm blood samples will be < 80 mg/dL and 10 will be > 250 mg/dL (by capillary YSI assay). Subject enrollment will continue until the required evaluable samples are collected.

The subjects will be 18 years and older. Each subject will make one visit, lasting approximately 1 hour at the clinical site. Study sessions will be conducted by study staff members who have been trained on all aspects of the protocol, the use of the T-PLUS system, data collection, and GCP.

Procedures: All subjects will complete the informed consent process before performing any study procedures.

- **For All Subjects**

Subjects will be assigned to use the T-PLUS meter and randomized to use one of three Contour Plus test strip lots throughout the visit.

The untrained subjects, who have never used the Ascensia Contour TV3 (Thunder V3), and Thunder PLUS meter systems previously, will be sequestered in such a way that they cannot observe or be influenced by the testing technique of other study participants. They will learn to use the T-PLUS meter by reading the User Guide (UG) and Quick Reference Guide (QRG). Each subject will perform one fingerstick self-test using a Microlet NEXT or similar lancing device and the T-PLUS meter. Immediately following the subject self-test, a Tenderlett™ or similar device will be used by the study staff on the subject's finger (preferably the same hand) to collect a capillary sample for testing on the YSI Glucose Analyzer.

Immediately following the Tenderlett lancing, subjects will lance their palm using the Microlet NEXT lancing device (with AST clear endcap) and will perform a self-test using the meter.

Immediately following the AST test, the staff person will perform a fingerstick test on the subject with the Microlet device and the T-PLUS meter.

All subject capillary and AST self-test results and study staff fingerstick results will be compared to YSI results from blood obtained from the Tenderlett fingerstick.

Hematocrit will be measured for all subjects.

All Subjects will then complete a questionnaire (Questionnaire 1) to provide feedback on the meter system and instructions for use (UG and QRG).

- **Additional Procedures for Subjects with Diabetes**

Subjects with diabetes will be given a venipuncture. The venous blood will be tested by the study staff using the T-PLUS system, , and the YSI lab analyzer. The venipuncture may be performed any time after the subject is enrolled (i.e. upon completion of informed consent and passed inclusion/exclusion criteria).

After all self-testing is completed, staff will demonstrate to the subjects with diabetes several features of the meter system that would not necessarily be experienced during the brief accuracy testing. These subjects will be given a second questionnaire (Questionnaire 2) to provide feedback about the new features of the system.

4.0 Inclusion/Exclusion Criteria and Subject Recruitment

4.1 Inclusion Criteria:

4.1.1 Males and females, 18 years of age and older

4.1.2 Ability to speak, read and understand English. Subjects must demonstrate ability to read a paragraph from the first page of the UG to qualify for the study.

4.1.3 Willing to complete all study procedures

4.2 Exclusion Criteria:

4.2.1 Hemophilia or any other bleeding disorder

4.2.2 Pregnancy (self-reported)

4.2.3 Physical, visual or neurological impairments that would make the person unable to perform testing with the BGMS.

4.2.4 Previous participation in a blood glucose monitoring study using the Ascensia Contour TV3 or Thunder V3 and Thunder PLUS BGMS.

4.2.5 Working for a medical laboratory, hospital or other clinical setting that involves training on or clinical use of blood glucose monitors.

4.2.6 Being in this trial during or soon after xylose absorption testing (Xylose in the blood is known to cause interference). However, subjects can be enrolled as soon as 2 days after the test is performed (25 gms of oral xylose administered during this test is cleared within 480 mins or 8 hours).

4.2.7 Working for a competitive medical device company, or having an immediate family member or someone who is not a family member but is living within the household of someone who works for such a company.

- Immediate family members are the subject's parents, spouse, children, and siblings, including the parent's spouse; step-children and adopted children and their spouses.
- A competitive medical device company is a company that provides a medical device or components of a device that is related to diabetes.

For example, people who are not eligible are those who work for companies that create or manufacture the following (or a company that is in a partnership with a company that provides such devices): lancing devices, blood glucose monitoring systems, continuous glucose monitoring systems, insulin pens, or systems related to the measurement of HbA1c.

People who are eligible are those who work for companies associated with products such as wound dressings, medications or dietary products.

4.2.8 A condition, which in the opinion of the investigator or designee, would put the person or study conduct at risk. The reason for exclusion will be clearly documented by investigator or designee on the subject disposition form.

4.3 Subject Recruitment

4.3.1 The following additional population criteria are study goals with the site recruiting to meet these goals:

1. Enrolment: At least 370 subjects
2. At least 70% of subjects will be younger than age 65.
3. Ten to fifteen % (10-15%) of subjects will be people without diabetes.
4. At least 20% of subjects (with diabetes) will have type 1 diabetes.

4.3.2 There must be at least 350 total evaluable fingerstick self-test results and at least 350 total evaluable AST palm self-test results. ISO 15197:2013 criteria requires at least 100 evaluable results each for fingerstick self-test results, AST palm self-test results, study staff performed fingerstick blood and venous blood tests.

4.3.3 In Addition, per FDA SMBG:2018, at least 10 unaltered evaluable subject fingerstick and AST palm blood samples will be < 80 mg/dL and 10 will be > 250 mg/dL (by capillary YSI assay). Subject enrollment will continue until the required evaluable samples are collected

4.3.4 No laboratory tests (e.g., pregnancy) are required to assure qualification. Subjects' verbal responses will be accepted and recorded on forms which will serve as source documents for the electronic data capture (EDC) system.

4.3.5 The site study staff will perform the following when the subject arrives at the clinical trial site:

1. Complete the informed consent process.
2. Assign a unique number to each subject.

4.3.6 The study staff will document subject demographics (including both screen-failed and enrolled subjects), diabetes history, medical conditions and medications information. Subjects will also be asked when they last ate, took diabetes medications, and performed vigorous exercise.

4.3.7 If the subject does not have diabetes, the outside of the subject folder will be labeled to indicate that venipuncture should NOT be performed.

4.3.8 According to the randomization schedule, subjects will be assigned to use one of three Contour Plus test strip lots (R,B or G) throughout the visit.

5.0 Materials and Methods

5.1 Resources Supplied by Investigational Site

5.1.1 *Staffing*

1. Principal Investigator and, as needed, Sub-Investigator(s)
2. Study Coordinator
3. Two or more study staff to conduct the study
4. Staff person(s) to measure hematocrit, prepare samples for YSI analysis, and perform YSI analysis of venous and capillary blood (plasma) and serum controls
5. Data entry person for EDC data entry (Note: This requires EDC training.)

5.1.2 *Other*

1. Final, IRB approved, informed consent forms (ICF), and other necessary supporting documents, as appropriate
2. Computer with internet connection for site and monitor access to EDC system
3. Facility with adequate space for conducting all testing. Site facilities for conducting subject visits must allow sufficient privacy so subjects cannot observe other subjects' BGMS testing procedures. Testing area must also be conducive to allow subjects to focus on testing (i.e. background distraction to be kept to a minimum).
4. YSI 2300 analyzers, reagents, and supplies. Up to date maintenance documentation will be required.
5. Supplies to treat any hypoglycemic events
6. Venipuncture supplies
7. Laboratory supplies including: disposable pipets, gloves, alcohol wipes, band aids, gauze, parafilm, disposable benchtop covers
8. Tenderlett™ or similar single-use lancing device
9. Micro collection tubes with lithium heparin anticoagulant (no gel separator)
10. Hand warmers
11. Biohazardous bags and sharps containers
12. Microhematocrit tubes without anticoagulant (for testing venous blood)
13. Microhematocrit tubes with anticoagulant (for testing capillary blood)

5.2 Resources Supplied by Ascensia

5.2.1 *Staffing*

1. Ascensia Study Manager
2. Study Monitor(s)

5.2.2 *Materials*

1. Protocol, EDC system, paper forms (CRF) and questionnaires, as needed
2. ICF template
3. CONTOUR PLUS test strips - 3 lots labeled as RED, GREEN, BLUE (R, G, B) and Contour Plus Test Strips Inserts. Randomization table for the assignment of strip lot will be provided to the site.
4. Investigational T-PLUS meters (Note: Meters will have Bluetooth turned ON throughout the study and will be provided to the sites with Bluetooth turned ON). Note: The meters are labeled in its proposed brand name only – Contour Plus Blue.
5. Investigational T-PLUS User Guides (UG) and Quick Reference Guides (QRG). Note: UG's and QRG's refer to the proposed meter brand name only – Contour Plus Blue
6. Contour-PLUS (Normal level equivalent to Level 2) control solution and Contour -Plus control solution inserts.
7. Microlet NEXT lancing device systems including clear, AST endcap. (All subjects will be provided new lancing devices and lancets; lancing devices will not be shared among subjects.)
8. Thermometer / hygrometer
9. Statspin™ microcentrifuge and tube rotors
10. Microhematocrit tube reader (or equivalent hematocrit measurement system), hematocrit rotors, sealant
11. Ascensia serum glucose traceability controls (four levels)
12. Disinfectant wipes for meters (listed in Appendix A)
13. Package delivery forms and envelopes (UPS or Fed Ex)

5.3 **Site Staff Pre-Study Orientation**

The Ascensia Study Manager, or designee, will review all study documents, procedures (including procedures for data collection and management), GCP, and usage of the T-PLUS BGMS with the site staff during Site Initiation Visit. All site staff involved in subject testing sessions will successfully perform at least one control solution test with the BGMS. Study-specific EDC training will also be provided to study staff as necessary and training will be documented by the site.

5.4 **Safety and Disinfection Procedures:**

1. Universal precautions will be used throughout the study.
2. Only study staff will perform disinfection procedures. Subjects will not perform disinfection.
3. A new T-PLUS meter will be provided to each subject. At the end of the subject's visit, the meters will be disinfected and stored until they are returned to Ascensia.

4. A new Microlet NEXT or similar lancing device will be provided to each subject. At the end of the subject's visit, the lancing device will either be discarded or offered to the subject to take home.
5. The lancets used in the study (Tenderlett and Microlet lancets) will be disposed of in a biohazard sharps container after use.
6. Microlet lancets must be changed for each lancing by the subject. (Insert, remove, and discard into sharps container.)
7. Disposable exam gloves will be worn by site staff during study procedures where the risk of transmission of disease is present. These procedures include performing blood glucose measurements, sample processing, and laboratory procedures involving blood samples. A new pair of gloves will be used when working with each subject and will be changed between subjects.
8. All test strips are single-use and will be appropriately discarded after use.
9. Disposable bench top covers should cover the work area and must be changed between subjects.
10. A marketed BGM device may be used for determining the subject's glucose level during the study as needed for safety reasons or to ascertain the glucose level for study reasons. The meter will be disinfected after use with each subject.

5.5 Study Staff Quality Control Testing of Meters

Prior to enrollment of the first subject, the study staff will perform testing with Contour Plus level 2 control solutions on all T-PLUS meters. All staff control testing will be done out of sight of the study subjects. If the results are out of range, troubleshooting will be performed (see meter UGs) and the test will be repeated. If the meter tests are out of range after troubleshooting is complete, the meter will be removed from the study. This will be documented on the Problem Reporter form, as described in Section 5.13 'Device Problem Reporting.'

5.6 Tests (See Table 5.1 for test scheme.)

Each subject will be provided with all the materials needed for self-testing during the visit: one T-PLUS meter, a Microlet NEXT or similar lancing device, lancets, Contour PLUS test strips, associated meter UGs and QRGs. The subject will be given time to review the UG and QRG to learn how to use the system but no training will be provided.

The subject will wash his/her hands thoroughly with warm water and soap, rinse and dry well.

5.6.1 *Fingerstick Testing*

1. The subject will perform a fingerstick using the Microlet NEXT lancing device and test his/her blood on T-PLUS meter. The study staff person will record the time and test result of the successful meter test on the appropriate form. For instructions on handling error codes and repeat testing, refer to protocol section 5.9 'Errors'.
2. Immediately, but at least within 5 minutes of the fingerstick self-test, the study staff person will lance the subject's finger (preferably the same hand as above) with a Tenderlett device

in order to collect enough blood for testing on the YSI Analyzer (approx. 200–300 uL). The time of this Tenderlett lancing will be recorded.

3. If adequate blood is not obtained from the Tenderlett lancing, 2 additional Tenderlett lancings are allowed (total of up to 3 Tenderlett lancings). The blood is to be combined into one tube for YSI analysis.
4. The blood sample will be centrifuged as soon as possible after sample collection but not longer than 15 minutes after the meter test. The study staff will record the time of centrifugation.
5. In order to reduce glycolysis of the plasma, it is recommended that the plasma be transferred from the microtainer tube to a micro/dispo tube or similar container, prior to analysis on the YSI Analyzer.
6. The plasma sample will be assayed on the YSI Analyzer: 1 sip = 1 result from black probe and 1 result from white probe. See 5.11.3 for details on testing of plasma samples.
7. Immediately following the YSI test of the subject plasma sample, a serum control close to the level of the subject plasma will be tested. (No re-calibration is required). The serum control test result should be within the range shown in Table 5.2. See 5.11.32 for details.
8. Additional details regarding processing of blood samples for the YSI Analyzer are found in Appendix B.
9. If the time from meter test to blood centrifugation exceeds 15 minutes, the test is considered non-evaluable. This will not be considered a protocol deviation. Times of meter tests and centrifugation will be recorded.

5.6.2 ***AST (palm) testing***

1. Only proceed to AST testing if an adequate blood sample is obtained from Tenderlett lancing for YSI analysis.
2. The same meter and test strip lot used for fingerstick testing will be used for AST testing.
3. Immediately following Step 5.6.1, the subject will perform an AST (palm) stick using the lancing device (new lancet), preferably on the same hand as the fingerstick lancing, and test his/her blood on the meter.
4. If adequate blood is not obtained from the Microlet palm lancings for the meter test, up to 2 additional Microlet palm lancings are allowed (total of up to 3 AST palm lancings).
5. The time of the successful AST meter test will be recorded. The time interval between the blood centrifugation and the AST meter test should be as soon as possible, and within 15 minutes or less.
6. If the time from meter test to blood centrifugation exceeds 15 minutes, the test is considered non-evaluable. This will not be considered a protocol deviation. Times of meter tests and centrifugation will be recorded.

5.6.3 ***Study Staff test***

1. Immediately following the AST testing, the study staff person will perform a fingerstick lancing using the same Microlet lancing device (new lancet) on the subject, preferably on the same hand as the other fingerstick lancing.
2. The staff person will test the fingerstick blood using the same meter and test strip lot used by the subject.
3. The study staff will record the time of the meter test. The time interval between the blood centrifugation and the final staff meter test should be as soon as possible, and within 15 minutes or less.
4. If the time from meter test to blood centrifugation exceeds 15 minutes, the test is considered non-evaluable. This will not be considered a protocol deviation. Times of meter tests and centrifugation will be recorded.

5.7 **Venous Blood Testing by Study Staff *(Only subjects with diabetes)***

5.7.1 The venipuncture and subsequent test may be performed at any time during the subject visit.

5.7.2 A venipuncture will be performed by a trained site phlebotomist. The subject's blood will be collected into one 4 mL heparin-coated Vacutainer tube.

5.7.3 *The study staff person will place one drop of venous blood onto Parafilm®, then immediately perform one test with the same lot of test strips and the same meter used for capillary tests. Record the time of the successful venous meter test (refer to Section 5.9 for information on handling error codes and repeat testing).*

5.7.4 *The venous blood will be centrifuged as soon as possible before or after the meter test (within 15 minutes or less). Record the centrifugation time. If the time from meter test to blood centrifugation exceeds 15 minutes, the test is considered non-evaluable. This will not be considered a protocol deviation. Times of meter tests and centrifugation will be recorded.*

5.7.5 *The venous plasma sample will be assayed on the YSI Analyzer: 1 sip = 1 result from black probe and 1 result from white probe. See 5.11.3 for details on testing of plasma samples and Appendix B regarding processing blood samples for the YSI analyser.*

5.7.6 *Up to three venipuncture attempts are allowed per subject, if subject and study staff agree, in order to obtain sufficient venous blood. If the venipuncture is ultimately unsuccessful, this will be recorded and will not be considered a protocol deviation.*

Table 5.1 – Subject Testing Schematic

Step	Test type	Operator	Description
1	Fingerstick Self-test	Subject	▪ Subject lances finger – Microlet NEXT lancing device. ▪ Test meter with 1 lot of test strip (Repeat 2 more times, only if needed). ▪ Record time of successful meter test.
2	Tenderlett deep stick	Study staff	▪ Immediately following Step 1 meter test (≤ 5 min), staff lances subject fingertip (up to 3 lancings allowed). ▪ Record time of lancing. ▪ Collect blood in Li Heparin tube (approx. 200 – 300 uL). ▪ Centrifuge blood and record time (should be within 15 min of meter test in step 1).
3	AST palm Self-test	Subject	▪ Immediately following Step 2, subject lances palm – Microlet NEXT lancing device. ▪ Test with same test strip lot and meter as above. (Repeat 2 more times, only if needed.) ▪ Record time of successful meter test (should be within 15 min of centrifugation in Step 2).
4	Study staff test	Study staff	▪ Immediately following Step 3, staff lances subject fingertip - Microlet NEXT lancing device. ▪ Test with same test strip lot and meter as above. (Repeat 2 more times, only if needed.) ▪ Record time of successful meter test (should be within 15 min of centrifugation in Step 2).
5	Venous test **(only subjects with diabetes)**	Study staff	▪ May be done prior to Steps 1-4. ▪ Staff performs venipuncture on subjects with diabetes (up to 3 attempts allowed). ▪ Collect blood in 4 ml Vacutainer tube with heparin anticoagulant. ▪ The next two sub-steps may be performed in either order; they should be within 15 minutes maximum of each other. o Staff tests blood (out of view of subject) with same meter and strip lot as fingerstick test. o Centrifuge blood and record time (should be within 15 min of venous blood meter test).
6	Hematocrit	Study staff	▪ Fill 2 micro-hematocrit tubes (only 1 tube to be measured). ▪ Blood is from capillary or venous blood.
7	Questionnaire 1	Study staff	▪ Study staff administers Questionnaire 1 to all subjects.
8	Questionnaire 2 (**only subjects with diabetes**)	Study staff	▪ Study staff demonstrates BGMS features to subjects with diabetes. ▪ Study staff administers Questionnaire 2 to subjects with diabetes.

5.8 General

5.8.1 If adequate blood is not obtained from any of the lancings (the subject or staff Microlet lancings, Microlet AST lancing) up to 2 additional lancings are allowed in each case.

5.8.2 If adequate blood is not obtained from the staff Tenderlett lancing, up to 2 additional lancings are allowed in each case (up to a total of 3 fingertip Tenderlett lancings). The blood from each lancing should be combined into one Microtainer tube.

5.8.3 If adequate blood is not obtained from the first venipuncture attempt, up to 2 additional attempts are allowed if subject and study staff agree (total of up to 3 venipuncture attempts).

5.8.4 **If the time from meter test to blood centrifugation exceeds 15 minutes, the test is considered non-evaluable.** This will not be considered a protocol deviation. Times of meter tests and centrifugation will be recorded.

5.8.5 Hand warmers may be used throughout the testing to increase blood flow.

5.8.6 It is preferred that all fingerstick and AST lancings are from the same hand if possible, throughout the study.

5.8.7 Study staff will record all meter results on the appropriate form. If meters have turned off, meter memory will be reviewed and the results will be obtained from meter memory.

5.8.8 Study staff will record in the comments section of the meter testing form, any obvious testing errors by the subject or staff as appropriate for staff tests, and any abnormalities observed in the testing procedure or BGMS.

5.8.9 Note that once the fingerstick testing is started in 5.6.1, the subjects will be advised not to eat or use medications until the study staff test testing is completed in 5.6.3, to avoid changing glucose levels. The subject can eat/take medicines at any time to avoid or treat hypoglycemia, hyperglycemia or an adverse event. If the subject eats or takes medications, the subject's participation in the study will be discontinued.

5.9 Errors

5.9.1 If the meter reports an error code, the instructions shown in the UG should be followed. Re-testing is recommended (per the UG). No more than a total of 3 attempts are allowed.

5.9.2 If the subject feels that he/she made an error while testing, without any prompting from the study staff, he/she will repeat the test. The reason for the repeated test will be documented in the comments section of the form. No more than a total of 3 attempts are allowed.

5.9.3 If the study staff feels that he/she made an error while performing staff fingerstick or venous testing, he/she will repeat the test. The reason for the repeated test will be documented in the comments section of the form. No more than a total of 3 attempts are allowed.

5.9.4 The study staff will record all non-numeric codes - QNS (Quantity not sufficient), NO (Not obtainable), and meter error codes as appropriate for the meter tests.

5.10 Hematocrit

Two hematocrit tubes will be filled with either the subject's venous or capillary blood, and then centrifuged. The hematocrit will be measured from only one tube; the second tube will be collected as a backup in case the first tube is not measureable (i.e., tube spun out, broken tube, clay seal

compromised, etc.). Subject hematocrit measurement should be within 0-70% in order for the subject BG results to be considered evaluable for the ISO 15197 analysis. For the analysis according to the FDA SMBG:2018, all subjects will be considered evaluable.

Note: Microhematocrit tubes with anticoagulant will be used for capillary blood hematocrit measurements collected directly off the fingertip and microhematocrit tubes without anticoagulant will be used for venous blood.

Additional details regarding hematocrit measurements are found in Appendix C.

5.11 **YSI Glucose Analyzer**

5.11.1 *YSI Analyzer Operation and Control Tests*

The YSI Glucose Analyzer will be maintained and operated according to the instructions in the manufacturer's operating manual.

Before the study begins, the YSI will be set up and appropriate maintenance will be performed. The YSI clock will be set to 24 hour format using an accurate time device, such as site central clock or atomic clock.

A set of four glucose control sera[3] that have been assayed by a method traceable to one proposed for use as a national glucose reference method developed through the combined efforts of the Center for Disease Control, the National Bureau of Standards, the American Association for Clinical Chemistry, and the FDA will be provided by Ascensia and used to verify the accuracy of the YSI Glucose Analyzer[4]. These controls will be run for pre-study and daily control tests, and will be within the lower and upper limits of the target levels noted in Table 5.2. The procedure for proper thawing and handling of the serum controls is found in Attachment 1.

Table 5.2: YSI serum control target values and limits, mg/dL

LOT#	LEVEL	TARGET	Lower Limit	Upper Limit
7AP204	2	48.02	45.52	50.52
7AP304	3	95.89	91.10	100.69
7AP404	4	193.19	183.53	202.85
7AP505	5	388.88	369.44	408.32

5.11.2 *Pre Study and Study Control Tests*

Pre-study control tests: The four glucose control sera below will be used to verify the accuracy of each YSI analyzer before the study begins (Pre-study testing). Six (6) pre-study runs will be

[3] Caution: The glucose traceability controls are human serum-based and must be handled using universal precautions.

[4] Neese J, Duncan P, Bayse D, *et al.* Development and evaluation of a hexokinase/glucose-6-phosphate dehydrogenase procedure for use as a national glucose reference method. HEW Publication No. (CDC) 77-8330. 1976. Atlanta, Centers for Disease Control.

performed on each YSI that will be used in the trial: 2 runs per day for 3 days. The Ascensia Study Manager or designee will review the YSI pre-study glucose control data before the study begins.

Study control tests: Any YSI used on a given day to test subject blood samples will be monitored by assaying the 4 glucose controls at the beginning of the day. The controls will be tested: 1 sip = 1 result from white probe and 1 result from black probe. The YSI will be calibrated before each serum control sample is tested.

The sequence of control testing will be:

1. Calibrate the YSI
2. Test Control 2 (1 sip)
3. Calibrate
4. Test Control 3 (1 sip)
5. Calibrate
6. Test Control 4 (1 sip)
7. Calibrate
8. Test Control 5 (1 sip)

Site staff will keep a maintenance log for each YSI, including daily operational checks, maintenance and membrane changes. A full set of serum controls should also be tested after YSI parts are changed (such as buffers, probes, or membranes).

5.11.3 *Testing of Subject Plasma Samples*

The subject plasma samples (capillary and venous) will be tested using 1 sip per test (=2 results, 1 from white and 1 from black probe) with calibration of the YSI before the first sip. A serum control (1 sip) will be tested after each subject plasma sample to verify the YSI Analyzer has remained in control during the plasma sample test. The control with target value close to the concentration of the subject plasma sample will be tested. The average of the black and white probe results will be verified to be within the ranges shown in Table 5.2.

If the control result is out of range, the YSI will be recalibrated and the control sample will be retested. If the control result is now within the appropriate range (Table 5.2), the control sample is confirmed as appropriate for use. The YSI analyzer will then be re-calibrated, the plasma will be re-tested, and the control will be tested per the protocol's plasma testing procedure already described.

If the control result is not within the appropriate range after the plasma sample has been tested twice, the results for this subject sample will be non-evaluable.

The sequence of testing for each subject sample will be:

1. Calibrate the YSI
2. Test plasma sample (1 sip)
3. Test control (1 sip)
4. Verify control result (mean of black and white probe results) is within limits in Table 5.1. If it is, stop and record result.
5. If not, repeat Steps 1, 3 and 4 to verify control sample is satisfactory for use.
6. When control result is shown to be within range in Table 5.2, repeat Steps 1-3.

If the control is again outside the limits, the results for this subject sample are non-evaluable.

Glucose values of the YSI replicates (both controls and plasma) should be within 4 mg/dL or 4% of each other (i.e. 4 mg/dL for values < 100 mg/dL and 4% for values ≥ 100 mg/dL). If they are not, an additional assay should be run.

5.11.4 *Recording YSI Results*

The YSI source documents will be labeled as follows throughout the study to facilitate matching glucose results.

Sample identification will include:

1) Ascensia serum traceability control level or
2) Subject ID plus either 'capillary' or 'venous' blood

The YSI source documents (tapes) will be affixed to plain white paper and photocopied to preserve the image on the thermal paper, and will be stored in individual subject's study file for source data verification.

NOTE: If glucose results are not to be used due to operator errors, insufficient sample, etc., this should be clearly marked on the YSI source document.

The YSI operator will record all non-numeric codes - QNS (Quantity not sufficient), NO (Not Obtainable), and error codes as appropriate for the YSI tests.

5.12 Subject Questionnaires

Site staff will administer two questionnaires to subjects. Questionnaire 1 will be given to all subjects and Questionnaire 2 will be given only to subjects with diabetes.

5.12.1 Questionnaire 1: Evaluation of Instructions for Use. (All subjects)

Staff will administer a questionnaire to all subjects who perform fingerstick self-testing after the meter testing is completed.

The questionnaire will include statements for which a numerical score or rating will be provided by subjects (1=strongly disagree, 2=disagree, 3=neutral, 4=agree, 5=strongly agree). These numerically scored statements will be tabulated into frequency distributions. The following statements will be subject to the pass/fail criteria listed in the study objectives section:

- I find it easy to do a fingerstick blood test with this meter.
- The meter display is easy to see and read.
- It was easy to understand my test results.
- The instructions (User Guide and Quick Reference Guide) were easy to understand.
- The instructions clearly explain how to run a test.
- The instructions clearly explain what to do if an error message is displayed by the meter. *Note: an error message will be demonstrated by the study staff if the subject does not experience an error message during his/her testing.*

Per ISO 15197:2013, Section 8.8.2, the questionnaire will also contain a section whereby the subject can provide unrestricted comments on their experience about using the BGMS. This

questionnaire will be administered by the staff who will record the subject's responses. The subject will be given an example questionnaire to assist with providing the responses.

5.12.2 Questionnaire 2: BGMS Features. (Only subjects with diabetes)

After all testing and the first questionnaire have been completed, the study staff will demonstrate to only the subjects with diabetes several features of the BGMS that would not necessarily be experienced during the brief accuracy testing. This will ensure that the subjects are able to understand the features, and answer questions in the questionnaire.

There are no acceptance criteria for this questionnaire. This questionnaire may be 1) administered by the staff who will record the subject's responses or 2) completed by the subjects and verified by the study staff, as per site procedures. In the first scenario, the subject will be given an example questionnaire to assist with providing the responses.

5.13 Device Problem Reporting

Any functional problems with the T-Plus BGMS will be documented by the study staff and reported timely to Ascensia. The study staff should be specific about describing the problem and the sequence of events that led to it. All information will be documented on the Problem Reporter Form, including the meter type, serial number and test strip lot. Malfunctioning BGMs will be replaced and this will be documented for study tracking. If appropriate, such meters will be disinfected and shipped back to Ascensia for evaluation.

5.14 Temperature and Humidity Monitoring

The study staff will measure the temperature and humidity of the meter and YSI testing area(s) twice a day (AM and PM) using an audited thermometer/hygrometer supplied by Ascensia, and record the results on the appropriate form.

5.15 Miscellaneous

Ascensia representatives may observe subject visits as part of study staff training, study monitoring or for troubleshooting problems with the investigational devices. This will be done under supervision of the investigator.

Ascensia representatives may assist with disinfection of the meters, and perform centrifugation of blood samples and measurement of hematocrit.

Ascensia representatives will not train or interact in any way with the subjects. The presence of Ascensia representatives will be noted in the ICF.

Subjects will receive nominal compensation for their time and inconvenience.

6.0 Risk / Benefit Assessment

Risks associated with this study include those associated with obtaining blood from finger, palm and venipuncture.

The following risks are anticipated and will not be reported as Adverse Events (AE). (See section 7.0 for further details.)

1) Bleeding: Mild bleeding similar to being stuck with a pin
2) Swelling (Edema): A mild swelling similar to a mosquito bite
3) Bruising: Less than one inch across (fingerstick) or less than 2 inches across (venipuncture)
4) Redness (Erythema): Mild reddening or darkening of the skin in the immediate area around the puncture site
5) Soreness: Mild discomfort at the puncture site resolving (going away) within one to two days (fingerstick or palm) or within hours (venipuncture)

The following risks are unlikely, but possible, and will be reported as anticipated Adverse Events:

1) Bleeding: Continued bleeding requiring changing a bandage
2) Bruising: A worsening bruise that is firm to the touch
3) Redness (Erythema): Bright redness or skin darkening spreading across the area (fingerstick or palm) or across the arm (venipuncture)
4) Soreness: Worsening pain and/or pain that interferes with activity
5) Infection: Bright redness or skin darkening spreading across the area with warmness and/or swelling, and/or red streaking
6) Nerve injury: Numbness or shooting pain in the forearm
7) Numbness in the area of the puncture wound
8) Dizziness
9) Fainting

Subjects should be instructed to report such events to the investigator or designee.

Benefits

A benefit associated with the study is the sense of well-being gained by contributing to the development of improved or new blood glucose monitoring systems, which may be beneficial to people with diabetes or other associated diseases or conditions.

7.0 Adverse Event Management and Medical Device Reporting

The procedures to be performed under this protocol are considered to be low risk.

7.1 Description and Classification

7.1.1. An AE during a clinical evaluation is "any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device." It is not dependent on whether the event is considered to be related to the investigational product or the study. An AE includes events not seen at the beginning of the study, or worsened if present at the beginning. Adverse events can be classified as presented in Table 7.1.

7.1.2. The next classification to consider is whether or not the AE is Anticipated or Unanticipated. In order for an AE to be considered 'anticipated' it must be listed in the protocol and ICF prior to any occurrence.

7.1.3. For studies on products that have not yet been cleared for market, it is important that all AEs be documented and included in the study file.

7.1.4. An unanticipated adverse device effect (UADE) is: Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with a device, if that effect, problem or death was not previously identified in nature, severity or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety or welfare of subjects [21CFR812.3(s)].

7.1.5. All AEs observed by the Investigator or reported by the subject will be documented on the AE form (including all symptoms) and included in the study file.

7.2 Events related to hypoglycemia and hyperglycemia

Hypoglycemia and hyperglycemia can be a common occurrence with subjects who have diabetes.

If it is found that a subject has a hypoglycemic event, as determined by the Principal Investigator (PI) during a study visit, the subject will be treated by the site staff accordingly. Appendices D and E note each site's treatment plan.

Classification of the hypoglycemic or hyperglycemic event is at the discretion of the PI or designee. If the hypoglycemic or hyperglycemic event is considered by the PI as an AE, it will be considered to be an anticipated AE and will be recorded on the AE Form.

Subjects who are hypo- or hyperglycemic may continue with fingerstick testing if they so choose, and if the PI determines that it is safe. The PI will define what is considered to be a hypoglycemic or hyperglycemic state.

Table 7.1: Classification of Adverse Event

Serious*	Non-Serious
An adverse event that leads to: a) death; b) a serious deterioration in health of the subject that, 1) resulted in a life-threatening illness or injury, 2) resulted in a permanent impairment of a body structure or a body function, 3) required in-patient hospitalization or prolongation of existing hospitalization, 4) resulted in medical or surgical intervention to prevent permanent impairment to body structure or a body function; c) fetal distress or a congenital abnormality or birth defect. May be anticipated or unanticipated. **EN ISO 14155. Clinical investigation of medical devices for human subjects – Good Clinical Practice*	All adverse events not reported as serious. At each visit, all adverse events observed by the investigator/designate or reported by subject must be evaluated and recorded on an adverse event form. A non-serious adverse event is further classified with respect to severity and relationship to the trial product. A non-serious adverse event may be anticipated or unanticipated. Further classification with regard to severity • Mild: Transient symptoms, easily tolerated, no interference with subjects daily activities. • Moderate: Marked symptoms, moderate interference with subjects daily activities, and tolerable. • Severe: Considerable interference with subject's daily activities, not tolerable.
Relationship to trial product: • Definitely Related: Causal relationship has been established and documented • Probably Related: Good reasons and sufficient documentation to assume causal relationship have been established • Possibly Related: Causal relationship is likely and cannot be excluded • Probably Not Related: Good reasons and sufficient documentation not established to assume causal relationship • Not Related: The event is most likely related to an etiology other than the trial treatment	

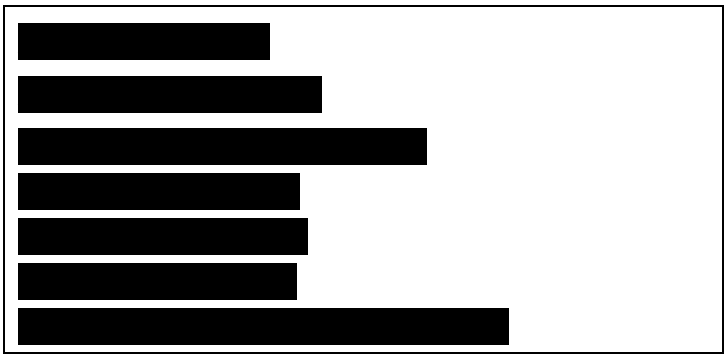
7.3 Procedure for Reporting an Adverse Event

Adverse events will be documented during this study by completing the AE Form. The Investigator or designee will sign and date an AE Form for each AE experienced by any subject. During the visit, AEs will be evaluated by a member of the study staff. The nature of each event and date of onset, outcome, course, maximum intensity and action taken with respect to treatment should be established. Details of any corrective treatment should be recorded on the AE Form. Investigators should follow-up on the status of subjects experiencing an ongoing AE until the event has been resolved, or until the condition has stabilized.

7.3.1. The Investigator or designee will notify the Study Manager or Monitor within 24 hours of any Serious Adverse Event that occurred during the study. The sponsor will promptly review all information relevant to the safety of the investigational device.

7.3.2. The investigator or designee must notify the sponsor and reviewing IRB by phone or fax of Unanticipated Serious Device-Related Adverse Events within 24 hours of learning of the event, followed by a written a report within 10 working days after learning of the event.

7.3.3. Upon receipt of a report of an UADE by the Ascensia Study Manager or Monitor, the report will be immediately forwarded to:



7.3.4. The sponsor (Ascensia) must report UADEs to the FDA, all participating investigators, and reviewing IRBs within 10 working days after the sponsor first receives notice of the event.

7.3.5. Regardless of the above definitions, any additional adverse experience that the investigator considers serious, and/or of concern in relationship to the study must be documented and reported to the Study Manager or Monitor within 24 hours.

7.4 Medical Device Reporting

The Microlet NEXT lancing device system is an Ascensia marketed product in the US. If an AE occurs that is directly caused by the marketed product, the AE should be reported immediately, but in any case within 24 hours, to the Customer Service Department at 1-800-348-8100. Also contact the Ascensia representative listed in Section 12.0.

8.0 Study Termination and Subject Withdrawal Criteria

8.1 Criteria to Withdraw / Discontinue a Subject from Study

Subjects who experience an AE will be withdrawn from the study. Subjects may be withdrawn from the study at their own request for any reason, or at the discretion of the PI (or designee) for one of the following reasons:

1) Illness of a subject
2) Subject non-compliance with protocol requirements
3) Other, at the discretion of the investigator

In such cases, the subject will be withdrawn from further study participation. Any evaluable data collected from withdrawn subjects will be analyzed and results will be retained for safety assessments. If available, data will be retained in analyses related to the objectives of the study unless there is a valid reason to believe that the data may be biased, incorrect, or confounded.

9.0 Regulatory

9.1 Regulatory Status of the Investigational Devices

In addition to the regulations outlined in the Ascensia Diabetes Care Global Clinical Affairs standard operating procedures, this protocol also complies with the clinical trial regulations of British Standard BS EN 13612:2002, "Performance evaluation of in vitro diagnostic medical devices."

The T-Plus meters, which is the focus of this study, is an investigational devices. For studies involving an investigational device, the sponsor is responsible for determining whether submission of an IDE application to FDA is required before a study may proceed. The IDE regulations (21 CFR 812) describe three types of device studies: significant risk (SR), nonsignificant risk (NSR), and exempt studies.

Ascensia has determined that the T-Plus study is a **Non-Significant Risk (NSR) study** which must follow the abbreviated IDE requirements of 21 CFR 812.2(b), including informed consent and IRB review, and do not require submission of an IDE application to FDA. As a result of this conclusion, Ascensia has not had correspondence with the FDA regarding determination of study classification and risk level.

Determination of the NSR classification is based on the following:

- T-Plus meters are not intended to be implantable devices.
- T-Plus meters are not purported or represented to be for use in supporting or sustaining human life
- T-Plus meters are not intended to provide substantial importance in diagnosing, curing, mitigating, or treating diabetes, or otherwise preventing impairment of human health.
- T-Plus meters do not present a potential for serious risk to the health, safety, or welfare of a subject.

9.2 Investigational Review Board (IRB) Approval

Prior to study initiation, an IRB must review this protocol, the ICF, and any other supporting study documents which impact subject safety. The investigational site may not begin the study until the IRB has given its written and dated approval via a letter that identifies the version/date of the protocol and ICF. A copy of the IRB approval letter and approved ICF must be provided to the Investigator and to Ascensia prior to the Study Initiation Visit.

9.3 Informed Consent Requirement

Each subject must provide informed consent before she/he can participate in the study. The informed consent process fully apprises the subjects of the risks and benefits to them and to society for participating in the study. The ICF will clearly state that designated study personnel will be able to view the subject's medical records. The ICF will also state that Ascensia representatives may observe some subjects as part of study staff training, study monitoring or for troubleshooting problems with the investigational devices.

If the subject understands and agrees to participate in the study, she/he will sign the ICF. All subjects will be given a signed copy of the ICF. If a subject has a question about his/her rights, he/she may contact a member of the IRB at any time during or after study participation.

9.4 Study Documentation Procedures

The investigator will keep study records for a minimum of three years. Alternatively, other arrangements may be made with Ascensia for study document storage.

9.5 Study Monitoring

The study will be monitored by Ascensia representatives. A monitoring plan will be completed by the Study Manager/designee prior to the study. The Study Manager or designee will conduct a study initiation visit, combined with 1st monitoring visit, at least 3 interim monitoring visits and a close-out visit. Note: the latter two visits may occur within a continuous timeframe. Study monitoring will be performed on site as well as by remote monitoring as per the monitoring plan. Per site/sponsor agreement, selected source documents will be scanned and sent to the sponsor for remote monitoring by the sponsor. Some of the critical data points that will be monitored 100% which include, but not limited to:

- Meter results
- YSI control and subject plasma results
- Centrifugation times

Ascensia representatives may observe some subject visits as part of study monitoring. This will be done under supervision of the investigator. Ascensia representatives will maintain subject confidentiality and will not interfere with rights of human subjects, safety or bias study conduct.

9.6 Investigator's Report of Study Closure

Ascensia representatives will send a letter to the site informing them that the study is closed. The study will be considered closed when the data has been locked for data analysis.

The Investigator or designee will submit a report summarizing subject disposition and other study details, as appropriate, to the Ascensia Study Manager and the reviewing IRB. This report will be completed within 3 months of the study closure date.

In addition, the Ascensia Study Manager, or designee, will report the completion of the study to the IRB within 6 months of study closure.

9.7 Registration of Trial

The clinical trial will be registered with www.clinicaltrials.gov as required.

9.8 Clinical Study Reports (CSR)

Two CSRs will be written by the sponsor for T-PLUS: one report will evaluate data according to ISO 15197:2013 criteria and one report according to FDA SMBG:2018 criteria.

10.0 Data Collection and Management

A unique number will identify each subject. The unique number will be the only identifying information entered. A master list of subject names, with their subject IDs, will be kept by the Investigator at the study site. The Investigator will retain all signed IC documents.

Clinical data will be primarily collected through an EDC system used by Ascensia. Study data will be recorded on forms that will serve as source documents for the EDC system. The YSI tapes will be the source documents for these test results. These original Forms will be retained by the site.

In addition to data collection, the EDC system will be used for data cleaning as well as monitoring operations. Site and sponsor users will be trained on the system prior to the study start, and their access to EDC system will be contingent upon successful completion of training requirements. Study personnel will complete and sign all appropriate source document forms in compliance with Good Clinical Practice (GCP). Source documents (Forms) should be completed legibly, in black or blue ink. If it is necessary to make corrections, a single line should be drawn through the original entry, the new entry written in, and the correction initialed and dated by the individual making the correction.

The original forms will be retained by the Investigator for a minimum of three years.

YSI source documents (tapes) will be photocopied to preserve the image on the thermal paper. Site staff or designees who photocopy the YSI tapes will certify (with signature, date, and YSI ID) that the copy is a true and accurate representation of the original.

11.0 Data Analysis

The statistical analysis plan for this study is described in document GCA-SAP-2019-001-01.

In general, meter results satisfying the pertinent objective criteria will be considered “successes” in the Bernoulli sense.

All fingerstick and AST meter test results will be compared to the capillary YSI laboratory results.

All venous blood meter test results will be compared to the venous blood YSI laboratory results.

11.1 Primary Objective, 2.1.1, and Secondary Objectives 2.2.1, 2.2.2 (ISO 15197)

The percentages of results from PWD subjects satisfying the ISO 15197 accuracy definition:

> **95% of glucose results shall fall within ±15mg/dL of laboratory method (LabBG) at glucose concentration <100 mg/dL and within ±15% at glucose concentrations ≥100 mg/dL**

will be computed.

If n = 315 PWD subjects (90% of 350), the critical value is 0.95*315 = 299.25 ≈ 300. Thus, the critical value is associated with testing the hypothesis:

H0: Pr{ISO Accurate result} < 0.9678

against the alternate:

H1: Pr{ISO Accurate result} ≥ 0.9678

(Pardo, 2014) [5]

The power to reject H0 at Pr{ISO Accurate result} = 0.9678 is approximately 0.9490.

Other Secondary Accuracy Objectives

Internal Accuracy Objectives: 2.2.8, 2.2.9

2.2.8 Criterion 1

Define criterion 1:

T-Plus Meter result with ±12.5mg/dL if LabBG < 100mg/dL, or

T-Plus Meter result within ±12.5% of corresponding Lab result (LabBG), if LabBG ≥ 100mg/dL

Test the hypothesis:

H0: p=Pr{meter result satisfies criterion 1} < 0.95

H1: p=Pr{meter result satisfies criterion 1} ≥ 0.95

The critical value, Xc (minimum number of X "successes") is determined to be the smallest number such that Pr{X≥Xc|p=0.95} ≤ 0.95. For n = 315, Xc = 294 (power ≈ 0.9263).

2.2.9 Criterion 2

Define criterion 2:

T-Plus Meter result with ±15mg/dL if YSI < 100mg/dL, or

T-Plus Meter result within ±15% of corresponding YSI result, if YSI ≥ 100mg/dL

Test the hypothesis:

H0: p=Pr{meter result satisfies criterion 2} < 0.95

H1: p=Pr{meter result satisfies criterion 2} ≥ 0.95

[5] Pardo, S., (2014) Equivalence and noninferiority Testing for Quality, Manufacturing, and Test Engineers, Chapman&Hall/CRC, Boca Raton

The critical value, Xc (minimum number of X "successes") is determined to be the smallest number such that $Pr\{X \geq Xc | p=0.95\} \leq 0.95$. If n = 315, Xc = 294 (power ≈ 0.9263). If n = 350, Xc = 327 (power ≈ 0.9246)

11.2 Secondary Objectives 2.2.4, 2.2.5 (FDA SMBG:2018)

a. At least 95% of n results will fall within ±15% of the corresponding YSI result

b. At least 99% of n results will fall within ±20% of the corresponding YSI result

These criteria, with n = 350, are equivalent to testing the hypotheses:

H0: Pr{T-Plus meter result within ±15% of LabBG} < 0.9664

H1: Pr{T-Plus meter result within ±15% of LabBG} ≥ 0.9664

with $Pr\{\text{reject } H0 | p=0.9664\} \approx 0.9488$

and:

H0: Pr{T-Plus meter result within ±20% of LabBG} < 0.9960

H1: Pr{T-Plus meter result within ±20% of LabBG} ≥ 0.9960

with $Pr\{\text{reject } H0 | p=0.9960\} \approx 0.9466$

11.3 Non-Accuracy Objective: Subject Questionnaire 1

To test the hypotheses for each questionnaire statement:

H0 : Pr{subject response is at least 3, or " Neutral" to 'Strongly Agree"} < 0.90

H1: Pr{subject response is at least 3, or " Neutral" to 'Strongly Agree"} ≥ 0.90

The minimum number of "successes", Xc, to reject H0 is determined such that

$Pr\{\text{Reject } H0 | p=0.90\} \leq 0.95$.

Thus, with n = 315, Xc = 276, with power ≈ 0.9299.

11.4 Other Statistical Computations

Refer to statistical analysis plan, GCA-SAP-2019-001-01 .

12.0 Administration

All investigator and site staff communications regarding the study should be directed to the Ascensia Study Manager. Please contact the Ascensia Study Managers noted below for any questions or problems concerning the clinical trial.

[redacted]
[redacted]
[redacted] Pragathi.Shelat@Ascensia.com

[redacted]

[redacted]
[redacted]
[redacted]
[redacted] Maria.Viggiani@Ascensia.com

13.0 Sponsor Regulatory Binder

Documents to be collected prior to the start of the study include, but are not limited to:

1) CVs of Investigator and Sub-Investigators
2) Signature of PI on page 2 of protocol
3) IRB approval letter
4) IRB-approved ICF
5) Financial Disclosures for PI and Sub- Investigators
6) Clinical Trials Agreement (including Investigator Agreements for PIs and Sub-Investigators)
7) Site staff GCP training documentation

Data / documents to be collected during or after completion of the study include, but are not limited to:

1) EDC database
2) Progress Notes
3) Signature and Delegation Log (for all study staff)
4) Investigator's Report of Study Closure and IRB acknowledgement
5) Study-related training documentation

Appendix A - Instructions for Cleaning and Disinfecting Meters

Single-use items, such as test strips, test strip vials or lancing devices, shall not be used with multiple subjects and will be discarded in biohazardous containers after use. Blood glucose meters will be for single subject-use, and will be cleaned and disinfected before returning to Ascensia by study staff only. Study staff will disinfect meters using the EPA-registered disinfectant noted in the UG and below that is suitable for use on hard, non-porous surfaces. Ascensia will provide the disinfectant.

EPA Registration	Manufacturer	Product Name	Active Ingredient
67619-12	Clorox Professional Products Company	Clorox (Bleach) Germicidal Wipes	0.55% sodium hypochlorite

1. Follow the manufacturer's directions printed on the container for handling the Clorox Germicidal Wipes. Wear gloves.
2. Before disinfecting, carefully clean the meter with germicidal wipes until all soil is removed and dry as necessary with a clean paper towel.
3. Note: For proper disinfection, you must keep all meter surfaces wet for 60 seconds.
4. Using a new germicidal wipe, carefully wipe all outer surfaces of your meter until wet.
5. After wiping for 60 seconds, use a clean paper towel to dry the meter surfaces and the test strip port.
6. Dispose of all materials used in the cleaning and disinfecting procedure according to current laboratory biohazard procedures.

References

Protection of laboratory workers from occupationally acquired infections; approved guideline – third edition. CLSI document M29-A3. ISBN1-56238-567-4.

Rutala W, Weber D, Healthcare Infection Control Practices Advisory Committee. Guideline for disinfection and sterilization in healthcare facilities, 2008. CDC.

FDA, Processing/Reprocessing Medical Devices in Health Care Settings: Validation Methods and Labeling, May 2, 2011.

Appendix B - Instructions for Collection/Processing of Blood for YSI

Capillary Blood Collection

1. The study staff will perform a deep finger puncture using a Tenderlett, or similar, lancing device, on the subject's finger.
2. The study staff will express the blood and collect approximately 200 - 300 µL in a blood collection tube (e.g. BD Microtainer with lithium heparin). A hand warmer may be used to improve blood flow and facilitate sample collection. Detailed instructions are as follows:
   - Typically the best finger to choose is the middle finger. It is recommended to practice the finger massaging technique prior to puncture.
   - Give subject a hand-warmer to hold for several minutes prior to puncture. Note that the hand-warmer should be moved to the subject's wrist when ready for puncture and kept there during the whole collection procedure.
   - Angle finger with tip slightly downward and squeeze fingertip gently prior to puncture.
   - Consider placement of finger puncture device: Choose a puncture site that will allow you to use your dominant hand to collect the blood. The device should be aligned so that the opening (where the blade comes out) is perpendicular to the grooves of the fingerprint and so that the opening is placed to the side of the finger (not too close to the nail).
   - While continuing to hold the fingertip, squeeze firmly, hold device firmly against side of finger and trigger the blade. Then turn the finger so that the nail is facing up.
   - Remove pressure from fingertip (to allow capillaries to fill with blood) and collect the first drop.
   - Begin massaging motion – continuous message of sides of the finger – this is critical.
   - Continue massaging finger from base to tip as you continue to collect the blood drops into the tube. If blood flow reduces it may help to press on the nail a bit in between massaging the finger. Having the subject stand up and drop their hand may also help.

Processing of Capillary or Venous Blood for YSI Analysis

1. Once collected, process the microtainer tube for laboratory glucose analysis as follows:
   - Label all tubes with the subject number and a C or a V (Capillary or Venous) as appropriate using a Sharpie marker.
   - Gently invert the microtainer tube several times to mix the anticoagulant.
   - Centrifuge the whole blood immediately to separate the plasma from the red blood cells. Centrifugation must occur within 15 minutes of first meter blood test. Timing of all meter testing/centrifugation will be recorded on the appropriate CRF.
   - It is recommended that after centrifugation, the plasma is transferred from the microtainer tube to a micro/dispo tube or similar container. Ensure the container is labeled and the cap is secured.
   - The capped plasma sample may be kept at room temperature for up to 4 hours before testing.

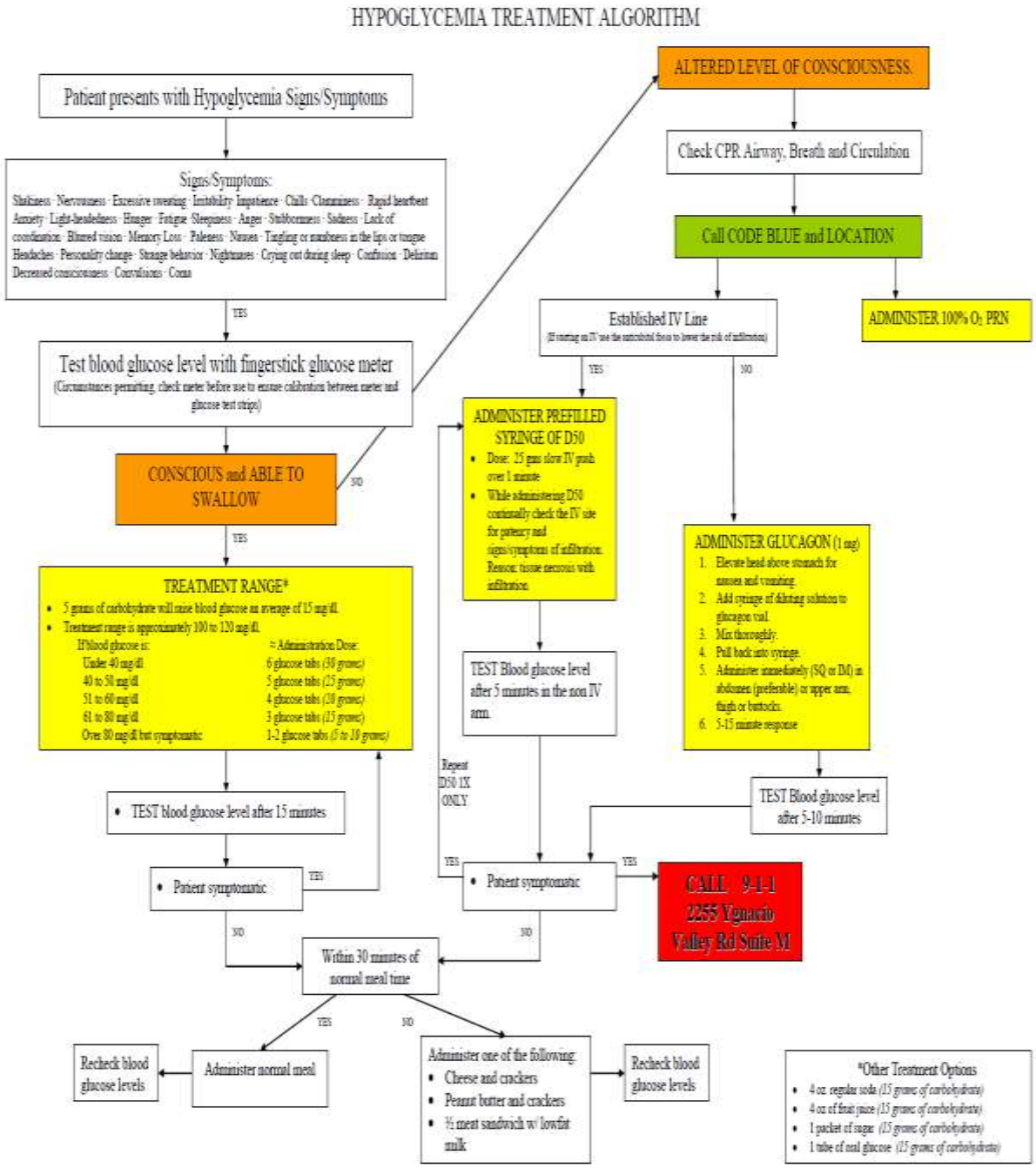
2. If testing on the YSI Analyzer is not completed within 4 hours of sample collection, the plasma should be refrigerated for up to 24 hours, after which it should be frozen until the sample is tested.
3. Shipment of samples to Ascensia for glucose determinations may be recommended if YSI Analyzers do not meet the accuracy guidelines for the Ascensia traceability control sera. Arrangements will be made to ship the samples with dry ice via prepaid express mail should it become necessary.
4. Glucose values of the YSI replicates (controls and plasma) should be within 4 mg/dL or 4% of each other; if not, an additional assay should be run.
5. Insufficient (or suspected to be insufficient) samples, missing samples, and other sample issues should be recorded in the CRF and on the YSI printout as appropriate. If a sample is hemolyzed, record in the CRF.

Appendix C - Instructions for Hematocrit Sample Preparation & Testing

Staff performing the hematocrit will be trained in this procedure during the initiation visit by the study manager or designee.

1. Two hematocrit tubes will be obtained from each subject's blood. One tube will be measured and the result recorded on the form. The second tube will be measured only if the first tube was not adequate for measurement. Note:
   - Sample that is collected from venous blood will be collected in **un-treated micro-hematocrit tubes**.
   - Samples collected from capillary fingerstick blood will be collected in **treated micro-hematocrit tubes.**
2. Place a microhematocrit tube against the surface of the blood drop. Do not place the opening of the tube flush against the skin or it will be occluded.
3. Fill the microhematocrit tube about halfway. Do not over-fill. Then immediately place one of the microhematocrit tube openings in the clay sealant. Press into the clay 2-3 times to make sure enough sealant is transferred.
4. Place the sealed microhematocrit tube into the micro-centrifuge. Note: it is very important to place the microhematocrit tube in centrifuge rotor with clay side pointed outward. Spin for approx. 2 mins. Use dialed setting on StatSpin centrifuge.
5. Measure hematocrit using StatSpin Crit-reader or other hematocrit reader approved by the sponsor.
6. Instructions for the StatSpin Crit-reader:
   - After collection and centrifugation of hematocrit, place tube rotor in StatSpin Crit-reader, with clay side to the right.
   - Slide the marker on the crit-reader to the exact line where the clay and red blood cells meet (this is the "0" line). Press set button until "0" shows in the display.
   - Then move marker all the way over to the other end of the tube to the exact line where the plasma ends – press the set button until "100" shows in the display.
   - Now you are ready to read the hematocrit result. Slide the marker to the exact line where the red blood cells meet the plasma.
   - The result in the display is the hematocrit percentage for that sample.
7. An alternative to Step 6 is manual reading of the hematocrit by the operator using Manual StatSpin Micro Hematocrit Capillary Tube Reader.
8. Record hematocrit result on the appropriate form.

Appendix D - Hypoglycemia Protocol at the trial site



Attachment 1: Proper Handling of Ascensia YSI Serum Controls

Ascensia R&D completed characterization of six-level serum controls purchased from Bio-Techne® (formerly Bionostics). During the characterization, staff observed that sample stratification during thawing can occur if the control vials are not handled judiciously. The recommended procedure is as follows.

1. Thaw the frozen control amber glass vials at 2-8ºC for 2-3 days in the refrigerator. Each vial holds approximately 2.0 mL.
2. Before opening, mix the vials well by gently inverting them at least 10 times.
3. Prepare an aliquot of each level in capped microcentrifuge tubes. Cap tightly. We prefer Fisherbrand™ Microcentrifuge 0.5mL tubes (PN 02-681-333) with O-ring seal caps (PN 02-681-358).
4. Store any remaining control in the amber glass vials at 2-8ºC.
5. Before running a control sample on the YSI, mix the aliquot tubes by gently inverting them.
6. At the beginning of each day, remix the amber glass vials and refill the aliquot tubes. When not in use, store the aliquots at 2-8ºC.
7. The thawed control use life is two weeks. Discard all thawed control—aliquot tubes and amber glass vials—after two weeks.
8. Since slow thawing for 2-3 days is recommended, it is advisable to keep an extra thawed set available for testing.