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Subject: FINAL REPORT for ACE_011 PK
Attach: 04-26-11_Ace-011.doc

Hi Peter,

Attached is the final report after the final internal QA and audit. I have attached the validation protocol and the signed amendments in the appendix.

Let me know what the next steps are.

Cheers

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ITLE PAGE

VALIDATION REPORT

Study Title	Determination of ACE-011 in Human Whole Blood using the Theranos Field System
Celgene Study Number	ACE-011-DMPK-001
Principal Investigator	Surekha Gangakhedkar, M.S.
Sponsor	Celgene Corporation 86 Morris Avenue Summit, New Jersey 07901 USA
Test Site	Theranos, Inc 3200 Hillview Ave, Palo Alto, CA 94304
Test Site Study Number	CELG-0004
Study Initiation Date	December 13, 2010
Experimental Completion Date	January 11, 2011
Report Issued	March 1, 2011
Total Pages	77

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4 ABBREVIATIONS

This section provides abbreviations and definitions of terms and concepts that may be commonly used throughout this report.

ALQ	Above the Limit of Quantification
BLQ	Below the Limit of Quantification
COA	Certificate of Analysis
Conc.	Concentration
CV	Coefficient of Variance
DFE	Difference from Expected
ELISA	Enzyme-linked Immunosorbent Assay
LLOQ	Lower Limit of Quantification. The lowest concentration for which an accurate and precise measurement can be obtained.
N	Number of cartridge replicates
NA	Not Applicable
ng/mL	Nanogram per milliliter
NR	Not Reportable
OORH	Out of Range High. Result when the signal is below S_{Min}.
OORL	Out of Range Low. Result when the signal is above S_{Max}.
QC	Quality Control
r^2	Coefficient of Determination
RLU	Relative Light Units
RT	Room Temperature
S.D.	Standard Deviation
S_{Min}	Minimum Signal for concentration quantification; corresponds to the calculated signal for 1.15*Expected ULOQ using the 4 Parameter Logistic model
S_{Max}	Maximum Signal for concentration quantification; corresponds to the calculated signal for 0.80*Expected LLOQ using the 4 Parameter Logistic model
SOP	Standard Operating Procedure
µL	Microliter(s)
ULOQ	Upper Limit of Quantitation. The highest concentration for which an accurate and precise measurement can be obtained.

5 COMPLIANCE STATEMENT

Study Title: Determination of ACE-011 in Human Whole Blood using the Theranos Field System

This study was conducted in compliance with Theranos Standard Operating Procedures (SOPs) and in accordance with the principles of the Food and Drug Administration (FDA) Good Laboratory Practice Regulations (GLP) as set forth in Title 21 of the U.S. Code of Federal Regulations Part 58; and the FDA Guidance for Industry: Bioanalytical Method Validation, May 2001.

_____ _____

Surekha Gangakhedkar, M.S. Date
Principal Investigator
Theranos, Inc

6 QUALITY ASSURANCE STATEMENT

Study Title: Determination of ACE-011 in Human Whole Blood using the Theranos Field System

Principal Investigator: Surekha Gangakhedkar, M.S.

This bioanalytical method validation report has been audited by the Quality Assurance Unit of Theranos, Inc and has been found to accurately represent the method validated in this study. Within the scope of this audit and review, the reported results accurately reflect the raw data. The type of audit performed, the date the audit was performed, and the date the audit findings were reported to the principal investigator (study director) and management are summarized below.

Audit Type	QA Auditor	Audit Dates	Date Audit Findings Reported to Theranos Principal Investigator and Management
Draft Protocol	Don Vu	12/06/10	12/22/10
Study Records	Javier Quinonez	01/11/11	01/11/11
Draft Report	Javier Quinonez	01/11/11	01/11/11
Final Report	Sukhdev Singh Bainiwal	04/20/11-04/26/11	04/26/11

Javier Quinonez
Quality Assurance
Theranos, Inc

Date

7 RESPONSIBLE PERSONNEL

Surekha Gangakhedkar, M.S.	Assay Systems Manager, Principal Investigator
Kapil Gadkar, Ph.D.	Project Manager
Gary Frenzel, B.S.	VP Assay Systems
Tina Noyes, M.S.	Senior Scientist
Javier Quinonez	QA Auditor

8 ARCHIVE STATEMENT

All raw data (Theranos SOP-00063), this bioanalytical report, and required supporting information for this study will be held under the control of Theranos, Inc. 3200 Hillview Ave, Palo Alto, CA 94304, USA.

A copy of the final report will be sent to Celgene Corporation.

9 SIGNATURE PAGE

Study Title: Determination of ACE-011 in Human Whole Blood using the Theranos Field System

This report accurately describes the data obtained in the study. I have reviewed the study and agree that the data supports the conclusions stated herein.

Surekha Gangakhedkar, M.S. Principal Investigator Theranos, Inc	Date
Gary Frenzel, B.S. VP Assay Systems Theranos, Inc	Date

SIGNATURE OF FINAL REPORT REVIEW, CELGENE CORPORATION

Peter D Bryan, Ph.D. Associate Director – DMPK Celgene Corporation	Date

10 REPORT SUMMARY

10.1 Introduction

The objective of the study was to validate an ELISA method to quantify ACE-011 in human whole blood using the Theranos System 3.0. The validated assay method will be used to determine ACE-011 in human whole blood samples generated during clinical studies.

10.2 Methods

A competitive ELISA implemented on a fully automated point of care system was validated for quantifying ACE-011 in human whole blood. In this assay, the capture surface consists of rabbit anti-goat antibody. The samples (including standards and QCs) are added to the cartridge by the operator and the cartridge inserted into the reader. The Theranos System 3.0 automatically prepares an aliquot of the sample, alkaline-phosphatase labeled ACE-011 and the anti-ACE-011 antibody (goat anti-ActRIIa) and adds the mixture to the capture surface. After the removal of unbound reagents by multiple wash steps, a chemiluminescent substrate is added. The response (Relative Light Units) is inversely proportional to the amount of analyte present. Calibrations are established using a standard curve consisting of ACE-011 analyzed by regression analysis performed by Theranos System 3.0 proprietary software [SOP-00081]. Once a calibration is established (during method validation or after each new lot of cartridges are produced), the concentration of ACE-011 in unknown samples is determined using the Theranos System 3.0. On-board control samples contained in the individual cartridges are used to assess the performance of the assay in addition to the precision of the two sample replicates.

Method validation will be performed with guidance from the FDA 2001 Bioanalytical Method Validation Guidance and with reference to Viswanathan et al. (2007) and DeSilva et al. (2003).

10.3 Results

A summary of the data for the calibration over 3 days in 3 different whole blood samples and the validation experiments are provided in Table 1. A total of 67 different instruments were used to generate the data in this validation report, not including stability. The assay has been validated for the quantitative determination of ACE-011 in human whole blood from 40.0 to 4000.0 ng/mL. The results indicated the method is sensitive, selective, accurate, and reproducible. The cartridges are stable when stored at 4ºC for 12 weeks (longer term stability is on-going). In addition, ACE-011 is stable in human whole blood for at least approximately 14 minutes after loading the sample into the cartridge, before starting the run.

Table 1: Validation Summary of ACE-011 in Human Whole Blood

Report Title	Determination of ACE-011 in Human Whole Blood using the Theranos Field System
Report Number	Theranos Project Number: CELG-0004 Celgene No. ACE-011-DMPK-001
Analyte Name and Synonym	ACE-011 (ActRIIA-IgG_1)
Sample Volume	20 µL per cartridge
Analytical Method Type	Competitive ELISA
Sample Processing Method	None
Calibration Range	40.0 – 4000.0 ng/mL
Standard Curve Concentrations	0, 20*, 40, 80, 125, 250, 500, 1000, 2000, 4000 and 8000* ng/mL
Lower Limit Of Quantitation (LLOQ)	40.0 ng/mL
Upper Limit Of Quantitation (ULOQ)	4000.0 ng/mL
QC Concentrations	40, 120, 400, 3000 and 4000 ng/mL

* Anchor points

Experiment	**Result**	**Criteria Met**
Inter-Instrument Precision N=24 Instruments (CV%)	10.6%	Yes
Inter-Instrument Accuracy N=24 Instruments (% Recovery)	93.3%	Yes
Intra-Day Precision at 5 QC Levels N=6 per level (CV%)	6.0 to 19.7%	Yes
Intra-Day Accuracy at 5 QC Levels N=6 per level (% Recovery)	79.8 to 106.8%	Yes
Inter-Day Precision at 5 QC Levels N=18 per level (CV%)	11.2 to 14.5%	Yes
Inter-Day Accuracy at 5 QC Levels N=18 per level (% Recovery)	82.8 to 101.4%	Yes
High Concentration Hook Effect Test (12,000 ng/mL) N=5	100% OORH	Yes
Instrument Carryover Test (0 ng/mL run after 4000 ng/mL) N=9	100% OORL	Yes
Selectivity: Recovery at 1000 ng/mL (% Recovery)	19 out of 20 samples were 100±25% nominal	Yes
Selectivity: Recovery at 40 ng/mL (LLOQ) (% Recovery)	17 out of 20 samples were 100±25% nominal	Yes
Selectivity: Recovery at 0 ng/mL (un-spiked) (% Recovery)	19 out of 20 samples were OORL	Yes
Process Stability: Precision at 500 ng/mL N = 15 (CV%)	9.4 %	Yes
Process Stability: Accuracy at 500 ng/mL N = 15 (% Recovery)	93.1 %	Yes
Cartridge Stability Week 1-12 at 4°C: Precision (CV%)	7.1 to 20.4%	Yes
Cartridge Stability Week 1-12 at 4°C: Accuracy (% Recovery)	82.1 to 121.4%	Yes

10.4 Conclusion

An ELISA method (Celgene number ACE-011-DMPK-001) has been validated on a fully automated point-of-care system for the quantitative determination of ACE-011 in human whole blood from 40.0 to 4000.0 ng/mL. The Theranos System 3.0 consists of a customized cartridge, a reader instrument, and an integrated data management and analysis system. A total of 67 different instruments were used to generate the data in this validation report, not including stability data. The results indicate that the method is sensitive, accurate, reproducible, and selective for ACE-011 in human whole blood. The cartridges are stable for at least 12 weeks when stored in their sealed foil pouches in a refrigerator at 4°C (stability study is on-going). The on-board controls provide an indication of cartridge viability and can be used to identify cartridges exposed to unsuitable storage conditions. In addition, it was shown that ACE-011 is stable in spiked human whole blood for at least approximately 14 minutes after loading the sample into the cartridge, before starting the run. In conclusion, the Theranos System 3.0 is a suitable automated platform for accurately measuring ACE-011 in human whole blood.

11 MATERIALS AND EQUIPMENT

11.1 Chemicals and Reagents

HPLC grade Water, Baker
Phosphate Buffered Saline (PBS), Sigma
Sodium Azide, Sigma-Aldrich
Wash Buffer, Assay Designs
99% Pure BSA, Sigma
Heterophilic Blocking Reagent (HBR), Scantibodies
Rabbit anti-goat antibody, Southern Biotech
ACE-011, Provided by Sponsor
Goat anti-ActRIIa antibody, R&D Systems
Alkaline Phosphatase-SH Labeling Kit, Dojindo
PhosphoGlo Substrate, KPL
Pooled Human Serum, Bioreclamation (for cartridge stability)
Human Whole Blood, Stanford Blood Center (for all other experiments)

11.2 Sample-Processing Equipment

Equivalent equipment may be substituted on an as-needed basis.

Disposable Plastic 1.5 mL Microcentrifuge tubes, VWR
Eppendorf Pipette Tips for single and Multichannel pipettes
Eppendorf Single Channel Pipettes: 1-10µL, 20-200 µL
Finnpipette Novus Multichannel 20-300µL

11.3 Analytical Equipment

Theranos ACE-011 060 Cartridges and Theranos System 3.0

12 ANALYTE INFORMATION

Name:	ACE-011
Synonym	ActRIIa-IgG_1
Supplied Form	Pre-determined quantity 50 mg/mL
Lot Number:	09011-001
Storage Conditions:	-65°C or colder

13 DEFINITIONS

This section provides definitions of terms and concepts commonly used throughout this report.

Percent Difference from Expected (%DFE) $= \frac{\text{Signal- Expected Signal}}{\text{Expected Signal}} \times 100$

Precision = % Coefficient of Variation (%CV) $= \frac{\text{Standard Deviation}}{\text{Mean Concentration}} \times 100$

Accuracy = % Recovery $= \frac{\text{Determined Concentration}}{\text{Nominal Concentration}} \times 100$

Quality control samples at five concentration levels: 40.0, 120.0, 400.0, 3000.0 and 4000.0 ng/mL) were prepared. The 40.0 ng/mL concentration corresponds to the assay LLOQ and the 4000.0 ng/mL concentration corresponds to the assay ULOQ. The Low QC concentration was prepared at 3 times the targeted LLOQ concentration. Mid QC concentration was approximately in the middle of the calibration curve. High QC was approximately 75-80% of the targeted ULOQ concentration.

Calibration standard and QC concentrations were determined based upon the nominal concentrations.

All statistics in the data tables were calculated by Microsoft® Excel according to the equation applied by the Theranos System 3.0.

14 ACCEPTANCE CRITERIA

The validation acceptance criteria and the statistical data will be determined at a minimum to the agreed-upon method validation protocol (Appendix 22.1).

14.1 Run Acceptance Criteria

Within each cartridge, the standards or samples will be automatically assayed in duplicate. The RLU from both replicates will be used to construct the calibration curve. A back-calculated concentration will be obtained for each replicate. The cartridge concentration will be reported as the mean of the replicate concentrations. If both replicates are OORL or OORH the cartridge result will be OORL or OORH. If one replicate is OORH or OORL but the other replicate is quantifiable, the cartridge concentration will be reported as the concentration of the quantifiable replicate.

Cartridge acceptance criteria include:

(1) The on-board controls satisfy acceptance criteria: the response from both the controls should be within ±25% of the defined mean response and at least one of them should be within ±20% of the defined mean response. [1]

(2) The %CV from the two sample replicates is ≤ 25%.

The above metrics are evaluated by rounding to the nearest whole number.

If cartridge does not meet acceptance criteria, the result is reported as NR.

14.2 Method Acceptance

Calibration standards

For the validation run to be acceptable, a minimum of 75% of the total number of cartridge results in the calibration range over the 3 day calibration should be within 100±20% (100±25% at LLOQ and ULOQ standards) of their nominal values, and a minimum of six unique standard concentrations must be within the assay range. The calibration curve must contain one calibration standard at both the LLOQ and ULOQ of the range.

Intra-Day Accuracy and Precision

For method acceptance, the mean of back-calculated concentrations of the six (or more) replicates at each QC level for each day should not deviate more than ±20% (±25% for the LLOQ and ULOQ) from its corresponding nominal concentration. In addition, at least half of all the individual back-calculated concentrations from the six (or more) replicates for each QC level for each day must be within 100±20% (100±25% at the LLOQ and ULOQ) of their corresponding nominal values. The precision at each QC level for each day must not exceed

[1] The defined mean response is calculated as the average control signal for all cartridges in the 3 core runs.

20% (25% for LLOQ) when calculated as the %CV. The concentrations will be calculated using the whole blood calibration curve, which does not include the High, Mid and Low QC levels. No more than one QC outlier (Dixon test) may be excluded from the statistical calculations for a given validation run, and a maximum of two QC outliers may be excluded for the combined three core runs.

Inter-day Accuracy and Precision

Inter-day accuracy and precision will be evaluated over a period of three days. On each day, the QC levels specified in Table 2 will be spiked into a single whole blood sample. At least six replicate cartridges will be used per QC level on each of the days. For method acceptance, the mean of back-calculated concentrations of all the replicates from all three days at each QC level should not deviate more than ±20% (±25% for the LLOQ and ULOQ) from its corresponding nominal concentration. The precision of all the replicates from all three days at each QC level must not exceed 20% (25% for LLOQ) when calculated as the %CV. The concentration will be back-calculated using the whole blood calibration curve. No more than one QC outlier (Dixon test) may be excluded from the statistical calculations for a given validation run, and a maximum of two QC outliers may be excluded for the combined three core runs.

Selectivity

For the spiked samples, 14 out of 20 of the back-calculated concentrations from the individual whole blood samples must be within 25% of the corresponding nominal concentration. For the un-spiked samples, 14 of 20 must have a back-calculated concentration less than the target LLOQ. The concentration will be back-calculated using the whole blood calibration curve.

Inter-Instrument Precision

For acceptance, the %CV from 24 cartridges (run on 24 different instruments) with a mid-range concentration (500 ng/mL in a single whole blood sample) should be within 20%. The concentration will be back-calculated using the whole blood calibration curve.

High Concentration Hook Effect Test

Evaluate response of assay at a concentration of 12,000 ng/mL in whole blood with 5 replicate cartridges. The back-calculated concentration for this analyte level for all five replicates should be greater than ULOQ.

Instrument Carryover Test

The response of an un-spiked whole blood sample run immediately after a 4000 ng/mL spiked blood sample on the same instrument must be less than the LLOQ response to be considered acceptable.

Stability Test in Pre-built Cartridge

Stability will be evaluated using pooled serum calibrators (flash frozen and stored at -80°C)[2]. To establish acceptance, the mean back-calculated concentrations for each analyte level must be no more than ±20% from their nominal concentration. In addition, the precision (%CV) of all the replicates within the reportable range must not exceed 20%. Data not available as of the report date will be presented as an appendix to the validation report at a later date.

[2] Pooled serum calibrators are used for stability purposes to provide a uniform pooled matrix that can be stored frozen.

15 METHODS

15.1 Primary Stock Solution and 10X Calibrators

ACE-011 was provided at a concentration of 50 mg/mL. This stock solution was diluted serially in Assay Buffer (3% BSA in TBS with 0.05% sodium azide) to create a set of 10X calibrators and 10X QC standards as per the dilution series shown below. These standard solutions were aliquoted and stored at –80°C.

	1X	10x	Volume (uL)		
Calibrator #	ng/mL	ng/mL	Stock/Previous	Diluent	Total
1	8,000	80,000	10.0	6240.0	6250
2	4000	40,000	3000.0	3000.0	6000
3	2000	20,000	2400.0	2400.0	4800
4	1000	10,000	2600.0	2600.0	5200
5	500	5,000	3100.0	3100.0	6200
6	250	2,500	2600.0	2600.0	5200
7	125	1,250	3200.0	3200.0	6400
8	80	800	2304.0	1296.0	3600
9	40	400	1500.0	1500.0	3000
10	20	200	1000.0	1000.0	2000
11	0	0	0.0	2000.0	2000

	1X	10x	Volume (uL)		
Level	ng/mL	ng/mL	Stock/Previous	Diluent	Total
QC High	3,000	30,000	5.0	8295.0	8300
QC Mid	400	4,000	800.0	5200.0	6000
QC Low	120	1,200	1440.0	3360.0	4800

15.2 Preparation of Working Standards and Quality Control Samples in Human Whole Blood

To prepare working standards and QC samples, 1 part of each 10X solution was combined with 9 parts whole blood for each data point. For example, 25 µL of the 10X standard mixed with 225 µL whole blood for a total of 250 µL of spiked whole blood. Spikes were mixed into whole blood by pipetting up and down gently 8 times.

15.3 Sample Analysis on the Theranos System

Each cartridge was removed from its individual foil pouch and 20 uL of whole blood (calibrator, QC sample or other) was added to the sample well using a 20-200 uL single channel pipette. To initiate a sample run, the on-screen commands on the Theranos System 3.0 were followed and the cartridge was inserted into the reader drawer [SOP-00093].

16 ANALYTICAL METHOD SUMMARY

Typical ELISA parameters used in this method validation are listed in Table 2 below.

Table 2: Theranos ELISA Parameters

Parameter	Value of Parameter
Analyte	ACE-011
Matrix	Human whole blood
Calibration Standard Concentrations	0, 20*, 40, 80, 125, 250, 500, 1000, 2000, 4000 and 8000* ng/mL
Quality Control Concentrations	40.0, 120.0, 400.0, 3000.0 and 4000.0 ng/mL
Regression Type	weighted 4-Parameter Logistic
Sample Volume	20 µL whole blood per cartridge
Assay Procedure Summary	In this assay, the capture surface consists of rabbit anti-goat antibody. The samples (whole blood, serum or plasma), alkaline-phosphatase labeled ACE-011 and the anti-ACE-011 antibody (goat anti-ActRIIa) are added to the capture surface. After the removal of unbound reagents by multiple wash steps, a chemiluminescent substrate is added.

* Anchor points

17 RESULTS AND DISCUSSION

A total of three complete standard curves in three different whole blood samples on three days were performed during the method validation.

17.1 Standard Curve and Regression Analysis

Calibration standards were prepared with a different individual whole blood sample for each of three day runs. Standard calibration curves were determined for each of the three days and for the combined 3-Day data using weighted 4-Parameter Logistic regression and Theranos System 3.0. Table 3 shows the expected values for the on-board controls. Expected control values were determined by calculating the mean signal for all cartridges in the 3 core runs. Table 4 shows the 4-Parameter Logistic Regression parameters. Tables 5-7 show the raw data for the calibration runs for the three days. Table 8 shows the back-calculated concentrations for each of the 3 days. All concentration data shown in this report is derived from the combined 3-day calibration.

The acceptance criteria for the Calibration Standards was met with the percentage of cartridge replicates in the reportable range showing recovery within 100±20% (100±25% at the LLOQ and ULOQ) of the nominal concentration at 78% (70 of 90 cartridges).

17.2 Limits of Quantitation, S_{Min} and S_{Max}

For the experiments in the validation report, it is required to report concentrations outside the LLOQ and ULOQ range for evaluation of the acceptance criteria. The maximum and minimum signal (RLU) corresponding to the quantifiable range was determined by the 4 Parameter Logistic fit as S_{Max} and S_{Min} respectively. S_{Max} is the signal (RLU) corresponding to a concentration of 0.80 * Expected LLOQ and S_{Min} is the signal corresponding to a concentration of 1.15 * Expected ULOQ [Theranos SOP-00081]. For signal above S_{Max} the result was reported as OORL and for signal below S_{Min} the result was reported as OORH. This is applicable for the results of the pre-validation and validation only.

For in-study applications, all concentrations below the LLOQ will be reported as BQL and concentrations above the ULOQ will be reported as AQL.

17.3 Precision and Accuracy

Precision and accuracy of the method were determined by analyzing QC samples at three different concentrations within the standard curve range in addition to the LLOQ and ULOQ for each whole blood sample/day to validate reproducibility.

17.3.1 Intra-Day Accuracy and Precision

Intra-day accuracy and precision were evaluated for each of the three days. Six replicate cartridges were run per QC level, LLOQ and ULOQ on each of the days. The concentrations of

the QC levels were calculated using the whole blood calibration curve. Accuracy and precision raw data are shown in Tables 9-11, and the calculated concentration results are shown in Table 12. These results met the acceptance criteria with the mean concentrations of each level within 20% of nominal (25% at LLOQ and ULOQ) and 20% CV (25% for LLOQ) on each day, and with at least 50% of individual cartridge replicates at each level for each day meeting the above-stated accuracy criteria.

17.3.2 Inter-Day Accuracy and Precision

Inter-day accuracy and precision were evaluated over the three days using the QC levels and the LLOQ and ULOQ levels. Inter-day accuracy and precision results are shown in Table 13. These results met the acceptance criteria with the mean concentrations of each level within 20% of nominal (25% at LLOQ and ULOQ) and 20% CV (25% for LLOQ) on each day.

17.3.3 Inter-Instrument Accuracy and Precision

Inter-instrument accuracy and precision were evaluated over 24 different instruments at a mid-range concentration of 500 ng/mL spiked into a whole blood sample. Inter-instrument accuracy and precision raw data and concentration results are shown in Table 14 and 15. These results met the acceptance criteria, with the %CV over the 24 instruments less than 20%.

17.4 Matrix Specificity and Selectivity in Human Whole Blood

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. The selectivity test was performed by analyzing 20 different whole blood samples (10 male and 10 female) spiked with 1000 ng/mL, 40 ng/mL and without ACE-011. Table 16 shows the raw data and Table 17 shows the concentration results for the selectivity test. Of the 20 whole blood samples tested, 19 were below OORL when tested unspiked, 17 were within 25% of nominal spiked at 40 ng/mL and 19 were within 25% of nominal spiked at 1000 ng/mL. As an additional note, none of the individual whole blood samples failed to meet the acceptance criteria for more than 1 of the 3 levels. These results met the acceptance criteria of at least 14 of 20 spiked samples falling within 25% of the nominal concentration and at least 14 of the 20 unspiked samples reporting OORL.

17.5 Stability

Stability tests were used to evaluate the stability of the analyte during situations likely to be encountered during sample handling and analysis.

17.5.1 Process Stability

Stability of ACE-011 spiked in human whole blood at room temperature after addition to the Theranos 060 Cartridge sample well was evaluated. A sample of whole blood was spiked at 500 ng/mL and loaded into a number of cartridges using a stepper pipette. The cartridges were left sitting on the bench-top at room temperature over a span of up to 14 minutes before loading the

cartridge into the Theranos instruments. These cartridges were a subset of the cartridges used for the inter-instrument precision test. The raw data from the process stability test are shown in Table 18 and the concentration results are shown in Table 19. Figures 3 and 4 depict the data in graphical form. There was no significant trend in signal or concentration over the process stability test period. These results met the acceptance criteria.

17.5.2 Cartridge Stability

Stability tests are ongoing for manufactured cartridges stored at room temperature and 4ºC (the recommended storage condition). This stability test encompasses all the components of the cartridge including the capture surface, conjugate, substrate, and the on-board liquid controls. The cartridges were manufactured and packaged in individual sealed foil pouches and then some were stored at 4ºC in a glass-front refrigerator, and some at room temperature on the bench top. The stability tests are performed with 3 analyte levels of 3000 ng/mL (QCH), 120 ng/mL (QCL) and 0 ng/mL spiked into pooled serum, aliquoted and stored at -80ºC[3]. Time points to be tested include 0, 1, 2, 4, 8, 12, 24 and 48 weeks with three replicates for each analyte level for each of the time points. All cartridges include the on-board controls at 3000 ng/mL and 120 ng/mL in an assay buffer.

Tables 20-23 show the raw data and calculated concentration results gathered as of this report. Figures 5 and 6 depict the stability data in graphical form. At 4ºC, the recommended storage condition, the stability results meet the acceptance criteria for accuracy within 20% of nominal concentration[4] and precision within 20% CV through week 12. At room temperature, the stability results fail to meet the acceptance criteria at 2 weeks and beyond. The on-board controls also failed at and after 2 weeks at room temperature illustrating that the on-board controls are a good indication of cartridge viability. It is recommended that the cartridges are not allowed to remain unrefrigerated for a significant length of time.

17.6 High Concentration Hook Effect Test

The response of assay at very high concentrations was tested. A whole blood sample spiked with 12,000 ng/mL ACE-011 was tested with 5 replicate cartridges. The raw data are shown in Table 24 and the concentration results are shown in Table 25. The calculated concentrations for all 5 cartridges were OORH and the RLU for all 5 cartridges were less than the mean RLU (2465) corresponding to the ULOQ. These results met the acceptance criteria.

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[3] Pooled serum calibrators are used for stability purposes to provide a uniform pooled matrix that can be stored frozen.

[4] It was agreed to deviate from the method validation protocol which called for a comparison to the Day 0 recovered concentration and instead compare results to the nominal concentration. The Day 0 recovery for the 3000 ng/mL calibrator was 119%, therefore it was more accurate to compare stability results to the nominal concentration.

17.7 Instrument Carryover Test

To verify that instrument carryover is not a potential problem, an un-spiked whole blood sample was run on 9 different instruments immediately after a 4000 ng/mL spiked whole blood sample was run on the same instruments. The raw data are shown in Table 26 and the concentration results are shown in Table 27. The calculated concentrations for all 9 cartridges were OORL and the RLU were greater than the mean RLU (49768) corresponding to the LLOQ. These results met the acceptance criteria.

18 CONCLUSION

An ELISA method (Celgene number ACE-011-DMPK-001) has been validated for the quantitative determination of ACE-011 in human whole blood from 40.0 to 4000.0 ng/mL. The results indicated the method is sensitive, selective, accurate, and reproducible. The cartridges are stable when stored at 4°C for 12 weeks (longer term stability is on-going). In addition, ACE-011 is stable in human whole blood for at least approximately 14 minutes after loading the sample into the cartridge, before starting the run.

19 REFERENCE DOCUMENTS

- Method Validation Protocol "Determination of ACE-011 in Human Whole Blood using the Theranos Field Systems" Theranos Project Number: CELG-0004, Celgene Study Number: ACE-011-DMPK-001
- ACE-011 Assay Notebook
- Theranos Archival Processes SOP-00063
- Theranos Computational Biosciences SOP-00081
- Standard Laboratory Use of Theranos System 3.0 Reader and Cartridges SOP-00093
- Viswanathan et al. "Workshop/Conference Report — Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays." The AAPS Journal 2007; 9
- DeSilva, Binodh et al. "Recommendations for the Bioanalytical Method Validation of Ligand-binding Assays to Support Pharmacokinetic Assessments of Macromolecules." Pharmaceutical Research, Vol. 20, No. 11, November 2003
- FDA Guidance for Industry Bioanalytical Method Validation. May 2001

20 TABLES

Table 3: Expected Values for On-Board Controls, Signal (RLU)

		Within 20% DFE*		Within 25% DFE*	
Control Level	**Mean**	**Min**	**Max**	**Min**	**Max**
120 ng/mL	47267	37589	56975	35225	59339
3000 ng/mL	4577	3634	5508	3405	5736

* Allowable range is the minimum and maximum allowable %DFE rounded to the nearest whole number.

Table 4: Regression Analysis of ACE-011 Calibration in Human Whole Blood

Parameter	**Day 1**	**Day 2**	**Day 3**	**Combined 3 Day**
Min	1983.967	1945.124	1990.383	1869.051
Max	76417.858	75951.109	68926.901	77478.303
Slope	1.228	1.257	1.293	1.188
Ed50	65.215	63.966	75.839	58.189
LLOQ (ng/mL)	40.0	40.0	40.0	40.0
ULOQ (ng/mL)	4000.0	4000.0	4000.0	4000.0
Equation	RLU = Min+(Max-Min)/(1+(Conc/Ed50))^Slope			
S_{Max}	54503	54106	52403	52568
S_{Min}	2382	2287	2321	2286

Table 5: ACE-011 Calibration Replicate Data in Human Whole Blood Day 1, Signal (RLU)

[ACE-011] ng/mL	Cartridge Sample Replicates 1	2	Intra-Cartridge Mean	CV %	120 ng/mL Control RLU	% DFE	3000 ng/mL Control RLU	% DFE
8000	2249	2217	2233	1.0	49662	5.0	4506	-1.4
	1795	2131	1963	12.1	48203	1.9	4817	5.4
	2204	1951	2077	8.6	51398	8.7	5064	10.8
4000	2403	2593	2498	5.4	52549	11.1	4724	3.4
	2623	2495	2559	3.5	56637	19.8	4870	6.5
	3082	2319	2700	20.0	52880	11.8	4813	5.3
	2315	2532	2424	6.3	49734	5.2	4791	4.8
	2120†	2579	2350	13.8	46719	-1.2	4086	-10.6
	2352	2540	2446	5.4	46760	-1.1	4304	-5.8
2000	2755	3439	3097	15.6	47847	1.2	5063	10.8
	3068	3268	3168	4.5	41066	-13.1	4349	-4.9
	NR	NR	NR	-	61013	29.0*	6713	47.9*
1000	3595	3563	3579	0.6	47495	0.5	3996	-12.6
	4068	4138	4103	1.2	40499	-14.3	4373	-4.3
	3762	4067	3914	5.5	41769	-11.7	4189	-8.3
500	7103	6978	7040	1.2	40447	-14.5	4557	-0.3
	7504	7689	7596	1.7	46245	-2.2	5467	19.6
	7646	7837	7741	1.7	53186	12.5	5047	10.4
250	18788	14624	16706	17.6	48247	2.0	5709	24.9
	15722	13343	14532	11.6	56775	20.1	5355	17.2
	12513	13378	12945	4.7	41126	-13.0	4656	1.9
125	23021	24507	23764	4.4	47247	-0.1	5382	17.7
	20346	22832	21589	8.1	40769	-13.8	4676	2.3
	29208	27104	28156	5.3	46690	-1.3	5190	13.5
80	31113	30363	30738	1.7	41914	-11.4	3895	-14.8
	34197	36255	35226	4.1	52316	10.6	5126	12.1
	34228	38665	36446	8.6	53704	13.6	5354	17.1
40	47775	45604	46690	3.3	48889	3.4	4029	-11.9
	47254	48326	47790	1.6	42102	-11.0	4603	0.7
	53686†	54718‡	54202	1.3	44463	-6.0	4684	2.5
	48868	53889‡	51379	6.9	47394	0.2	4954	8.4
	54716‡	42336	48526	18.0	39714	-16.0	4231	-7.4
	47557	56957‡	52257	12.7	51034	7.9	4567	-0.1
20	57089	66045	61567	10.3	48576	2.7	4874	6.6
	66928	59600	63264	8.2	46302	-2.1	5353	17.1
	49084	53406	51245	6.0	43317	-8.4	4461	-2.4
0	70841	77712	74277	6.5	41781	-11.6	4426	-3.2
	72171	77054	74612	4.6	50172	6.1	5130	12.2
	NR	NR	NR	-	53661	13.5	5989	31.0*

N = 3 cartridges for regular calibration points, N = 6 cartridges for LLOQ and ULOQ
* Cartridge fails to meet internal acceptance criteria, concentration will not be reported (NR)
† RLU value for standard curve point below combined S_{Min}, ‡ RLU value for standard curve point above combined S_{Max},

Table 6: ACE-011 Calibration Replicate Data in Human Whole Blood Day 2, Signal (RLU)

[ACE-011] ng/mL	Cartridge Sample Replicates 1	2	Intra-Cartridge Mean	CV %	120 ng/mL Control RLU	% DFE	3000 ng/mL Control RLU	% DFE
8000	2299	2197	2248	3.2	43566	-7.9	4848	6.1
	1643	1975	1809	13.0	41324	-12.6	4761	4.2
	1848	1978	1913	4.8	38009	-19.6	4299	-5.9
4000	2111†	2558	2335	13.5	40924	-13.4	4045	-11.5
	2222†	2485	2354	7.9	49231	4.1	4972	8.8
	2473	2332	2403	4.2	50926	7.7	4821	5.5
	2337	2427	2382	2.7	59248	25.3	4515	-1.2
	2597	2337	2467	7.5	59220	25.2	4996	9.3
	2238†	2481	2360	7.3	57384	21.4	4301	-5.9
2000	3308	3310	3309	0.1	54294	14.8	4777	4.5
	2926	3113	3020	4.4	49680	5.1	4892	7.0
	2958	3531	3245	12.5	38592	-18.4	4134	-9.6
1000	4205	4728	4467	8.3	41383	-12.5	5185	13.4
	3939	4090	4014	2.7	53626	13.4	4641	1.5
	3781	3699	3740	1.6	38404	-18.8	4820	5.5
500	8114	7175	7644	8.7	42761	-9.6	4107	-10.2
	6088	6655	6372	6.3	39260	-17.0	4334	-5.2
	7315	6561	6938	7.7	50010	5.8	4202	-8.1
250	12122	12145	12133	0.1	52479	11.0	4400	-3.7
	11693	13297	12495	9.1	50797	7.4	5527	20.9
	12282	11991	12136	1.7	49700	5.1	4912	7.5
125	18764	23428	21096	15.6	54169	14.6	4290	-6.1
	28980	28274	28627	1.7	53011	12.1	4742	3.8
	23391	23946	23669	1.7	44567	-5.7	4304	-5.8
80	42238	40066	41152	3.7	58116	22.9	5149	12.6
	40103	46287	43195	10.1	44074	-6.8	5261	15.1
	N/A	N/A	-	-	N/A	-	N/A	-
40	41253	47407	44330	9.8	42491	-10.1	4443	-2.8
	41230	45206	43218	6.5	40768	-13.8	4452	-2.6
	50914	50348	50631	0.8	52808	11.7	4912	7.5
	50596	52882‡	51739	3.1	41551	-12.1	3881	-15.1
	47124	53378‡	50251	8.8	41650	-11.9	4152	-9.2
	46206	48804	47505	3.9	52610	11.3	3854	-15.7
20	58475	58315	58395	0.2	46134	-2.4	4027	-11.9
	55193	60335	57764	6.3	41759	-11.7	4825	5.6
	68673	62039	65356	7.2	46445	-1.8	4783	4.6
0	69169	67562	68366	1.7	44854	-5.1	4011	-12.2
	72089	60445	66267	12.4	46480	-1.7	4720	3.3
	74581	64696	69638	10.0	43365	-8.3	4718	3.2

N = 3 cartridges for regular calibration points, N = 6 cartridges for LLOQ and ULOQ
* Cartridge fails to meet internal acceptance criteria, concentration will not be reported (NR)
† RLU value for standard curve point below combined S_{Min}, ‡ RLU value for standard curve point above combined S_{Max},
N/A = Not Available (no results available for this cartridge)

Table 7: ACE-011 Calibration Replicate Data in Human Whole Blood Day 3, Signal (RLU)

[ACE-011] ng/mL	Cartridge Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
	1	2	Mean	CV %	RLU	% DFE	RLU	% DFE
8000	2210	2238	2224	0.9	45154	-4.5	5267	15.2
	1987	2216	2102	7.7	56674	19.9	4786	4.7
	1849	1879	1864	1.1	50811	7.5	4378	-4.2
4000	2433	2407	2420	0.8	44285	-6.3	4952	8.3
	2248†	2500	2374	7.5	44967	-4.9	4287	-6.2
	2665	2803	2734	3.6	48375	2.3	4663	2.0
	2427	2155†	2291	8.4	50822	7.5	4650	1.7
	2414	2489	2452	2.2	49930	5.6	4610	0.9
	2710	2607	2658	2.7	46827	-1.0	4288	-6.2
2000	2617	2012†	2315	18.5	36822	-22.1	4359	-4.6
	3756	2923	3339	17.6	51834	9.6	5286	15.7
	2871	2826	2848	1.1	38257	-19.1	4771	4.4
1000	4247	4813	4530	8.8	47156	-0.3	5191	13.6
	4315	2999	3657	25.4	43606	-7.8	4844	6.0
	5126	4465	4795	9.8	42911	-9.2	4132	-9.6
500	7261	7234	7247	0.3	52152	10.3	3614	-20.9
	7828	8283	8056	4.0	43250	-8.5	3759	-17.8
	6188	5646	5917	6.5	51757	9.5	5110	11.8
250	14716	14444	14580	1.3	48921	3.5	4567	-0.1
	18668	16044	17356	10.7	56113	18.7	4612	0.9
	12553	13149	12851	3.3	50389	6.6	4783	4.6
125	22898	23011	22954	0.3	42990	-9.1	4393	-3.9
	24167	27331	25749	8.7	55890	18.2	4912	7.5
	29215	25625	27420	9.3	50068	5.9	4753	4.0
80	25690	25270	25480	1.2	42501	-10.1	3742	-18.1
	NR	NR	NR	-	28835	-39.0*	4100	-10.3
	27764	32012	29888	10.1	42291	-10.6	3559	-22.1
40	55153‡	51339	53246	5.1	48215	2.0	4152	-9.2
	51241	48919	50080	3.3	37887	-19.9	5376	17.6
	51876	51260	51568	0.8	45086	-4.6	3932	-14.0
	53666‡	46016	49841	10.9	47894	1.3	4170	-8.8
	48955	53651‡	51303	6.5	43615	-7.8	3834	-16.1
	48281	54254‡	51268	8.2	37158	-21.4	3750	-18.0
20	57032	69005	63018	13.4	55679	17.8	4181	-8.5
	54016	52990	53503	1.4	48175	1.9	4255	-6.9
	58150	53330	55740	6.1	43827	-7.3	4266	-6.7
0	68818	64844	66831	4.2	51469	8.9	4849	6.1
	NR	NR	NR	-	31803	-32.7*	4094	-10.4
	72720	66887	69803	5.9	39374	-16.7	3757	-17.8

N = 3 cartridges for regular calibration points, N = 6 cartridges for LLOQ and ULOQ
* Cartridge fails to meet internal acceptance criteria, concentration will not be reported (NR)
† RLU value for standard curve point below combined S_{Min}, ‡ RLU value for standard curve point above combined S_{Max},

Table 8: Day 1, 2 and 3 Calibration, Back-Calculated Concentration (ng/mL)

[ACE-011] ng/mL	Day 1					Day 2					Day 3				
	Conc	% Rec.	Mean	CV %	% Rec.	Conc	% Rec.	Mean	CV %	% Rec.	Conc	% Rec.	Mean	CV %	% Rec.
4000	3307.4	82.7	3344.6	9.1	83.6	3008.4†	75.2	3473.9	9.4	86.8	3639.2	91.0	3489.9	5.1	87.2
	3024.7	75.6				3308.9†	82.7				3243.3†	81.1			
	3089.2	77.2				3787.2	94.7				2491.2	62.3*			
	3727.9	93.2				3887.5	97.2				3598.2†	90.0			
	2932.9†	73.3*				3524.8	88.1				3478.8	87.0			
	3573.9	89.3				3326.5†	83.2				2689.3	67.2*			
2000	1959.7	98.0	1860.0	7.6	93.0	1604.1	80.2	1762.0	10.1	88.1	2806.4†	140.3*	2240.5	25.1	112.0
	1760.4	88.0				1954.1	97.7				1683.1	84.2			
	NR	-				1727.9	86.4				2231.9	111.6			
1000	1384.1	138.4*	1145.0	5.7	114.5	971.5	97.2	1055.2	11.2	105.5	952.2	95.2	914.8	5.8	91.5
	1098.9	109.9				1139.0	113.9				1523.3	152.3*			
	1191.1	119.1				1281.1	128.1*				877.5	87.7			
500	523.9	104.8	489.5	6.2	97.9	476.5	95.3	535.9	11.1	107.2	505.6	101.1	475.6	8.9	95.1
	477.6	95.5				595.2	119.0				445.5	89.1			
	466.9	93.4				535.8	107.2				654.7	130.9*			
250	194.2	77.7*	241.4	9.0	96.6	276.2	110.5	273.6	1.6	109.4	223.5	89.4	241.1	10.3	96.4
	226.1	90.5				268.4	107.4				183.4	73.4*			
	256.7	102.7				276.2	110.5				258.7	103.5			
125	124.0	99.2	132.2	8.8	105.7	146.0	116.8	135.3	11.3	108.2	129.4	103.5	114.8	11.7	91.9
	140.4	112.3				96.6	77.3*				112.0	89.6			
	99.0	79.2*				124.5	99.6				103.0	82.4			
80	87.3	109.1	75.3	14.0	94.1	54.5	68.2*	-	-	-	113.1	141.3*	114.4	-	93.8
	71.1	88.9				50.2	62.7*				NR	-			
	67.6	84.5				NR	-				91.5	114.4			
40	42.5	106.2	40.5	4.2	101.2	47.6	119.1	41.7	14.6	104.4	34.0†	85.0	37.5	10.1	93.8
	40.3	100.8				49.8	124.6				36.2	90.5			
	OORL	-				35.2	88.0				33.6	84.1			
	38.3†	95.8				35.3†	88.2				43.8†	109.4			
	51.7†	129.2*				41.6†	104.0				38.2†	95.4			
	40.8†	101.9				40.9	102.3				39.4†	98.5			

NR = Not Reported, % Rec. = Percentage Recovery, † Concentration derived from only one replicate when other is OORL/OORH, as described in section 14.1.
* Individual cartridge fails to meet the accuracy criteria and was excluded from the 3-day calibration and from back-calculated mean, %CV and recovery.
A total of 70 cartridges of 90 (78%) met the accuracy criteria for the 3-day calibration.

Table 9: ACE-011 Quality Control Replicate Data in Whole Blood Day 1, Signal (RLU)

Level	[ACE-011] ng/mL	Cartridge Sample Replicates 1	2	Intra-Cartridge Mean	CV %	120 ng/mL Control RLU	% DFE	3000 ng/mL Control RLU	% DFE
ULOQ	4000	2403	2593	2498	5.4	52549	11.1	4724	3.4
		2623	2495	2559	3.5	56637	19.8	4870	6.5
		3082	2319	2700	20.0	52880	11.8	4813	5.3
		2315	2532	2424	6.3	49734	5.2	4791	4.8
		2120†	2579	2350	13.8	46719	-1.2	4086	-10.6
		2352	2540	2446	5.4	46760	-1.1	4304	-5.8
High	3000	3004	2848	2926	3.8	45336	-4.1	3896	-14.8
		2768	2523	2645	6.5	43816	-7.3	4825	5.6
		2449	2944	2696	13.0	53951	14.1	4608	0.8
		2628	2799	2713	4.5	42839	-9.4	4547	-0.5
		2360	2446	2403	2.5	53090	12.3	4807	5.2
		2471	2564	2518	2.6	50694	7.2	3787	-17.1
Mid	400	7745	7183	7464	5.3	50336	6.5	4651	1.7
		9393	9711	9552	2.4	51873	9.7	4774	4.4
		9770	11141	10456	9.3	44917	-5.0	5521	20.8
		9575	11921	10748	15.4	48583	2.8	5020	9.8
		7744	9558	8651	14.8	49210	4.1	5112	11.8
		8441	10028	9235	12.2	52912	11.9	4578	0.2
Low	120	30821	26724	28773	10.1	48420	2.4	4519	-1.1
		28612	25002	26807	9.5	39484	-16.5	4533	-0.8
		26392	25357	25874	2.8	41854	-11.5	4233	-7.4
		20811	24689	22750	12.1	53167	12.4	4388	-4.0
		28134	25609	26871	6.6	44364	-6.2	4122	-9.8
		28279	21639	24959	18.8	51263	8.4	4783	4.6
LLOQ	40	47775	45604	46690	3.3	48889	3.4	4029	-11.9
		47254	48326	47790	1.6	42102	-11.0	4603	0.7
		53686‡	54718‡	54202	1.3	44463	-6.0	4684	2.5
		48868	53889‡	51379	6.9	47394	0.2	4954	8.4
		54716‡	42336	48526	18.0	39714	-16.0	4231	-7.4
		47557	56957‡	52257	12.7	51034	7.9	4567	-0.1

N = 6 cartridges per level
† RLU value for standard curve point below combined S_{Min}, ‡ RLU value for standard curve point above combined S_{Max},

Table 10: ACE-011 Quality Control Replicate Data in Whole Blood Day 2, Signal (RLU)

Level	[ACE-011] ng/mL	Cartridge Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
		1	2	Mean	CV %	RLU	% DFE	RLU	% DFE
ULOQ	4000	2111†	2558	2335	13.5	40924	-13.4	4045	-11.5
		2222†	2485	2354	7.9	49231	4.1	4972	8.8
		2473	2332	2403	4.2	50926	7.7	4821	5.5
		2337	2427	2382	2.7	59248	25.3	4515	-1.2
		2597	2337	2467	7.5	59220	25.2	4996	9.3
		2238†	2481	2360	7.3	57384	21.4	4301	-5.9
High	3000	2520	2572	2546	1.4	44634	-5.6	5291	15.8
		3050	2321	2686	19.2	47306	0.1	4661	2.0
		2421	2016	2219	12.9	48140	1.8	4092	-10.5
		2515	2796	2655	7.5	55105	16.5	5016	9.7
		2804	2577	2691	6.0	46970	-0.7	5119	12.0
		2326	2891	2608	15.3	49117	3.9	4646	1.6
Mid	400	8043	8772	8407	6.1	42959	-9.1	4465	-2.3
		8177	7051	7614	10.5	50679	7.2	4239	-7.3
		7746	9241	8493	12.4	57450	21.5	5111	11.8
		8345	9623	8984	10.1	48172	1.9	4745	3.8
		10537	10007	10272	3.7	53681	13.5	3967	-13.2
		7474	7284	7379	1.8	39899	-15.6	4206	-8.0
Low	120	23829	24704	24267	2.6	49519	4.7	3945	-13.7
		25951	23536	24744	6.9	50884	7.6	4114	-10.0
		21554	20931	21242	2.1	41001	-13.3	5029	10.0
		24556	28511	26534	10.5	45089	-4.6	4284	-6.3
		26315	32235	29275	14.3	49986	5.7	5057	10.6
		23102	22380	22741	2.2	39188	-17.1	3813	-16.6
LLOQ	40	41253	47407	44330	9.8	42491	-10.1	4443	-2.8
		41230	45206	43218	6.5	40768	-13.8	4452	-2.6
		50914	50348	50631	0.8	52808	11.7	4912	7.5
		50596	52882‡	51739	3.1	41551	-12.1	3881	-15.1
		47124	53378‡	50251	8.8	41650	-11.9	4152	-9.2
		46206	48804	47505	3.9	52610	11.3	3854	-15.7

N = 6 cartridges per level
† RLU value for standard curve point below combined S_{Min}, ‡ RLU value for standard curve point above combined S_{Max},

Table 11: ACE-011 Quality Control Replicate Data in Whole Blood Day 3, Signal (RLU)

Level	[ACE-011] ng/mL	Cartridge Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
		1	2	Mean	CV %	RLU	% DFE	RLU	% DFE
ULOQ	4000	2433	2407	2420	0.8	44285	-6.3	4952	8.3
		2248†	2500	2374	7.5	44967	-4.9	4287	-6.2
		2665	2803	2734	3.6	48375	2.3	4663	2.0
		2427	2155†	2291	8.4	50822	7.5	4650	1.7
		2414	2489	2452	2.2	49930	5.6	4610	0.9
		2710	2607	2658	2.7	46827	-1.0	4288	-6.2
High	3000	2642	2442	2542	5.6	45899	-2.9	4476	-2.1
		3122	2681	2901	10.7	54945	16.2	5013	9.7
		2761	2754	2757	0.2	55856	18.1	3969	-13.2
		2607	2398	2502	5.9	54259	14.8	5159	12.9
		2320	2577	2448	7.4	40684	-14.0	4058	-11.2
		2758	2425	2591	9.1	39457	-16.5	4134	-9.5
Mid	400	10239	9687	9963	3.9	47345	0.1	4308	-5.7
		8758	9486	9122	5.6	51841	9.6	5214	14.1
		9862	9675	9769	1.4	50547	6.9	4926	7.8
		9895	10012	9953	0.8	47281	0.0	5141	12.5
		8312	8028	8170	2.5	50334	6.5	5352	17.1
		8198	8105	8152	0.8	45567	-3.6	5176	13.3
Low	120	24559	25649	25104	3.1	42500	-10.1	3741	-18.1
		22334	28579	25457	17.3	47783	1.1	4879	6.8
		26186	25169	25677	2.8	47978	1.5	4225	-7.6
		19715	20441	20078	2.6	44983	-4.9	3458	-24.3
		27888	27907	27898	0.0	41562	-12.1	3602	-21.2
		27452	25485	26468	5.3	42861	-9.4	4189	-8.4
LLOQ	40	55153‡	51339	53246	5.1	48215	2.0	4152	-9.2
		51241	48919	50080	3.3	37887	-19.9	5376	17.6
		51876	51260	51568	0.8	45086	-4.6	3932	-14.0
		53666‡	46016	49841	10.9	47894	1.3	4170	-8.8
		48955	53651‡	51303	6.5	43615	-7.8	3834	-16.1
		48281	54254‡	51268	8.2	37158	-21.4	3750	-18.0

N = 6 cartridges per level

† RLU value for standard curve point below combined S_{Min}, ‡ RLU value for standard curve point above combined S_{Max},

Table 12: Quality Control Sample Intra-Day Precision and Accuracy, Concentration (ng/mL)

[ACE-011] ng/mL	Day 1					Day 2					Day 3				
	Conc	% Rec	Mean	CV %	% Rec	Conc	% Rec	Mean	CV %	% Rec	Conc	% Rec	Mean	CV %	% Rec
4000	3307.4	82.7	3276.0	9.7	81.9	3008.4†	75.2	3473.9	9.4	86.8	3639.2	91.0	3190.0	15.3	79.8
	3024.7	75.6				3308.9†	82.7				3243.3†	81.1			
	3089.2	77.2				3787.2	94.7				2491.2	62.3*			
	3727.9	93.2				3887.5	97.2				3598.2†	90.0			
	2932.9†	73.3*				3524.8	88.1				3478.8	87.0			
	3573.9	89.3				3326.5†	83.2				2689.3	67.2*			
3000	2099.1	70.0*	2854.4	19.7	95.1	3058.5	101.9	3063.9	11.5	102.1	3124.3	104.1	3056.0	13.4	101.9
	2772.2	92.4				3099.5	103.3				2424.1	80.8			
	2771.8	92.4				3631.1†	121.0*				3302.5	110.1			
	2551.5	85.1				2757.6	91.9				3625.7	120.9*			
	3753.0	125.1*				2629.8	87.7				3016.3	100.5			
	3178.5	106.0				3206.9	106.9				2843.1†	94.8			
400	488.8	122.2*	383.8	16.1	96.0	424.1	106.0	425.0	13.7	106.3	347.1	86.8	385.1	11.3	96.3
	364.3	91.1				480.0	120.0				385.1	96.3			
	329.7	82.4				422.5	105.6				354.8	88.7			
	322.5	80.6				393.9	98.5				347.1	86.8			
	415.2	103.8				335.0	83.7				437.8	109.4			
	382.6	95.6				494.7	123.7*				438.8	109.7			
120	96.5	80.4	111.7	11.1	93.1	120.6	100.5	119.1	14.1	99.3	115.4	96.1	108.8	6.0	90.7
	106.3	88.6				117.9	98.2				115.4	96.2			
	110.8	92.4				142.7	118.9				112.0	93.3			
	132.1	110.1				107.9	89.9				102.6	85.5			
	105.6	88.0				94.9	79.0*				100.1	83.4			
	118.8	99.0				131.0	109.2				107.7	89.7			
40	42.5	106.2	42.7	12.2	106.8	47.6	119.1	41.7	14.6	104.4	34.0†	85.0	37.5	10.1	93.8
	40.3	100.8				49.8	124.6				36.2	90.5			
	OORL	-				35.2	88.0				33.6	84.1			
	38.3†	95.8				35.3†	88.2				43.8†	109.4			
	51.7†	129.2*				41.6†	104.0				38.2†	95.4			
	40.8†	101.9				40.9	102.3				39.4†	98.5			

N = 6 cartridges per level per day NR = Not Reported, % Rec. = Percentage Recovery

† Concentration derived from only one replicate when other is OORL/OORH, as described in section 14.1.

* Individual cartridge fails to meet the accuracy criteria.

Table 13: Quality Control Sample Inter-Day Precision and Accuracy, Concentration (ng/mL)

[ACE-011] ng/mL	Mean Conc.	CV %	% Recovery
4000	3313.3	11.6	82.8
3000	2991.4	14.5	99.7
400	398.0	13.9	99.5
120	113.2	11.2	94.4
40	40.5	13.1	101.4

N = 18 cartridges per level (6 cartridges per day per level)

Table 14: Inter-Instrument Replicate Data in Whole Blood at 500 ng/mL, Signal (RLU)

Instrument	Cartridge Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
	1	2	Mean	CV %	RLU	% DFE	RLU	% DFE
1	8114	7175	7644	8.7	42761	-9.6	4107	-10.2
2	7646	7837	7741	1.7	53186	12.5	5047	10.4
3	7828	8283	8056	4.0	43250	-8.5	3759	-17.8
4	8447	7965	8206	4.1	50906	7.7	3837	-16.0
5	6882	7612	7247	7.1	52234	10.5	4530	-0.9
6	9315	8148	8732	9.4	50905	7.7	4581	0.2
7	6254	7916	7085	16.6	56405	19.3	4304	-5.8
8	7801	7586	7694	2.0	52148	10.3	3994	-12.6
9	8607	8142	8375	3.9	52903	11.9	4307	-5.8
10	7497	7164	7331	3.2	51062	8.0	4201	-8.1
11	7449	8242	7846	7.1	49759	5.2	4324	-5.4
12	6752	7667	7210	9.0	36124	-23.6	4263	-6.7
13	6615	6664	6640	0.5	52356	10.7	4174	-8.7
14	8378	8628	8503	2.1	48152	1.8	3551	-22.3
15	6176	6778	6477	6.6	51265	8.4	4220	-7.7
16	7439	7512	7476	0.7	49027	3.7	3545	-22.5
17	8052	8074	8063	0.2	51908	9.8	3838	-16.0
18	7799	8153	7976	3.1	47989	1.5	4397	-3.8
19	8254	6551	7403	16.3	56814	20.2	3786	-17.2
20	8556	8559	8558	0.0	45939	-2.8	4206	-8.0
21	8051	8206	8129	1.3	49282	4.2	3942	-13.7
22	7859	7820	7840	0.3	54004	14.2	3613	-20.9
23	8452	9390	8921	7.4	54405	15.1	4757	4.1
24	8287	9249	8768	7.8	52854	11.8	4335	-5.2

N = 24 cartridges, N = 24 instruments

Table 15: Inter-Instrument Precision in Whole Blood at 500 ng/mL, Concentration (ng/mL)

Instrument	Conc. (ng/mL)	% Recovery	Inter-Instrument		
			Mean Conc. (ng/mL)	CV %	% Recovery
1	473.9	94.8	466.6	10.6	93.3
2	466.9	93.4			
3	445.5	89.1			
4	435.8	87.2			
5	507.5	101.5			
6	407.0	81.4			
7	531.0	106.2			
8	470.4	94.1			
9	425.4	85.1			
10	499.0	99.8			
11	461.0	92.2			
12	511.9	102.4			
13	563.3	112.7			
14	417.5	83.5			
15	583.1	116.6			
16	486.9	97.4			
17	444.5	88.9			
18	450.7	90.1			
19	502.6	100.5			
20	414.2	82.8			
21	440.3	88.1			
22	459.7	91.9			
23	395.9	79.2			
24	404.2	80.8			

N = 24 cartridges, N = 24 instruments

Table 16: Selectivity in Human Whole Blood, Signal (RLU)

Sample	Spike (ng/mL)	Cartridge Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
		1	2	Mean	CV %	RLU	% DFE	RLU	% DFE
F1	0	92081‡	87739‡	89910	3.4	58244	23.2	4691	2.6
		71284‡	67996‡	69640	3.3	52816	11.7	3828	-16.3
		50504	48240	49372	3.2	47328	0.1	4511	-1.3
	40	44779	49998	47388	7.8	43248	-8.5	5127	12.2
		40379	51852	46115	17.6	46693	-1.2	3898	-14.7
		42805	51197	47001	12.6	50693	7.2	4943	8.2
	1000	3823	5009	4416	19.0	51094	8.1	3580	-21.7
		4103	5016	4560	14.2	47226	-0.1	4422	-3.3
		3541	3869	3705	6.3	43274	-8.5	4059	-11.2
F2	0	77179‡	78665‡	77922	1.3	47980	1.5	4051	-11.4
		55303‡	62304‡	58804	8.4	44747	-5.4	4451	-2.6
		NR	NR	NR	-	42900	-9.3	3062	-33.0*
	40	45463	46414	45938	1.5	47084	-0.4	3802	-16.8
		41888	43362	42625	2.4	44815	-5.2	3519	-23.0
		45769	46872	46320	1.7	42031	-11.1	4783	4.6
	1000	5571	5230	5400	4.5	40132	-15.1	4166	-8.9
		4139	3153	3646	19.1	37638	-20.4	3423	-25.1
		NR	NR	NR	-	35734	-24.4	3238	-29.2*
F3	0	89184‡	64666‡	76925	22.5	42221	-10.7	4494	-1.7
		79202‡	78967‡	79085	0.2	49570	4.8	4303	-5.9
		59903‡	63667‡	61785	4.3	49340	4.4	4259	-6.8
	40	43443	46229	44836	4.4	38432	-18.7	3859	-15.6
		45835	54196‡	50015	11.8	36974	-21.8	3702	-19.0
		NR	NR	NR	-	35932	-24.0	2879	-37.0*
	1000	4010	4073	4041	1.1	53463	13.1	4212	-7.9
		5012	5213	5113	2.8	46188	-2.3	4386	-4.0
		3679	4943	4311	20.7	59168	25.1	4893	7.0
F4	0	89448‡	88218‡	88833	1.0	49286	4.2	4103	-10.2
		65695‡	61270‡	63482	4.9	46527	-1.6	4297	-6.0
		68620‡	76274‡	72447	7.5	45735	-3.3	3631	-20.6
	40	45612	42511	44061	5.0	50323	6.4	3953	-13.5
		42032	46580	44306	7.3	35428	-25.1	3773	-17.5
		55545‡	51061	53303	5.9	54071	14.4	4029	-11.8
	1000	4463	4699	4581	3.6	45371	-4.0	4170	-8.8
		6007	6828	6418	9.0	54422	15.1	4509	-1.3
		5154	4461	4807	10.2	48794	3.2	4911	7.4

* Cartridge fails to meet internal acceptance criteria, concentration will not be reported (NR)
‡ RLU value for standard curve point above combined S_{Max},

Table 16: Selectivity in Human Whole Blood, Signal (RLU), *Continued*

Sample	Spike (ng/mL)	Cartridge Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
		1	2	Mean	CV %	RLU	% DFE	RLU	% DFE
F5	0	55028‡	60568‡	57798	6.8	49876	5.5	4185	-8.4
		67612‡	70072‡	68842	2.5	51900	9.8	4298	-6.0
		57483‡	68335‡	62909	12.2	42299	-10.5	4544	-0.6
	40	37123	40403	38763	6.0	42582	-9.9	4275	-6.5
		38539	40810	39674	4.0	39098	-17.3	3978	-13.0
		41563	46042	43803	7.2	42258	-10.6	3638	-20.4
	1000	4283	4416	4349	2.2	48005	1.5	3480	-23.9
		4982	5098	5040	1.6	45773	-3.2	3532	-22.7
		4964	4570	4767	5.8	54590	15.5	3975	-13.0
F6	0	63667‡	70176‡	66922	6.9	44363	-6.2	3639	-20.4
		58147‡	58237‡	58192	0.1	44676	-5.5	3703	-19.0
		57329‡	73025‡	65177	17.0	38753	-18.0	4207	-7.9
	40	40106	47799	43953	12.4	42932	-9.2	3464	-24.2
		52170	53252‡	52711	1.5	43309	-8.4	4096	-10.4
		52122	50731	51427	1.9	50966	7.8	4420	-3.3
	1000	3530	3772	3651	4.7	41913	-11.4	3520	-23.0
		3369	4427	3898	19.2	45218	-4.4	3462	-24.3
		4970	4764	4867	3.0	42774	-9.5	3601	-21.2
F7	0	59894‡	60987‡	60440	1.3	44719	-5.4	3528	-22.8
		78025‡	78050‡	78038	0.0	46532	-1.6	3804	-16.8
		68269‡	66569‡	67419	1.8	46637	-1.4	4352	-4.8
	40	NR	NR	NR	-	209	-99.6*	3735	-18.3
		68421‡	49714	59067	22.4	45632	-3.5	4130	-9.6
		42159	40993	41576	2.0	42391	-10.3	4143	-9.4
	1000	3681	4960	4321	20.9	43169	-8.7	3964	-13.3
		4113	3493	3803	11.5	48727	3.1	3839	-16.0
		4129	4218	4173	1.5	40857	-13.6	3455	-24.4
F8	0	73656‡	68221‡	70938	5.4	50678	7.2	3781	-17.3
		75567‡	72151‡	73859	3.3	49157	4.0	4748	3.9
		65483‡	69691‡	67587	4.4	43789	-7.4	3630	-20.6
	40	42124	42829	42477	1.2	47115	-0.4	3848	-15.8
		56041‡	62415‡	59228	7.6	41834	-11.5	3740	-18.2
		46575	48993	47784	3.6	44626	-5.6	4337	-5.1
	1000	5452	5438	5445	0.2	56833	20.2	5308	16.1
		5193	5339	5266	2.0	49768	5.3	4047	-11.5
		4521	4583	4552	1.0	52987	12.1	3640	-20.4

* Cartridge fails to meet internal acceptance criteria, concentration will not be reported (NR)
‡ RLU value for standard curve point above combined S_{Max},

Table 16: Selectivity in Human Whole Blood, Signal (RLU), *Continued*

Sample	Spike (ng/mL)	Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
		1	2	Mean	CV %	RLU	% DFE	RLU	% DFE
F9	0	62370‡	66514‡	64442	4.5	47349	0.1	4467	-2.3
		64018‡	63780‡	63899	0.3	44127	-6.7	4443	-2.8
		69038‡	73566‡	71302	4.5	45383	-4.0	3651	-20.1
	40	39729	41843	40786	3.7	48781	3.2	4520	-1.1
		44863	40683	42773	6.9	40835	-13.6	3471	-24.1
		53397‡	51774	52585	2.2	46953	-0.7	4111	-10.1
	1000	3305	3862	3584	11.0	35601	-24.7	3857	-15.6
		4870	5050	4960	2.6	48797	3.2	4059	-11.2
		4694	3679	4186	17.2	48880	3.4	4711	3.1
F10	0	62503‡	58722‡	60613	4.4	44482	-5.9	4177	-8.6
		NR	NR	NR	-	45941	-2.8	3119	-31.8*
		50430	57977‡	54203	9.8	37266	-21.2	4075	-10.8
	40	49506	44987	47247	6.8	43505	-8.0	4087	-10.6
		47391	45890	46640	2.3	46089	-2.5	4603	0.7
		43354	53439‡	48397	14.7	40331	-14.7	4269	-6.6
	1000	5089	4882	4986	2.9	39395	-16.7	3568	-21.9
		3937	3812	3874	2.3	40977	-13.3	4087	-10.6
		3755	3732	3744	0.4	40888	-13.5	3561	-22.1
M1	0	57728‡	55753‡	56741	2.5	38234	-19.1	3615	-20.9
		62402‡	78222‡	70312	15.9	50214	6.2	4512	-1.3
		120781‡	121321‡	121051	0.3	42283	-10.6	4084	-10.6
	40	51882	53620‡	52751	2.3	45457	-3.9	4692	2.7
		46817	62705‡	54761	20.5	45279	-4.2	4145	-9.3
		52353	52220	52286	0.2	42092	-11.0	3765	-17.6
	1000	3815	4659	4237	14.1	51209	8.3	4449	-2.7
		3966	4193	4079	3.9	43512	-8.0	4456	-2.5
		3272	3870	3571	11.8	42188	-10.8	4303	-5.9
M2	0	51991	69872‡	60932	20.8	39317	-16.8	4083	-10.7
		77488‡	74576‡	76032	2.7	52761	11.6	3957	-13.4
		55091‡	60403‡	57747	6.5	39457	-16.5	3586	-21.5
	40	44885	44464	44674	0.7	42067	-11.0	3698	-19.1
		53075‡	48623	50849	6.2	46253	-2.2	4184	-8.5
		45613	50776	48195	7.6	42951	-9.2	3970	-13.1
	1000	3636	3965	3801	6.1	41609	-12.0	4728	3.4
		NR	NR	NR	-	32789	-30.7*	3392	-25.8*
		4813	4878	4845	1.0	52714	11.5	4769	4.3

* Cartridge fails to meet internal acceptance criteria, concentration will not be reported (NR)
‡ RLU value for standard curve point above combined S_{Max},

Table 16: Selectivity in Human Whole Blood, Signal (RLU), *Continued*

Sample	Spike (ng/mL)	Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
		1	2	Mean	CV %	RLU	% DFE	RLU	% DFE
M3	0	52059	52627‡	52343	0.8	44651	-5.6	3909	-14.5
		69257‡	56557‡	62907	14.3	41037	-13.2	3563	-22.0
		53381‡	61101‡	57241	9.5	41280	-12.7	3528	-22.8
	40	44896	48156	46526	5.0	48194	1.9	3422	-25.1
		47572	48435	48004	1.3	48839	3.3	5163	13.0
		50750	58207‡	54478	9.7	51685	9.3	5392	18.0
	1000	3725	3397	3561	6.5	40907	-13.5	4032	-11.8
		3669	3615	3642	1.0	47164	-0.3	4653	1.8
		3758	4325	4042	9.9	47069	-0.4	3572	-21.9
M4	0	71341‡	70720‡	71031	0.6	44003	-6.9	4292	-6.1
		65156‡	73656‡	69406	8.7	38678	-18.2	4091	-10.5
		70390‡	62967‡	66678	7.9	51306	8.5	4722	3.3
	40	41554	46009	43782	7.2	40340	-14.7	3570	-21.9
		41336	48775	45056	11.7	46474	-1.7	4301	-5.9
		40958	46510	43734	9.0	39781	-15.9	5203	13.8
	1000	3702	4100	3901	7.2	47490	0.4	4212	-7.8
		4159	3567	3863	10.8	52555	11.2	3841	-16.0
		3952	3599	3776	6.6	45698	-3.4	3759	-17.8
M5	0	59687‡	64118‡	61902	5.1	49373	4.4	3759	-17.8
		55595‡	63034‡	59314	8.9	39089	-17.3	3435	-24.9
		66382‡	61403‡	63893	5.5	40826	-13.7	3596	-21.3
	40	39615	41443	40529	3.2	48512	2.6	4598	0.6
		37613	39188	38400	2.9	39466	-16.5	4446	-2.7
		37260	47486	42373	17.1	42025	-11.1	3482	-23.8
	1000	4368	4197	4283	2.8	42042	-11.1	4136	-9.5
		3138	3455	3296	6.8	44816	-5.2	3905	-14.6
		5416	4632	5024	11.0	52596	11.2	4448	-2.7
M6	0	57252‡	66378‡	61815	10.4	52798	11.7	3840	-16.0
		72812‡	64053‡	68433	9.0	43358	-8.3	4328	-5.3
		72996‡	68529‡	70763	4.5	43026	-9.0	3622	-20.8
	40	NR	NR	NR	-	46364	-1.9	2874	-37.1*
		40204	41344	40774	2.0	52670	11.4	5019	9.8
		51293	58621‡	54957	9.4	48643	2.9	5276	15.4
	1000	4469	5991	5230	20.6	54435	15.1	4292	-6.1
		3643	4122	3883	8.7	46690	-1.3	3410	-25.4
		3958	3980	3969	0.4	41968	-11.2	3458	-24.3

* Cartridge fails to meet internal acceptance criteria, concentration will not be reported (NR)
‡ RLU value for standard curve point above combined S_{Max},

Table 16: Selectivity in Human Whole Blood, Signal (RLU), *Continued*

Sample	Spike (ng/mL)	Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
		1	2	Mean	CV %	RLU	% DFE	RLU	% DFE
M7	0	56530‡	56422‡	56476	0.1	49713	5.1	4229	-7.5
		61377‡	59798‡	60587	1.8	40573	-14.2	3638	-20.4
		66236‡	76306‡	71271	10.0	53359	12.9	4011	-12.2
	40	46289	49663	47976	5.0	50695	7.2	4771	4.4
		NR	NR	NR	-	43424	-8.2	3234	-29.2*
		57479‡	50535	54007	9.1	48054	1.6	4779	4.6
	1000	4431	3180	3806	23.2	48096	1.7	5376	17.6
		4638	4401	4520	3.7	51491	8.9	5321	16.4
		4531	5172	4851	9.3	43448	-8.1	4325	-5.4
M8	0	77385‡	65348‡	71366	11.9	51505	8.9	3711	-18.8
		57312‡	67137‡	62224	11.2	42541	-10.0	4008	-12.3
		NR	NR	NR	-	40170	-15.0	3300	-27.8*
	40	46473	41668	44071	7.7	40915	-13.5	5159	12.9
		56411‡	52950‡	54680	4.5	58006	22.7	5152	12.7
		50823	45931	48377	7.2	51363	8.6	4409	-3.5
	1000	5830	5738	5784	1.1	55430	17.2	3495	-23.5
		4239	4574	4407	5.4	36631	-22.5	4312	-5.6
		3680	3310	3495	7.5	53031	12.2	5635	23.3
M9	0	NR	NR	NR	-	20	-100.0*	4214	-7.8
		71063‡	77485‡	74274	6.1	45559	-3.6	3903	-14.6
		67992‡	61544‡	64768	7.0	48711	3.0	4869	6.5
	40	52359	54332‡	53346	2.6	47285	0.0	3419	-25.2
		54620‡	50787	52704	5.1	49694	5.1	5452	19.3
		48943	46533	47738	3.6	45058	-4.7	4340	-5.0
	1000	4413	5815	5114	19.4	53580	13.3	4631	1.3
		5550	4931	5241	8.4	49753	5.2	4774	4.4
		3646	4050	3848	7.4	37049	-21.6	3867	-15.4
M10	0	65140‡	62462‡	63801	3.0	49465	4.6	4097	-10.4
		64572‡	72131‡	68352	7.8	45656	-3.4	3643	-20.3
		58656‡	61359‡	60007	3.2	56093	18.6	4800	5.0
	40	50077	50003	50040	0.1	54082	14.4	3538	-22.6
		52860‡	46182	49521	9.5	49557	4.8	4433	-3.0
		51177	48463	49820	3.9	49148	3.9	4823	5.5
	1000	4174	5211	4692	15.6	47806	1.1	5586	22.2
		5104	4758	4931	5.0	39931	-15.5	3899	-14.7
		4732	5131	4932	5.7	46592	-1.5	3581	-21.6

* Cartridge fails to meet internal acceptance criteria, concentration will not be reported (NR)
‡ RLU value for standard curve point above combined S_{Max},

Table 17: Selectivity in Human Whole Blood, Concentration (ng/mL)

Sample	Spike (ng/mL)	Conc (ng/mL)	Mean	CV %	% Recovery	Meets Criteria
F1	0	OORL OORL 37.5	OORL	-	-	Yes
	40	41.3 44.8 42.4	42.8	4.1	107.1	Yes
	1000	1025.0 956.6 1310.1	1097.3	17.1	109.7	Yes
F2	0	OORL OORL NR	OORL	-	-	Yes
	40	43.9 51.0 43.2	46.0	9.4	115.1	Yes
	1000	737.6 1426.5 NR	1082.1	45.0	108.2	Yes
F3	0	OORL OORL OORL	OORL	-	-	Yes
	40	46.3 44.1† NR	45.2	3.3	113.0	Yes
	1000	1125.8 794.0 1075.0	998.3	17.9	99.8	Yes
F4	0	OORL OORL OORL	OORL	-	-	Yes
	40	47.9 47.5 34.5†	43.3	17.6	108.3	Yes
	1000	929.4 591.8 875.1	798.8	22.7	79.9	Yes
F5	0	OORL OORL OORL	OORL	-	-	Yes
	40	60.8 58.3 48.6	55.9	11.5	139.7	No
	1000	1003.8 809.7 879.2	897.5	11.0	89.8	Yes

NR = Not Reported.
† Concentration derived from only one replicate when other is OORL/OORH, as described in section 14.1

Table 17: Selectivity in Human Whole Blood, Concentration (ng/mL), *Continued*

Sample	Spike (ng/mL)	Conc (ng/mL)	Mean	CV %	% Recovery	Meets Criteria
F6	0	OORL OORL OORL	OORL	-	-	Yes
	40	48.7 32.6† 33.9	38.4	23.2	96.0	Yes
	1000	1340.7 1262.6 851.0	1151.5	22.8	115.1	Yes
F7	0	OORL OORL OORL	OORL	-	-	Yes
	40	NR 36.8† 53.5	45.1	26.1	112.9	No
	1000	1072.1 1270.6 1069.8	1137.5	10.1	113.8	Yes
F8	0	OORL OORL OORL	OORL	-	-	Yes
	40	51.4 OORL 40.4	45.9	16.9	114.7	Yes
	1000	728.1 762.1 936.9	809.0	13.9	80.9	Yes
F9	0	OORL OORL OORL	OORL	-	-	Yes
	40	55.4 50.9 33.3†	46.5	25.1	116.3	Yes
	1000	1410.3 828.4 1106.9	1115.2	26.1	111.5	Yes
F10	0	OORL NR 35.6	35.6	-	-	No
	40	41.5 42.5 49.4†	44.5	9.6	111.2	Yes
	1000	822.5 1207.4 1278.6	1102.8	22.2	110.3	Yes

NR = Not Reported.
† Concentration derived from only one replicate when other is OORL/OORH, as described in section 14.1

Table 17: Selectivity in Human Whole Blood, Concentration (ng/mL), *Continued*

Sample	Spike (ng/mL)	Conc (ng/mL)	Mean	CV %	% Recovery	Meets Criteria
M1	0	OORL OORL OORL	OORL	-	-	Yes
	40	33.1† 42.2† 32.5	35.9	15.1	89.8	Yes
	1000	1071.8 1111.3 1424.5	1202.5	16.1	120.3	Yes
M2	0	32.9 OORL OORL	OORL	-	-	Yes
	40	46.5 38.8† 39.8	41.7	10.1	104.2	Yes
	1000	1253.1 NR 855.7	1054.4	26.6	105.4	Yes
M3	0	32.8 OORL OORL	OORL	-	-	Yes
	40	42.8 39.9 35.0†	39.3	10.1	98.1	Yes
	1000	1407.1 1342.0 1141.0	1296.7	10.7	129.7	No
M4	0	OORL OORL OORL	OORL	-	-	Yes
	40	48.6 46.3 48.9	47.9	3.0	119.8	Yes
	1000	1201.7 1233.7 1268.6	1234.7	2.7	123.5	Yes
M5	0	OORL OORL OORL	OORL	-	-	Yes
	40	56.1 61.6 52.8	56.8	7.8	142.1	No
	1000	1028.2 1632.2 823.3	1161.2	36.2	116.1	Yes

NR = Not Reported.
† Concentration derived from only one replicate when other is OORL/OORH, as described in section 14.1

Table 17: Selectivity in Human Whole Blood, Concentration (ng/mL), *Continued*

Sample	Spike (ng/mL)	Conc (ng/mL)	Mean	CV %	% Recovery	Meets Criteria
M6	0	OORL OORL OORL	OORL	-	-	Yes
	40	NR 55.4 34.1†	44.8	33.7	111.9	Yes
	1000	802.5 1215.6 1159.2	1059.1	21.1	105.9	Yes
M7	0	OORL OORL OORL	OORL	-	-	Yes
	40	40.1 NR 35.4†	37.7	8.8	94.3	Yes
	1000	1356.8 948.3 862.1	1055.8	25.0	105.6	Yes
M8	0	OORL OORL NR	OORL	-	-	Yes
	40	48.0 OORL 39.4	43.7	14.0	109.3	Yes
	1000	672.1 986.9 1460.3	1039.8	38.2	104.0	Yes
M9	0	NR OORL OORL	OORL	-	-	Yes
	40	32.3† 35.0† 40.5	35.9	11.5	89.8	Yes
	1000	824.3 772.1 1230.3	942.3	26.6	94.2	Yes
M10	0	OORL OORL OORL	OORL	-	-	Yes
	40	36.2 43.4† 36.7	38.8	10.4	97.0	Yes
	1000	921.2 836.8 837.3	865.1	5.6	86.5	Yes

NR = Not Reported.
† Concentration derived from only one replicate when other is OORL/OORH, as described in section 14.1

Table 18: Process Sample Stability (Room Temperature) Whole Blood Spiked at 500 ng/mL, Signal (RLU)

Time Elapsed	Cartridge Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
Min:Sec	1	2	Mean	CV %	Mean	% DFE	Mean	% DFE
00:49	8447	7965	8206	4.1	50906	7.7	3837	-16.0
02:36	6882	7612	7247	7.1	52234	10.5	4530	-0.9
03:45	9315	8148	8732	9.4	50905	7.7	4581	0.2
05:07	7801	7586	7694	2.0	52148	10.3	3994	-12.6
06:15	8607	8142	8375	3.9	52903	11.9	4307	-5.8
06:39	7497	7164	7331	3.2	51062	8.0	4201	-8.1
06:54	7449	8242	7846	7.1	49759	5.2	4324	-5.4
08:29	6752	7667	7210	9.0	36124	-23.6	4263	-6.7
09:40	6615	6664	6640	0.5	52356	10.7	4174	-8.7
11:01	8052	8074	8063	0.2	51908	9.8	3838	-16.0
11:32	7799	8153	7976	3.1	47989	1.5	4397	-3.8
12:13	8254	6551	7403	16.3	56814	20.2	3786	-17.2
12:57	8051	8206	8129	1.3	49282	4.2	3942	-13.7
13:12	7859	7820	7840	0.3	54004	14.2	3613	-20.9
13:56	8287	9249	8768	7.8	52854	11.8	4335	-5.2

Table 19: Process Sample Stability (Room Temperature) Whole Blood Spiked at 500 ng/mL, Concentration (ng/mL)

Time Elapsed Min:Sec	Conc. (ng/mL)	% Recovery	Mean	CV %	% Recovery
00:49	435.8	87.2	465.6	9.4	93.1
02:36	507.5	101.5			
03:45	407.0	81.4			
05:07	470.4	94.1			
06:15	425.4	85.1			
06:39	499.0	99.8			
06:54	461.0	92.2			
08:29	511.9	102.4			
09:40	563.3	112.7			
11:01	444.5	88.9			
11:32	450.7	90.1			
12:13	502.6	100.5			
12:57	440.3	88.1			
13:12	459.7	91.9			
13:56	404.2	80.8			

Table 20: Cartridge Stability at 4°C, Signal (RLU)

		Cartridge Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
Week	[ACE-011] ng/mL	1	2	Mean	CV %	Mean	% DFE	Mean	% DFE
0	3000	2380	2551	2465	4.9	51926	9.8	5076	11.1
		2796	3072	2934	6.6	43839	-7.3	4573	0.0
		2640	2622	2631	0.5	49747	5.2	4728	3.4
	120	29392	27621	28507	4.4	38730	-18.1	3644	-20.3
		24779	28318	26549	9.4	45208	-4.4	4544	-0.6
		29441	32403	30922	6.8	57337	21.3	4480	-2.0
	0	86557‡	82599‡	84578	3.3	45583	-3.6	4819	5.4
		85460‡	90474‡	87967	4.0	50445	6.7	4302	-5.9
		74353‡	62383‡	68368	12.4	43652	-7.7	4881	6.8
1	3000	3226	3634	3430	8.4	52163	10.3	5122	12.1
		2474	2900	2687	11.2	62161	31.5*	5574	22.0
		2888	3505	3197	13.7	53508	13.2	4280	-6.4
	120	25180	30311	27745	13.1	41495	-12.2	3913	-14.4
		31185	25970	28577	12.9	49477	4.6	4812	5.3
		32143	28821	30482	7.7	40514	-14.3	4382	-4.1
	0	81642‡	75745‡	78693	5.3	47306	0.1	4511	-1.3
		102424‡	106377‡	104400	2.7	52394	10.8	5157	12.8
		68936‡	57996‡	63466	12.2	42322	-10.5	4503	-1.5
2	3000	2497	2620	2559	3.4	46307	-2.1	3449	-24.5
		2683	2979	2831	7.4	48263	2.1	4701	2.9
		3080	2995	3037	2.0	57351	21.3	4556	-0.3
	120	31193	27374	29284	9.2	48891	3.4	3541	-22.5
		28096	27402	27749	1.8	50073	5.9	3905	-14.6
		26410	26825	26618	1.1	45410	-4.0	3660	-19.9
	0	67998‡	66113‡	67056	2.0	48283	2.1	3785	-17.2
		76997‡	80070‡	78534	2.8	40125	-15.1	4513	-1.3
		100818‡	91998‡	96408	6.5	44277	-6.4	4231	-7.4
4	3000	3283	3202	3242	1.8	40496	-14.4	3574	-21.8
		2779	2174†	2477	17.3	55883	18.2	4598	0.6
		2563	3391	2977	19.7	29899	-36.8*	2575	-43.7*
	120	24261	22571	23416	5.1	46672	-1.3	4029	-11.8
		27098	23706	25402	9.4	36665	-22.5	4235	-7.3
		26203	26497	26350	0.8	55987	18.4	3580	-21.7
	0	70654‡	69664‡	70159	1.0	43219	-8.6	4197	-8.2
		71295‡	84439‡	77867	11.9	52216	10.4	4111	-10.1
		83013‡	94563‡	88788	9.2	47604	0.7	4487	-1.8

† RLU value for standard curve point below combined S_{Min}, ‡ RLU value for standard curve point above combined S_{Max},
* Cartridge fails to meet internal acceptance criteria, concentration would not normally be reported (NR). Because this is a stability study, concentration is calculated even if the cartridge fails acceptance criteria, but will not be included in the mean or % recovery for the level.

Table 20: Cartridge Stability at 4°C, Signal (RLU), *Continued*

		Cartridge Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
Week	[ACE-011] ng/mL	1	2	Mean	CV %	Mean	% DFE	Mean	% DFE
8	3000	3232	2838	3035	9.2	40857	-13.6	3644	-20.3
		3029	2569	2799	11.6	38286	-19.0	3625	-20.7
		2737	2809	2773	1.9	42699	-9.7	3013	-34.1*
	120	32198	26833	29515	12.9	48935	3.5	4202	-8.1
		23329	22753	23041	1.8	40678	-14.0	4074	-10.9
		22494	22775	22634	0.9	42669	-9.8	3414	-25.3
	0	82152‡	88739‡	85446	5.5	49384	4.4	3916	-14.3
		71571‡	81237‡	76404	8.9	46895	-0.8	3485	-23.8
		60117‡	68781‡	64449	9.5	54252	14.7	3683	-19.4
12	3000	2898	2597	2748	7.7	41147	-13.0	3095	-32.3*
		3045	3182	3113	3.1	44796	-5.3	4041	-11.6
		3455	3327	3391	2.7	39287	-16.9	4232	-7.4
	120	25916	27718	26817	4.8	36820	-22.1	3720	-18.6
		27940	26308	27124	4.3	38562	-18.4	3827	-16.3
		33502	30133	31817	7.5	51823	9.6	4421	-3.3
	0	60759‡	57185‡	58972	4.3	34565	-26.9*	3669	-19.7
		59199‡	59114‡	59157	0.1	40196	-15.0	3696	-19.1
		67659‡	73078‡	70368	5.4	38815	-17.9	3995	-12.6

‡ RLU value for standard curve point above combined S_{Max},
* Cartridge fails to meet internal acceptance criteria, concentration would not normally be reported (NR). Because this is a stability study, concentration is calculated even if the cartridge fails acceptance criteria, but will not be included in the mean or % recovery for the level.

Table 21: Cartridge Stability at 4°C, Concentration (ng/mL)

Week	Nominal [Ace-011] ng/mL	Conc. ng/mL	Mean	CV %	% Recovery To Day 0	% Recovery To Nominal
0	3000	4118.8	3573.3	15.9	100.0	119.1
		2985.0				
		3616.2				
	120	116.3	116.4	12.2	100.0	97.0
		130.7				
		102.2				
	0	OORL	OORL	-	-	-
		OORL				
		OORL				
1	3000	2300.4	2463.0	9.3	68.9	82.1
		3549.6*				
		2625.7				
	120	122.9	115.0	8.1	98.8	95.8
		117.3				
		104.8				
	0	OORL	OORL	-	-	-
		OORL				
		OORL				
2	3000	3820.9	3267.4	15.9	91.4	108.9
		3187.0				
		2794.5				
	120	112.1	120.8	7.1	103.8	100.7
		121.2				
		129.3				
	0	OORL	OORL	-	-	-
		OORL				
		OORL				
4	3000	2501.8	2884.3	18.8	80.7	98.1
		3266.8†				
		3061.9*				
	120	157.2	142.8	9.2	122.6	119.0
		139.9				
		131.3				
	0	OORL	OORL	-	-	-
		OORL				
		OORL				

* Cartridge fails to meet internal acceptance criteria, concentration would not normally be reported (NR). Because this is a stability study, concentration is calculated even if the cartridge fails acceptance criteria, but will not be included in the mean or % recovery for the level.

† Concentration derived from only one replicate when other is OORL/OORH, as described in section 14.1.

Table 21: Cartridge Stability at 4°C, Concentration (ng/mL), *Continued*

Week	Nominal [Ace-011] ng/mL	Conc. ng/mL	Mean	CV %	% Recovery To Day 0	% Recovery To Nominal
8	3000	2830.1	3062.4	10.7	85.7	102.1
		3294.8				
		3282.3*				
	120	111.4	145.6	20.4	125.1	121.4
		160.6				
		164.9				
	0	OORL	OORL	-	-	-
		OORL				
		OORL				
12	3000	3368.3*	2502.8	10.1	70.0	83.4
		2680.9				
		2324.7				
	120	128.0	117.1	14.4	100.6	97.6
		125.7				
		97.6				
	0	OORL*	OORL	-	-	-
		OORL				
		OORL				

* Cartridge fails to meet internal acceptance criteria, concentration would not normally be reported (NR). Because this is a stability study, concentration is calculated even if the cartridge fails acceptance criteria, but will not be included in the mean or % recovery for the level.

Table 22: Cartridge Stability at Room Temperature, Signal (RLU)

		Cartridge Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
Week	[ACE-011] ng/mL	1	2	Mean	CV %	Mean	% DFE	Mean	% DFE
0	3000	2380	2551	2465	4.9	51926	9.8	5076	11.1
		2796	3072	2934	6.6	43839	-7.3	4573	0.0
		2640	2622	2631	0.5	49747	5.2	4728	3.4
	120	29392	27621	28507	4.4	38730	-18.1	3644	-20.3
		24779	28318	26549	9.4	45208	-4.4	4544	-0.6
		29441	32403	30922	6.8	57337	21.3	4480	-2.0
	0	86557‡	82599‡	84578	3.3	45583	-3.6	4819	5.4
		85460‡	90474‡	87967	4.0	50445	6.7	4302	-5.9
		74353‡	62383‡	68368	12.4	43652	-7.7	4881	6.8
1	3000	1905†	2840	2373	27.9*	31077	-34.3*	3873	-15.3
		2751	2634	2693	3.1	51732	9.4	3750	-17.9
		2404	2625	2514	6.2	49873	5.5	4015	-12.2
	120	20982	25678	23330	14.2	40426	-14.5	3412	-25.3
		31560	32623	32091	2.3	52388	10.8	4013	-12.2
		27352	26601	26976	2.0	41197	-12.9	3100	-32.2*
	0	93890‡	90733‡	92311	2.4	47997	1.5	4076	-10.8
		83414‡	85095‡	84255	1.4	41477	-12.3	3396	-25.7*
		67883‡	71350‡	69616	3.5	45150	-4.5	2710	-40.7*
2	3000	2051†	2051†	2051	0.0	30303	-35.9*	2256	-50.6*
		2047†	1809†	1928	8.8	28612	-39.5*	2278	-50.2*
		1878†	1233†	1555	29.3*	35157	-25.6*	3067	-32.9*
	120	25185	21858	23522	10.0	35529	-24.9	3356	-26.6*
		15625	15807	15716	0.8	28218	-40.3*	2529	-44.7*
		16567	18597	17582	8.2	30747	-35.0*	2069	-54.7*
	0	57470‡	81993‡	69732	24.9	44424	-6.0	3282	-28.2*
		47641	43794	45718	5.9	32563	-31.1*	2154	-52.9*
		57573‡	53481‡	55527	5.2	32832	-30.6*	2763	-39.5*
4	3000	1617†	1515†	1566	4.6	27492	-41.9*	2721	-40.5*
		2031†	2172†	2102	4.7	34543	-26.9*	2648	-42.1*
		1684†	1732†	1708	2.0	28709	-39.3*	2593	-43.3*
	120	17028	18112	17570	4.4	30558	-35.4*	2653	-42.0*
		16045	11800	13922	21.6	27640	-41.5*	2718	-40.5*
		11883	14922	13403	16.0	23217	-50.9*	1737	-62.0*
	0	43522	61163‡	52343	23.8	33367	-29.4*	1925	-57.9*
		36090	33735	34913	4.8	24220	-48.8*	1934	-57.7*
		42539	47717	45128	8.1	32847	-30.5*	2212	-51.6*

† RLU value for standard curve point below combined S_{Min}, ‡ RLU value for standard curve point above combined S_{Max},
* Cartridge fails to meet internal acceptance criteria, concentration would not normally be reported (NR). Because this is a stability study, concentration is calculated even if the cartridge fails acceptance criteria, but will not be included in the mean or % recovery for the level.
Note: Week 0 is the same data for both storage conditions; storage stability testing was initiated at Week 0.

Table 22: Cartridge Stability at Room Temperature, Signal (RLU), *Continued*

		Cartridge Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
Week	[ACE-011] ng/mL	1	2	Mean	CV %	Mean	% DFE	Mean	% DFE
8	3000	874†	779†	827	8.1	12094	-74.4*	1017	-77.8*
		827†	865†	846	3.2	15258	-67.7*	1391	-69.6*
		1088†	985†	1036	7.0	15622	-67.0*	1280	-72.0*
	120	6737	6340	6539	4.3	46915	-0.8	1277	-72.1*
		11370	9803	10586	10.5	15343	-67.5*	1323	-71.1*
		7258	5289	6274	22.2	50	-99.9*	707	-84.5*
	0	37012	30165	33588	14.4	19640	-58.5*	2503	-45.2*
		107†	113†	110	3.4	13621	-71.2*	1146	-74.9*
		25964	33699	29831	18.3	15299	-67.6*	1330	-70.9*
12	3000	740†	553†	646	20.5	9832	-79.2*	931	-79.6*
		713†	774†	744	5.8	9918	-79.0*	844	-81.5*
		510†	482†	496	4.0	8560	-81.9*	997	-78.2*
	120	4306	5260	4783	14.1	8247	-82.6*	1044	-77.2*
		9938	6454	8196	30.1*	12938	-72.6*	1044	-77.1*
		9798	6208	8003	31.7*	11588	-75.5*	544	-88.1*
	0	13786	14682	14234	4.5	9158	-80.6*	1351	-70.4*
		3†	3115†	1559	141.1*	763	-98.4*	117	-97.4*
		20133	20109	20121	0.1	12711	-73.1*	947	-79.3*

† RLU value for standard curve point below combined S_{Min},
* Cartridge fails to meet internal acceptance criteria, concentration would not normally be reported (NR). Because this is a stability study, concentration is calculated even if the cartridge fails acceptance criteria, but will not be included in the mean or % recovery for the level.
Note: Week 0 is the same data for both storage conditions; storage stability testing was initiated at Week 0.

Table 23: Cartridge Stability at Room Temperature, Concentration (ng/mL)

Week	Nominal [Ace-011] ng/mL	Conc. ng/mL	Mean	CV %	% Recovery To Day 0	% Recovery To Nominal
0	3000	4118.8	3573.3	15.9	100.0	119.1
		2985.0				
		3616.2				
	120	116.3	116.4	12.2	100.0	97.0
		130.7				
		102.2				
	0	OORL	OORL	-	-	-
		OORL				
		OORL				
1	3000	3141.8*	3719.8	9.6	104.1	124.0*
		3467.6				
		3972.0				
	120	160.3	128.0	35.6	110.0	106.7
		95.8				
		126.6*				
	0	OORL	OORL	-	-	-
		OORL*				
		OORL*				
2	3000	OORH*	-	-	-	-
		OORH*				
		OORH*				
	120	157.1*	-	-	-	-
		275.4*				
		237.3*				
	0	OORL*	-	-	-	-
		49.4*				
		OORL*				
4	3000	OORH*	-	-	-	-
		OORH*				
		OORH*				
	120	236.6*	-	-	-	-
		336.0*				
		348.0*				
	0	54.6†*	-	-	-	-
		83.1*				
		51.0*				

† Concentration derived from only one replicate when other is OORL/OORH, as described in section 14.1.
* Cartridge fails to meet internal acceptance criteria, concentration would not normally be reported (NR). Because this is a stability study, concentration is calculated even if the cartridge fails acceptance criteria, but will not be included in the mean or % recovery for the level.
Note: Week 0 is the same data for both storage conditions; storage stability testing was initiated at Week 0.

Table 23: Cartridge Stability at Room Temperature, Concentration (ng/mL), *Continued*

Week	Nominal [Ace-011] ng/mL	Conc. ng/mL	Mean	CV %	% Recovery To Day 0	% Recovery To Nominal
8	3000	OORH*	-	-	-	-
		OORH*				
		OORH*				
	120	889.7*	-	-	-	-
		470.9*				
		981.3*				
	0	90.4*	-	-	-	-
		OORH*				
		111.2*				
12	3000	OORH*	-	-	-	-
		OORH*				
		OORH*				
	120	1402.1*	-	-	-	-
		705.9*				
		735.4*				
	0	315.3*	-	-	-	-
		2675.4*				
		195.4*				

* Cartridge fails to meet internal acceptance criteria, concentration would not normally be reported (NR). Because this is a stability study, concentration is calculated even if the cartridge fails acceptance criteria, but will not be included in the mean or % recovery for the level.
Note: Week 0 is the same data for both storage conditions; storage stability testing was initiated at Week 0.

Table 24: High Concentration Hook Effect Test at 12,000 ng/mL, Signal (RLU)

	Cartridge Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
Cartridge	**1**	**2**	**Mean**	**CV %**	**Mean**	**% DFE**	**Mean**	**% DFE**
1	1279†	1589†	1434	15.3	48443	2.5	4801	5.0
2	1386†	1577†	1481	9.1	53278	12.7	3729	-18.4
3	1417†	1621†	1519	9.5	54351	15.0	4966	8.7
4	1430†	1551†	1490	5.7	49445	4.6	4391	-3.9
5	1600†	1414†	1507	8.7	47251	-0.1	4006	-12.4

† RLU value for standard curve point below combined S_{Min},

Table 25: High Concentration Hook Effect Test at 12,000 ng/mL, Concentration (ng/mL)

Cartridge	Conc. ng/mL
1	OORH
2	OORH
3	OORH
4	OORH
5	OORH

All Cartridges tested at 12,000 ng/mL produced RLU lower than the mean RLU corresponding to ULOQ (mean RLU at ULOQ of 4000 ng/mL = 2456).

Table 26: Instrument Carryover Test, Signal (RLU)

Cartridge	Cartridge Sample Replicates 1	2	Intra-Cartridge Mean	CV %	120 ng/mL Control Mean	% DFE	3000 ng/mL Control Mean	% DFE
1	71709‡	82422‡	77066	9.8	39468	-16.5	4399	-3.8
2	74445‡	69102‡	71773	5.3	45561	-3.6	4772	4.4
3	63038‡	59067‡	61052	4.6	41790	-11.6	3685	-19.4
4	60541‡	66187‡	63364	6.3	45972	-2.8	4788	4.8
5	76452‡	79921‡	78186	3.1	43852	-7.3	4780	4.6
6	81220‡	77226‡	79223	3.6	56121	18.7	4755	4.0
7	80695‡	81689‡	81192	0.9	46882	-0.8	4861	6.4
8	80593‡	79944‡	80269	0.6	50381	6.6	4491	-1.7
9	57960‡	69693‡	63827	13.0	41486	-12.3	4160	-9.0

‡RLU value for standard curve point above combined S_{Max},

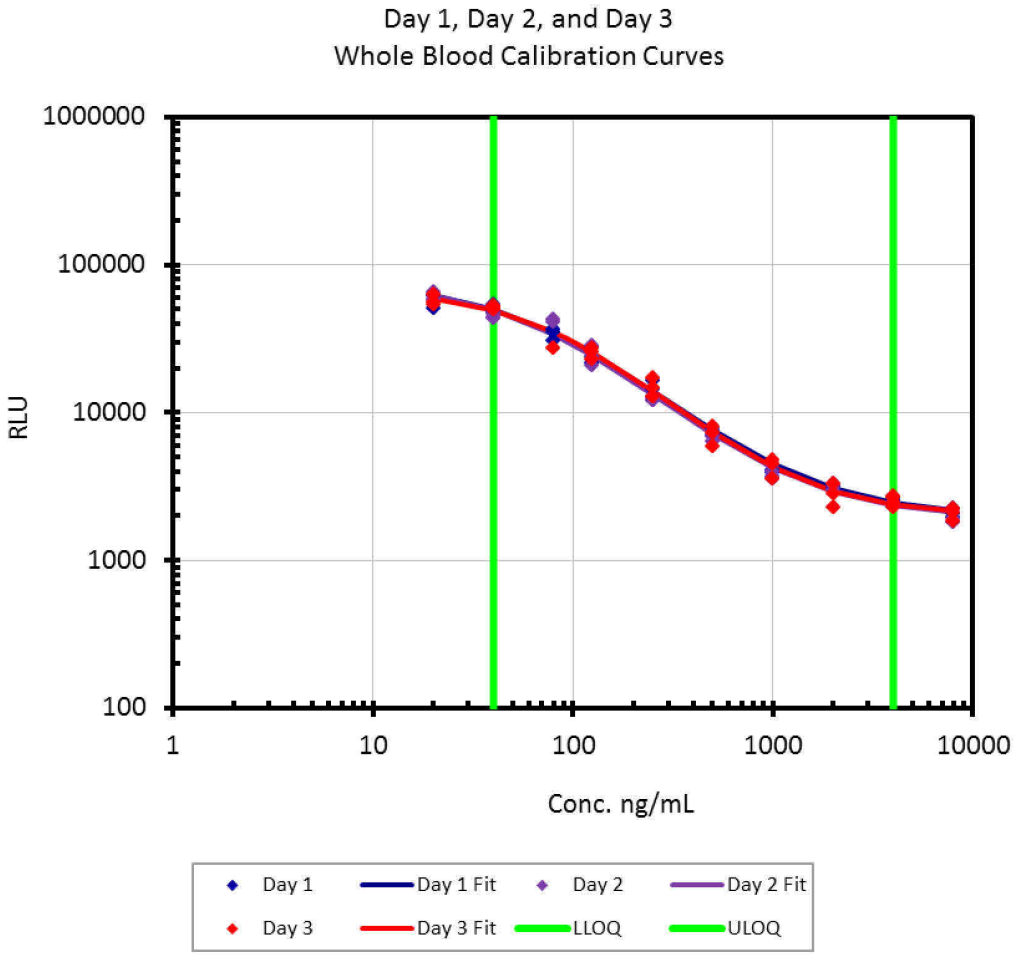
Table 27: Instrument Carryover Test, Concentration (ng/mL)

Cartridge	Conc. ng/mL
1	OORL
2	OORL
3	OORL
4	OORL
5	OORL
6	OORL
7	OORL
8	OORL
9	OORL

All cartridges tested at 0 ng/mL on 9 instruments immediately following a cartridge with a 4000 ng/mL sample produced RLU higher than the mean S_{Max} (52568)

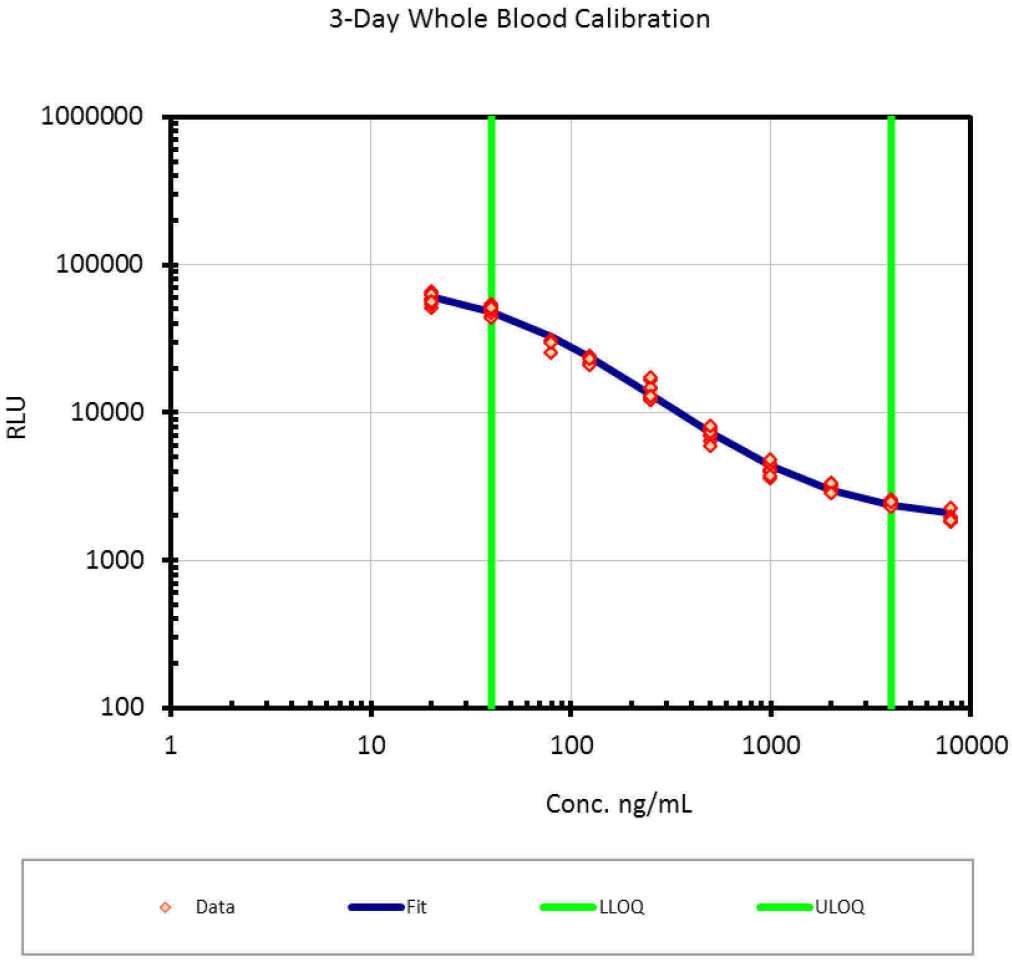
21 FIGURES

Figure 1: Standard Curves for Day 1, 2 and 3 Calibrations in Whole Blood



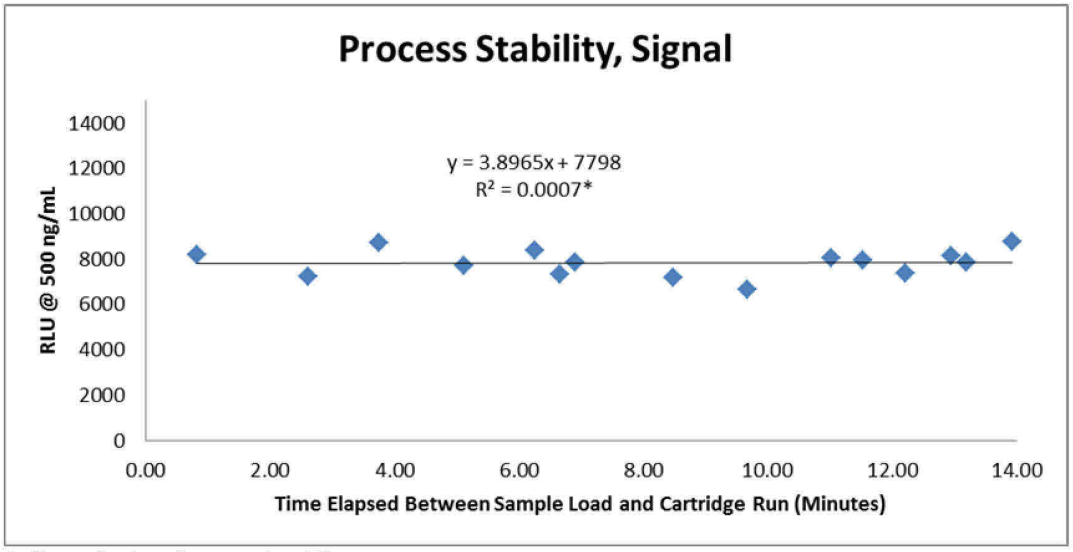
Superimposed standard curves for day 1, 2 and 3 to illustrate reproducibility of standard curve in 3 different whole blood samples. (All whole blood concentrations were calculated from the combined mean standard curve shown in Figure 2.)

Figure 2: Mean Standard Curve for 3-Day Calibration in Whole Blood



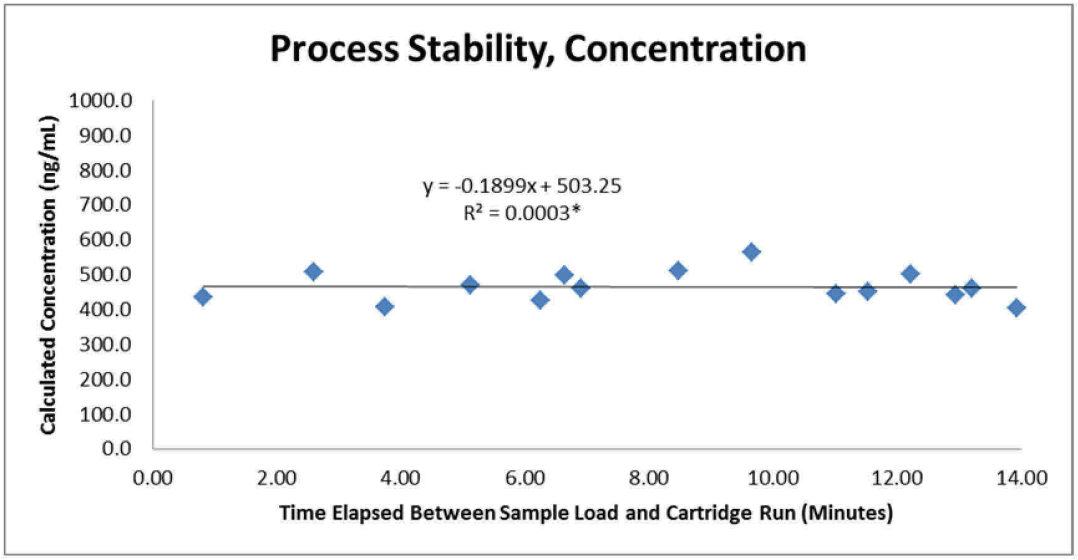
The combined 3 day mean standard curve was used to calculate all whole blood concentrations.

Figure 3: Process Stability at Room Temperature, Signal (RLU)



* Correlation is not significant

Figure 4: Process Stability at Room Temperature, Concentration (ng/mL)



* Correlation is not significant

Figure 5: Cartridge Stability at 4°C, Signal (RLU)

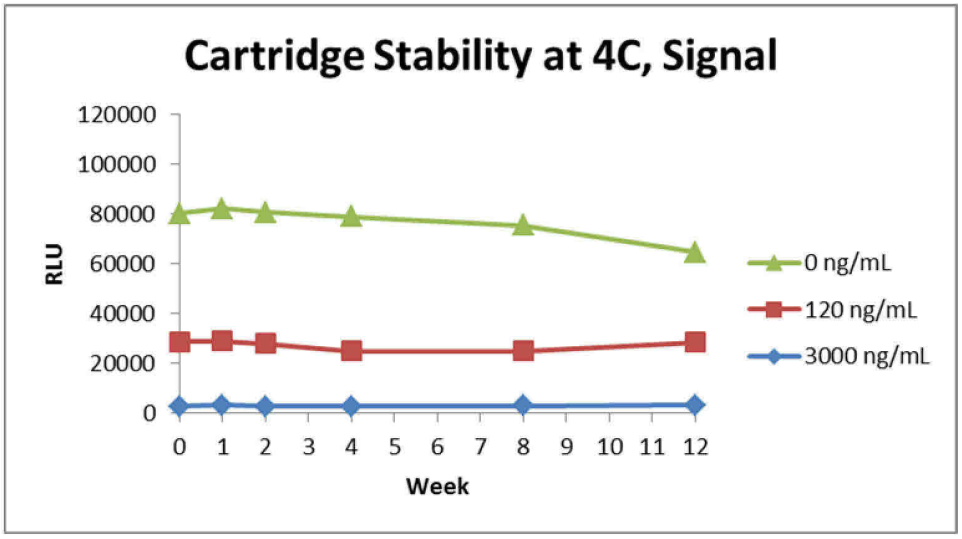
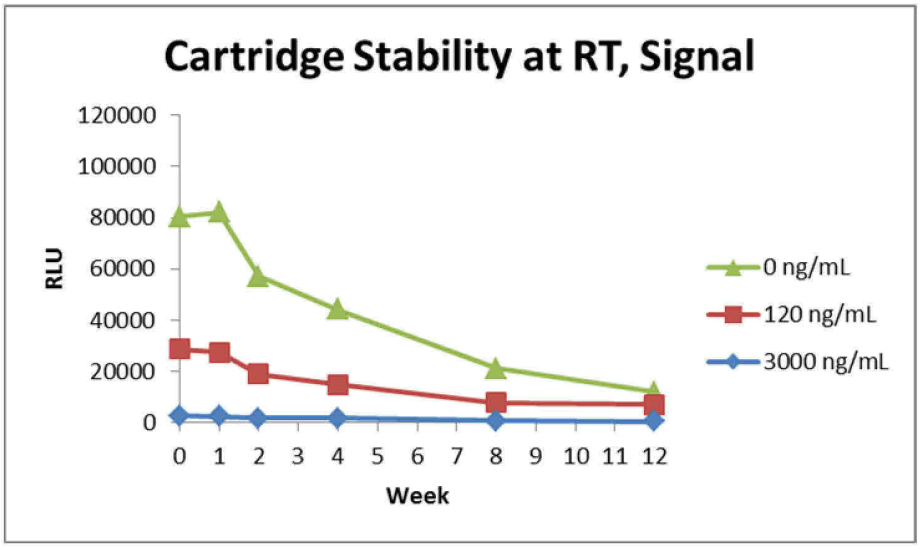


Figure 6: Cartridge Stability at Room Temperature, Signal (RLU)



22 APPENDICES

22.1 Protocol

A copy of the original protocol is included here.

Method Validation Protocol

Determination of ACE-011 in Human Whole Blood using the Theranos Field Systems

CELG-0004 12/23/2010 DLY

Theranos Project Number: ~~Celgene Contract ID 8919 for CO#2 to SOW:CELG-002~~
Celgene Study Number: ACE-011-DMPK-001

Sponsor
Celgene Corporation
86 Morris Avenue
Summit, NJ 07901

THERANOS CONFIDENTIAL Page 1

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Theranos Validation Protocol

PROTOCOL TITLE:
Determination of ACE-011 in Human Whole Blood using the Theranos Field Systems

THERANOS PROJECT NUMBER:
~~Celgene Contract ID 8919 for CO#2 to SOW:CELG-002~~
CELG-0004

12/23/2010
DLY

BIOANALYTICAL TEST FACILITY:
Theranos
3200 Hillview Ave,
Palo Alto, Ca 94304

SPONSOR:
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86 Morris Avenue
Summit, NJ 07901

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PROTOCOL APPROVAL

This protocol has been approved by:

Surekha Gangakhedkar
Principal Investigator
Theranos

12-6-10
Date:

Reviewed by:

Gary Frenzel
VP Assay Systems
Theranos

12-13-10
Date:

Sponsor Representative:

Peter D Bryan, Ph.D.
Associate Director – DMPK
Celgene Corporation

06 Dec 10
Date:

Theranos Quality Assurance:

Don Vu
Quality Assurance Representative
Theranos

12/06/10
Date:

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Introduction

The purpose of this protocol is to outline the responsibilities of Theranos and the Sponsor, and agree upon the conditions set forth herein with regard to Theranos Project No.: ~~Celgene Contract ID 8919 for CO#2 to SOW:CELG-002~~ CELG0004. Changes made to this protocol will be made by an approved amendment by Theranos and Celgene Corporation. The principal investigator from Theranos serves as the study director for the validation study.

12/23/2010
DLY

Objective

The objective of the study is to validate an ELISA method to quantify ACE-011 using the Theranos Assay System in human whole blood. The validated assay method will be used to determine ACE-011 in human whole blood samples generated during clinical studies.

Reference Standards

Reference standards as stock solution, will be supplied by the Sponsor. A Certificate of Analysis (C of A) containing lot numbers and expiration dates for the reference standards will be provided by the Sponsor. All precautions in the handling, storage, and disposal of ACE-011 and other assay components will be according to the C of A and the vendor's recommendations.

Test Article information:

Name of Test Article: ACE-011

Source: Acceleron Pharma

Lot No.: 09011-001

Expiration Date: Will be provided by the Sponsor

Purity: Will be provided by the Sponsor

Storage Conditions: -65°C or colder

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Experimental Procedures

A competitive ELISA will be validated for quantifying ACE-011 in human whole blood according to this bioanalytical method validation protocol performed on a Theranos cartridge. In this assay, the capture surface consists of rabbit anti-goat antibody. The samples (including standards and QCs), alkaline-phosphatase labeled ACE-011 and the anti-ACE-011 antibody (goat anti-ACTRIIa) are added to the capture surface. After the removal of unbound reagents by multiple wash steps, a chemiluminescent substrate is added. The response (Relative luminescence units) is inversely proportional to the amount of analyte present. Calibrations are analyzed using Theranos proprietary software.

Method validation will be performed to comply with the FDA 2001 Bioanalytical Method Validation Guidance.

Note: All cartridges will have two on board controls with ACE-011 at 3000 ng/mL and 120 ng/mL as plate system suitability controls.

1. Calibration Standards:

Calibration standards will be prepared within the concentration range of 40-4000 ng/mL with three individual whole blood samples. Anchor points below the LLOQ (40 ng/mL) and above the ULOQ (4000 ng/mL) will be used. For each validation run to be acceptable, a minimum of 75% of the total number of calibration standards in the calibration range should be within 100±20% (100±25% at LLOQ and ULOQ standards) of their nominal values, and a minimum of six unique standard concentrations must be within the assay range. The calibration curve must contain at least one calibration standard at both the LLOQ and ULOQ of the range.

- Calibration standards will consist of ACE-011 spiked into 3 individual whole blood samples (11 point standard curve). For each of the whole blood samples, 3 replicate cartridges will be run at each analyte level.

Table 1: Calibrator concentrations for the ACE-011 Standard Curve

ACE-011 Standard Curve	
Calibrator	**Concentration (ng/mL)**
1	8000
2	4000 (**ULOQ**)
3	2000
4	1000
5	500
6	250
7	125
8	80
9	40 (**LLOQ**)
10	20
11	0

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2. Quality Control Samples:

Quality control (validation) samples (LLOQ, QCL, QCM and QCH) will be spiked into three individual whole blood samples. The QC samples will be used to assess the accuracy and precision of the assay.

Table 2: QC levels for experiments in Validation protocol

ACE-011 QC levels		
	QC Level	**Concentration (ng/mL)**
1	ULOQ	4000
2	QCH	3000
3	QCM	400
4	QCL	120
5	LLOQ	40

3. Intra-day Accuracy and Precision:

Intra-day accuracy and precision will be evaluated for each of the three days. On each day, the QC levels specified in Table 2 will be spiked into a single whole blood sample. At least six replicate cartridges will be used per QC level on each of the days.

For method acceptance, the mean of back-calculated concentrations of the six (or more) replicates at each QC level for each day should not deviate more than ±20% (±25% for the LLOQ and ULOQ) from its corresponding nominal concentration. In addition, at least half of all the individual back-calculated concentrations from the six (or more) replicates for each QC level for each day must be within 100±20% (100±25% at the LLOQ and ULOQ) of their corresponding nominal values. The precision at each QC level for each day must not exceed 20% (25% for LLOQ) when calculated as the %CV.

The concentration will be back-calculated using the whole blood calibration curve. No more than one QC outlier (Dixon test) may be excluded from the statistical calculations for a given validation run, and a maximum of two QC outliers may be excluded for the combined three core runs.

4. Inter-day Accuracy and Precision:

Inter-day accuracy and precision will be evaluated over a period of three days. On each day, the QC levels specified in Table 2 will be spiked into a single whole blood sample. At least six replicate cartridges will be used per QC level on each of the days.

For method acceptance, the mean of back-calculated concentrations of all the replicates from all three days at each QC level should not deviate more than ±20% (±25% for the LLOQ and ULOQ) from its corresponding nominal concentration. The precision of all the replicates from all

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three days at each QC level must not exceed 20% (25% for LLOQ) when calculated as the %CV.

The concentration will be back-calculated using the whole blood calibration curve. No more than one QC outlier (Dixon test) may be excluded from the statistical calculations for a given validation run, and a maximum of two QC outliers may be excluded for the combined three core runs.

5. System Suitability Controls

All cartridges will include two on board controls with ACE-011 at 3000 and 120 ng/mL as system suitability controls. These controls are assayed to determine if the results from the cartridge are acceptable. For acceptance, the response from both the controls should be within ±25% of the defined mean response and at least one of them should within ±20% of the defined mean response.

6. Selectivity (matrix interference):

Twenty (20) individual whole blood samples (10 male, 10 female) will be tested unspiked and spiked at LLOQ (40 ng/mL) and 1000 ng/mL. Samples will be run in triplicate cartridges. For the spiked samples, 14 out of 20 of the back-calculated concentrations from the individual whole blood samples must be within 25% of the corresponding nominal concentration. In addition, the mean of the back-calculated concentrations of the individual whole blood samples must be within 25% of the corresponding nominal concentration. For the unspiked samples, 14 of 20 must have a back-calculated concentration less than the LLOQ. The concentration will be back-calculated using the whole blood calibration curve.

7. Instrument Precision:

To establish instrument precision, %CV across 24 cartridges (run on 24 instruments) with a mid range concentration (500 ng/mL in a single whole blood sample) should be within 20% of the nominal concentration. The concentration will be back-calculated using the whole blood calibration curve.

8. Test to evaluate high dose hook effect:

Evaluate response of assay at a concentration of 12,000 ng/mL in whole blood with 5 replicate cartridges. The back-calculated concentration for this analyte level for all five replicates should be greater than ULOQ.

9. Instrument Carryover:

A blank matrix sample will be assayed after the highest matrix standard (ULOQ) from each core validation run on three instruments to assess instrument carryover. The response of the instrument carryover sample must be less than of the mean LLOQ response to be considered

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acceptable.

10. Stability Test in Pre-built Cartridge:

Cartridge stability will be tested for cartridges stored at room temperature and 4°C. The stability tests will be performed with 3 analyte levels of 3000 ng/mL (QCH), 120 ng/mL (QCL) and 0 ng/mL spiked into pooled serum. Time points to be tested include 0, 1, 2, 4, 8, 12, 24 and 48 weeks with three replicates for each analyte level for each of the time points. All cartridges will include the on board controls. To establish acceptance, the mean back-calculated concentrations for each analyte level must be no more than ±20% from their Day 0 back-calculated concentration. In addition, the precision (%CV) of all the replicates must not exceed 20%. Data beyond 4 weeks will be presented as an appendix to the validation report at a later date.

For a process stability test, the stability of the spiked whole blood sample after loading into the cartridge will tested for a period of up to 10 minutes. Data from the experiments to determine instrument precision will be utilized for the process stability test evaluation.

11. Validation Report:

The validation report will follow the format recommended by Celgene.

22.2 Protocol Alteration Form

Method Validation Protocol Alteration Form

Determination of ACE-011 in Human Whole Blood using the Theranos Field Systems

Theranos Project No.: CELG-0004 Alteration No.: 1
Celgene Study No.: ACE-011-DMPK-001

Requested By: Daniel Young
Date requested: 15th January 2011

TYPE of ALTERATION

{x} Amendment (a-c)
{ } Deviation
{ } Clarification

SPONSER NOTIFICATION: Alteration Form

EFFECTIVE DATE OF CHANGE: 10th December 2010

DESCRIPTION OF CHANGE

a. Page 1: change Theranos Project number from "Celgene Contract ID 8919 for CO#2 to SOW:CELG-002" to "CELG-0004"

b. Page 2: change Theranos Project number from "Celgene Contract ID 8919 for CO#2 to SOW:CELG-002" to "CELG-0004"

c. Page 4: change Theranos Project number from "Celgene Contract ID 8919 for CO#2 to SOW:CELG-002" to "CELG-0004"

JUSTIFICATION

This is a correction to indicate the correct Project Number.

IMPACT ON THE STUDY

(a-c) No impact on the study

SIGNATURES

Sponsor Approval : _____ Date: 19 Jan 11

Study Director : _____ Date: 4/27/2011

Management : _____ Date: 4/26/2011

Quality Assurance Review: _____ Date: 4/27/2011

Method Validation Protocol Alteration Form

Determination of ACE-011 in Human Whole Blood using the Theranos Field Systems

Theranos Project No.: CELG-0004
Celgene Study No.: ACE-011-DMPK-001

Requested By: Daniel Young
Date requested: 6th April 2011

TYPE of ALTERATION

{x} Amendment (a)
{ } Deviation
{x} Clarification (b)

SPONSER NOTIFICATION: Alteration Form

EFFECTIVE DATE OF CHANGE: 6th April 2011

DESCRIPTION OF CHANGE

a) Page 8:

change *"To establish acceptance, the mean back-calculated concentrations for each analyte level must be no more than ±20% from their Day 0 back-calculated concentration"*

to *"To establish acceptance, the mean back-calculated concentrations for each analyte level must be no more than ±20% from their nominal concentration"*

b) Page 7:

change "*For the spiked samples, 14 out of 20 of the back-calculated concentrations from the individual whole blood samples must be within 25% of the corresponding nominal concentration. In addition, the mean of the back-calculated concentrations of the individual whole blood samples must be within 25% of the corresponding nominal concentration.*"

to "*the mean back-calculated concentrations from the three replicates of each whole blood*

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sample will be used for evaluation of selectivity criteria and at least 14 out of these 20 mean back calculated concentrations should be within 25% of the nominal concentration."

JUSTIFICATION

a) Amendment made with approval of Study Director from Celgene to eliminate the undesired dependence on Day 0 concentrations for evaluation of criteria at subsequent time points.

b) Clarification of acceptance criteria

IMPACT ON THE STUDY

a) Allows appropriate evaluation of acceptance criteria for stability study

b) No impact on the study

SIGNATURES

Sponsor Approval : _____ Date: 7 Apr 11

Study Director : _____ Date: 4/27/11

Management : _____ Date: 4/26/2011

Quality Assurance Review: _____ Date: 4/27/2011