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
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-004
Study Initiation Date	December 13, 2010
Experimental Completion Date	January 10, 2011
Report Issued	January 10, 2011
Total Pages	72

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4 ABBREVIATIONS

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

COA	Certificate of Analysis
Conc.	Concentration
CV	Coefficient of Variance
DFE	Difference from Expected
ELISA	Enzyme-linked Immunosorbent Assay
LLOQ	Lower Limit of Quantification
N	Number of cartridge replicates
NA	Not Applicable
NC	Not Calculated
ng/mL	Nanogram per milliliter
NR	Not Reportable
OORH	Out of Range High
OORL	Out of Range Low
QC	Quality Control
r^2	Coefficient of Determination
RLU	Relative Light Units
RT	Room Temperature
S.D.	Standard Deviation
SOP	Standard Operating Procedure
µL	Microliter(s)
ULOQ	Upper Limit of Quantitation

5 GLP COMPLIANCE STATEMENT

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

This study was conducted in compliance with 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	01/10/11
Surekha Gangakhedkar Principal Investigator Theranos, Inc	Date

6 QUALITY ASSURANCE STATEMENT

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

Principal Investigator: Surekha Gangakhedkar

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	01/10/11	01/10/11
Draft Report	Javier Quinonez	01/10/11	01/10/11

Javier Quinonez — 01/03/11

Quality Assurance
Theranos, Inc — Date

7 RESPONSIBLE PERSONNEL

Surekha Gangakhedkar	Assay Systems Manager, Principal Investigator
Gary Frenzel	VP Assay Systems
Tina Noyes	Senior Scientist
Javier Quinonez	QA Auditor

8 ARCHIVE STATEMENT

All raw data, 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	01/10/11
Surekha Gangakhedkar Principal Investigator Theranos, Inc	Date
Gary Frenzel	01/10/11
Gary Frenzel 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 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.

10.2 Methods

A competitive ELISA will be validated for quantifying ACE-011 in human whole blood 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 Light 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.

10.3 Results

A summary of the data for ACE-011 test results for the 3 core runs in 3 different whole blood samples and additional experiments are provided in Table 1.

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-004 Celgene No. ACE-011-DMPK-001
Analyte Name and Synonym	ACE-011 (ActRIIA-IgG1)
Sample Volume	20 µL
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 %)	10.1%	Yes
Inter-Instrument Accuracy N=24 Instruments (% Recovery)	93%	Yes
Intra-Day Precision at 5 QC Levels N=6 per level (CV%)	5.9 to 19.8%	Yes
Intra-Day Accuracy at 5 QC Levels N=6 per level (% Recovery)	81 to 110%	Yes
Inter-Day Precision at 5 QC Levels N=18 per level (CV%)	10.2 to 16.4%	Yes
Inter-Day Accuracy at 5 QC Levels N=18 per level (% Recovery)	82 to 105%	Yes
High Dose 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)	20 out of 20 samples were 100±25%	Yes
Selectivity: Recovery at 40 ng/mL (LLOQ) (% Recovery)	17 out of 20 samples were 100±25%	Yes
Selectivity: Recovery at 0 ng/mL (un-spiked) (% Recovery)	20 out of 20 samples were OORL	Yes
Process Stability: Precision at 500 ng/mL N = 15 (CV %)	8.8 %	Yes
Process Stability: Accuracy at 500 ng/mL N = 15 (% Recovery)	93 %	Yes
Cartridge Stability Week 1-4 at 4°C: Precision (CV %)	7.1 to 18.8%	Yes
Cartridge Stability Week 1-4 at 4°C: Accuracy (% Recovery)	82 to 119%	Yes

10.4 Conclusion

An ELISA method (Celgene No. ACE-011-DMPK-001) has been validated for the quantification of ACE-011 in human whole blood from 40.0 to 4000.0 ng/mL.

The results indicate the method is sensitive, selective, accurate, and reproducible.

11 MATERIALS AND EQUIPMENT

11.1 Chemicals and Reagents

HPLC grade Water, Baker
Phosphate Buffered Saline (PBS), Sigma
Wash Buffer, Assay Designs
99% Pure BSA, Sigma
Heterophilic Blocking Reagent (HBR), Scantibodies
Rabbit anti-goat antibody, Southern Biotech
ACE-011, Provided by Sponsor
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.

Plastic tubes: 1.5 mL
Pipette Tips for single and Multichannel pipettes
Pipettes: Single channels: 1-10µL, 20-200 µL; Multichannel 20-300µL

11.3 Analytical Equipment

Theranos ACE-011 060 Cartridges and System

12 ANALYTE INFORMATION

Name:	ACE-011
Synonym	ActRIIA-IgG1
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 ≤3 times the concentration of the lowest calibration standard. Mid QC concentration was approximately in the middle (linear) of the calibration curve. High QC was approximately 75-80% of the highest calibration standard concentration.

Calibration standard and QC concentrations were determined based upon the actual concentrations of the stock solutions.

All statistics in the data tables are calculated by Microsoft® Excel according to the calibration rules and equation applied by the Theranos System.

14 ACCEPTANCE CRITERIA

The validation acceptance criteria and the statistical data will be determined at a minimum to this protocol.

14.1 Run Acceptance Criteria

Within each cartridge, the standards or samples will be automatically assayed in two replicates. 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.

Cartridge acceptance criteria include:

(1) The % CV from the two sample replicates is $\leq 25\%$.

(2) 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 within ±20% of the defined mean response.

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

14.2 Method Acceptance

Calibration standards

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.

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 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 LLOQ. The concentration will be back-calculated using the whole blood calibration curve.

Instrument Precision

For acceptance, 24 cartridges (run on 24 difference 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.

High Dose 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 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

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 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.

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 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, 25µL of the 10X standard mixed with 225µL whole blood for a total of 250µL of spike whole blood. Spikes were mixed into whole blood by pipetting up and down gently 8 times.

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	4-Parameter Logistic
Sample Volume	20 µL whole blood
Extraction 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 3 complete validation runs in 3 different whole blood samples on 3 days were performed during the method validation.

17.1 Standard Curve and Regression Analysis

Calibration standard concentrations 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 using 4-Parameter Logistic regression using Theranos proprietary software. In addition, a standard calibration curve was determined using data from all three days. Expected values for the on-board controls were calculated as the mean RLU using all the runs for three days.

Table 3 shows the expected values for the on-board controls. Tables 4-6 show the 4-Parameter Logistic Regression parameters for each of the three days. Table 7 shows the 4-Parameter Logistic Regression parameters using data from all 3 days. Tables 8-10 show the raw data for the calibration runs for the three days and Table 11 summarizes the back-calculated concentrations for the runs for the three days.

The acceptance criteria for the Calibration Standards is met each day with the percentage of individual cartridges showing recovery within 100±20% (100±25% at the LLOQ and ULOQ) of nominal as follows: Day 1; 83%, Day 2; 76%, Day 3; 75%

17.2 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.

17.2.1 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 on each of the days. The concentrations of the QC levels were calculated using the same day's whole blood calibration curve. Intra-day accuracy and precision raw data and calculated concentration results are shown in Tables 12 through 15. These results met the acceptance criteria.

17.2.2 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 raw data and calculated concentration results are shown in Tables 16 and 17. These results met the acceptance criteria.

17.2.3 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. Intra-instrument accuracy and precision raw data and concentration results are shown in Table 18 and 19. These results met the acceptance criteria.

17.3 Lower and Upper Limit of Quantification, SMin and SMax

The lower limit of quantification (LLOQ) of the assay was 40.0 ng/mL and the upper limit of quantification (ULOQ) was 4000.0 ng/mL. The maximum and minimum signal (RLU) corresponding to the calibrated range was determined by the 4 Parameter Logistic fit as SMax and SMin respectively, and any results that fell above SMax were reported as "OORH" while results that fell below SMin were reported as "OORL".

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 twenty individual lots of normal human whole blood spiked with 1000 ng/mL, 40 ng/mL and without ACE-011. Table 20 shows the raw data and Table 21 shows the concentration results for the selectivity test, with a summary in Table 22. All of the 20 samples showed OORL (below LLOQ) results un-spiked, 17 of the 20 samples showed a recovery between 75-125% spiked at 40.0 ng/mL (LLOQ), and all 20 of the samples showed recovery between 75-125% spiked at 1000.0 ng/mL. These results met the acceptance criteria.

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 in human whole blood at room temperature after addition to the Theranos 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 23 and the concentration results are shown in Table 24. Figures 5 and 6 depict the data in graphical form. These results met the acceptance criteria.

17.5.2 Cartridge Stability

Stability tests are ongoing for manufactured cartridges stored at room temperature and 4°C (the recommended storage condition). This stability test encompasses all the components of the cartridge including the capture surface, conjugate, and the onboard liquid controls.

The cartridges were manufactured and packaged in individual sealed foil pouches and then stored at either 4°C in a glass-front refrigerator, or 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 -80°C. 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 at 3000 ng/mL and 120 ng/mL in assay buffer.

Tables 25 through 28 show the raw data and calculated concentration results gathered as of this report. Figures 7 and 8 depict the stability data in graphical form. At 4°C, the recommended storage condition, the stability results meet the acceptance criteria through week 4. 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 indicating 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 Dose 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 29 and the concentration results are shown in Table 30. The calculated concentrations for all 5 cartridges were OORH and the RLU were less than the SMin. These results met the acceptance criteria.

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 31 and the concentration results are shown in Table 32. The calculated concentrations for all 9 cartridges were OORL and the RLU were greater than the SMax. These results met the acceptance criteria.

18 CONCLUSION

An ELISA method (Celgene number ACE-011-DMPK-001) has been validated for the quantification of ACE-011 in Human Whole Blood from 40.0 - 4000.0 ng/mL. The results indicate the method is sensitive, selective, accurate, and reproducible. The cartridges are stable when stored at 4 degrees (current stability data available for 4 weeks; longer study is on-going). In addition, ACE-011 is stable in human whole blood for at least 10 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-004, Celgene Study Number: ACE-011-DMPK-001

- ACE-011 Assay Notebook

20 TABLES

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

Parameter	Value
120 ng/mL Onboard Control: Expected Value (RLU)	47267
3000 ng/mL Onboard Control: Expected Value (RLU)	4577

Table 4: Regression Analysis of Day 1 ACE-011 Calibration in Human Whole Blood

Parameter	Value
Min	1983.96712949276
Max	76417.8579939314
Slope	1.22759742248462
Ed50	65.2149302620573
LLOQ (ng/mL)	40.0
ULOQ (ng/mL)	4000.0
Equation	RLU = Min+(Max-Min)/(1+(Conc/Ed50))^Slope
SMax (Expected RLU at LLOQ)	54503
SMin (Expected RLU at ULOQ)	2382

Table 5: Regression Analysis of Day 2 ACE-011 Calibration in Human Whole Blood

Parameter	**Value**
Min	1945.12382586302
Max	75951.1087062286
Slope	1.25658397094462
Ed50	63.9656971481931
LLOQ (ng/mL)	40.0
ULOQ (ng/mL)	4000.0
Equation	RLU = Min+(Max-Min)/(1+(Conc/Ed50))^Slope
SMax (Expected RLU at LLOQ)	54106
SMin (Expected RLU at ULOQ)	2287

Table 6: Regression Analysis of Day 3 ACE-011 Calibration in Human Whole Blood

Parameter	**Value**
Min	1990.38258329355
Max	68926.9009417742
Slope	1.29265551374546
Ed50	75.8393131455105
LLOQ (ng/mL)	40.0
ULOQ (ng/mL)	4000.0
Equation	RLU = Min+(Max-Min)/(1+(Conc/Ed50))^Slope
SMax (Expected RLU at LLOQ)	52403
SMin (Expected RLU at ULOQ)	2321

Table 7: Regression Analysis of 3-Day ACE-011 Calibration in Human Whole Blood

Parameter	**Value**
Min	2014.04465757502
Max	69872.237599832
Slope	1.30878573744094
Ed50	75.1058097981751
LLOQ (ng/mL)	40.0
ULOQ (ng/mL)	4000.0
Equation	RLU = Min+(Max-Min)/(1+(Conc/Ed50))^Slope
SMax (Expected RLU at LLOQ)	53136
SMin (Expected RLU at ULOQ)	2324

Table 8: ACE-011 Calibration in Human Whole Blood Day 1, Signal (RLU)

[ACE-011] ng/mL	Sample Replicates 1	2	Intra-Cartridge Mean	CV %	Inter-Cartridge Mean	CV %	120 ng/mL Control RLU	% DFE	3000 ng/mL Control RLU	% DFE
8000	2249	2217	2233	1	2091	6.5	49662	5	4506	-1
	1795	2131	1963	12			48203	2	4817	5
	2204	1951	2077	9			51398	9	5064	11
4000	2403	2593	2498	5	2496	4.9	52549	11	4724	3
	2623	2495	2559	4			56637	20	4870	7
	3082	2319	2700	20			52880	12	4813	5
	2315	2532	2424	6			49734	5	4791	5
	2120	2579	2350	14			46719	-1	4086	-11
	2352	2540	2446	5			46760	-1	4304	-6
2000	2755	3439	3097	16	3132	1.6	47847	1	5063	11
	3068	3268	3168	4			41066	-13	4349	-5
	NR	NR	NR	-			NR	-	NR	-
1000	3595	3563	3579	1	3865	6.9	47495	0	3996	-13
	4068	4138	4103	1			40499	-14	4373	-4
	3762	4067	3914	5			41769	-12	4189	-8
500	7103	6978	7040	1	7459	5.0	40447	-14	4557	0
	7504	7689	7596	2			46245	-2	5467	20
	7646	7837	7741	2			53186	12	5047	10
250	18788	14624	16706	18	14728	12.8	48247	2	5709	25
	15722	13343	14532	12			56775	20	5355	17
	12513	13378	12945	5			41126	-13	4656	2
125	23021	24507	23764	4	24503	13.7	47247	0	5382	18
	20346	22832	21589	8			40769	-14	4676	2
	29208	27104	28156	5			46690	-1	5190	14
80	31113	30363	30738	2	34137	8.8	41914	-11	3895	-15
	34197	36255	35226	4			52316	11	5126	12
	34228	38665	36446	9			53704	14	5354	17
40	47775	45604	46690	3	50141	5.8	48889	3	4029	-12
	47254	48326	47790	2			42102	-11	4603	1
	53686	54718	54202	1			44463	-6	4684	2
	48868	53889	51379	7			47394	0	4954	8
	54716	42336	48526	18			39714	-16	4231	-7
	47557	56957	52257	13			51034	8	4567	0
20	57089	66045	61567	10	58692	11.1	48576	3	4874	7
	66928	59600	63264	8			46302	-2	5353	17
	49084	53406	51245	6			43317	-8	4461	-2
0	70841	77712	74277	7	74444	0.3	41781	-12	4426	-3
	72171	77054	74612	5			50172	6	5130	12
	NR	NR	NR	-			NR	-	NR	-

N = 3 cartridges for regular calibration points, N = 6 cartridges for LLOQ and ULOQ
NR = Not Reported, % DFE = Percentage Difference From Expected Value

Table 9: ACE-011 Calibration in Human Whole Blood Day 2, Signal (RLU)

[ACE-011] ng/mL	Sample Replicates 1	2	Intra-Cartridge Mean	CV %	Inter-Cartridge Mean	CV %	120 ng/mL Control RLU	% DFE	3000 ng/mL Control RLU	% DFE
8000	2299	2197	2248	3	1990	11.5	43566	-8	4848	6
	1643	1975	1809	13			41324	-13	4761	4
	1848	1978	1913	5			38009	-20	4299	-6
4000	2111	2558	2335	14	2383	2.0	40924	-13	4045	-11
	2222	2485	2354	8			49231	4	4972	9
	2473	2332	2403	4			50926	8	4821	5
	2337	2427	2382	3			59248	25	4515	-1
	2597	2337	2467	7			59220	25	4996	9
	2238	2481	2360	7			57384	21	4301	-6
2000	3308	3310	3309	0	3191	4.8	54294	15	4777	5
	2926	3113	3020	4			49680	5	4892	7
	2958	3531	3245	12			38592	-18	4134	-10
1000	4205	4728	4467	8	4074	9.0	41383	-12	5185	13
	3939	4090	4014	3			53626	13	4641	2
	3781	3699	3740	2			38404	-19	4820	5
500	8114	7175	7644	9	6985	9.1	42761	-10	4107	-10
	6088	6655	6372	6			39260	-17	4334	-5
	7315	6561	6938	8			50010	6	4202	-8
250	12122	12145	12133	0	12255	1.7	52479	11	4400	-4
	11693	13297	12495	9			50797	7	5527	21
	12282	11991	12136	2			49700	5	4912	7
125	18764	23428	21096	16	24464	15.6	54169	15	4290	-6
	28980	28274	28627	2			53011	12	4742	4
	23391	23946	23669	2			44567	-6	4304	-6
80	42238	40066	41152	4	42173	3.4	58116	23	5149	13
	40103	46287	43195	10			44074	-7	5261	15
	NR	NR	NR	-			NR	-	NR	-
40	41253	47407	44330	10	47946	7.4	42491	-10	4443	-3
	41230	45206	43218	7			40768	-14	4452	-3
	50914	50348	50631	1			52808	12	4912	7
	50596	52882	51739	3			41551	-12	3881	-15
	47124	53378	50251	9			41650	-12	4152	-9
	46206	48804	47505	4			52610	11	3854	-16
20	58475	58315	58395	0	60505	7.0	46134	-2	4027	-12
	55193	60335	57764	6			41759	-12	4825	6
	68673	62039	65356	7			46445	-2	4783	5
0	69169	67562	68366	2	68090	2.5	44854	-5	4011	-12
	72089	60445	66267	12			46480	-2	4720	3
	74581	64696	69638	10			43365	-8	4718	3

N = 3 cartridges for regular calibration points, N = 6 cartridges for LLOQ and ULOQ
NR = Not Reported, % DFE = Percentage Difference From Expected Value

Table 10: ACE-011 Calibration in Human Whole Blood Day 3, Signal (RLU)

[ACE-011] ng/mL	Sample Replicates 1	2	Intra-Cartridge Mean	CV %	Inter-Cartridge Mean	CV %	120 ng/mL Control RLU	% DFE	3000 ng/mL Control RLU	% DFE
8000	2210	2238	2224	1	2063	8.9	45154	-5	5267	15
	1987	2216	2102	8			56674	20	4786	5
	1849	1879	1864	1			50811	7	4378	-4
4000	2433	2407	2420	1	2488	6.9	44285	-6	4952	8
	2248	2500	2374	7			44967	-5	4287	-6
	2665	2803	2734	4			48375	2	4663	2
	2427	2155	2291	8			50822	7	4650	2
	2414	2489	2452	2			49930	6	4610	1
	2710	2607	2658	3			46827	-1	4288	-6
2000	2617	2012	2315	18	2834	18.1	36822	-22	4359	-5
	3756	2923	3339	18			51834	10	5286	16
	2871	2826	2848	1			38257	-19	4771	4
1000	4247	4813	4530	9	4663	4.0	47156	0	5191	14
	NR	NR	NR	-			NR	-	NR	-
	5126	4465	4795	10			42911	-9	4132	-10
500	7261	7234	7247	0	7073	15.3	52152	10	3614	-21
	7828	8283	8056	4			43250	-9	3759	-18
	6188	5646	5917	6			51757	9	5110	12
250	14716	14444	14580	1	14929	15.2	48921	3	4567	0
	18668	16044	17356	11			56113	19	4612	1
	12553	13149	12851	3			50389	7	4783	5
125	22898	23011	22954	0	25374	8.9	42990	-9	4393	-4
	24167	27331	25749	9			55890	18	4912	7
	29215	25625	27420	9			50068	6	4753	4
80	25690	25270	25480	1	27684	11.3	42501	-10	3742	-18
	NR	NR	NR	-			NR	-	NR	-
	27764	32012	29888	10			42291	-11	3559	-22
40	55153	51339	53246	5	51218	2.4	48215	2	4152	-9
	51241	48919	50080	3			37887	-20	5376	18
	51876	51260	51568	1			45086	-5	3932	-14
	53666	46016	49841	11			47894	1	4170	-9
	48955	53651	51303	6			43615	-8	3834	-16
	48281	54254	51268	8			37158	-21	3750	-18
20	57032	69005	63018	13	57420	8.7	55679	18	4181	-9
	54016	52990	53503	1			48175	2	4255	-7
	58150	53330	55740	6			43827	-7	4266	-7
0	68818	64844	66831	4	68317	3.1	51469	9	4849	6
	NR	NR	NR	-			NR	-	NR	-
	72720	66887	69803	6			39374	-17	3757	-18

N = 3 cartridges for regular calibration points, N = 6 cartridges for LLOQ and ULOQ
NR = Not Reported, % DFE = Percentage Difference From Expected Value

Table 11: Day 1, 2 and 3 Calibration, Back-Calculated Concentration (ng/mL)

	Day 1				Day 2				Day 3			
[ACE-011] ng/mL	Conc	Mean	CV %	% Rec.	Conc	Mean	CV %	% Rec.	Conc	Mean	CV %	% Rec.
8000	OORH	OORH			OORH	OORH			OORH	OORH		
	OORH				OORH				OORH			
	OORH				OORH				OORH			
4000	3828	3270	19.8	82	2883	3373	10.3	84	3753	3238	17.0	81
	3436				3192				3284			
	1998				3707				2457			
	3544				3811				3702			
	3311				3436				3562			
	3502				3211				2668			
2000	2129	2009	8.5	100	1513	1663	10.1	83	2794	2207	26.1	110
	1888				1845				1641			
	NR				1632				2186			
1000	1467	1293	12.2	129	923	1071	13.4	107	933	898	5.6	90
	1157				1078				NR			
	1256				1211				862			
500	551	515	6.1	103	464	519	10.6	104	510	538	19.1	108
	503				575				452			
	491				519				651			
250	208	241	13.6	96	275	273	1.6	109	235	233	16.2	93
	241				268				195			
	273				275				270			
125	134	131	16.8	105	150	126	19.4	101	139	124	11.4	99
	151				101				121			
	108				129				111			
80	95	82	13.7	103	58	56	5.8	70	122	111	14.8	138
	78				54				NR			
	74				NR				99			
40	47	43	17.9	108	51	44	16.5	109	34	38	11.8	96
	44				53				37			
	33				38				34			
	38				36				46			
	57				39				39			
	45				44				41			
20	OORL	OORL			OORL	OORL			OORL	OORL		
	OORL				OORL				OORL			
	38				OORL				OORL			
0	OORL	OORL			OORL	OORL			OORL	OORL		
	OORL				OORL				NR			
	NR				OORL				OORL			

% Rec. = Percentage Recovery

Table 12: Intra-Day Precision, Day 1 Signal (RLU)

Level	[ACE-011] ng/mL	Sample Replicates 1	2	Intra-Cartridge Mean	CV %	Inter-Cartridge Mean	CV %	120 ng/mL Control RLU	% DFE	3000 ng/mL Control RLU	% DFE
ULOQ	4000	2403	2593	2498	5	2496	4.9	52549	11	4724	3
		2623	2495	2559	4			56637	20	4870	7
		3082	2319	2700	20			52880	12	4813	5
		2315	2532	2424	6			49734	5	4791	5
		2120	2579	2350	14			46719	-1	4086	-11
		2352	2540	2446	5			46760	-1	4304	-6
High	3000	3004	2848	2926	4	2650	6.8	45336	-4	3896	-15
		2768	2523	2645	7			43816	-7	4825	6
		2449	2944	2696	13			53951	14	4608	1
		2628	2799	2713	4			42839	-9	4547	-1
		2360	2446	2403	3			53090	12	4807	5
		2471	2564	2518	3			50694	7	3787	-17
Mid	400	7745	7183	7464	5	9351	12.9	50336	6	4651	2
		9393	9711	9552	2			51873	10	4774	4
		9770	11141	10456	9			44917	-5	5521	21
		9575	11921	10748	15			48583	3	5020	10
		7744	9558	8651	15			49210	4	5112	12
		8441	10028	9235	12			52912	12	4578	0
Low	120	30821	26724	28773	10	26006	7.8	48420	2	4519	-1
		28612	25002	26807	10			39484	-16	4533	-1
		26392	25357	25874	3			41854	-11	4233	-7
		20811	24689	22750	12			53167	12	4388	-4
		28134	25609	26871	7			44364	-6	4122	-10
		28279	21639	24959	19			51263	8	4783	5
LLOQ	40	47775	45604	46690	3	50141	5.8	48889	3	4029	-12
		47254	48326	47790	2			42102	-11	4603	1
		53686	54718	54202	1			44463	-6	4684	2
		48868	53889	51379	7			47394	0	4954	8
		54716	42336	48526	18			39714	-16	4231	-7
		47557	56957	52257	13			51034	8	4567	0

N = 6 cartridges per level
% DFE = Percentage Difference From Expected Value

Table 13: Intra-Day Precision, Day 2 Signal (RLU)

Level	[ACE-011] ng/mL	Sample Replicates 1	2	Intra-Cartridge Mean	CV %	Inter-Cartridge Mean	CV %	120 ng/mL Control RLU	% DFE	3000 ng/mL Control RLU	% DFE
ULOQ	4000	2111	2558	2335	14	2383	2.0	40924	-13	4045	-11
		2222	2485	2354	8			49231	4	4972	9
		2473	2332	2403	4			50926	8	4821	5
		2337	2427	2382	3			59248	25	4515	-1
		2597	2337	2467	7			59220	25	4996	9
		2238	2481	2360	7			57384	21	4301	-6
High	3000	2520	2572	2546	1	2567	7.0	44634	-6	5291	16
		3050	2321	2686	19			47306	0	4661	2
		2421	2016	2219	13			48140	2	4092	-10
		2515	2796	2655	7			55105	17	5016	10
		2804	2577	2691	6			46970	-1	5119	12
		2326	2891	2608	15			49117	4	4646	2
Mid	400	8043	8772	8407	6	8525	12.2	42959	-9	4465	-2
		8177	7051	7614	10			50679	7	4239	-7
		7746	9241	8493	12			57450	22	5111	12
		8345	9623	8984	10			48172	2	4745	4
		10537	10007	10272	4			53681	14	3967	-13
		7474	7284	7379	2			39899	-16	4206	-8
Low	120	23829	24704	24267	3	24800	11.4	49519	5	3945	-14
		25951	23536	24744	7			50884	8	4114	-10
		21554	20931	21242	2			41001	-13	5029	10
		24556	28511	26534	11			45089	-5	4284	-6
		26315	32235	29275	14			49986	6	5057	11
		23102	22380	22741	2			39188	-17	3813	-17
LLOQ	40	41253	47407	44330	10	47946	7.4	42491	-10	4443	-3
		41230	45206	43218	7			40768	-14	4452	-3
		50914	50348	50631	1			52808	12	4912	7
		50596	52882	51739	3			41551	-12	3881	-15
		47124	53378	50251	9			41650	-12	4152	-9
		46206	48804	47505	4			52610	11	3854	-16

N = 6 cartridges per level
% DFE = Percentage Difference From Expected Value

Table 14: Intra-Day Precision, Day 3 Signal (RLU)

Level	[ACE-011] ng/mL	Sample Replicates 1	2	Intra-Cartridge Mean	CV %	Inter-Cartridge Mean	CV %	120 ng/mL Control RLU	% DFE	3000 ng/mL Control RLU	% DFE
ULOQ	4000	2433	2407	2420	0.8	2488	6.9	44285	-6	4952	8
		2248	2500	2374	7.5			44967	-5	4287	-6
		2665	2803	2734	3.6			48375	2	4663	2
		2427	2155	2291	8.4			50822	7	4650	2
		2414	2489	2452	2.2			49930	6	4610	1
		2710	2607	2658	2.7			46827	-1	4288	-6
High	3000	2642	2442	2542	5.6	2624	6.6	45899	-3	4476	-2
		3122	2681	2901	10.7			54945	16	5013	10
		2761	2754	2757	0.2			55856	18	3969	-13
		2607	2398	2502	5.9			54259	15	5159	13
		2320	2577	2448	7.4			40684	-14	4058	-11
		2758	2425	2591	9.1			39457	-17	4134	-10
Mid	400	10239	9687	9963	3.9	9188	9.3	47345	0	4308	-6
		8758	9486	9122	5.6			51841	10	5214	14
		9862	9675	9769	1.4			50547	7	4926	8
		9895	10012	9953	0.8			47281	0	5141	12
		8312	8028	8170	2.5			50334	6	5352	17
		8198	8105	8152	0.8			45567	-4	5176	13
Low	120	24559	25649	25104	3.1	25114	10.6	42500	-10	3741	-18
		22334	28579	25457	17.3			47783	1	4879	7
		26186	25169	25677	2.8			47978	1	4225	-8
		19715	20441	20078	2.6			44983	-5	3458	-24
		27888	27907	27898	0.0			41562	-12	3602	-21
		27452	25485	26468	5.3			42861	-9	4189	-8
LLOQ	40	55153	51339	53246	5.1	51218	2.4	48215	2	4152	-9
		51241	48919	50080	3.3			37887	-20	5376	18
		51876	51260	51568	0.8			45086	-5	3932	-14
		53666	46016	49841	10.9			47894	1	4170	-9
		48955	53651	51303	6.5			43615	-8	3834	-16
		48281	54254	51268	8.2			37158	-21	3750	-18

N = 6 cartridges per level
% DFE = Percentage Difference From Expected Value

Table 15: Intra-Day Precision, Concentration (ng/mL)

[ACE-011] ng/mL	Day 1				Day 2				Day 3			
	Conc	Mean	CV %	% Rec	Conc	Mean	CV %	% Rec	Conc	Mean	CV %	% Rec
4000	3828	3270	19.8	82	2883	3373	10.3	84	3753	3238	17.0	81
	3436				3192				3284			
	1998				3707				2457			
	3544				3811				3702			
	3311				3436				3562			
	3502				3211				2668			
3000	2280	3180	19.6	106	2934	2961	12.4	99	3159	2956	11.3	99
	3115				3028				2385			
	3144				3531				3369			
	2830				2636				2941			
	4072				2505				3050			
	3639				3128				2834			
400	514	405	15.8	101	415	416	13.1	104	357	394	10.7	98
	385				467				394			
	349				413				364			
	341				387				357			
	438				331				445			
	404				481				446			
120	105	121	10.8	101	125	123	13.5	103	124	118	5.9	98
	115				122				124			
	120				147				121			
	143				112				111			
	115				99				108			
	129				135				116			
40	47	44	18.4	110	51	44	16.5	109	34	38	11.8	96
	44				53				37			
	33				38				34			
	38				36				46			
	57				39				39			
	45				44				41			

N = 6 cartridges per level per day

% Rec. = Percentage Recovery

Table 16: Inter-Day Precision, Signal (RLU)

[ACE-011] ng/mL	Mean RLU	CV %
4000	2456	5.2
3000	2583	6.4
400	9021	11.6
120	25720	8.2
40	49768	5.9

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

Table 17: Inter-Day Precision, Concentration (ng/mL)

[ACE-011] ng/mL	Mean Conc	CV %	% Recovery
4000	3294	15.2	82
3000	3032	14.7	101
400	405	12.8	101
120	121	10.2	101
40	42	16.4	105

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

Table 18: Inter-Instrument Precision at 500 ng/mL, Signal (RLU)

Instrument	Sample Replicates		Intra-Cartridge		Inter-Cartridge		120 ng/mL Control		3000 ng/mL Control	
	1	2	Mean	CV %	Mean RLU	CV %	RLU	% DFE	RLU	% DFE
1	8114	7175	7644	9	7830	8.3	42761	-10	4107	-10
2	7646	7837	7741	2			53186	12	5047	10
3	7828	8283	8056	4			43250	-9	3759	-18
4	8447	7965	8206	4			50906	8	3837	-16
5	6882	7612	7247	7			52234	10	4530	-1
6	9315	8148	8732	9			50905	8	4581	0
7	6254	7916	7085	17			56405	19	4304	-6
8	7801	7586	7694	2			52148	10	3994	-13
9	8607	8142	8375	4			52903	12	4307	-6
10	7497	7164	7331	3			51062	8	4201	-8
11	7449	8242	7846	7			49759	5	4324	-5
12	6752	7667	7210	9			36124	-24	4263	-7
13	6615	6664	6640	1			52356	11	4174	-9
14	8378	8628	8503	2			48152	2	3551	-22
15	6176	6778	6477	7			51265	8	4220	-8
16	7439	7512	7476	1			49027	4	3545	-22
17	8052	8074	8063	0			51908	10	3838	-16
18	7799	8153	7976	3			47989	1	4397	-4
19	8254	6551	7403	16			56814	20	3786	-17
20	8556	8559	8558	0			45939	-3	4206	-8
21	8051	8206	8129	1			49282	4	3942	-14
22	7859	7820	7840	0			54004	14	3613	-21
23	8452	9390	8921	7			54405	15	4757	4
24	8287	9249	8768	8			52854	12	4335	-5

N = 24 cartridges, N = 24 instruments
% DFE = Percentage Difference From Expected Value

Table 19: Inter-Instrument Precision at 500 ng/mL, Concentration (ng/mL)

			Inter-Instrument		
Instrument	**Conc**	**% Recovery**	**Mean Conc**	**CV %**	**% Recovery**
1	473	95	464	10	93
2	464	93			
3	444	89			
4	435	87			
5	502	100			
6	408	82			
7	524	105			
8	468	94			
9	426	85			
10	494	99			
11	459	92			
12	506	101			
13	554	111			
14	418	84			
15	572	114			
16	483	97			
17	444	89			
18	449	90			
19	497	99			
20	415	83			
21	440	88			
22	458	92			
23	398	80			
24	406	81			

N = 24 cartridges, N = 24 instruments

Table 20: Selectivity in Human Whole Blood, Signal (RLU)

Sample	HC	Spike (ng/mL)	Sample Replicates		Intra-Cartridge		Inter-Cartridge		120 ng/mL Control		3000 ng/mL Control	
			1	2	Mean	CV %	Mean	CV %	RLU	% DFE	RLU	% DFE
F1	44	0	92081	87739	89910	3	69641	29	58244	23	4691	3
			71284	67996	69640	3			52816	12	3828	-16
			50504	48240	49372	3			47328	0	4511	-1
		40	44779	49998	47388	8	46835	1	43248	-9	5127	12
			40379	51852	46115	18			46693	-1	3898	-15
			42805	51197	47001	13			50693	7	4943	8
		1000	3823	5009	4416	19	4227	11	51094	8	3580	-22
			4103	5016	4560	14			47226	0	4422	-3
			3541	3869	3705	6			43274	-8	4059	-11
F2	39	0	77179	78665	77922	1	61914	24	47980	1	4051	-11
			55303	62304	58804	8			44747	-5	4451	-3
			NR	NR	NR	-			NR	-	NR	-
		40	45463	46414	45938	1	44961	5	47084	0	3802	-17
			41888	43362	42625	2			44815	-5	3519	-23
			45769	46872	46320	2			42031	-11	4783	5
		1000	5571	5230	5400	4	4319	22	40132	-15	4166	-9
			4139	3153	3646	19			37638	-20	3423	-25
			NR	NR	NR	-			NR	-	NR	-
F3	45	0	89184	64666	76925	23	72598	13	42221	-11	4494	-2
			79202	78967	79085	0			49570	5	4303	-6
			59903	63667	61785	4			49340	4	4259	-7
		40	43443	46229	44836	4	46527	6	38432	-19	3859	-16
			45835	54196	50015	12			36974	-22	3702	-19
			NR	NR	NR	-			NR	-	NR	-
		1000	4010	4073	4041	1	4488	12	53463	13	4212	-8
			5012	5213	5113	3			46188	-2	4386	-4
			3679	4943	4311	21			59168	25	4893	7
F4	47	0	89448	88218	88833	1	74921	17	49286	4	4103	-10
			65695	61270	63482	5			46527	-2	4297	-6
			68620	76274	72447	7			45735	-3	3631	-21
		40	45612	42511	44061	5	47224	11	50323	6	3953	-14
			42032	46580	44306	7			35428	-25	3773	-17
			55545	51061	53303	6			54071	14	4029	-12
		1000	4463	4699	4581	4	5269	19	45371	-4	4170	-9
			6007	6828	6418	9			54422	15	4509	-1
			5154	4461	4807	10			48794	3	4911	7

% DFE = Percentage Difference From Expected
NR = Not Reported

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

Sample	HC	Spike (ng/mL)	Sample Replicates		Intra-Cartridge		Inter-Cartridge		120 ng/mL Control		3000 ng/mL Control	
			1	2	Mean	CV %	Mean	CV %	RLU	% DFE	RLU	% DFE
F5	43	0	55028	60568	57798	7	63183	9	49876	5	4185	-8
			67612	70072	68842	3			51900	10	4298	-6
			57483	68335	62909	12			42299	-11	4544	-1
		40	37123	40403	38763	6	40747	7	42582	-10	4275	-6
			38539	40810	39674	4			39098	-17	3978	-13
			41563	46042	43803	7			42258	-11	3638	-20
		1000	4283	4416	4349	2	4719	7	48005	2	3480	-24
			4982	5098	5040	2			45773	-3	3532	-23
			4964	4570	4767	6			54590	15	3975	-13
F6	49	0	63667	70176	66922	7	63430	7	44363	-6	3639	-20
			58147	58237	58192	0			44676	-6	3703	-19
			57329	73025	65177	17			38753	-18	4207	-8
		40	40106	47799	43953	12	49363	10	42932	-9	3464	-24
			52170	53252	52711	1			43309	-8	4096	-10
			52122	50731	51427	2			50966	8	4420	-3
		1000	3530	3772	3651	5	4139	16	41913	-11	3520	-23
			3369	4427	3898	19			45218	-4	3462	-24
			4970	4764	4867	3			42774	-10	3601	-21
F7	43	0	59894	60987	60440	1	68632	13	44719	-5	3528	-23
			78025	78050	78038	0			46532	-2	3804	-17
			68269	66569	67419	2			46637	-1	4352	-5
		40	NR	NR	NR	-	45358	27	NR	-	NR	-
			68421	49714	59067	22			45632	-3	4130	-10
			42159	40993	41576	2			42391	-10	4143	-9
		1000	3681	4960	4321	21	4099	7	43169	-9	3964	-13
			4113	3493	3803	12			48727	3	3839	-16
			4129	4218	4173	2			40857	-14	3455	-24
F8	46	0	73656	68221	70938	5	70795	4	50678	7	3781	-17
			75567	72151	73859	3			49157	4	4748	4
			65483	69691	67587	4			43789	-7	3630	-21
		40	42124	42829	42477	1	49830	17	47115	0	3848	-16
			56041	62415	59228	8			41834	-12	3740	-18
			46575	48993	47784	4			44626	-6	4337	-5
		1000	5452	5438	5445	0	5088	9	56833	20	5308	16
			5193	5339	5266	2			49768	5	4047	-11
			4521	4583	4552	1			52987	12	3640	-20

% DFE = Percentage Difference From Expected
NR = Not Reported

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

Sample	HC	Spike (ng/mL)	Sample Replicates		Intra-Cartridge		Inter-Cartridge		120 ng/mL Control		3000 ng/mL Control	
			1	2	Mean	CV %	Mean	CV %	RLU	% DFE	RLU	% DFE
F9	42	0	62370	66514	64442	5	66548	6	47349	0	4467	-2
			64018	63780	63899	0			44127	-7	4443	-3
			69038	73566	71302	4			45383	-4	3651	-20
		40	39729	41843	40786	4	45382	14	48781	3	4520	-1
			44863	40683	42773	7			40835	-14	3471	-24
			53397	51774	52585	2			46953	-1	4111	-10
		1000	3305	3862	3584	11	4243	16	35601	-25	3857	-16
			4870	5050	4960	3			48797	3	4059	-11
			4694	3679	4186	17			48880	3	4711	3
F10	51	0	62503	58722	60613	4	60390	10	44482	-6	4177	-9
			NR	NR	NR	-			NR	-	NR	-
			50430	57977	54203	10			37266	-21	4075	-11
		40	49506	44987	47247	7	47428	2	43505	-8	4087	-11
			47391	45890	46640	2			46089	-3	4603	1
			43354	53439	48397	15			40331	-15	4269	-7
		1000	5089	4882	4986	3	4201	16	39395	-17	3568	-22
			3937	3812	3874	2			40977	-13	4087	-11
			3755	3732	3744	0			40888	-14	3561	-22
M1	51	0	57728	55753	56741	2	82701	41	38234	-19	3615	-21
			62402	78222	70312	16			50214	6	4512	-1
			120781	121321	121051	0			42283	-11	4084	-11
		40	51882	53620	52751	2	53266	2	45457	-4	4692	3
			46817	62705	54761	21			45279	-4	4145	-9
			52353	52220	52286	0			42092	-11	3765	-18
		1000	3815	4659	4237	14	3962	9	51209	8	4449	-3
			3966	4193	4079	4			43512	-8	4456	-3
			3272	3870	3571	12			42188	-11	4303	-6
M2	49	0	51991	69872	60932	21	64904	15	39317	-17	4083	-11
			77488	74576	76032	3			52761	12	3957	-13
			55091	60403	57747	7			39457	-17	3586	-22
		40	44885	44464	44674	1	47906	6	42067	-11	3698	-19
			53075	48623	50849	6			46253	-2	4184	-8
			45613	50776	48195	8			42951	-9	3970	-13
		1000	3636	3965	3801	6	4018	18	41609	-12	4728	3
			NR	NR	NR	-			NR	-	NR	-
			4813	4878	4845	1			52714	11	4769	4

% DFE = Percentage Difference From Expected
NR = Not Reported

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

Sample	HC	Spike (ng/mL)	Sample Replicates		Intra-Cartridge		Inter-Cartridge		120 ng/mL Control		3000 ng/mL Control	
			1	2	Mean	CV %	Mean	CV %	RLU	% DFE	RLU	% DFE
M3	50	0	52059	52627	52343	1	57497	9	44651	-6	3909	-14
			69257	56557	62907	14			41037	-13	3563	-22
			53381	61101	57241	10			41280	-13	3528	-23
		40	44896	48156	46526	5	49669	9	48194	2	3422	-25
			47572	48435	48004	1			48839	3	5163	13
			50750	58207	54478	10			51685	9	5392	18
		1000	3725	3397	3561	7	3748	7	40907	-13	4032	-12
			3669	3615	3642	1			47164	0	4653	2
			3758	4325	4042	10			47069	0	3572	-22
M4	54	0	71341	70720	71031	1	69038	3	44003	-7	4292	-6
			65156	73656	69406	9			38678	-18	4091	-10
			70390	62967	66678	8			51306	9	4722	3
		40	41554	46009	43782	7	44190	2	40340	-15	3570	-22
			41336	48775	45056	12			46474	-2	4301	-6
			40958	46510	43734	9			39781	-16	5203	14
		1000	3702	4100	3901	7	3847	2	47490	0	4212	-8
			4159	3567	3863	11			52555	11	3841	-16
			3952	3599	3776	7			45698	-3	3759	-18
M5	46	0	59687	64118	61902	5	61703	4	49373	4	3759	-18
			55595	63034	59314	9			39089	-17	3435	-25
			66382	61403	63893	6			40826	-14	3596	-21
		40	39615	41443	40529	3	40434	5	48512	3	4598	1
			37613	39188	38400	3			39466	-17	4446	-3
			37260	47486	42373	17			42025	-11	3482	-24
		1000	4368	4197	4283	3	4201	21	42042	-11	4136	-10
			3138	3455	3296	7			44816	-5	3905	-15
			5416	4632	5024	11			52596	11	4448	-3
M6	49	0	57252	66378	61815	10	67003	7	52798	12	3840	-16
			72812	64053	68433	9			43358	-8	4328	-5
			72996	68529	70763	4			43026	-9	3622	-21
		40	NR	NR	NR	-	47865	21	NR	-	NR	-
			40204	41344	40774	2			52670	11	5019	10
			51293	58621	54957	9			48643	3	5276	15
		1000	4469	5991	5230	21	4360	17	54435	15	4292	-6
			3643	4122	3883	9			46690	-1	3410	-25
			3958	3980	3969	0			41968	-11	3458	-24

% DFE = Percentage Difference From Expected
NR = Not Reported

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

Sample	HC	Spike (ng/mL)	Sample Replicates		Intra-Cartridge		Inter-Cartridge		120 ng/mL Control		3000 ng/mL Control	
			1	2	Mean	CV %	Mean	CV %	RLU	% DFE	RLU	% DFE
M7	57	0	56530	56422	56476	0	62778	12	49713	5	4229	-7
			61377	59798	60587	2			40573	-14	3638	-20
			66236	76306	71271	10			53359	13	4011	-12
		40	46289	49663	47976	5	34453	84	50695	7	4771	4
			NR	NR	NR	-			NR	-	NR	-
			57479	50535	54007	9			48054	2	4779	5
		1000	4431	3180	3806	23	4392	12	48096	2	5376	18
			4638	4401	4520	4			51491	9	5321	16
			4531	5172	4851	9			43448	-8	4325	-5
M8	51	0	77385	65348	71366	12	62823	13	51505	9	3711	-19
			57312	67137	62224	11			42541	-10	4008	-12
			NR	NR	NR	-			NR	-	NR	-
		40	46473	41668	44071	8	49043	11	40915	-13	5159	13
			56411	52950	54680	4			58006	23	5152	13
			50823	45931	48377	7			51363	9	4409	-4
		1000	5830	5738	5784	1	4562	25	55430	17	3495	-24
			4239	4574	4407	5			36631	-23	4312	-6
			3680	3310	3495	7			53031	12	5635	23
M9	48	0	NR	NR	NR	-	68154	8	NR	-	NR	-
			71063	77485	74274	6			45559	-4	3903	-15
			67992	61544	64768	7			48711	3	4869	7
		40	52359	54332	53346	3	51262	6	47285	0	3419	-25
			54620	50787	52704	5			49694	5	5452	19
			48943	46533	47738	4			45058	-5	4340	-5
		1000	4413	5815	5114	19	4734	16	53580	13	4631	1
			5550	4931	5241	8			49753	5	4774	4
			3646	4050	3848	7			37049	-22	3867	-15
M10	50	0	65140	62462	63801	3	64053	7	49465	5	4097	-10
			64572	72131	68352	8			45656	-3	3643	-20
			58656	61359	60007	3			56093	19	4800	5
		40	50077	50003	50040	0	49794	1	54082	14	3538	-23
			52860	46182	49521	10			49557	5	4433	-3
			51177	48463	49820	4			49148	4	4823	6
		1000	4174	5211	4692	16	4852	3	47806	1	5586	22
			5104	4758	4931	5			39931	-16	3899	-15
			4732	5131	4932	6			46592	-1	3581	-22

% DFE = Percentage Difference From Expected
NR = Not Reported

Table 21: **Selectivity in Human Whole Blood, Concentration (ng/mL)**

Sample	HC	Spike (ng/mL)	Conc	Mean	CV %	% Recovery
F1	44	0	OORL OORL 40	OORL		
		40	44 48 45	46	4.3	115
		1000	980 917 1245	1048	16.6	105
F2	39	0	OORL OORL NR	OORL		
		40	47 55 46	50	10.0	124
		1000	715 1359 NR	1037	43.9	104
F3	45	0	OORL OORL OORL	OORL		
		40	50 47 NR	49	3.5	122
		1000	1073 767 1027	956	17.3	96
F4	47	0	OORL OORL OORL	OORL		
		40	52 51 36	46	19.2	116
		1000	891 580 841	771	21.7	77
F5	43	0	OORL OORL OORL	OORL		
		40	66 64 53	61	11.9	152
		1000	960 781 845	862	10.5	86

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

Sample	HC	Spike (ng/mL)	Conc	Mean	CV %	% Recovery
F6	49	0	OORL OORL OORL	OORL		
		40	53 34 35	41	25.6	102
		1000	1274 1203 819	1099	22.3	110
F7	43	0	OORL OORL OORL	OORL		
		40	NR 39 58	49	28.1	121
		1000	1024 1209 1021	1085	9.9	108
F8	46	0	OORL OORL OORL	OORL		
		40	56 OORL 43	49	18.1	124
		1000	706 737 898	781	13.2	78
F9	42	0	OORL OORL OORL	OORL		
		40	60 55 35	50	27.1	125
		1000	1341 798 1056	1065	25.5	107
F10	51	0	OORL NR 37	OORL		
		40	44 46 53	48	10.3	120
		1000	793 1149 1216	1053	21.6	105

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

Sample	HC	Spike (ng/mL)	Conc	Mean	CV %	% Recovery
M1	51	0	OORL OORL OORL	OORL		
		40	34 45 34	38	17.1	94
		1000	1024 1060 1354	1146	15.8	115
M2	49	0	34 OORL OORL	OORL		
		40	50 37 42	43	15.8	108
		1000	1192 NR 824	1008	25.8	101
M3	50	0	34 OORL OORL	OORL		
		40	46 43 37	42	11.2	104
		1000	1337 1275 1088	1233	10.5	123
M4	54	0	OORL OORL OORL	OORL		
		40	53 50 53	52	3.3	129
		1000	1144 1174 1207	1175	2.7	117
M5	46	0	OORL OORL OORL	OORL		
		40	61 67 57	62	8.2	155
		1000	983 1552 794	1109	35.6	111

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

Sample	HC	Spike (ng/mL)	Conc	Mean	CV %	% Recovery
M6	49	0	OORL OORL OORL	OORL		
		40	NR 60 36	48	36.4	120
		1000	775 1157 1104	1012	20.5	101
M7	57	0	OORL OORL OORL	OORL		
		40	43 NR 37	40	9.8	100
		1000	1294 909 829	1011	24.6	101
M8	51	0	OORL OORL NR	OORL		
		40	52 32 42	42	23.3	105
		1000	654 945 1387	995	37.1	100
M9	48	0	NR OORL OORL	OORL		
		40	34 37 43	38	13.1	94
		1000	795 747 1171	904	25.7	90
M10	50	0	OORL OORL OORL	OORL		
		40	38 40 39	39	1.8	97
		1000	884 806 807	832	5.4	83

Table 22: Summary of Selectivity in Human Whole Blood, Concentration (ng/mL)

Sample	HC	Spike (ng/mL)	Mean	CV %	% Recovery
F1	44	0	OORL	-	-
		40	46	4.3	115
		1000	1048	16.6	105
F2	39	0	OORL	-	-
		40	50	10.0	124
		1000	1037	43.9	104
F3	45	0	OORL	-	-
		40	49	3.5	122
		1000	956	17.3	96
F4	47	0	OORL	-	-
		40	46	19.2	116
		1000	771	21.7	77
F5	43	0	OORL	-	-
		40	61	11.9	152
		1000	862	10.5	86
F6	49	0	OORL	-	-
		40	41	25.6	102
		1000	1099	22.3	110
F7	43	0	OORL	-	-
		40	49	28.1	121
		1000	1085	9.9	108
F8	46	0	OORL	-	-
		40	49	18.1	124
		1000	781	13.2	78
F9	42	0	OORL	-	-
		40	50	27.1	125
		1000	1065	25.5	107
F10	51	0	OORL	-	-
		40	48	10.3	120
		1000	1053	21.6	105

Summary of Selectivity in Human Whole Blood, Concentration (ng/mL), *Continued*

Sample	HC	Spike (ng/mL)	Mean	CV %	% Recovery
M1	51	0	OORL	-	-
		40	38	17.1	94
		1000	1146	15.8	115
M2	49	0	OORL	-	-
		40	43	15.8	108
		1000	1008	25.8	101
M3	50	0	OORL	-	-
		40	42	11.2	104
		1000	1233	10.5	123
M4	54	0	OORL	-	-
		40	52	3.3	129
		1000	1175	2.7	117
M5	46	0	OORL	-	-
		40	62	8.2	155
		1000	1109	35.6	111
M6	49	0	OORL	-	-
		40	48	36.4	120
		1000	1012	20.5	101
M7	57	0	OORL	-	-
		40	40	9.8	100
		1000	1011	24.6	101
M8	51	0	OORL	-	-
		40	42	23.3	105
		1000	995	37.1	100
M9	48	0	OORL	-	-
		40	38	13.1	94
		1000	904	25.7	90
M10	50	0	OORL	-	-
		40	39	1.8	97
		1000	832	5.4	83

Table 23: Process Stability, Signal (RLU)

Time Elapsed	Sample Replicates		Intra-Cartridge		120 ng/mL Control		3000 ng/mL Control	
H:M:S	1	2	Mean	CV %	Mean	% DFE	Mean	% DFE
0:00:49	8447	7965	8206	4	50906	8	3837	-16
0:02:36	6882	7612	7247	7	52234	10	4530	-1
0:03:45	9315	8148	8732	9	50905	8	4581	0
0:05:07	7801	7586	7694	2	52148	10	3994	-13
0:06:15	8607	8142	8375	4	52903	12	4307	-6
0:06:39	7497	7164	7331	3	51062	8	4201	-8
0:06:54	7449	8242	7846	7	49759	5	4324	-5
0:08:29	6752	7667	7210	9	36124	-24	4263	-7
0:09:40	6615	6664	6640	1	52356	11	4174	-9
0:11:01	8052	8074	8063	0	51908	10	3838	-16
0:11:32	7799	8153	7976	3	47989	1	4397	-4
0:12:13	8254	6551	7403	16	56814	20	3786	-17
0:12:57	8051	8206	8129	1	49282	4	3942	-14
0:13:12	7859	7820	7840	0	54004	14	3613	-21
0:13:56	8287	9249	8768	8	52854	12	4335	-5

% DFE = Percentage Difference From Expected

Table 24: Process Stability, Concentration (ng/mL)

Time Elapsed H:M:S	Conc.	% Recovery	Mean	CV %	% Recovery
0:00:49	435	87	463	8.8	93
0:02:36	502	100			
0:03:45	408	82			
0:05:07	468	94			
0:06:15	426	85			
0:06:39	494	99			
0:06:54	459	92			
0:08:29	506	101			
0:09:40	554	111			
0:11:01	444	89			
0:11:32	449	90			
0:12:13	497	99			
0:12:57	440	88			
0:13:12	458	92			
0:13:56	406	81			

Table 25: Cartridge Stability at 4°C, Signal (RLU)*

		Sample Replicates		Intra-Cartridge		Inter-Cartridge		120 ng/mL Control		3000 ng/mL Control	
Week	[ACE-011] ng/mL	1	2	Mean	CV %	Mean	CV %	Mean	% DFE	Mean	% DFE
0	3000	2380	2551	2465	5	2677	8.9	51926	10	5076	11
		2796	3072	2934	7			43839	-7	4573	0
		2640	2622	2631	0			49747	5	4728	3
	120	29392	27621	28507	4	28659	7.6	38730	-18	3644	-20
		24779	28318	26549	9			45208	-4	4544	-1
		29441	32403	30922	7			57337	21	4480	-2
	0	86557	82599	84578	3	80304	13.0	45583	-4	4819	5
		85460	90474	87967	4			50445	7	4302	-6
		74353	62383	68368	12			43652	-8	4881	7
1	3000	3226	3634	3430	8	3105	12.2	52163	10	5122	12
		2474	2900	2687	11			62161	31	5574	22
		2888	3505	3197	14			53508	13	4280	-6
	120	25180	30311	27745	13	28935	4.8	41495	-12	3913	-14
		31185	25970	28577	13			49477	5	4812	5
		32143	28821	30482	8			40514	-14	4382	-4
	0	81642	75745	78693	5	82187	25.2	47306	0	4511	-1
		102424	106377	104400	3			52394	11	5157	13
		68936	57996	63466	12			42322	-10	4503	-1
2	3000	2497	2620	2559	3	2809	8.5	46307	-2	3449	-25
		2683	2979	2831	7			48263	2	4701	3
		3080	2995	3037	2			57351	21	4556	0
	120	31193	27374	29284	9	27883	4.8	48891	3	3541	-23
		28096	27402	27749	2			50073	6	3905	-15
		26410	26825	26618	1			45410	-4	3660	-20
	0	67998	66113	67056	2	80666	18.3	48283	2	3785	-17
		76997	80070	78534	3			40125	-15	4513	-1
		100818	91998	96408	6			44277	-6	4231	-7
4	3000	3283	3202	3242	2	2899	13.4	40496	-14	3574	-22
		2779	2174	2477	17			55883	18	4598	1
		2563	3391	2977	20			29899	-37	2575	-44
	120	24261	22571	23416	5	25056	6.0	46672	-1	4029	-12
		27098	23706	25402	9			36665	-22	4235	-7
		26203	26497	26350	1			55987	18	3580	-22
	0	70654	69664	70159	1	78938	11.9	43219	-9	4197	-8
		71295	84439	77867	12			52216	10	4111	-10
		83013	94563	88788	9			47604	1	4487	-2

% DFE – Percentage Difference From Expected

* NOTE: Because this is a stability study, signal (RLU) data for the cartridges is reported even if it fails acceptance criteria.

Table 26: Cartridge Stability at Room Temperature, Signal (RLU)*

		Sample Replicates		Intra-Cartridge		Inter-Cartridge		120 ng/mL Control		3000 ng/mL Control	
Week	[ACE-011] ng/mL	1	2	Mean	CV %	Mean	CV %	Mean	% DFE	Mean	% DFE
0	3000	2380	2551	2465	5	2677	8.9	51926	10	5076	11
		2796	3072	2934	7			43839	-7	4573	0
		2640	2622	2631	0			49747	5	4728	3
	120	29392	27621	28507	4	28659	7.6	38730	-18	3644	-20
		24779	28318	26549	9			45208	-4	4544	-1
		29441	32403	30922	7			57337	21	4480	-2
	0	86557	82599	84578	3	80304	13.0	45583	-4	4819	5
		85460	90474	87967	4			50445	7	4302	-6
		74353	62383	68368	12			43652	-8	4881	7
1	3000	1905	2840	2373	28	2527	6.4	31077	-34	3873	-15
		2751	2634	2693	3			51732	9	3750	-18
		2404	2625	2514	6			49873	5	4015	-12
	120	20982	25678	23330	14	27466	16.0	40426	-15	3412	-25
		31560	32623	32091	2			52388	11	4013	-12
		27352	26601	26976	2			41197	-13	3100	-32
	0	93890	90733	92311	2	82061	14.0	47997	2	4076	-11
		83414	85095	84255	1			41477	-12	3396	-26
		67883	71350	69616	4			45150	-5	2710	-41
2	3000	2051	2051	2051	0	1845	14.0	30303	-36	2256	-51
		2047	1809	1928	9			28612	-39	2278	-50
		1878	1233	1555	29			35157	-26	3067	-33
	120	25185	21858	23522	10	18940	21.5	35529	-25	3356	-27
		15625	15807	15716	1			28218	-40	2529	-45
		16567	18597	17582	8			30747	-35	2069	-55
	0	57470	81993	69732	25	56992	21.2	44424	-6	3282	-28
		47641	43794	45718	6			32563	-31	2154	-53
		57573	53481	55527	5			32832	-31	2763	-40
4	3000	1617	1515	1566	5	1792	15.5	27492	-42	2721	-40
		2031	2172	2102	5			34543	-27	2648	-42
		1684	1732	1708	2			28709	-39	2593	-43
	120	17028	18112	17570	4	14965	15.2	30558	-35	2653	-42
		16045	11800	13922	22			27640	-42	2718	-41
		11883	14922	13403	16			23217	-51	1737	-62
	0	43522	61163	52343	24	44128	19.8	33367	-29	1925	-58
		36090	33735	34913	5			24220	-49	1934	-58
		42539	47717	45128	8			32847	-31	2212	-52

% DFE – Percentage Difference From Expected

* NOTE: Because this is a stability study, signal (RLU) data for the cartridges is reported even if it fails acceptance criteria.

Table 27: Cartridge Stability at 4°C, Concentration (ng/mL)

Week	[ACE-011] ng/mL	Mean	CV %*	% Recovery*	Number of cartridges with NR Results
0	3000	3573	15.9	119	0
	120	116	12.2	97	0
	0	OORL	-	-	0
1	3000	2463	9.3	82	1
	120	115	8.1	96	0
	0	OORL	-	-	0
2	3000	3267	15.9	109	0
	120	121	7.1	101	0
	0	OORL	-	-	0
4	3000	2884	18.8	96	1
	120	143	9.2	119	0
	0	OORL	-	-	0

N = 3 per level per time point

Table 28: Cartridge Stability at Room Temperature, Concentration (ng/mL)

Week	[ACE-011] ng/mL	Mean	CV %*	% Recovery*	Number of cartridges with NR Results
0	3000	3573	15.9	119	0
	120	116	12.2	97	0
	0	OORL	-	-	0
1	3000	3549	3.3	118	1
	120	128	35.6	107	1
	0	OORL	-	-	2
2	3000	-	-	-	3
	120	-	-	-	3
	0	-	-	-	3
4	3000	-	-	-	3
	120	-	-	-	3
	0	-	-	-	3

N = 3 per level per time point

* Note: non-reportable cartridge concentration results are excluded from calculation of % CV and % Recovery.

Note: Week 0 is the same data for both storage conditions; storage stability testing was initiated at Week 0.

Table 29: High Dose Hook Effect Test, Signal (RLU)

	Sample Replicates		Intra-Cartridge		Inter-Cartridge		120 ng/mL Control		3000 ng/mL Control	
Cartridge	**1**	**2**	**Mean**	**CV %**	**Mean**	**CV %**	**Mean**	**% DFE**	**Mean**	**% DFE**
1	1279	1589	1434	15	1486	2.2	48443	2	4801	5
2	1386	1577	1481	9			53278	13	3729	-18
3	1417	1621	1519	10			54351	15	4966	9
4	1430	1551	1490	6			49445	5	4391	-4
5	1600	1414	1507	9			47251	0	4006	-12

% DFE = Percentage Difference From Expected

Table 30: High Dose Hook Effect Test, Concentration (ng/mL)

Cartridge	Conc.
1	OORH
2	OORH
3	OORH
4	OORH
5	OORH

Table 31: Instrument Carryover Test, Signal (RLU)

	Sample Replicates		Intra-Cartridge		Inter-Cartridge		120 ng/mL Control		3000 ng/mL Control	
Cartridge	**1**	**2**	**Mean**	**CV %**	**Mean**	**CV %**	**Mean**	**% DFE**	**Mean**	**% DFE**
1	71709	82422	77066	9.8	72884	11.1	39468	-17	4399	-4
2	74445	69102	71773	5.3			45561	-4	4772	4
3	63038	59067	61052	4.6			41790	-12	3685	-19
4	60541	66187	63364	6.3			45972	-3	4788	5
5	76452	79921	78186	3.1			43852	-7	4780	5
6	81220	77226	79223	3.6			56121	19	4755	4
7	80695	81689	81192	0.9			46882	-1	4861	6
8	80593	79944	80269	0.6			50381	7	4491	-2
9	57960	69693	63827	13.0			41486	-12	4160	-9

% DFE = Percentage Difference From Expected

Table 32: Instrument Carryover Test, Concentration (ng/mL)

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

21 FIGURES

Figure 1: Standard Curve for Day 1 Calibration in Whole Blood

N = 3 cartridges for regular points, N = 6 cartridges for LLOQ and ULOQ

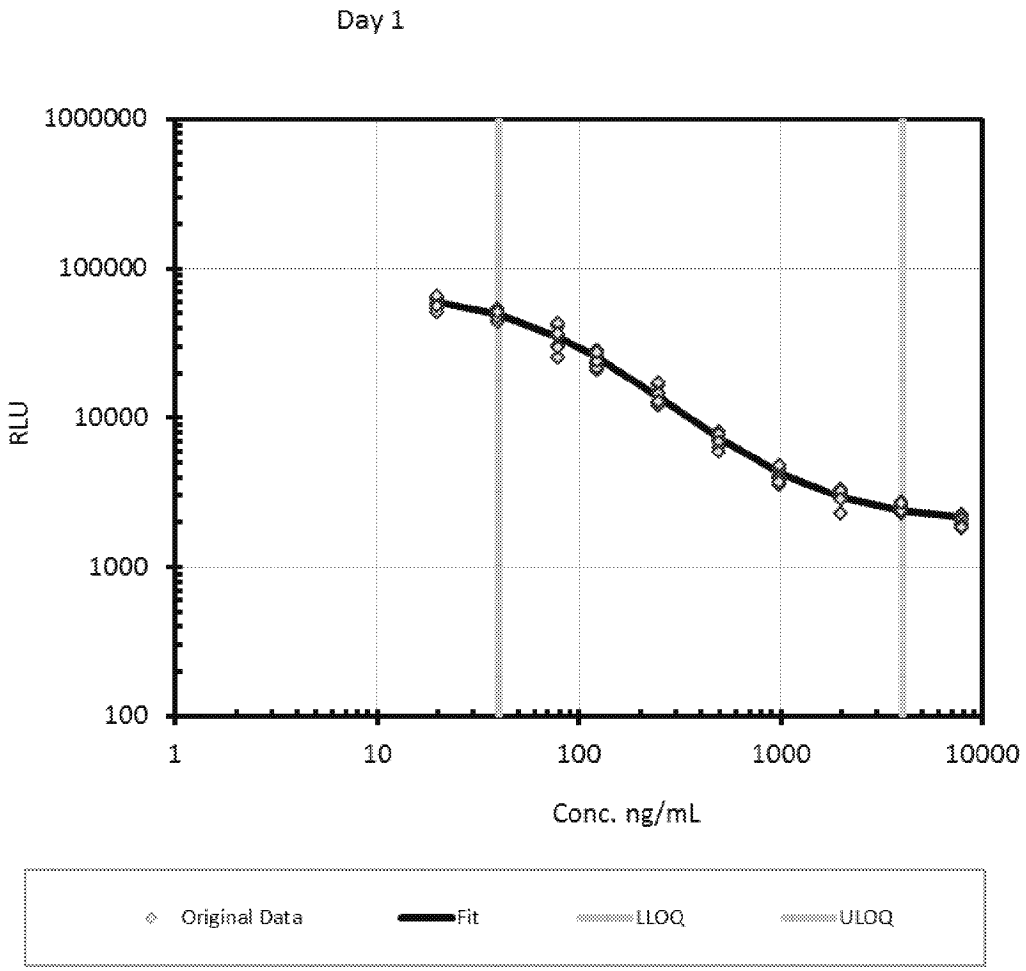


Figure 2: Standard Curve for Day 2 Calibration in Whole Blood

N = 3 cartridges for regular points, N = 6 cartridges for LLOQ and ULOQ

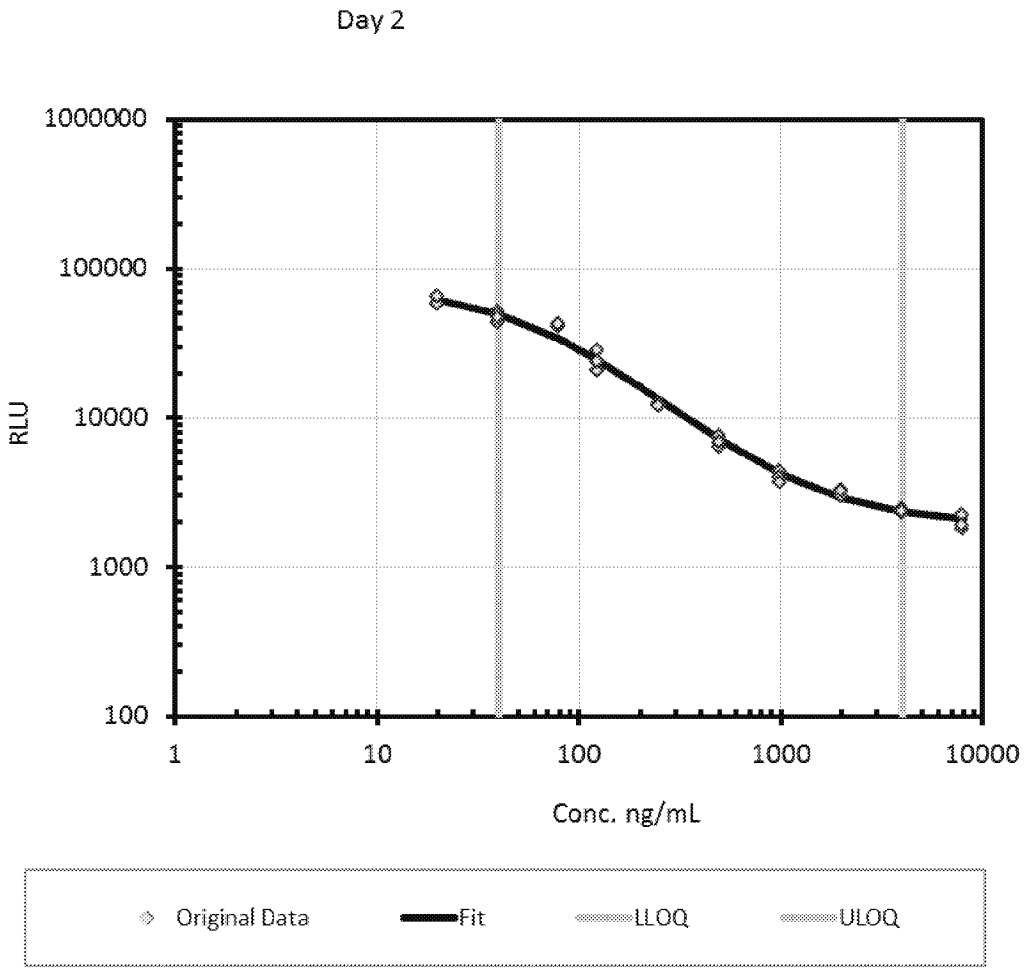


Figure 3: Standard Curve for Day 3 Calibration in Whole Blood

N = 3 cartridges for regular points, N = 6 cartridges for LLOQ and ULOQ

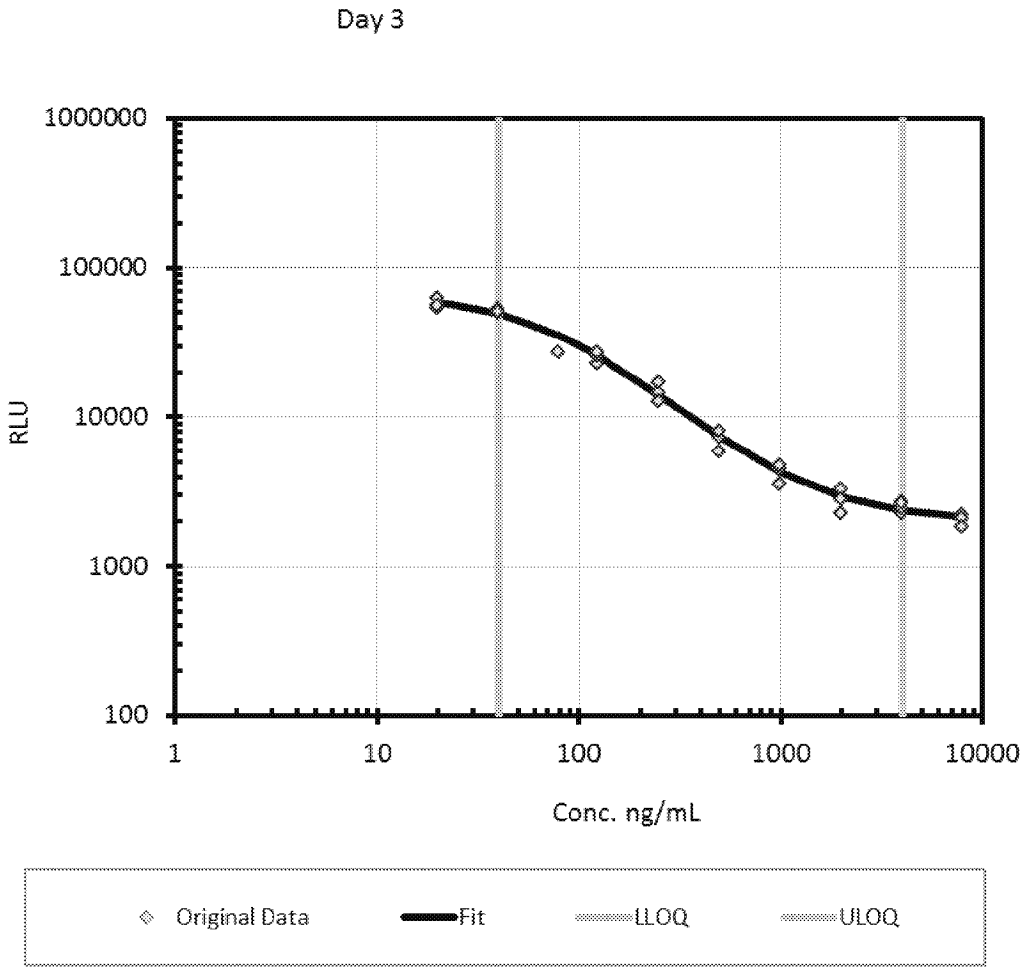


Figure 4: Standard Curve for 3-Day Calibration in Whole Blood

N = 9 cartridges for regular points, N = 18 cartridges for LLOQ and ULOQ

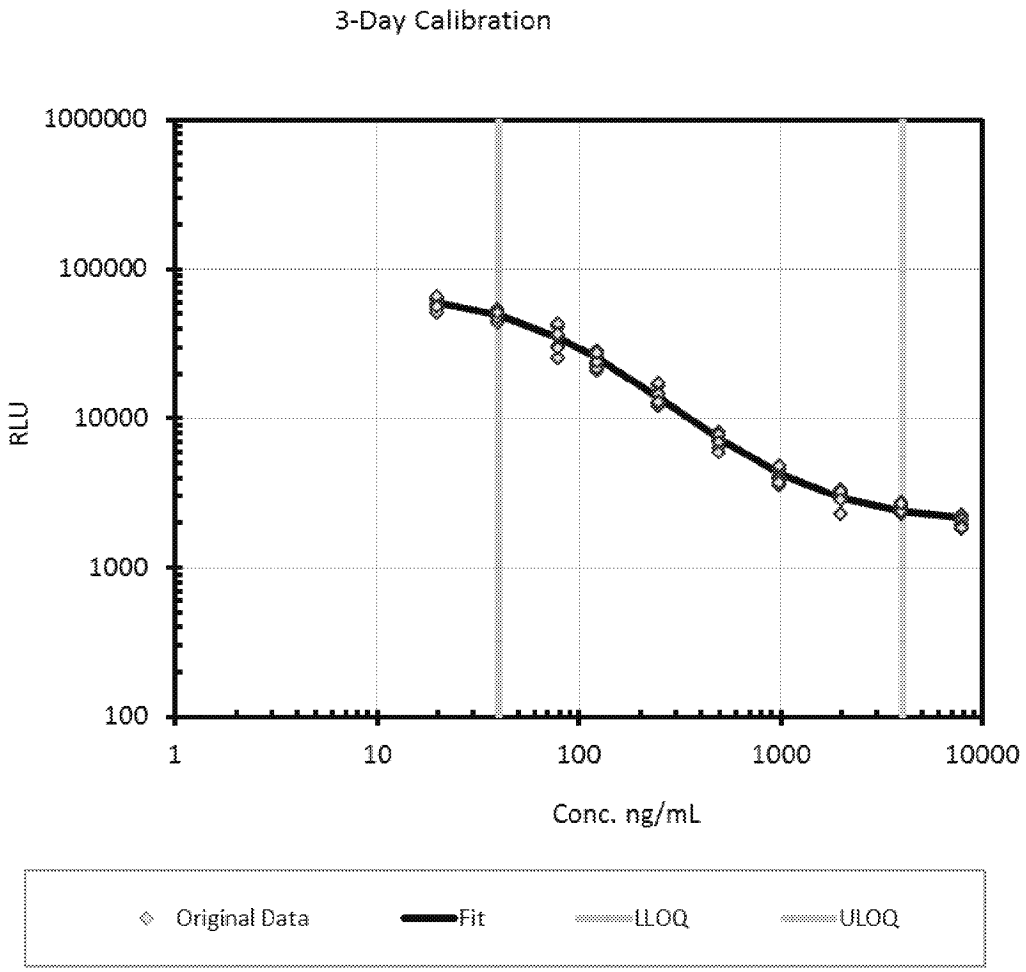


Figure 5: Process Stability, Signal (RLU)

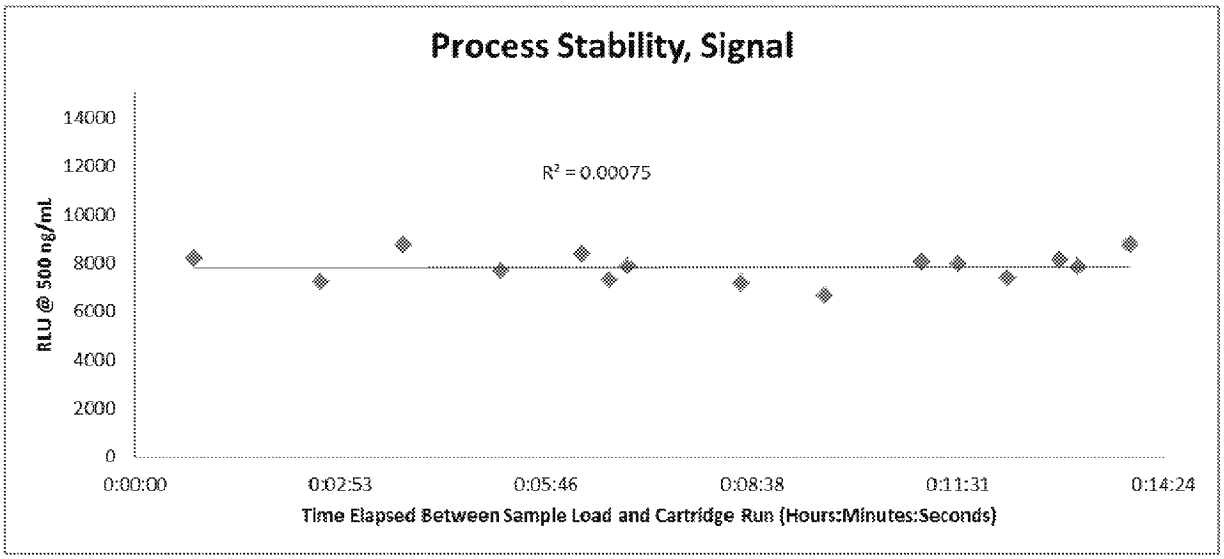


Figure 6: Process Stability, Concentration (ng/mL)

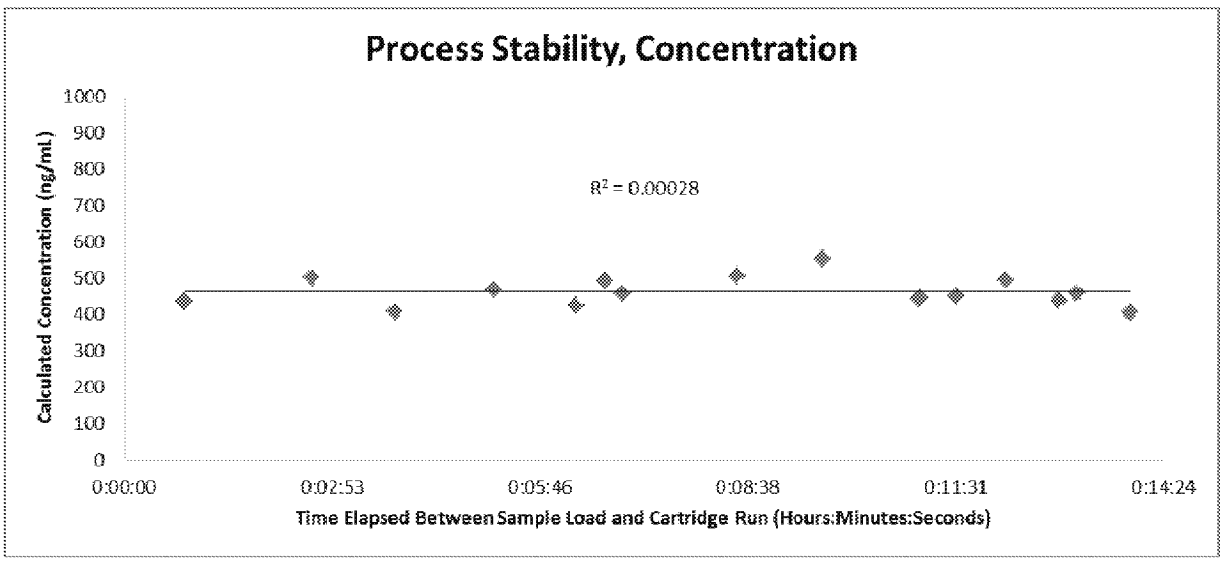


Figure 7: Cartridge Stability at 4°C, Signal (RLU)

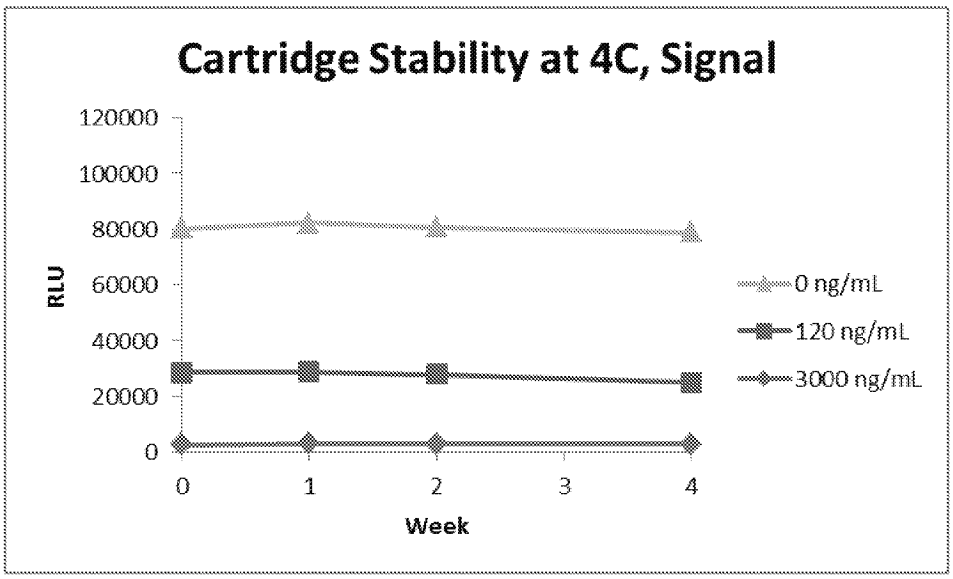
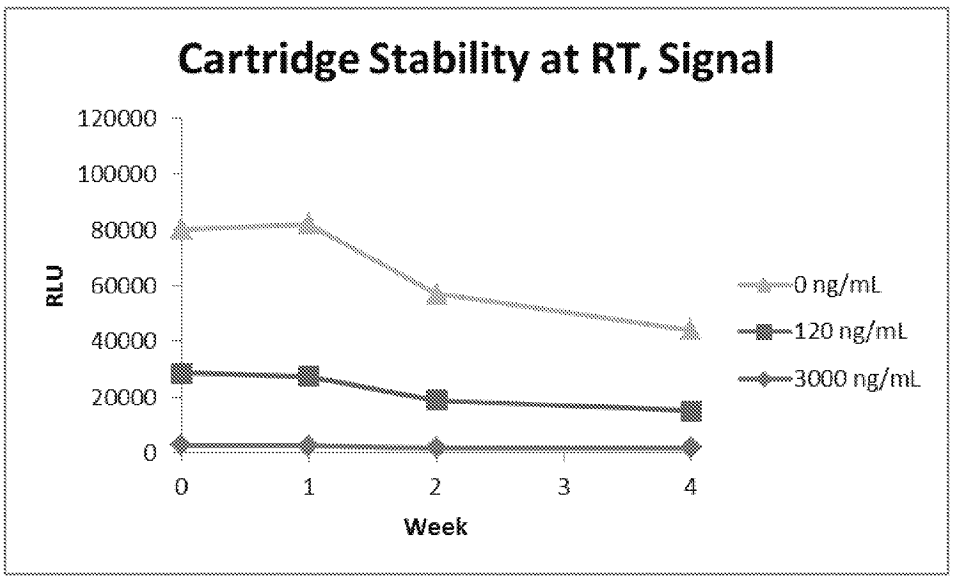


Figure 8: Cartridge Stability at Room Temperature, Signal (RLU)



22 APPENDICES

22.1 Protocol

A copy of the original protocol is included here.

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Method Validation Protocol

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

CELG-004 12/27/2010 DLY

Theranos Project Number: ~~Celgene Contract ID 8919 for CO#2 to SOW:CELG-003~~
Celgene Study Number: ACE-011-DMPK-001

Sponsor
Celgene Corporation
86 Morris Avenue
Summit, NJ 07901

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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-004

12/23/2010
DLY

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

SPONSOR:
Celgene Corporation
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.~~ CELG-004 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.

12/27/2010
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.