STATISTICAL PLAN APPROVAL FORM

Title: i-STAT 500® Analyzer for Glucose, Hematocrit and Sodium Clinical Evaluation Statistical Plan (and Acceptance Criteria)
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ABBOTT i-STAT 500® Analyzer for Glucose, Hematocrit and Sodium

Clinical Evaluation Statistical Plan (and Acceptance Criteria)

I. Protocol Reference

Clinical Evaluation of i-STAT 500® Analyzer for Glucose, Hematocrit and Sodium (CS-2016-0003).

II. General Information

This plan will document additional statistical information and acceptance criteria that are not contained in the clinical evaluation protocol.

All data analyses will be performed, and tables and listings of data will be provided by APOC Statistics using validated statistical analysis software.

Additional subgroups of some testing sites and/or sample types may be created and analyzed.

If revised or additional analyses are required, a description of the additional or revised analyses and justifications for the changes will be documented and approved by the same functional areas as the original approvers to the protocol.

The clinical monitor will review all test results and may request that certain observations be excluded from analysis if there is an assignable cause, i.e., control or validity criteria failure, instrument errors or problems, acknowledged technologist error, and/or noncompliance with the study protocol. All results tested according to the protocol and not excluded from this study will be eligible for analysis.

The statistical analysis output along with listings containing each observation collected for this study will be completed. The listings will be created for data included in the analysis, and data excluded from the analysis. The excluded listing will also include the reasons for exclusion.

It is expected that specimen collection will be completed at different times for each of the three assays (sodium, glucose and hematocrit) during the study. The data for a given assay may be analyzed against acceptance criteria once the specimen collection for that assay has been completed and may be used for different regulatory submissions (i.e. FDA response or 510(k) submission). Once specimen collection is determined to be completed, additional specimens will not be collected for that assay unless requested by a regulatory agency.

Interim data for a given assay (before specimen collection is completed) will not be evaluated against acceptance criteria. Interim data monitoring will be conducted to ensure the data meets validity criteria and minimum sample size requirements.

III. Statistical Procedures

Refer to the clinical evaluation protocol for the objective of the study, study design, and for additional details on data handling conventions. This section contains additional details about the analyses in the clinical study plan, as well as other additional analyses needed for the regulatory submission(s).

A. Method Comparison

1. Glucose

a. Study Design

Method comparison testing will be performed using whole blood capillary specimens prospectively collected from routine patient care. This testing will be performed at a minimum of 3 sites. Testing of approximately 120 native and altered specimens will be performed in singlicate on both the i-STAT 500 analyzer and the i-STAT 1 Wireless analyzer. One (1) i-STAT 500 and one (1) i-STAT 1 Wireless analyzer will be used for this testing. Each site should collect and test a minimum of 40 specimens until sufficient specimens are collected and tested for study analysis. A minimum of one lot of i-STAT EC4+ cartridges will be used for this testing.

b. Analysis Variables

The analysis variable for the method comparison study is the i-STAT EC4+ cartridge glucose concentration values (mg/dL).

c. Sample Size

The sample size is based on the recommendation of CLSI EP09-A3.

A minimum of 120 capillary whole blood specimens covering the measuring interval will be collected from a minimum of 3 sites. Each site should collect a minimum of 40 specimens.

Minimum of 3 sites
- Within each site:
  - Number of lots = 1
  - i-STAT 1 Wireless analyzers = 1
  - i-STAT 500 analyzers = 1
  - Replicates per specimen = 1

A suggested distribution of specimen target levels for glucose by site is presented in the chart below:

Glucose (mg/dL)	~ # Sample /Site	Max # Samples /Site
≤ 50	2	4
> 50-80	4	8
> 80-120	8	14
> 120-200	12	20
> 200-300	6	12
> 300-400	4	8
> 400-500	2	4
>500	2	4
Total	**40**	

If more than the maximum allowed number of samples is collected for a given glucose range within a site, the glucose result from samples with the earlier collection date and time will be included in the analysis. The glucose results for samples collected beyond the maximum allowed number of samples will not be used for this analysis.

d. Statistical Analysis Method

Perform the following analyses for the single replicate of the i-STAT 500 result versus the single replicate of the i-STAT 1 Wireless result. Analyses will be done with outliers and without outliers if any outliers are identified.

- For each site as well as all sites combined, calculate the correlation coefficient and perform the Deming regression and Passing-Bablok regression analyses on the concentration values (the i-STAT 500 result as dependent variable versus the i-STAT 1 Wireless result as independent variable) and provide estimates and 95% CIs of the slope and intercept from the regression analysis.

- For each site as well as all sites combined, generate scatter plots of the concentration values of the i-STAT 500 result (y-axis) versus the concentration values of the i-STAT 1 Wireless result (x-axis). The line of identity ($y = x$) and descriptive statistics will be presented in the scatter plots.

- For each site as well as all sites combined, generate Bland-Altman bias plots and Bland-Altman percent bias plots. Calculate the difference between the i-STAT 500 concentration value (y) and the i-STAT 1 Wireless concentration value (x), $y - x$. Also calculate $(y + x) / 2$. Plot the difference between y and x, $y - x$, against their mean $(y + x) / 2$ (Bland-Altman bias plot); Plot the

percent difference between y and x, $100 \times (y - x) / x$, against their mean $(y + x) / 2$ (Bland-Altman percent bias plot).

- Using the regression equation for each site separately and for all sites combined, calculate the predicted bias (difference) between the i-STAT 500 results and the i-STAT 1 Wireless results at the medical decision points (45, 120, 180 mg/dL) and their 95% CIs. The calculation of predicted bias and its 95% CI calculation are based on CLSI EP09-A3.

e. Level of Significance

Two-sided confidence intervals will be calculated at a 95% confidence level.

f. Data Handling Convention

All results from this testing that are performed according to the protocol and not excluded by the reviewers will be eligible for analysis. Reasons for any excluded results will be captured in the study database.

- Exclude the specimen from analysis if no results can be obtained from either the i-STAT 1 Wireless or the i-STAT 500.
- Exclude test results if there is an assignable cause.
- Exclude the specimen if the glucose concentration value is outside of the measuring interval on either analyzer.
- Exclude a second valid result obtained due to a repeat test of the same specimen for another analyte. These results will be excluded with the reason "Additional test for other analyte. Repeat testing produces results for all analytes on cartridge."
- Exclude a result obtained from a subject that is for a bucket that is already filled with the reason "Not part of analysis."

g. Outliers

The outlier criteria below are based on CLSI document EP09-A3 Appendix B. The generalized Extreme Studentized Deviate (ESD) technique will be used to detect the outlier.

The generalized Extreme Studentized Deviate (ESD) technique assumes that the distribution of the vast majority of data points is normal (Gaussian), can be used when the number of outliers is unknown and becomes more robust as the number of samples increases. To perform this technique:

i Set the significance level $\alpha = 0.01$, which will be used to detect outliers.

ii No more than 5% of sample results can be flagged as outliers. Set the upper bound on number of potential outliers (h) at this 5% level, rounding down to a whole number ($h = 6$ for 120 samples).

iii Determine if one or more suspect results could be outliers based upon the generalized Extreme Studentized Deviate (ESD) test.

1) Compute the average bias across all the specimens ($\bar{d}$) and the SD corresponding to the bias.
2) Find the maximum scaled difference (ESD_1) as follows:

$$ESD_1 = \max\left|d_j - \bar{d}\right| / SD \quad \text{for } j = 1, 2, \ldots n$$

3) Repeat this calculation to obtain the ESD_i for all potential outliers ($i = 1, 2, \ldots h$). Each subsequent calculation of ESD_i is performed after removing the previously identified potential outlier from the dataset. Thus at each iteration the number of results is reduced by one, then $\bar{d}$, SD, and the maximum scaled difference (ESD_i) are computed again (ie, to look for outlier 2, the number of samples remaining is $j = 1, 2, \ldots n\text{-}1$).
4) For $i = 1,2, \ldots, h$, compute the following critical values:

$$\lambda_i = \frac{t_{v,p}\,(n-1)}{\sqrt{(n-i+1)\left(v + t_{v,p}^2\right)}}$$

where
n = the initial number of samples in the dataset
$i = 1, 2, \ldots h$
$v = n - i - 1$

$$p = \frac{\alpha}{2(n-i+1)}$$

$t_{v,p} = 100p$ percentage point from Student's t distribution with v, degrees of freedom and probability = p.

iv The number of outliers is determined by finding the largest i such that $ESD_i > \lambda_i$.

h. Acceptance Criteria

For method comparison, acceptance criterion will be applied to Passing-Bablok regression result for the single replicate result from the i-STAT 500 vs. the single replicate result from the i-STAT 1 Wireless for the entire measuring interval. Samples across the measuring interval of 20 – 700 mg/dL shall have a regression slope of 1.0 ± 0.05 and correlation coefficient (r) ≥ 0.95 (DIR-001, CartP_121).

There is no acceptance criterion for other analyses. The information is intended to be reported in regulatory submission(s) and/or product labeling.

2. Hematocrit

a. Study Design

Method comparison testing will be performed using whole blood capillary specimens prospectively collected from routine patient care. This testing will be performed at a minimum of 3 sites. Testing of approximately 120 native and altered specimens will be performed in singlicate on both the i-STAT 500 analyzer and the i-STAT 1 Wireless analyzer. One (1) i-STAT 500 and one (1) i-STAT 1 Wireless analyzer will be used for this testing. Each site should collect and test a minimum of 40 specimens until sufficient specimens are collected and tested for study analysis. A minimum of one lot of i-STAT EC4+ cartridges will be used for this testing.

b. Analysis Variables

The analysis variable for the method comparison study is the i-STAT EC4+ cartridge hematocrit values (%PCV).

c. Sample Size

The sample size is based on the recommendation of CLSI EP09-A3.

A minimum of 120 whole blood capillary specimens covering the measuring interval will be collected from a minimum of 3 sites. Each site should collect a minimum of 40 specimens.

Minimum of 3 sites
- Within each site:
  - Number of lots = 1
  - i-STAT 1 Wireless analyzers = 1
  - i-STAT 500 analyzers = 1
  - Replicates per specimen= 1

An estimated distribution of specimen target levels for hematocrit by site is presented in the chart below:

Hct (%PCV)	~# Sample /Site	Target Proportion
< 27	6	15%
27 – 35	10	25%
36 – 50	20	50%
> 50	4	10%
Total	**40**	

No hematocrit range shall have greater than 10% above the target proportion. Hematocrit results from samples that would cause a given hematocrit range to have a greater proportion of samples beyond the maximum allowed proportion will be excluded from this analysis. The hematocrit results from samples with earlier collection dates and times will be included.

d. Statistical Analysis Method

Perform the following analyses for the single replicate of the i-STAT 500 result versus the single replicate of the i-STAT 1 Wireless result. Analyses will be done with outliers and without outliers if any outliers are identified.

- For each site as well as all sites combined, calculate the correlation coefficient and perform the Deming regression and Passing-Bablok regression analyses on the concentration values (the i-STAT 500 result as dependent variable versus the i-STAT 1 Wireless result as independent variable) and provide estimates and 95% CIs of the slope and intercept from the regression analysis.

- For each site as well as all sites combined, generate scatter plots of the concentration values of the i-STAT 500 result (y-axis) versus the concentration values of the i-STAT 1 Wireless result (x-axis). The line of identity ($y = x$) and descriptive statistics will be presented in the scatter plots.

- For each site as well as all sites combined, generate Bland-Altman bias plots and Bland-Altman percent bias plots. Calculate the difference between the i-STAT 500 concentration value (y) and the i-STAT 1 Wireless concentration value (x), $y - x$. Also calculate $(y + x) / 2$. Plot the difference between y and x, $y - x$, against their mean $(y + x) / 2$ (Bland-Altman bias plot); Plot the percent difference between y and x, $100 \times (y - x) / x$, against their mean $(y + x) / 2$ (Bland-Altman percent bias plot).

- Using the regression equation for each site separately and for all sites combined, calculate the predicted bias (difference) between the i-STAT 500

results and the i-STAT 1 Wireless results at the medical decision points (33, 53, 56, 70 %PCV) and their 95% CIs. If gender information is collected, the medical decision point of 53 %PCV will be analyzed with only females and the medical decision point of 56 %PCV will be analyzed with only males. The calculation of predicted bias and its 95% CI calculation are based on CLSI EP09-A3.

e. Level of Significance

Two-sided confidence intervals will be calculated at a 95% confidence level.

f. Data Handling Convention

All results from this testing that are performed according to the protocol and not excluded by the reviewers will be eligible for analysis. Reasons for any excluded results will be captured in the study database.

- Exclude the specimen from analysis if no results can be obtained from either the i-STAT 1 Wireless or the i-STAT 500.
- Exclude test results if there is an assignable cause.
- Exclude any specimen if the hematocrit value is outside of the measuring interval on either analyzer.
- Exclude a second valid result obtained due to a repeat test of the same specimen for another analyte. These results will be excluded with the reason "Additional test for other analyte. Repeat testing produces results for all analytes on cartridge."
- Exclude a result obtained from a subject that is for a bucket that is already filled with the reason "Not part of analysis."

g. Outliers

The outlier criteria below are based on CLSI document EP09-A3 Appendix B. The generalized Extreme Studentized Deviate (ESD) technique will be used to detect outliers.

The generalized Extreme Studentized Deviate (ESD) technique assumes that the distribution of the vast majority of data points is normal (Gaussian), can be used when the number of outliers is unknown and becomes more robust as the number of samples increases. To perform this technique:

i Set the significance level $\alpha = 0.01$, which will be used to detect outliers.

ii No more than 5% of sample results can be flagged as outliers. Set the upper bound on number of potential outliers (h) at this 5% level, rounding down to a whole number ($h = 8$ for 160 samples).

iii Determine if one or more suspect results could be outliers based upon the generalized Extreme Studentized Deviate (ESD) test.

1) Compute the average bias across all the specimens ($\bar{d}$) and the SD corresponding to the bias.
2) Find the maximum scaled difference (ESD_1) as follows:

$$ESD_1 = \max\left|d_j - \bar{d}\right| / SD \quad \text{for } j = 1, 2, \ldots n$$

3) Repeat this calculation to obtain the ESD_i for all potential outliers ($i = 1, 2, \ldots h$). Each subsequent calculation of ESD_i is performed after removing the previously identified potential outlier from the dataset. Thus at each iteration the number of results is reduced by one, then $\bar{d}$, SD, and the maximum scaled difference (ESD_i) are computed again (ie, to look for outlier 2, the number of samples remaining is $j = 1, 2, \ldots n\text{-}1$).
4) For $i = 1,2, \ldots, h$, compute the following critical values:

$$\lambda_i = \frac{t_{v,p}\,(n-1)}{\sqrt{(n-i+1)\left(v + t_{v,p}^2\right)}}$$

where
n = the initial number of samples in the dataset
$i = 1, 2, \ldots h$
$v = n - i - 1$

$$p = \frac{\alpha}{2(n-i+1)}$$

$t_{v,p} = 100p$ percentage point from Student's t distribution with v, degrees of freedom and probability = p.

iv The number of outliers is determined by finding the largest i such that $ESD_i > \lambda_i$.

h. Acceptance Criteria

For method comparison, acceptance criterion will be applied to Passing-Bablok regression result for the single replicate result from the i-STAT 500 vs. the single replicate result from the i-STAT 1 Wireless for the entire measuring interval. Samples across the measuring interval shall have a regression slope of 1.0 ± 0.054 and correlation coefficient (r) ≥ 0.95 (DIR-001, CartP_145).

There is no acceptance criterion for other analyses. The information is intended to be reported in regulatory submission(s) and/or product labeling.

3. Sodium

a. Study Design

Method comparison testing will be performed using whole blood capillary specimens prospectively collected from routine patient care. This testing will be performed at a minimum of 3 sites. Testing of approximately 120 native and altered specimens will be performed in singlicate on both the i-STAT 500 analyzer and the i-STAT 1 Wireless analyzer. One (1) i-STAT 500 and one (1) i-STAT 1 Wireless analyzer will be used for this testing. Each site should collect and test a minimum of 40 specimens until sufficient specimens are collected and tested for study analysis. A minimum of one lot of i-STAT EC4+ cartridges will be used for this testing.

b. Analysis Variables

The analysis variable for the method comparison study is the i-STAT EC4+ cartridge sodium concentration values (mmol/L).

c. Sample Size

The sample size is based on the recommendation of CLSI EP09-A3.

A minimum of 120 capillary whole blood specimens covering the measuring interval will be collected from a minimum of 3 sites. Each site should collect a minimum of 40 specimens.

Minimum of 3 sites
- Within each site:
  - Number of lots = 1
  - i-STAT 1 Wireless analyzers = 1
  - i-STAT 500 analyzers = 1
  - Replicates per specimen = 1

A suggested distribution of specimen target levels for sodium by site is presented in the chart below:

Sodium (mmol/L)	~# Sample /Site	Max # Samples /Site
≤ 130	8	14
> 130-140	16	24
> 140-150	12	20
> 150	4	8
Total	**40**	

If more than the maximum allowed number of samples is collected for a given sodium range within a site, the sodium result from samples with the earlier collection date and time will be included in the analysis. The sodium results for samples collected beyond the maximum allowed number of samples will not be used for this analysis.

d. Statistical Analysis Method

Perform the following analyses for the single replicate of the i-STAT 500 result versus the single replicate of the i-STAT 1 Wireless result. Analyses will be done with outliers and without outliers if any outliers are identified.

- For each site as well as all sites combined, calculate the correlation coefficient and perform the Deming regression and Passing-Bablok regression analyses on the concentration values (the i-STAT 500 result as dependent variable versus the i-STAT 1 Wireless result as independent variable) and provide estimates and 95% CIs of the slope and intercept from the regression analysis.

- For each site as well as all sites combined, generate scatter plots of the concentration values of the i-STAT 500 result (y-axis) versus the concentration values of the i-STAT 1 Wireless result (x-axis). The line of identity (y = x) and descriptive statistics will be presented in the scatter plots.

- For each site as well as all sites combined, generate Bland-Altman bias plots and Bland-Altman percent bias plots. Calculate the difference between the i-STAT 500 concentration value (y) and the i-STAT 1 Wireless concentration value (x), y – x. Also calculate (y + x) / 2. Plot the difference between y and x, y – x, against their mean (y + x) / 2 (Bland-Altman bias plot); Plot the percent difference between y and x, 100 × (y – x) / x, against their mean (y + x) / 2 (Bland-Altman percent bias plot).

- Using the regression equation for each site separately and for all sites combined, calculate the predicted bias (difference) between the i-STAT 500

results and the i-STAT 1 Wireless results at the medical decision points (115, 135, 150 mmol/L) and their 95% CIs. The calculation of predicted bias and its 95% CI calculation are based on CLSI EP09-A3.

e. Level of Significance

Two-sided confidence intervals will be calculated at a 95% confidence level.

f. Data Handling Convention

All results from this testing that are performed according to the protocol and not excluded by the reviewers will be eligible for analysis. Reasons for any excluded results will be captured in the study database.

- Exclude the specimen from analysis if no results can be obtained from either the i-STAT 1 Wireless or the i-STAT 500.
- Exclude test results if there is an assignable cause.
- Exclude the specimen if the sodium concentration value is outside of the measuring interval on either analyzer.
- Exclude a second valid result obtained due to a repeat test of the same specimen for another analyte. These results will be excluded with the reason "Additional test for other analyte. Repeat testing produces results for all analytes on cartridge."
- Exclude a result obtained from a subject that is for a bucket that is already filled with the reason "Not part of analysis."

g. Outliers

The outlier criteria below are based on CLSI document EP09-A3 Appendix B. The generalized Extreme Studentized Deviate (ESD) technique will be used to detect the outlier.

The generalized Extreme Studentized Deviate (ESD) technique assumes that the distribution of the vast majority of data points is normal (Gaussian), can be used when the number of outliers is unknown and becomes more robust as the number of samples increases. To perform this technique:

i Set the significance level $\alpha = 0.01$, which will be used to detect outliers.

ii No more than 5% of sample results can be flagged as outliers. Set the upper bound on number of potential outliers (h) at this 5% level, rounding down to a whole number ($h = 6$ for 120 samples).

iii Determine if one or more suspect results could be outliers based upon the generalized Extreme Studentized Deviate (ESD) test.

5) Compute the average bias across all the specimens ($\bar{d}$) and the SD corresponding to the bias.

6) Find the maximum scaled difference (ESD_1) as follows:

$$ESD_1 = \max\left|d_j - \bar{d}\right| / SD \quad \text{for } j = 1, 2, \ldots n$$

7) Repeat this calculation to obtain the ESD_i for all potential outliers ($i = 1, 2, \ldots h$). Each subsequent calculation of ESD_i is performed after removing the previously identified potential outlier from the dataset. Thus at each iteration the number of results is reduced by one, then $\bar{d}$, SD, and the maximum scaled difference (ESD_i) are computed again (ie, to look for outlier 2, the number of samples remaining is $j = 1, 2, \ldots n\text{-}1$).

8) For $i = 1,2, \ldots, h$, compute the following critical values:

$$\lambda_i = \frac{t_{v,p}\,(n-1)}{\sqrt{(n-i+1)\left(v + t_{v,p}^2\right)}}$$

where
n = the initial number of samples in the dataset
$i = 1, 2, \ldots h$
$v = n - i - 1$

$$p = \frac{\alpha}{2(n-i+1)}$$

$t_{v,p} = 100p$ percentage point from Student's t distribution with v, degrees of freedom and probability = p.

iv The number of outliers is determined by finding the largest i such that $ESD_i > \lambda_i$.

h. Acceptance Criteria

For method comparison, acceptance criterion will be applied to Passing-Bablok regression result for the single replicate result from the i-STAT 500 vs. the single replicate result from the i-STAT 1 Wireless for the entire measuring interval. Samples across the measuring interval of 100 – 180 mmol/L shall have a regression slope of 1.0 ± 0.05 and correlation coefficient (r) ≥ 0.95 (DIR-001, CartP_74).

There is no acceptance criterion for other analyses. The information is intended to be reported in regulatory submission(s) and/or product labeling.

IV. References

1. Clinical and Laboratory Standards Institute (CLSI). *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition,* CLSI document EP09-A3 (ISBN 1-56238-887-8 [Print]; ISBN 1-56238-888-6[Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania, 19087, USA, 2013.

2. Kristian Linnet. *Performance of Deming regression analysis in case of misspecified analytical error ratio in method comparison studies*, Clin Chem. Vol 44 No. 5 1998; 1024-1031.

3. Passing, H, Bablok W. *A new biometrical procedure for testing the equality of measurements from two different analytical methods*. J Clin Chem Clin Biochem. 1983;21:709-720.

4. CS-2016-0003 V2 Capillary Protocol: Clinical Evaluation of i-STAT 500® Analyzer for Glucose, Hematocrit and Sodium.