



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY**

I Background Information:

A 510(k) Number

K210633

B Applicant

Abbott Ireland Diagnostics Division

C Proprietary and Established Names

Amylase2

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
JFJ	Class II	21 CFR 862.1070 - Amylase Test System	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Amylase

C Type of Test:

Quantitative Enzymatic/Colorimetric

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Amylase2 assay is used for the quantitation of amylase in human serum, plasma, or urine on the ARCHITECT c System.

The Amylase2 assay is to be used primarily as an aid in the diagnosis and treatment of pancreatitis (inflammation of the pancreas).

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

ARCHITECT c8000 clinical chemistry analyzer

IV Device/System Characteristics:

A Device Description:

The Amylase2 assay is comprised of a reagent kit that is run on the ARCHITECT c System. Each kit has two reagents: R1 and R2. R1 contains α -glucosidase 16.000 KU/L. R2 contains Ethylidene-4-NP-G7 (EPS) 6.501 g/L, both R1 and R2 have sodium azide as preservative. The Amylase2 assay is calibrated by the user using one of two methods:

- Calibration method, using the Consolidated Chemistry Calibrator.
- Calibration Factor method, using a fixed calibration factor value (4412) to calculate result

There are separate assay parameter files that the user must upload for use with each of the calibration methods. The Instructions for Use (IFU) provides information on calibration set up for the two methods.

B Principle of Operation:

The Amylase2 assay is a two-part reaction. Ethylidene-4-NP-G7 (EPS) is hydrolyzed by α -amylase to form 4,6-ethylidene- α -(1,4)-D-glucopyranosyl-Gx and 4-nitrophenyl- α -(1,4)-glucopyranosyl-G(7-x). The 4-nitrophenyl- α -(1,4)-glucopyranosyl-G(7-x) is then hydrolyzed into glucose monomers and the assay chromophore (4-nitrophenol) by α -glucosidase. The resulting change in absorbance at 404 nm is proportional to the α -amylase concentration in the sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Amy

B Predicate 510(k) Number(s):

K981653

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K210633</u>	<u>K981653</u>
Device Trade Name	Amylase2	Amy
General Device Characteristic Similarities		
Intended Use/Indications For Use	The assay is used for the quantitation of amylase.	Same
Measurand	Amylase	Same
Method	Enzymatic	Same
Specimen type	Serum, Plasma, Urine	Same
General Device Characteristic Differences		
Traceability	IRMM/IFCC-456	Molar extinction coefficient of CNP (non-IFCC method)
Calibration Method	Calibrator or Calibration factor method	Calibration factor Method
Assay range	Serum/Plasma: Analytical measuring interval: 3–3010 U/L Extended measuring interval: 3010- 5959 U/L Urine: Analytical measuring interval: 3–3010 U/L Extended measuring interval: 3010-8600 U/L	Serum/Plasma/Urine: Analytical measuring interval: 3–3010 U/L Flex Rate Linearity: 6554 U/L

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition.

CLSI EP07-A3 Interference Testing in Clinical Chemistry; Approved Guideline —Third Edition.

CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision studies were performed according to CLSI EP05-A3.

Serum precision was assessed by testing two levels of controls and three contrived serum panels (target concentrations of 4 U/L, 170 U/L, and 2800 U/L) samples in duplicates, two times per day, for 20 days, three lots of reagent, three instruments for a total of 80 replicates per sample for each lot. The results of the precision studies are summarized below for one representative lot. The within-laboratory SD and %CV were estimated using the within-run, between-run, and between-day variance components.

Serum, Calibration method :

Sample Type	n	Mean U/L	Repeatability		Within-Lab Precision	
			SD U/L	CV (%)	SD U/L	CV (%)
Serum QC 1	80	77	0.5	0.6	1.5	2.0
Serum QC 2	80	413	3.3	0.8	6.7	1.6
Panel A	80	4	0.3	7.4	0.5	11.0
Panel B	80	162	0.8	0.5	3.3	2.1
Panel C	80	2629	14.8	0.6	51.6	2.0

Serum, Calibration Factor method :

Sample Type	n	Mean U/L	Repeatability		Within-Lab Precision	
			SD U/L	CV (%)	SD U/L	CV (%)
Serum QC 1	80	76	0.5	0.6	1.5	2.0
Serum QC 2	80	411	3.3	0.8	6.7	1.6
Panel A	80	4	0.3	6.5	0.4	9.7
Panel B	80	161	0.8	0.5	3.4	2.1
Panel C	80	2615	14.7	0.6	51.2	2.0

Urine Precision was assessed by testing two levels of controls and five contrived human urine panels (target concentrations of 5 U/L, 16 U/L, 500 U/L, 2000 U/L, and 2800 U/L) in duplicates, two times per day, for 20 days, 3 lots of reagent, 3 instruments for a total of 80 replicates per sample for each lot. The within-laboratory SD and %CV were estimated using the within-run, between-run, and between-day variance components. The results of the urine precision studies are summarized below for one representative lot.

Urine, Calibration method:

Sample Type	n	Mean U/L	Repeatability		Within-Lab Precision	
			SD U/L	CV (%)	SD U/L	CV (%)
Urine QC 1	80	55	0.4	0.8	0.5	1.0
Urine QC 2	80	180	0.8	0.4	1.3	0.7
Panel A	80	6	0.3	5.4	0.5	7.9
Panel B	80	18	0.4	2.1	0.4	2.4
Panel C	80	538	2.7	0.5	5.3	1.0
Panel D	80	1891	10.2	0.5	20.4	1.1
Panel E	80	2625	12.2	0.5	20.3	0.8

Urine, Calibration Factor method:

Sample Type	n	Mean U/L	Repeatability		Within-Lab Precision	
			SD U/L	CV (%)	SD U/L	CV (%)
Urine QC 1	80	55	0.4	0.7	0.6	1.0
Urine QC 2	80	179	0.8	0.4	1.3	0.7
Panel A	80	6	0.4	6.5	0.5	8.4
Panel B	80	18	0.4	2.1	0.5	2.6
Panel C	80	535	2.7	0.5	5.3	1.0
Panel D	80	1881	10.1	0.5	20.2	1.1
Panel E	80	2611	12.2	0.5	20.1	0.8

Reproducibility:

Reproducibility studies were performed according to the CLSI EP05-A3 guideline.

Serum:

Testing was conducted using five control samples, one lot of reagent, one lot of calibrator, one lot of controls, and three instruments. Each instrument was operated by a different technician. Samples were tested in three replicates, two times per day for five days, for a total of 90 replicates. The results of the precision studies are summarized below for one representative lot. The within-laboratory SD and %CV were estimated using the within-run, between-run, and between-day variance components.

Sample Type	n	Mean U/L	Repeatability		Within-Lab Precision		Reproducibility	
			SD U/L	CV (%)	SD U/L	CV (%)	SD U/L	CV (%)
QC 1	90	74	0.4	0.6	0.6	0.8	0.6	0.9
QC 2	90	416	2.5	0.6	3.0	0.7	3.1	0.7
QC 3	90	37	0.4	1.0	0.5	1.3	0.6	1.6
QC 4	90	113	0.8	0.7	0.8	0.7	0.9	0.8
QC 5	90	351	1.2	0.4	1.3	0.4	1.6	0.4

Urine:

Testing was conducted using four controls, one lot of reagent, one lot of calibrator, one lot of controls, and three instruments. Each instrument was operated by a different technician. Samples were tested in three replicates, two times per day for five days, for a total of 90 replicates. The results of the precision studies are summarized below for one representative lot. The within-laboratory SD and %CV were estimated using the summation of the within-run, between-run, and between-day variance components.

Sample Type	n	Mean U/L	Repeatability		Within-Lab Precision		Reproducibility	
			SD U/L	CV (%)	SD U/L	CV (%)	SD U/L	CV (%)
QC 1	90	54	0.5	1.0	0.5	1.0	0.6	1.1
QC 2	90	171	1.2	0.7	1.2	0.7	1.6	0.9
QC 3	90	66	0.4	0.7	0.5	0.8	0.9	1.3
QC 4	90	232	1.6	0.7	2.2	1.0	2.4	1.0

2. Linearity:

In this study, one contrived low level amylase sample with a concentration at or below the limit of quantitation (LoQ) was mixed with one high level contrived amylase sample with a concentration at or above the upper limit of the analytical measuring interval to create 14 samples/pools for serum and urine. Samples were tested in duplicate using one reagent lot and one instrument. The mean results were plotted against the expected results, below is the linear regression analysis.

Serum:

- Calibration method: $y = 0 + 1.0102x$
- Calibration factor method: $y = 0 + 1.0095x$

Urine:

- Calibration method: $y = 0 + 0.9886x$
- Calibration factor method: $y = 0 + 0.9898x$

Auto Dilution study:

The sponsor conducted a study to validate the auto-dilute feature available for both serum and urine extended range. Five human serum samples were prepared by spiking α -amylase stock solution into a serum pool to obtain the target concentration values of 3800, 4500, 5200, 5900, and 6600 U/L. Five urine samples were prepared by spiking α -amylase stock solution into a urine pool to obtain the target concentration values of 3800, 4500, 5200, 5900, and 6600 U/L. Three runs were performed, each sample was tested in replicates of five on two instruments for a total of 30 replicates. The percent dilution recovery (% Dilution Recovery) relative to the assigned sample concentration was calculated for the automated dilution with calibration method and calibration factor method. Results of this study supported the sponsor's claim that samples with amylase concentrations above 3010 U/L can be auto diluted 1:2 (i.e. 1:1.98) to obtain results up to 5959 U/L for serum and auto diluted 1:3 (i.e. 1:2.86) to obtain results up to 8600 U/L for urine.

Manual Dilution study:

The sponsor conducted a study to validate the manual-dilution available for both serum and urine extended range. For serum, three sets of manually diluted samples (1:2 dilution with saline) were prepared for each sample by two technicians. Each sample was tested in replicates of five on one instrument for a total of 30 replicates. The percent dilution recovery (% Dilution Recovery) relative to the assigned sample concentration was calculated for the manual dilution with calibration method and calibration factor method. Results of this study supported the sponsor's claim that samples with amylase concentrations above 3010 U/L can be diluted manually 1:2 to obtain results up to 5959 U/L for serum and diluted manually 1:3 to obtain results up to 8600 U/L for urine.

3. Analytical Specificity/Interference:

Interference studies were performed in accordance with the CLSI EP07-A3 guideline.

Serum:

Studies were conducted using two samples with amylase concentration of 50 U/L and 200 U/L. Each sample had one aliquot for test sample and one for reference sample. Samples were run in 10 replicates on one reagent lot, one instrument. The difference between the mean concentration of the test and reference samples was calculated and the % difference was calculated. The sponsor defined Amylase2 interference if the two-sided 95% confidence interval (CI) values around the % difference was within or equal to $\pm 10\%$. The results for the Calibration Factor method were generated using the absorbance data from the Calibrator method, two-sided 95% CI around the % difference for amylase samples targeted at 50 U/L and 200 U/L. The results for Calibration factor method ranged from -10% to 5% and calibration method from -10% to 6%. The following table lists the concentrations of each substance at which no significant interference was found:

Interferent	Highest concentration tested that did not show significant interference
Conjugated Bilirubin	60 mg/dL
Unconjugated Bilirubin	60 mg/dL
Hemoglobin	1000 mg/dL
Total Protein	15 g/dL
Triglycerides	1500 mg/dL
Acetaminophen	160 mg/L
Acetylcysteine	150 mg/L
Acetylsalicylic acid	30 mg/L
Ampicillin-Na	80 mg/L
Ascorbic acid	60 mg/L
Biotin	4250 ng/mL
Ca-dobesilate	60 mg/L
Cefotaxime	53 mg/dL
Cefoxitin	6600 mg/L
Cyclosporine	2 mg/L
Doxycycline	20 mg/L
Ibuprofen	220 mg/L
Icodextrin	3.6 mg/dL
Levodopa	8 mg/L
Methyldopa	25 mg/L
Metronidazole	130 mg/L
Pancreozymin	314 pg/mL
Phenylbutazone	330 mg/L
Rifampicin	50 mg/L
Sodium heparin	4 U/mL
Theophylline	60 mg/L

Urine:

The study was conducted using two urine samples with amylase concentration at 450 U/L and 1400 U/L. Each sample had two aliquots and tested for the test sample (spiked with potentially interfering substances) and for the reference sample. Samples were run in 10 replicates, 1 reagent lot, 1 instrument. The difference between the mean concentration of the test and reference samples and the % difference was calculated for calibration method and calibration factor method. The sponsor defined Amylase2 interference if the two-sided 95% Confidence Interval (CI) values around the % difference was within or equal to $\pm 10\%$, at the target amylase levels of 450 U/L and 1400 U/L. The results for the Calibration Factor method were generated using the absorbance data from the Calibrator method. Two-sided 95% CI around the % difference for amylase samples ranged from -1% to 1% for both calibration method and calibration factor method.

The following table lists the concentrations of each substance at which no significant interference was found:

Interferent	Highest concentration tested that did not show significant interference
Ascorbate	150 mg/dL
Boric acid	250 mg/dL
Glucose	1000 mg/dL
Protein	50 mg/dL
Acetaminophen	16 mg/dL
Acetylcysteine	15 mg/dL
Biotin	4250 ng/mL
Ibuprofen	22 mg/dL
Sodium Carbonate	1.25 g/dL
Sodium Fluoride	400 mg/dL
Sodium Oxalate	60 mg/dL

Acetic acid (8.5N), hydrochloric acid (6N) and nitric acid (6N) were found to interfere at amylase levels of 450 U/L and 1400 U/L.

The following limitation for acidified urine samples is included in the Amylase2 reagent package insert: “*Amylase2 testing should not be performed on acidified urine samples due to enzyme instability.*”

Icodextrin interference: Treatment with icodextrin can result in decreased amylase results at therapeutically relevant interferent concentrations and samples from patients with this treatment should not be tested.

Ascorbate interference: The following interference beyond +/-10% was observed at the 200 mg/dL level of Ascorbate and added to labeling:

Interferent	Interferent Level	Analyte Level	% Interference (95% CI)
Ascorbate	200 mg/dL	450 U/L	-21% (-21%, -20%)
Ascorbate	200 mg/dL	1400 U/L	-18% (-19%, -17%)

4. Assay Reportable Range:

The assay has an analytical measuring interval of 3-3010 U/L for serum, plasma, and urine specimens with an extended measuring interval of 3010-5959 U/L for serum and 3010-8600 U/L for urine.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

The Amylase2 assay is traceable to IRMM/IFCC-456 reference material.

6. Detection Limit:

Detection capability studies of the Amylase 2 assay on the ARCHITECT c8000 with serum and urine for limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) was conducted following the recommendations in CLSI EP17-A2. Data analysis was conducted using both the calibration method and calibration factor method.

LoB

The 95th percentile of the zero-analyte sample concentrations based on $n \geq 60$ replicates using each of three lots of reagent and two instruments was calculated and considered the estimated LoB.

LoD

The LoD was determined parametrically using the lowest concentration at which the analyte could be detected with 95% probability based on $n \geq 60$ replicates of low-analyte level samples tested using each of three lots of reagent and two instruments.

LoQ

The LoQ was defined as the lowest concentration at which a maximum allowable precision of 20 %CV was met and was determined from $n \geq 60$ replicates of low-analyte level samples using each of three lots of reagent and two instruments.

The results of the detection limit studies are presented in the table below:

Matrix	LoB	LoD	LoQ	Analytical Measuring Interval
Serum	0 U/L	1 U/L	2 U/L	3-3010 U/L
Urine	0 U/L	1 U/L	3 U/L	3-3010 U/L

7. Assay Cut-Off:

Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was conducted by testing 124 serum samples and 103 urine samples. Two of 124 of serum samples and seven of 103 of urine samples were spiked with amylase stock solution. For the candidate device, samples were tested using three lots of reagents and one lot each of calibrator and controls on two ARCHITECT c8000 instruments. For the predicate device, samples were tested using one lot each of reagents, calibrator, and controls on two ARCHITECT c8000 instruments. Each sample was tested in duplicate using both methods and testing occurred over three days. A Passing-Bablok evaluation was performed by comparing the first replicate result from the candidate device versus the mean result from the predicate device. The results are summarized below:

Sample	n	Slope	Intercept	r	Test range (per predicate)
Serum, calibration method	124	0.98	-1.00	1.0	6-2788
Serum, calibration factor method	124	0.96	-1.00	1.0	6-2788
Urine, calibration method	103	0.93	-1.00	1.00	4-2916
Urine, calibration factor method	103	0.93	-1.00	1.00	4-2916

2. Matrix Comparison:

A matrix comparison study was performed to evaluate the suitability of other blood specimen types for use with the Amylase2 assay. A total of 65 samples were tested using one lot of the Amylase2 reagent, one lot of the Consolidated Chemistry Calibrator, one lot of commercially available controls, and one instrument in triplicate. The first replica result of the test tube type was used for comparison to the mean of the replica for the control tube type (serum). The results are shown below:

Calibration method:

Specimen Type	N	Min	Max	r	Intercept	Slope
Lithium heparin (plasma)	65	20	2638	1.00	-0.82	1.01
Sodium heparin (plasma)	65	20	2526	1.00	-0.89	1.01
Lithium heparin (separator tube, plasma)	65	20	2417	1.00	-0.57	1.01
Serum (separator tube)	65	21	2565	1.00	0.33	1.00

Calibration factor method:

Specimen Type	N	Min	Max	r	Intercept	Slope
Lithium heparin (plasma)	65	20	2602	1.00	-0.67	1.00
Sodium heparin (plasma)	65	20	2492	1.00	-0.75	1.00
Lithium heparin (separator tube, plasma)	65	20	2385	1.00	-0.85	1.01
Serum (separator tube)	65	21	2531	1.00	0.33	1.00

The study results support the sponsor's claim that serum separator tube, lithium heparin, lithium heparin serum separator tube and sodium heparin tube are acceptable collection methods for serum and plasma samples to be used with this assay.

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable

2. Clinical Specificity:

Not applicable

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

Sample Type	Range U/L
Serum ¹	28-100
Urine Random ¹	
Male	16-491
Female	21-447
Timed Urine ²	1-17 U/hour
Adult (24-hour urine) ³	170-2000

1. Junge W, Wortmann W, Wilke B, et al. Development and evaluation of assays for the determination of total and pancreatic amylase at 37 degrees C according to the principle recommended by the IFCC. Clin Biochem 2001;34(8):607-615.
2. Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 2nd ed. Philadelphia, PA: WB Saunders; 1994.
3. Wu AHB, editor. Tietz Clinical Guide to Laboratory Tests. 4th ed. St. Louis, MO: Saunders Elsevier; 2006:102.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.