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

A. 510(k) Number:

k182474

B. Purpose for Submission:

New devices

C. Measurand:

α-Amylase
Pancreatic α-Amylase
Bilirubin Total
Bilirubin Direct

D. Type of Test:

α-Amylase assays: Quantitative, enzymatic assays
Bilirubin (Total and Direct) assays: Colorimetric, photometric assays

E. Applicant:

BioSystems S.A.

F. Proprietary and Established Names:

alpha-AMYLASE DIRECT
alpha-AMYLASE EPS
alpha-AMYLASE PANCREATIC
BILIRUBIN TOTAL
BILIRUBIN DIRECT

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
JFJ	II	21 CFR § 862.1070 Amylase test system	Clinical Chemistry (75)
CIG	II	21 CFR § 862.1110 Bilirubin (total or direct) test system	

H. Intended Use:

1. Intended use(s):

   See Indications for use below

2. Indication(s) for use:

   alpha-AMYLASE DIRECT: Reagent for the measurement of alpha-amylase concentration in human serum, plasma or urine. The obtained values are useful as an aid in the diagnosis and of acute and chronic pancreatitis. This reagent is for use in the BioSystems BA analyzers. Only for in vitro use in the clinical laboratory.

   alpha-AMYLASE EPS: Reagent for the measurement of alpha-amylase concentration in human serum, plasma or urine. The obtained values are useful as an aid in the diagnosis of acute and chronic pancreatitis. This reagent is for use in the BioSystems BA analyzers. Only for in vitro use in the clinical laboratory.

   alpha-AMYLASE PANCREATIC: Reagent for the measurement of pancreatic α-amylase concentration in human serum, plasma or urine. The obtained values are useful as an aid in the diagnosis of acute and chronic pancreatitis. This reagent is for use in the BioSystems BA analyzers. Only for in vitro use in the clinical laboratory.

   BILIRUBIN TOTAL: Reagent for the measurement of total bilirubin concentration in human serum or plasma. Measurements of the levels of bilirubin are used in the diagnosis and treatment of liver, hemolytic hematological and metabolic disorders, including hepatitis and gall bladder block. This reagent is for use in the BioSystems BA analyzers. Only for in vitro use in the clinical laboratory.

   BILIRUBIN DIRECT: Reagent for the measurement of direct bilirubin concentration in human serum or plasma. Measurements of the levels of bilirubin are used in the diagnosis and treatment of liver, hemolytic hematological and metabolic disorders, including hepatitis and gall bladder block. This reagent is for use in the BioSystems BA analyzers. Only for in vitro use in the clinical laboratory.

3. Special conditions for use statement(s):

   These devices are intended for prescription use and in vitro diagnostic use only.

4. Special instrument requirements:

   BioSystems BA400

I. Device Description:

α-AMYLASE DIRECT:

MES 50 mmol/L, calcium chloride 5 mmol/L, sodium chloride 300 mmol/L, sodium thiocyanate 450 mmol/L, CNP-G3 2.25 mmol/L, pH 6.1.

α-AMYLASE EPS:

A: 2 x 60 mL. HEPES 50 mmol/L, calcium chloride 0.075 mmol/L, sodium chloride 90 mmol/L, magnesium chloride 13 mmol/L, α -glucosidase > 4 U/mL, pH 7.1.

B: 2 x 15 mL. HEPES 50 mmol/L, 4-Nitrophenyl-maltoheptaoside-ethylidene 18 mmol/L, pH 7.1.

α-AMYLASE PANCREATIC:

A: 2 x 60 mL. HEPES 50 mmol/L, calcium chloride 0.075 mmol/L, sodium chloride 90 mmol/L, magnesium chloride 13 mmol/L, α-glucosidase > 4 U/mL, pH 7.1, monoclonal antibodies (mouse) 50 mg/L.

B: 2 x 15 mL. HEPES 50 mmol/L, 4-Nitrophenyl-maltoheptaoside-ethylidene 18 mmol/L, pH 7.1.

BILIRUBIN DIRECT:

A: Phosphoric acid 90 mmol/L, HEDTA 4.5 mmol/L, sodium chloride 50 mmol/L, pH 1.5.

B: 3,5-dichlorophenyl diazonium 1.5 mmol/L.

BILIRUBIN TOTAL:

A: Hydrochloric acid 170 mmol/L, cetrimide 40 mmol/L, pH 0.9.

B: 3,5-dichlorophenyl diazonium 1.5 mmol/L.

J. Substantial Equivalence Information:

1. Predicate device name(s):

AMYL-α-Amylase EPS, BILD2- Bilirubin Direct Gen.2, BILT2- Bilirubin Total Gen.2

2. Predicate 510(k) number(s):

k100853

3. Comparison with predicate:

Similarities and Differences		
Item	Candidate Device alpha-AMYLASE DIRECT (k182474)	Predicate Device AMYL-α-Amylase EPS (k100853)
Intended Use	Reagent for the measurement of α-amylase concentration in human serum, plasma or urine.	Same
Measurand	α-amylase	Same
Sample Type	Serum, plasma or urine	Same
Assay Technology	Direct Substrate	IFCC
Assay Range	8.20-1300 U/L	3-1500 U/L
Reagent storage	Store at 2-8 ºC until the expiry date marked in the label.	Same

Similarities and Differences		
Item	Candidate Device alpha-AMYLASE EPS (k182474)	Predicate Device AMYL-α-Amylase EPS (k100853)
Intended Use	Reagent for the measurement of α-amylase concentration in human serum, plasma or urine.	Same
Measurand	α -amylase	Same
Sample Type	Serum, plasma or urine	Same
Assay Technology	IFCC	Same
Assay Range	14.3-1300 U/L	3-1500 U/L
Reagent storage	Store at 2-8 ºC until the expiry date	Same

Similarities and Differences		
Item	Candidate Device alpha-AMYLASE PANCREATIC (k182474)	Predicate Device AMYL-α-Amylase EPS Pancreatic (k100853)
Intended Use	Reagent for the measurement of pancreatic α-amylase concentration in human serum, plasma or urine.	Same
Measurand	Pancreatic α -amylase	Same
Sample Type	Serum, plasma or urine	Same
Assay Technology	Immuno-inhibition	Same
Assay Range	7.49-1300 U/L	3-1500 U/L
Reagent storage	Store at 2-8 ºC until the expiry date	Same

Similarities and Differences		
Item	Candidate Device BILIRUBIN DIRECT (k182474)	Predicate Device BILD2- Bilirubin Direct Gen.2 (k100853)
Intended Use	Reagent for the measurement of direct bilirubin concentration in human serum or plasma.	Same
Measurand	Bilirubin	Same
Sample Type	Serum, plasma	Same
Assay Technology	Dichloropheny diazonium	Same
Assay Range	0.14-15 mg/dL	0.09-17 mg/dL
Reagent storage	Store at 2-8 ºC until the expiry date	Same

Similarities and Differences		
Item	Candidate Device BILIRUBIN TOTAL (k182474)	Predicate Device BILT2- Bilirubin Total Gen.2 (k100853)
Intended Use	Reagent for the measurement of total bilirubin concentration in human serum or plasma.	Same
Measurand	Bilirubin	Same
Sample Type	Serum, plasma	Same
Assay Technology	Dichloropheny diazonium	Same
Assay Range	0.26-38 mg/dL	0.1-38 mg/dL
Reagent storage	Store at 2-8 ºC until the expiry date	Same

K. Standard/Guidance Document Referenced (if applicable):

CLSI - EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Third Edition.

CLSI - EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.

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

L. Test Principle:

The alpha-AMYLASE DIRECT reagent is a photometric assay in which the α-amylase catalyzes the hydrolysis of 2-chloro-4-nitrophenyl-malto-trioside (CNPG3) to 2-chloro-4-nitrophenol (CNP). The catalytic concentration is determined from the rate of 2-chloro-4-nitrophenol formation, measured at 405 nm.

The alpha-AMYLASE EPS reagent is a photometric assay in which the α-amylase catalyzes the hydrolysis of 4-nitrophenyl-maltoheptaoside-ethylidene to smaller oligosaccharides which are hydrolyzed by α-glucosidase liberating 4-nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405 nm.

The alpha-AMYLASE PANCREATIC reagent is a photometric assay in which pancreatic α-amylase catalyzes the hydrolysis of 4-nitrophenyl-maltoheptaoside-ethylidene to smaller oligosaccharides which are hydrolyzed by α-glucosidase liberating 4- nitrophenol. The catalytic concentration is determined from the rate of 4- nitrophenol formation, measured at 405 nm. Specific antibodies inhibit the salivary isoenzyme and thus allow the measurement of pancreatic α-amylase.

The BILIRUBIN DIRECT reagent is a photometric assay in which the direct bilirubin in the sample reacts with 3,5-dichlorophenyl diazonium salt forming a colored complex that can be measured by spectrophotometry at 535 nm.

The BILIRUBIN TOTAL reagent is a photometric assay in which both the direct and indirect bilirubin in the sample reacts with 3,5-dichlorophenyl diazonium salt in the presence of cetrimide forming a colored complex that can be measured by spectrophotometry at 535 nm.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision studies were performed according to the CLSI EP5-A3 guideline. For alpha-AMYLASE DIRECT, alpha-AMYLASE EPS and alpha-AMYLASE PANCREATIC, three levels of commercial biochemistry control serum (human serum matrix) or Urine, Level I, II and III, and for BILIRUBIN DIRECT and BILIRUBIN TOTAL, four levels of commercial biochemistry control serum (human serum matrix) Level I, II , III and IV were tested on two runs per day, two measurements per run, for twenty days with total of 80 observations. The results of the precision studies are summarized in the tables below:

alpha-AMYLASE DIRECT:

Matrix	Samples	n	Mean (U/L)	Repeatability		Total	
				SD	CV%	SD	CV%
Serum	QC 1	80	53.3	0.8	1.6	1.6	2.2
	QC 2	80	201	1.0	0.5	0.5	0.9
	QC 3	80	1061	6.7	0.6	0.6	1.5
Urine	QC 1	80	92	2.3	2.5	2.3	2.5
	QC 2	80	407	10.1	2.5	10.2	2.5
	QC 3	80	2078	12.6	0.6	35.4	1.7

alpha-AMYLASE EPS:

Matrix	Samples	n	Mean (U/L)	Repeatability		Total	
				SD	CV%	SD	CV%
Serum	QC 1	80	54.3	0.9	1.7	1.9	3.5
	QC 2	80	207	4.5	2.2	4.9	2.4
	QC 3	80	1090	4.3	0.4	16.9	1.6
Urine	QC 1	80	103	2.3	2.2	2.8	2.7
	QC 2	80	384	4.7	1.2	7.8	2.0
	QC 3	80	2161	26.1	1.2	36.6	1.7

alpha-AMYLASE PANCREATIC:

Matrix	Samples	n	Mean (U/L)	Repeatability		Total	
				SD	CV%	SD	CV%
Serum	QC 1	80	52.7	0.6	1.0	1.2	2.2
	QC 2	80	156	2.1	1.4	2.2	1.4
	QC 3	80	1058	6.1	0.6	11.7	1.1
Urine	QC 1	80	66.2	1.4	2.1	1.6	2.5
	QC 2	80	366	5.4	1.5	6.2	1.7
	QC 3	80	2259	43.8	1.9	45.2	2.0

BILIRUBIN DIRECT:

Matrix	Samples	n	Mean (mg/dL)	Repeatability		Total	
				SD	CV%	SD	CV%
Serum	QC 1	80	0.35	0.03	7.7	0.03	8.0
	QC 2	80	0.61	0.03	4.1	0.03	5.0
	QC 3	80	1.68	0.03	2.0	0.05	2.9
	QC 4	80	15.1	0.09	0.6	0.19	1.3

BILIRUBIN TOTAL:

Matrix	Samples	n	Mean (mg/dL)	Repeatability		Total	
				SD	CV%	SD	CV%
Serum	QC 1	80	1.03	0.09	8.5	0.09	8.7
	QC 2	80	2.08	0.07	3.3	0.07	3.3
	QC 3	80	4.90	0.05	0.9	0.09	1.8
	QC 4	80	30.9	0.13	0.4	0.33	1.1

b. Linearity/assay reportable range:

Linearity studies were conducted according to the CLSI EP06-A guideline. Eleven levels of dilutions were prepared by mixing different proportions of samples with high and low concentration of each analyte.

Samples were analyzed on one BA-400 instrument in duplicate. The results of the regression analysis are shown below:

Analyte	Matrix	Slope	Intercept	r^2	Range Conc. tested	Claimed range
alpha-AMYLASE DIRECT (U/L)	Serum	1.011	-3.50	1.000	0.0 - 1400	8.18 - 1300
	Urine	1.006	1.58	1.000	0.0 - 2700	19.2 - 2600
alpha-AMYLASE EPS (U/L)	Serum	0.981	3.48	1.000	0.0 - 1400	14.3 - 1300
	Urine	0.982	9.72	1.000	0.0 - 2700	19.1 - 2600
alpha-AMYLASE PANCREATIC (U/L)	Serum	0.982	2.13	1.000	0.0 - 1300	8.18 - 1300
	Urine	0.990	4.46	1.000	0.0 - 2700	19.6 - 2600
BILIRUBIN DIRECT (mg/dL)	Serum	0.999	0.003	1.000	0.08 - 22.80	0.14 - 15.00
BILIRUBIN TOTAL (mg/dL)	Serum	0.972	0.327	0.999	0.00 - 38.00	0.32 - 38.00

The linearity studies support the sponsor's claimed measuring ranges as described in the table above.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability:

alpha-AMYLASE DIRECT reagent: Values assigned and traceable to an internal BioSystems Master Calibrator (BMC).

alpha-AMYLASE-EPS reagent: Values assigned and traceable to C-SER/IFCC; International Federation of Clinical Chemistry and Laboratory Medicine.

alpha-AMYLASE-PANCREATIC reagent: Values assigned and traceable to C-SER/IFCC; International Federation of Clinical Chemistry and Laboratory Medicine.

BILIRUBIN DIRECT Reagent: Values assigned and traceable to an internal BioSystems Master Calibrator (BMC).

BILIRUBIN TOTAL Reagent: Values assigned and traceable to SRM 916 (NIST) Standard Reference Materials.

d. Detection limit:

Detection capabilities studies for each analyte were evaluated based upon the CLSI EP17-A2 guideline.

Limit of blank (LoB) studies were performed by testing 5 blank samples in replicates of 4 over 3 days, on two lots for a total of 120 observations. LoB was defined as the highest result that can reasonably be expected from a blank sample for a given error probability with α=0.05.

Limit of detection (LoD) studies were performed by testing 5 pool of human serum or urine with analyte concentrations close to expected detection limit for each analyte. Samples were tested in replicates of 4 over 3 days, on two lots for a total of 120 observations. The LoD was estimated based on a probability of error of 5% (β=0.05).

Limit of quantitation (LoQ) studies were performed using 4 to 5 pools of human serum or urine with analytes concentrations closed to the expected LoQ of the corresponding assay. Samples were tested in replicates of 4 over 3 days, on two lots for a total of 120 observations. The sponsor defines LoQ as the lowest concentration of a measurand with an imprecision ≤10%.

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

Assays	Matrix	LoB	LoD	LoQ	Claimed Range
alpha-AMYLASE DIRECT (U/L)	Serum	0.50	1.64	8.18	8.18 - 1300
	Urine	1.36	5.14	19.2	19.2 - 2600
alpha-AMYLASE EPS (U/L)	Serum	1.53	4.63	14.3	14.3 - 1300
	Urine	4.48	8.90	19.1	19.1 - 2600
alpha-AMYLASE PANCREATIC (U/L)	Serum	2.26	4.95	8.14	8.18 - 1300
	Urine	3.06	6.42	19.6	19.6 - 2600
BILIRUBIN DIRECT (mg/dL)	Serum	0.07	0.10	0.14	0.14 - 15.00
BILIRUBIN TOTAL (mg/dL)	Serum	0.14	0.21	0.32	0.32 - 38.00

e. Analytical specificity:

Interference studies were performed in accordance with the CLSI EP07-A2 guideline. For alpha-AMYLASE DIRECT, alpha-AMYLASE EPS, alpha-AMYLASE PANCREATIC, specimens consist on human serum and urine representing low and high concentrations of the analyte, with and without added increasing concentration of the substance tested for interference. Interference studies for BILIRUBIN TOTAL and BILIRUBIN DIRECT were conducted using serum samples. Samples were spiked

with increasing concentrations of the potential interferents and analyzed in duplicate. Interference was calculated as: (b-a)/a, where a and b are the concentration of measurand found without (a) and with (b) interferent. The sponsor states that interference is considered to be non-significant if the difference between the samples with and without interferent are within 10%.

The results of the interference studies are summarized in the table below.

Analyte	Interferent	Highest concentration tested that did not show significant interference
alpha-AMYLASE DIRECT	Bilirubin	30 mg/dL
	Hemoglobin	200 mg/dL
	Triglycerides	1756 mg/dL
alpha-AMYLASE EPS	Bilirubin	30 mg/dL
	Hemoglobin	604mg/dL
	Triglycerides	659 mg/dL
alpha-AMYLASE PANCREATIC	Bilirubin	30 mg/dL
	Hemoglobin	500 mg/dL
	Triglycerides	1625 mg/dL
BILIRUBIN DIRECT	Hemoglobin	25 mg/dL
	Triglycerides	975 mg/dL
BILIRUBIN TOTAL	Hemoglobin	500 mg/dL
	Triglycerides	512 mg/dL

Exogenous interferences:

Analyte	Matrix	Interferent	Highest concentration tested that did not show significant interference
alpha-AMYLASE DIRECT	Serum	Acetaminophen	20 mg/dL
		Ascorbic Acid	30 mg/dL
		Acetylsalicylic Acid	70 mg/dL
	Urine	Ascorbic Acid	30 mg/dL
alpha-AMYLASE EPS	Serum	Acetaminophen	20 mg/dL
		Ascorbic Acid	30 mg/dL
		Acetylsalicylic Acid	70 mg/dL
	Urine	Ascorbic Acid	30 mg/dL
alpha-AMYLASE PANCREATIC	Serum	Acetaminophen	20 mg/dL
		Ascorbic Acid	30 mg/dL
		Acetylsalicylic Acid	70 mg/dL
	Urine	Ascorbic Acid	30 mg/dL
BILIRUBIN DIRECT	Serum	Acetaminophen	20 mg/dL
		Ascorbic Acid	30 mg/dL
BILIRUBIN TOTAL	Serum	Acetaminophen	20 mg/dL
		Ascorbic Acid	30 mg/dL

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison studies were conducted by testing native human serum, plasma and urine samples (as applicable) on the α-AMYLASE DIRECT, α-AMYLASE EPS, α-AMYLASE PANCREATIC, BILIRUBIN DIRECT and BILIRUBIN TOTAL candidate assays on the BA400 analyzer and the predicate devices.

The results of the regression analysis are summarized below:

Analyte	Specimen Type	N	Slope	Intercept	R	Test range*	Claimed Measuring Range
alpha-AMYLASE DIRECT (U/L)	Serum	83	0.994	4.74	0.999	17.5-1285	8.18-1300
	Li Heparin Plasma	110	0.951	2.75	0.995	19.3-1295	
	Urine	136	1.029	21.65	0.998	79-2572	19.2-2600
alpha-AMYLASE EPS (U/L)	Serum	136	1.075	0.04	0.997	13.9-1287	14.3-1300
	Li Heparin Plasma	111	1.109	-5.317	0.999	19.3-1217	
	Urine	136	0.932	2.77	0.993	86.7-2363	19.1-2600
alpha-AMYLASE PANCREATIC (U/L)	Serum	110	1.000	-1.20	0.999	12.0-1289	8.18-1300
	Li Heparin Plasma	115	1.011	-3.176	0.998	12.0-1283	
	Urine	81	1.063	-1.30	1.000	12.0-2410	19.6-2600
BILIRUBIN DIRECT (mg/dL)	Serum	125	1.005	0.02	0.997	0.33-15.0	0.14-15
	Li Heparin Plasma	142	1.029	0.01	0.997	0.12-14.0	
BILIRUBIN TOTAL (mg/dL)	Serum	108	1.015	0.09	0.997	0.55-35.1	0.32-38
	K_3 EDTA plasma	106	1.02	0.02	0.998	0.28-28.8	

*Concentrations tested based on the results reported by the predicate devices.

Method comparison data supports that serum and lithium heparin plasma are acceptable samples types for the BILIRUBIN DIRECT assay. Studies also support that serum and K_3 EDTA plasma samples are acceptable sample types for the

BILIRUBIN TOTAL assay. The alpha-AMYLASE DIRECT, alpha-AMYLASE EPS, alpha-AMYLASE PANCREATIC assays can be used with serum, lithium heparin plasma and urine specimens.

b. Matrix comparison:

Not applicable. Different sample types were assessed in other analytical studies described above.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

The following reference ranges are cited from the literature:

alpha-AMYLASE DIRECT[1]:
Serum and plasma: 22 - 80 U/L
Urine: <321 U/L

alpha-AMYLASE EPS[2,3]:
Serum and plasma: 28 - 100 U/L
Urine: 16 - 491U/L

alpha-AMYLASE PANCREATIC[2,3]:
Serum and Plasma: 13-53 U/L
Urine: 7 - 356 U/L

BILIRUBIN DIRECT[1,4]:
Adults: 0 - 0.3 mg/dL

BILIRUBIN TOTAL[1]:
Adults: 0 - 2.0 mg/dL

1- Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 5th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2012.

2- IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37ºC. Part 8. Reference procedure for the measurement of catalytic concentration of α-amylase. Clin Chem Lab Med 2006; 44: 1146-1155.

3- Junge W, Werner W, Wilke B et al. Development and evaluation of assays for the determination of total and pancreatic amylase at 37ºC according to the principle recommended by the IFCC. Clin Biochem 2001;34:607-615.

4- McPherson RA, Pincus MR. Henry's Clinical Diagnosis and Management by Laboratory Methods. 20st ed. Saunders Elsevier, 2001:1427.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 809.10.

O. Conclusion:

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