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

A. 510(k) Number:
k062503

B. Purpose for Submission
New device

C. Measurand:
Bile acid cholylglycine

D. Type of Test:
Enzymatic Colorimetric Assay

E. Applicant:
Catachem, Inc.

F. Proprietary and Established Names:
Bile Acids (Liquid) Reagents
Bile Acids Calibrator
Bile Acids Controls

G. Regulatory Information:
1. Regulation Section
   21 CFR §862.1177, Cholylglycine test system
   21 CFR §862. 1150, Calibrator
   21 CFR §862. 1660, Quality Control Material
2. Classification:
   Class II (Reagent, Calibrator)
   Class I Reserved (Control)
3. Product Code:
   KWW; JIS; JJX
4. Panel
   Clinical Chemistry (75)

H. Intended Use:
1. Intended use(s)
   See Indications for Use below.

2. Indication(s) for use:
   Catachem, Inc. Bile Acids assay is an In-Vitro diagnostic enzymatic assay for the quantitative determination of total bile acids in human serum. Measurement of bile acids in human serum, and increased reported levels are representative of specific liver disease.

   Bile Acids Calibrator
   The bile acids assay contains a calibrator designed for the calibration of the Bile Acids method.

Bile Acids Controls

The Bile Acids assay also contains Control Level-I and Control Level-II. These controls are designed to monitor the performance of the Bile Acids assay.

3. Special conditions for use statement(s):
   For Prescription Use only.

4. Special instrument requirements:
   COBAS MIRA analyzer

I. Device Description:

The DiscretPak Liquid Bile Acids consists of:

1. Two reagents - Enzyme Color Reagent containing buffer, pH 7.8, Nitrotetrazolium blue 0.6 mmol/L, 3-α-hydroxysteroid dehydrogenase ≥800 units/L, Diaphorase ≥5000 units/L and preservatives and stabilizer. The Bile Acids Activator Reagent is an aqueous solution, pH ≤ 3.5 with preservative and stabilizer.
2. Calibrator and Control Level-I and Control Level-II are bovine serum based and contain pure bile acid with preservative and stabilizer.

J. SUBSTANTIAL EQUIVALENCE INFORMATION

1. Predicate device name(s):
   Bile Acids Reagents
2. Predicate 510(k) number(s):
   k872296
3. Comparison with predicate:
4.

Similarities		
Item	**Device**	**Predicate**
Intended Use	For the quantitative enzymatic determination of bile acids in serum.	For the quantitative enzymatic determination of bile acids in serum.
Method Principle	1. Bile acids are oxidized to 3-Keto hydroxyl bile acids by the enzyme 3-α-hydroxysteroid dehydrogenase with reduction of NAD to NADH. 2. NADH is oxidized to NAD by the action of the enzyme diaphorase where NBT dye is reduced to form a formazan dye with maximum absorbance at 540 nm. The intensity of the color produced is proportional to the concentration of the bile acids in the serum sample.	1. Bile acids are oxidized to 3-Keto hydroxyl bile acids by the enzyme 3-α-hydroxysteroid dehydrogenase with reduction of NAD to NADH. 2. NADH is oxidized to NAD by the action of the enzyme diaphorase where NBT dye is reduced to form a formazan dye with maximum absorbance at 540 nm. The intensity of the color produced is proportional to the concentration of the bile acids in the serum sample.
Applications	Manual and automated.	Manual and automated.
Calibration	Bile acids concentration in the serum sample is calculated using a calibrator.	Bile acids concentration in the serum sample is calculated using a calibrator.

Similarities		
Item	**Device**	**Predicate**
Quality Control	The reliability of test results is monitored by routine use of bile acids control sera.	The reliability of test results is monitored by routine use of bile acids control sera.
Expected Values	0-8.0 µmol/L	0-8.0 µmol/L
Analytical Range	1.0-200 µmol/L	1.9-200 µmol/L
Linear Range	Bile acids method linear to 200 µmol/L	Bile acids method linear to 200 µmol/L

Differences		
Item	**Device**	**Predicate**
Reagents	Two liquid reagents, ready for use.	Two lyophilized reagents, requires preparation
Working Reagent Stability	60 days at 2-8 °C	One week at 2-8 °C
Manual Method Serum Sample Dilution	0.05 ml serum sample plus 0.5 ml reagent for a total sample dilution of 1:11	0.2 ml serum sample plus 0.5 ml reagent for a total sample dilution of 1:3.5
Method Sensitivity	0.000958 O.D. / µmol/L, automated-method, COBAS MIRA analyzer, 1:13 sample dilution.	0.000665 O.D. / µmol/L, automated- method, COBAS MIRA analyzer, 1:13 sample dilution.
Interference Substances	Ascorbic acid ≤50 mg/dl Bilirubin ≤1.7 mg/dl Hemoglobin ≤300 mg/dl Lipemia (triglycerides) ≤1000 mg/dl	Ascorbic acid Not reported Bilirubin Not reported Hemoglobin Not reported triglycerides Not reported

K. STANDARD/GUIDANCE DOCUMENT REFERENCED (IF APPLICABLE)

CLSI EP05-A2: Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline-Second Edition.

CLSI EP06-A: Evaluation of the linearity of Quantitative Analytical Methods; Approved Guideline.

CLSI EP07-A: Interference Testing in Clinical Chemistry; Proposed Guideline.

CLSI EP09-A2: Method Comparison and bias Estimation Using Patient Samples; Approved Guideline-Second Edition.

CLSI EP17-A: Protocol for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

L. Test Principle

In this Bile Acids procedure, 3α-hydroxy Bile Acids are converted to corresponding 3-keto hydroxy Bile Acids by the action of the enzyme 3α-hydroxysteroid dehydrogenase (3∝-HSDH) with concomitant reduction of NAD+ to NADH. The NADH thus produced is subsequently oxidized to NAD+ in a diaphorase-catalyzed reaction where nitrotetrazolium

blue (NBT) is reduced to form a formazan dye, which has an absorption maximum at 540 nm. The intensity of the color produced is directly proportional to the concentration of Bile Acids in the serum sample.

M. Performance Characteristics (if/when applicable):

1. Analytical Performance:

a. Precision /Reproducibility:

Serum samples prepared in-house were spiked with bile acids to obtain four different concentrations in µmol/L. These samples were assayed on the analyzer COBAS MIRA PLUS for a period of seven days 3 times per day. Results were tabulated and the statistics were worked out to arrive at the results claimed in the device.

SAMPLE TYPE	BILE ACIDS	WITHIN-RUN		DAY-DAY	
	MEAN	SD	CV	SD	CV
	µmol/L	µmol/L	%	µmol/L	%
SERUM	6.0	0.23	3.85	0.26	4.51
SERUM	25	0.77	3.0	1.23	5.01
SERUM	150	2.84	1.86	7.30	5.08

b. Linearity/ Assay reportable range.

The measuring range of this device is 1 – 200 µmol/L. Linearity was evaluated following the recommendations of CLSI EP6-A on an automated analyzer.

Two serum samples were prepared with 99% pure bile acids (cholic acid) to obtain a lower concentration and a high concentration. Seven additional serum samples were prepared by combining the low (2.1µmol/L) and the high (228.5µmol/L) serum samples. A total of 9 serum dilutions and concentrations spanning the desired mesauring range (2-200µmol/L) were assayed in random order in replicates of four. The resulting linear regression statistics were: Observed = 1.0037(Expected) – 1.6699.

Device Bile Acids Linearity-Serum	
EXPECTED VALUES	OBSERVED VALUES
2.1	2.10
30.1	29.80
58.7	55.55
87.0	85.15
115.3	112.70
146.6	143.20
171.9	171.40
200.2	200.85
228.5	228.50

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

Projected and real life stability studies of the bile acid reagents were conducted. Accelerated stability testing of the bile acids reagents were done at 30 °C and 37 °C to project the shelf life of reagents. Real life stability tests are in progress at 2-8 °C. Based on

the accelerated experiments the sponsor projects reagents to be stable for 12 months. These stability experiments were done using reagent formulations packaged ready for use by the consumer. Specific concentrations of the essential components in the reagent formulation are disclosed in the bile acids package insert.

The device bile acids require a Calibrator to compute the bile acids concentration of the unknown serum samples. The device bile acids also require a Control Level-I and Level-II to monitor the quality of the assay. These three required components are value-assigned by performing multiple determinations using an automated analyzer. The Calibrator and Control Level I and Level II are traceable to an in-house reference master lot which was cross referenced with the predicate bile acids assay calibrator and assessed for agreement. The device bile acids calibrator has a nominal value of 100 µmol/L and the controls level I and II have nominal values of 50 and 150 µmol/L respectively.

d. Detection Limit

Limits of Quantitation (LoQ) and Limits of Detection (LoD)
The lower limit of detection and quantitation was established following the guidelines of CLSI (Formerly NCCLS) EP17-A. The criterion for limits of quantitation is the concentration of the analyte which has imprecision ≤ 20% cv.

The lower limit of sensitivity of the device bile acids was determined based on the LoQ. To establish the LoQ, serum bile acids assays were done in between zero-baseline and the established linear low (2 µmol/L). Three runs were conducted on an automated analyzer, using replicates of 20 in each case. The assays revealed a LoQ value of 1.7 µmol/L. The LoD was determined to be 0.9 µmol/L.

Device Bile Acids Limits of Quantitation and Detection-Serum Samples	
LoD Results	**LoQ Results**
0.001 OD, 0.9 µmol/L	1.7 µmol/L, % CV 20.0

e. Analytical Specificity
Interference studies were conducted following the guidelines of CLSI EP07-A.

Potential substances which may affect the accuracy of the device were evaluated using serum samples. The study included ascorbic acid, bilirubin, hemoglobin and lipemia (triglycerides). The level of interference was considered acceptable if there was no more than ± 10% difference between the iterferant result and the reference result.

Interfering Substance	**Concentration mg/dL**
Ascorbic Acid	≤ 50 mg/dL
Bilirubin	≤ 1.7 mg/dL
Hemoglobin	≤ 300 mg/dL
Lipemia(Triglycerides)	≤ 1000 mg/dL

f. Assay Cut-off

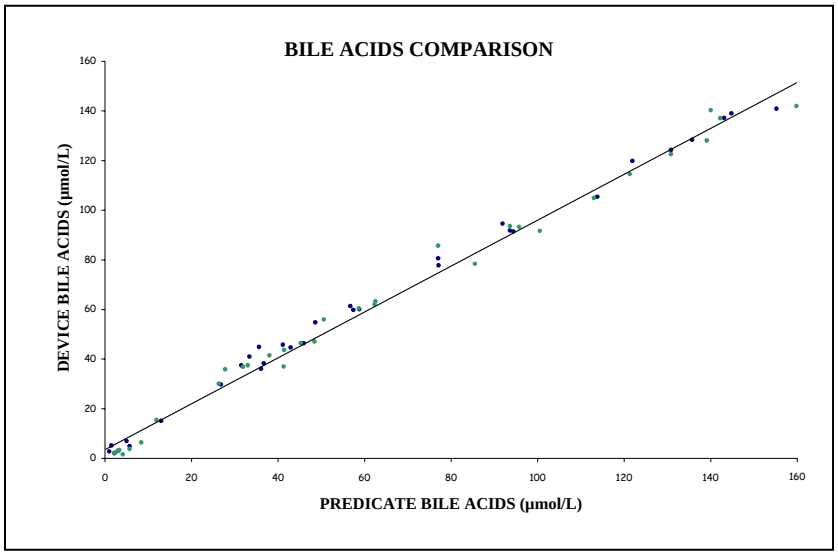
Not applicable

2. Comparison Studies:

a. Method comparison with predicate device:

Method comparison studies were performed following the guidelines of CLSI EP09-A2 on an automated analyzer.

A correlation of the device bile acids with the predicate was conducted. Serum samples (n=36) obtained from a local hospital and 30 spiked serum samples (> 60 µmol/L) prepared in-house were used in the comparison study. The total number of samples was 66 and the observed analytical range was 1.0-159.8µmol/L.



BILE ACIDS COMPARISON REGRESSION ANALYSIS

N	= 66	
Range	= 1.0-159.8	µmol/L.
Mean of X	= 50.95	µmol/L.
Mean of Y	= 48.50	µmol/L.
Y	= 0.924X + 4.3	
R	= 0.9958	
Sy.x	= 4.079	

b. Matrix comparison

Not applicable.

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 Ranges:

Expected Values 0.0-8.0 µmoles/L

(1. Mashige F, Osuga T, Tanka N, Yamanaka M: Continuous –flow determination of bile acids in serum and its clinical application. Clin Chem 24: 1150, 1978. & 2. Mashige F, Tanaka N, Maki A, Kamei S, Yamanaka M: Direct spectrophotometry of total bile acids in serum, Clin Chem 27:1352, 1981.)

N. Proposed Labeling:

The labeling is sufficient to and satisfies the requirements of 21 CFR Part 809.10

O. Conclusion:

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