

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE**

A. 510(k) Number:

K032027

B. Analyte:

Aspartate Aminotransferase (AST)

C. Type of Test:

Enzymatic method test cassette, for the quantitative determination of aspartate aminotransferase in serum or whole blood

D. Applicant:

Cholestech Corporation

E. Proprietary and Established Names:

Cholestech LDX™ AST Test

F. Regulatory Information:

1. Regulation section:
21 CFR § 862.1100, Aspartate amino transferase (AST/SGOT) test system
2. Classification:
Class II
3. Product Code:
CIS - Hydrazone colorimetry, AST/SGOT
4. Panel:
Clinical Chemistry (75)

G. Intended Use:

1. Indication(s) for use:
The Cholestech LDX aspartate aminotransferase (AST) test is for the *in vitro* quantitative determination of AST in whole blood or serum on the Cholestech LDX Analyzer.
AST measurements are used in the diagnosis and treatment of certain liver diseases (e.g., viral hepatitis and cirrhosis), and heart disease.
For *in vitro* diagnostic use only.
2. Special condition for use statement(s):
For professional use and point-of-care.
3. Special instrument Requirements:
For professional use with the Cholestech LDX Analyzer.

H. Device Description:

The Cholestech LDX AST cassette is designed to measure aspartate aminotransferase (AST) activity using the Cholestech LDX Analyzer (K901900). Whole blood (venous or fingerstick) or serum is applied to the cassette where cells are filtered out and plasma can enter the reaction center of the cassette. On the reaction pad, a combination of enzymatic methodology and solid-phase technology result in a color reaction that can be measured by reflectance photometry.

I. Substantial Equivalence Information:

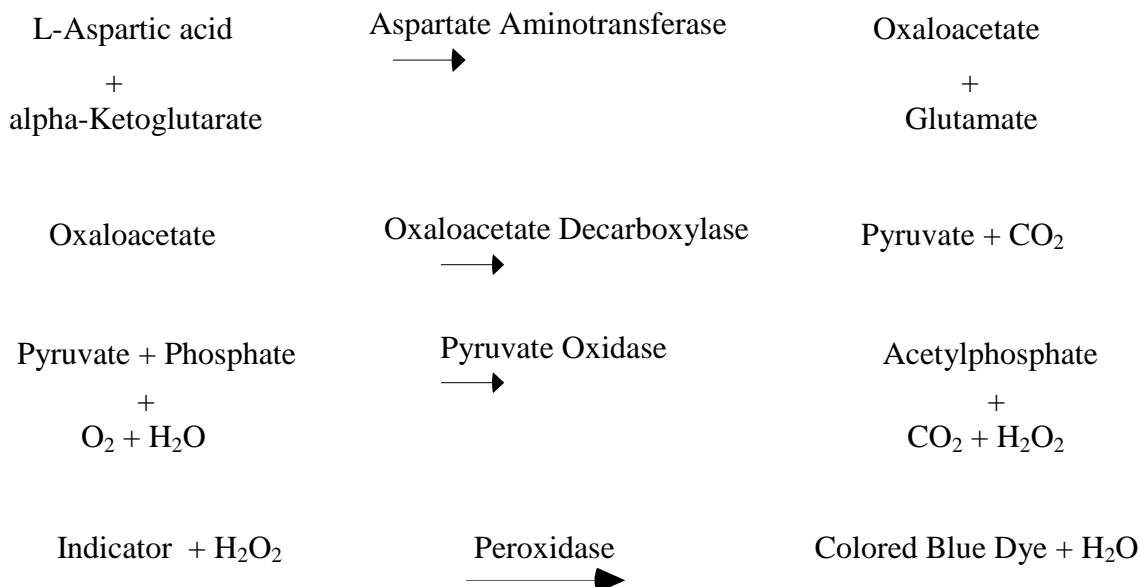
1. Predicate device name(s):
Synchron CX® AST
2. Predicate K number(s):
K952427
3. Comparison with predicate:

| Similarities | | |
|--------------------------|--|---|
| Item | Device | Predicate |
| Technology | Enzymatic methodology | Enzymatic rate method |
| Assay Range | 10-400 U/L | 5-400 U/L |
| Testing Environment | Professional, Point-of-Care | Professional Use, conventional laboratory |
| Differences | | |
| Item | Device | Predicate |
| Instrument required | LDX Analyzer | Synchron CX Clinical System |
| Sample Type | Whole blood (capillary and venous) and serum | Serum or plasma |
| Calibration Requirements | No calibration performed by the user; test information is encoded on the magnetic stripe of the cassette, and the stripe is read by the LDX Analyzer each time a cassette is run | Calibration required via the use of the Synchron Enzyme Validator Set; under typical operating conditions, the AST reagent cartridge must be calibrated every 5 days, and also with certain parts replacements or maintenance procedures. |
| Assay Indicator | Enzymatic production of hydrogen peroxide, which reacts with an indicator _{red} and horseradish peroxidase to produce an indicator _{ox} (blue dye) | Reduction of oxaloacetate to malate by malate dehydrogenase with the concurrent oxidation of NADH to NAD (the indicator) |

J. Standard/Guidance Document referenced (if applicable):**K. Test Principle:**

The Cholestech LDX System combines enzymatic methodology and solid-phase technology to measure aspartate aminotransferase (AST) activity. Samples used for testing can be whole blood from a fingerstick (collected in a lithium heparin coated capillary tube), venous whole blood or serum. The sample is applied to a Cholestech LDX AST cassette. The cassette is then placed into the Cholestech LDX Analyzer where a system on the cassette separates the plasma from the cells. The plasma flows to both sides of the cassette and is transferred to the AST reaction pad. The Cholestech LDX Analyzer then measures AST by an enzymatic method in which aspartic acid

aminotransferase catalyzes the transfer of amino groups from L-Aspartic acid to alpha-Ketoglutarate producing oxaloacetate and glutamate. Oxaloacetate Decarboxylase converts the Oxaloacetate to Pyruvate by the removal of CO₂. Pyruvate oxidase, in the presence of oxygen, oxidizes the pyruvate to acetylphosphate and hydrogen peroxide. In a reaction involving hydrogen peroxide and catalyzed by horseradish peroxidase, an indicator dye (2-(3,5- di-tert-butyl-4-hydroxyphenyl)-4,5-bis(4-dimethylaminophenyl)imidazole) is oxidized to form a blue color at a rate proportional to the AST concentration of the sample. The resultant color in the reaction is measured by reflectance photometry.



L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

To establish within run and total precision, two replicates of each control level (low with a mean value of 31 U/L, and high with a mean value of 106 U/L) and on a high frozen serum sample were assayed twice a day for 20 days for a total of n=4 per day and n=80 over the course of the precision study at each level. The values are summarized below:

| | Within-Run | | | Total Precision | | |
|-----------------------------------|------------|------|------|-----------------|------|------|
| | Mean (U/L) | SD | %CV | Mean (U/L) | SD | %CV |
| Commercial Control Level 1 (Low) | 31 | 1.9 | 6.1% | 31 | 2.7 | 8.8% |
| Commercial Control Level 2 (High) | 106 | 3.8 | 3.5% | 106 | 4.7 | 4.4% |
| Frozen serum sample (V. High) | 277 | 10.6 | 3.8% | 277 | 14.5 | 5.2% |

Due to the instability of whole blood samples, precision was tested by running 20 replicates of a single sample on 20 analyzers with the following results:

Mean (U/L) 58
SD (U/L) 2.8
%CV 4.8

b. Linearity/assay reportable range:

Two samples were prepared to contain “low” and “high” concentrations of AST, and the activity of those samples was determined in replicates of n=10. Mean results from those measurements were calculated and these became the base solutions for preparing the test solutions below:

Pool 1 - 100% Low
Pool 2 - 75% Low, 25% High
Pool 3 - 50% Low, 50% High
Pool 4 - 25% Low, 75% High
Pool 5 - 100% High

Each pool was quantitated in replicates of n=3 and mean results of each pool were calculated and analyzed using least-squares linear regression. Observed results were plotted vs. calculated results and the results are as presented below.

| | Expected Results (U/L) | Observed Results (U/L) |
|----------------|---------------------------|---------------------------|
| Pool 1 | n/a | 23.3 |
| Pool 2 | 138.2 | 124.5 |
| Pool 3 | 253.1 | 251.0 |
| Pool 4 | 368.0 | 322.0 |
| Pool 5 | n/a | 482.9 |
| Slope | 0.9719 | |
| Intercept | -5.245 | |
| R ² | 0.9886 | |

See also method comparison, below.

c. Traceability (controls, calibrators, or method):

A brown magnetic stripe on each cassette contains the calibration information required for the Cholestech LDX Analyzer to convert the reflectance reading to the AST concentration in U/L, 37°C for both serum and whole blood. This magnetic strip contains coefficients that define the dose response curve of the assay allowing the conversion of the signal to an AST dose without the customer needing to run calibrators. The coefficients are specific for each lot of cassettes. Cholestech claims that most customers utilize either whole blood or fingerstick samples, so the coefficients are set to accurately quantitate whole blood. If needed, there is also a serum correction factor on the cassette that corrects for any differences between

serum and whole blood for customers that choose to run serum. In addition, the Lot expiration date and the timing sequence specific for the product are encoded on the magnetic strip.

d. Detection limit:

Cholestech claims a 10-400 U/L range of detection.

The 10 U/L assay range lower limit is based on Cholestech's precision acceptance criteria for low-activity samples. Using a 3 U/L S.D. criterion, a low end cutoff value of 10 was determined as the lowest activity accepted. All activity measurements below 10 are reported as "<10 U/L" on the instrument readout.

The applicant bases the 400 U/L limit for the upper limit on the fact that the change in reflectance readings becomes less pronounced as the AST concentration increases. The precision and accuracy of the test diminishes to an unacceptable degree above 400 U/L.

e. Analytical specificity:

To determine the effect of potentially interfering substances, each of two samples (with low and high [AST]) was spiked with a concentrated solution of the potentially interfering substance and AST activity was measured. Percent recovery was determined using non-spiked samples as a baseline. The table below shows the concentrations of the potentially interfering substances tested. Tests containing all substances resulted in $\geq 95\%$ recovery. The impact of triglycerides was evaluated by comparing the results of the LDX AST test to the IFCC reference AST assay. The difference in measured AST activity between the two methods was plotted vs. the triglyceride concentration (0- 500 mg/dL).

The substances listed below were tested for interference with the AST test. Less than 10% interference was seen at the levels shown.

Substance Concentration (mg/dL)

| | | | |
|------------------|-----|-------------------------|------|
| Hemoglobin | 75 | Gemfibrozil (Lopid) | 15 |
| Bilirubin | 5 | Oxytetracycline | 4 |
| Ditaurobilirubin | 5 | Probucol (Lorelco) | 32.5 |
| Ascorbic Acid | 1 | Nicotinic Acid (Niacin) | 10 |
| Urea | 500 | Clofibrate (Atromid) | 80 |
| Uric Acid | 15 | Lovastatin (Mevacor) | 4 |
| Creatinine | 30 | L-Dopa (Levodopa) | 0.2 |
| Glutathione | 1 | Cimetidine (Tagamet) | 5 |
| Lactate | 100 | Nitrofurantoin | 1 |
| Fructose | 30 | Gentisic Acid | 0.5 |
| Lactose | 100 | Methyl dopa | 0.2 |
| Cysteine* | 7 | Sulfamethoxazole | 16 |

| | | | |
|---------------|------|-------------------------|-----|
| Glucose | 1200 | Pravastatin (Pravachol) | 0.8 |
| Pyruvic acid | 0.2 | Simvastatin (Zocor) | 4.0 |
| Triglycerides | 450 | Fluvastatin (Lescol) | 4.0 |
| Glucose | 1200 | | |

*Note: 7 mg/dL Cysteine was determined from linear regression of multiple runs.

Hematocrit interference was tested by obtaining a venous whole blood and plasma samples from two single individuals. The plasma sample was mixed with the whole blood to produce samples with varying hematocrits. Each sample was analyzed for AST activity in replicates of 4. Hematocrits up to 50% do not affect AST activity results.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device: (reference method)

AST concentrations of 109 serum samples were determined using the LDX AST test cassette and the IFCC reference method. Using unweighted Deming regression analysis, the following result was obtained:

| vs. IFCC reference method | Number of Pairs | Slope | y Intercept | Correlation coefficient | Range of Values |
|--|--------------------|-------|-------------|----------------------------|--------------------|
| serum | 109 | 0.97 | 1.6 | 0.983 | 12-396 |

b. Matrix comparison:

AST concentrations of 46 whole blood and 21 fingerstick samples were determined using the LDX AST test cassette and plotted versus the corresponding serum measurements of AST concentration. The results are as follows:

| Sample Type (vs. serum) | Number of Pairs | Slope | y Intercept | Correlation coefficient | Range of Values |
|------------------------------------|--------------------|-------------------------|-----------------------|----------------------------|--------------------|
| venous whole blood | 46 | 1.08 | 0.3 | 0.998 | 13-343 |
| fingerstick | 21 | .86 (0.625 to 1.094) | 4.4 (-1.5 to 10.3) | 0.934 | 13-65 |

(see fingerstick comparison data below)

| Sample Comparison - LDX (U/L) | |
|-------------------------------|-------------|
| Serum | Fingerstick |
| 18 | 17 |
| 17 | 16 |
| 30 | 30 |
| 20 | 24 |
| 26 | 25 |
| 22 | 26 |
| 35 | 31 |
| 24 | 15 |
| 54 | 45 |
| 22 | 25 |
| 13 | 17 |
| 28 | 33 |
| 16 | 21 |
| 22 | 19 |
| 29 | 28 |
| 20 | 22 |
| 19 | 22 |
| 15 | 22 |
| 22 | 30 |
| 65 | 63 |
| 31 | 33 |

3. Clinical studies:

a. Clinical sensitivity:

Not applicable.

b. Clinical specificity:

Not applicable. No outside clinical investigators were used in these studies. Fingerstick samples were collected at the sponsor's facility and tested on site. See matrix comparison above for fingerstick accuracy data.

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

The sponsor suggests that fingerstick samples be applied to the cassette immediately after collection, and that the following protocol for obtaining fingerstick samples be used:

Fingerstick Procedure

1. The patient should sit quietly for five minutes before the blood sample is collected.
2. Put a capillary plunger into the end of a Cholestech capillary tube with the red mark. Set aside.

3. Choose a spot that is on the side of one of the center fingers of either hand. The fingers and hands should be warm to the touch. To warm the hand, you can:
 - a. Wash the patient's hand with warm water, or...
 - b. Apply a warm (not hot) compress to the hand for several minutes, or...
 - c. Gently massage the finger from the base to the tip several times to bring the blood to the fingertip.
4. Clean the site with an alcohol swab. Dry thoroughly before pricking the finger.
5. Firmly prick the selected site with a lancet.
6. Squeeze the finger gently to obtain a large drop of blood. Wipe away this first drop of blood as it may contain tissue fluid.
7. Squeeze the finger gently again while holding it downward until a second large drop of blood forms. Do not milk the finger. The puncture should provide a free-flowing drop of blood.
8. Hold the capillary tube horizontally by the end with the plunger. Touch the end of the tube to the drop of blood without touching the skin. The tube will fill by capillary action to the black mark. Do not collect air bubbles. If it is necessary to collect another drop of blood, massage the finger again from base to tip.

Note: A warm hand and good blood flow from the puncture site are essential in order to collect a good capillary sample. Fill the capillary tube in under 10 seconds.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

Reference interval, 37°C 10-30 U/L
 (sponsor references Burtis CA, Ashwood ER (Ed), Tietz Textbook of Clinical Chemistry, Third Edition, W.B. Saunders Co., Philadelphia, PA, (1999).)

M. Conclusion:

I recommend that the Cholestech LDX™ AST Test is substantially equivalent to the legally marketed predicate device.