

Cholestech
LDX[®]

ALT (GPT)

Alanine Aminotransferase Test Cassette

CLIA-WAIVED—This test is waived under CLIA '88 regulations. If a laboratory modifies the test system instructions, then the test is considered highly complex and subject to all CLIA requirements.

IVD For professional *in vitro* diagnostic use.

Catalog No. 11-772

Indications For Use:

The Cholestech LDX alanine aminotransferase test is for the *in vitro* quantitative determination of alanine aminotransferase (ALT) in whole blood on the Cholestech LDX Analyzer.

Introduction

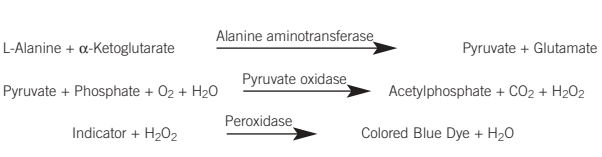
Alanine aminotransferase is an enzyme that catalyzes the conversion of alanine to pyruvate and is found in muscle, the liver and other tissues. ALT is most prevalent in the liver. ALT levels are a reflection of alterations in liver function and therefore are a valuable measurement of damage to the liver. Liver damage may be due to chronic alcohol or drug ingestion, or infection. There are a number of lipid-lowering drugs available to treat hyperlipidemia. A side effect of such therapy can be a persistent increase in serum ALT (to more than 3 times the upper limit of normal) in about 1% of patients receiving lipid-lowering therapy. It is suggested that patients undergoing lipid-lowering drug therapy should be tested for ALT before (baseline) and shortly after initiation of therapy and then periodically thereafter to determine the ALT levels.

Now alanine aminotransferase can be measured from a single drop of blood using rapid, accurate technology.

Test Principle

The Cholestech LDX System combines enzymatic methodology and solid-phase technology to measure ALT. Samples used for testing can be whole blood from a fingerstick (collected in a lithium heparin-coated capillary tube) or venipuncture. The sample is applied to a Cholestech LDX ALT Cassette. The cassette is then placed into the Cholestech LDX Analyzer, where a unique system on the cassette separates the plasma from the blood cells. The plasma flows to both sides of the cassette and is transferred to the ALT reaction pad.

The Cholestech LDX Analyzer measures alanine aminotransferase by an enzymatic method based on the method formulation of Katsuyama et al.^{1, 2} Alanine aminotransferase catalyzes the transfer of amino groups from L-Alanine to α-Ketoglutarate, producing pyruvate and glutamate. Pyruvate oxidase, in the presence of oxygen, oxidizes the pyruvate to acetylphosphate and hydrogen peroxide. In a reaction catalyzed by horseradish peroxidase, the peroxide reacts with an indicator dye to form a blue color at a rate proportional to the ALT concentration of the sample. The resultant color in the reaction is measured by reflectance photometry.



A brown magnetic stripe on each cassette contains the calibration information required for the Cholestech LDX Analyzer to convert the reflectance reading to the ALT concentration in U/L, 99°F / 37°C.

Alanine Aminotransferase Cassette

Each ALT cassette contains a minimum of:

L-Alanine, µg	ALT
α-Ketoglutaric Acid, µg	91.9
Sodium Phosphate Monobasic, µg	6.4
Pyruvate Oxidase (Aerococcus viridans), U	9.6
Peroxidase (horseradish), U	2.4
2-(3,5 di-tert-butyl-4-hydroxyphenyl)-4,5-bis(4-dimethylaminophenyl)imidazole, µg	3.6
	6.1

Nonreactive ingredients: Buffers and stabilizers

Cassette Storage and Stability

Cassettes **must** be stored in the sealed foil pouches.

Cassettes may be used until the date printed on the pouch when stored in a refrigerator (36–46°F / 2–8°C). Or the cassettes may be stored for up to 30 days at room temperature (less than 86°F / 30°C).

Once the cassettes have been stored at room temperature, they should not be returned to the refrigerator.

- Do not use a cassette beyond the printed expiration date.
- Do not use a cassette that has been stored at room temperature for more than 30 days.
- Do not reuse cassettes.

Cassette Handling

Cassettes should sit at room temperature for 10 minutes before opening the pouch. Use the cassette as soon as the pouch is opened.

Sample Type

Please Note:

The Cholestech LDX System is CLIA-waived for fingerstick or venous whole blood unprocessed samples only. If you run serum or plasma on the Cholestech LDX, you will be classified as moderately complex and will have to comply with the regulations for moderate complexity. See the Cholestech LDX System User Manual for a summary of these regulations.

Sample Requirement

- Sample Volume: 35 µL of whole blood.

Fingerstick whole blood:

- Collect the sample from a fingerstick into a Cholestech LDX Capillary Tube. (See the Fingerstick Procedure below.)
- Place the blood into the cassette *immediately* after collection.
- Blood from the fingerstick should flow freely. Too much squeezing of the finger may produce inaccurate results.

Venous whole blood:

- Collect blood into a green-top tube (lithium heparin anticoagulant).

NOTE: Do not use a tube with any other additives because it may cause inaccurate results.

- Whole blood should be used within 30 minutes. The blood sample may be taken directly from the tube after mixing.
- Use a 35 µL MiniPet™ Pipette and tips, or a micropipetter that is calibrated to deliver 35 µL, to place whole blood into the cassette.
- Samples should be at room temperature before testing.
- Mix all samples by inverting gently 7–8 times before testing.
- Do not use the Cholestech LDX Capillary Tubes for measuring venous whole blood.

PRECAUTION: All blood samples, containers, capillary tubes and materials that have come in contact with blood should be handled as if capable of transmitting infectious disease and discarded into a biohazard waste container after use.

Calibration

No calibration is performed by the user. Test information is encoded on the brown stripe of the cassette. The brown stripe is read by the Cholestech LDX each time a cassette is run.

An optics check should be run on the Analyzer each day that patient samples are tested. See the Cholestech LDX System User Manual for instructions.

TEST PROCEDURE

Materials Provided:

ALT cassettes

Additional Materials Required:

- Cholestech LDX Analyzer and power supply
- Alcohol swabs and gauze for cleaning puncture site
- Lancets for capillary blood collection
- Cholestech LDX Capillary Tubes (with lithium heparin anticoagulant)
- Cholestech LDX Capillary Plungers
- Gloves
- Biohazard waste containers
- Quality control material
- 35 µL MiniPet Pipette and tips or micropipetter that will deliver 35 µL for use with venipuncture samples and quality control material
- Vacuum collection tubes, needles, tube holders and sample tubes if the sample is to be collected by venipuncture

Running a Test

- Remove the cassette pouch from the refrigerator.
- Let the cassette pouch sit at room temperature for 10 minutes.
- Remove the cassette from its pouch. Hold the cassette by the short sides only. Do not touch the black bar or the brown stripe. Place the cassette on a flat surface.

NOTE: Gloves should be worn whenever working with blood and quality control samples.

- Press **RUN**. In a few seconds the screen will read:

```
Selftest
running.
```

```
Selftest OK
```

- The cassette drawer will open. The screen will read:

```
Load cassette
and press RUN
```

- Place the sample into the cassette well. Use a Cholestech LDX Capillary Tube for fingerstick samples. Use the 35 µL MiniPet Pipette for other samples.

NOTE: Fingerstick samples must be applied *immediately* after collection or the blood may clot.

An optics check should be run on the Analyzer each day that patient samples are tested. See the Cholestech LDX System User Manual for instructions.

- Keep the cassette flat after the sample has been applied. Place the cassette into the drawer of the Analyzer *immediately*. The black bar must face the Analyzer. The brown stripe must be on the right.

- Press **RUN**. The drawer will close. During the test the screen will read:

```
ALT
Running***
```

- Put everything that touched the blood sample or control in a biohazard waste container.
- When the test is complete, the Analyzer will beep. The screen will read:

```
ALT=###
warnings
```

- When the results are outside the measuring range, the screen will read:

```
ALT < 10 or
ALT > 400
```

- If there is a problem with the test, a message will appear on the screen. See the *Troubleshooting & Maintenance* section of the Cholestech LDX System User Manual if this happens.

Please call Cholestech Technical Service at 800-733-0404 if you have any questions about the operation of the Cholestech LDX System.

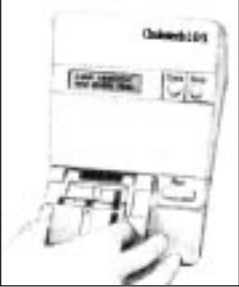
- When the drawer opens, remove the cassette. Put it in a biohazard waste container. Leave the Analyzer drawer empty when not in use.
- Record the results.
- To run another cassette, press **RUN**. The screen will read:

```
Load cassette
and press RUN
```

- Repeat the test procedure.
- Otherwise, after 4 minutes a beep will sound and the screen will read:

```
System timeout
RUN to continue
```

If the **RUN** button is not pushed within 15 seconds, the drawer will close. Then the screen will go blank.



Fingerstick Procedure

- The patient should sit quietly for 5 minutes before the blood sample is collected.
- Put a capillary plunger into the end of a Cholestech LDX Capillary Tube with the red mark. Set aside.
- 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:
 - Wash the patient's hand with warm water, or...
 - Apply a warm (not hot) compress to the hand for several minutes, or...
 - Gently massage the finger from the base to the tip several times to bring to blood to the fingertip.
- Clean the site with an alcohol swab. Dry thoroughly **before pricking the finger**.
- Firmly prick the selected site with a lancet.
- Squeeze the finger gently to obtain a large drop of blood. Wipe away this first drop of blood, as it may contain tissue fluid.
- 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.
- 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.

Using the MiniPet Pipette

Use this procedure to apply a venous sample or a control sample to the cassette. Any pipette that can deliver 35 µL may be used.

- Firmly attach the pipette tip to the end of the 35 µL MiniPet Pipette.
- To fill the pipette, push the plunger down as far as you can. Place the pipette tip into the sample and slowly release the plunger. Do not allow air to be drawn into the tip.
- Dispense the sample into the cassette sample well by pressing the plunger again. Move the pipette tip out of the sample well before releasing the plunger.
- Remove the pipette tip and throw it away in a biohazard waste container.

IMPORTANT: If the plunger is released before the pipette tip is out of the sample well, it will remove the sample just dispensed.

IMPORTANT: Keep the cassette horizontal at all times after applying the sample.

QUALITY CONTROL

Quality control should be run routinely to show that your system is giving accurate results. We recommend the following quality control procedures for the Cholestech LDX System.

Choice of Materials

A high and a low control is preferred. Cholestech-recommended controls work well with the Cholestech LDX System. If you use other controls, you will need to set ranges for the Cholestech LDX System.

Handling

- Follow the instructions that come with your controls.
- Check the expiration date before use. Do not use if expired.
- See “Running a Test” above for the procedure.

External Quality Control

External controls must also be used to demonstrate that the reagents and assay procedure perform properly.

Liquid ALT Level 1 and Level 2 Controls are available from Cholestech. Controls must be tested:

- With each new shipment of cassettes (even if cassettes are from the same lot previously received).
- With each new lot of cassettes.
- As otherwise required by your laboratory’s standard quality control procedures.
- If you are not running the Cholestech LDX under CLIA-waived status, or if your local or state regulations require more frequent testing of quality control material, then quality control must be performed in compliance with those regulations.

Good Laboratory Practice principles suggest that external controls must be run whenever the laboratory director has any question about test system integrity or operator technique (e.g., when reagents may have been stored or handled in a way that can degrade their performance or when operators have not performed a particular test in recent weeks).

If the controls do not perform as expected, repeat the test or contact Cholestech Technical Service before testing patient samples.

The quality control results should be in range before testing patient samples. See the Cholestech LDX System User Manual if they are not. Please call Cholestech Technical Service at 800-733-0404 if you have any questions about quality control.

RESULTS

ALT test results will show on the screen when the test is complete.

LIMITATIONS

- The measuring range of ALT is 10–400 U/L, 99°F / 37°C. Results outside this range will appear as <10 U/L or >400 U/L.
- Samples with ALT outside the measuring range should be sent to a laboratory for testing.
- Performance of the Cholestech LDX System has not been tested on samples from newborns or pediatric patients.
- Hemolysis, breakdown of the red blood cells, should be avoided as this will increase the level of ALT and cause inaccurate results.

Some substances may cause false results with enzymatic tests. The substances listed below were tested for interference with the ALT test. Less than 10% interference was seen at the levels shown.

Substance Concentration (mg/dL)			
Hemoglobin	75	Gemfibrozil	15
L-Dopa	0.2	Bilirubin	5
Ascorbic Acid	1	Probucol	32.5
Urea	500	Nicotinic Acid	10
Fructose	30	Clofibrate	80
Uric Acid	15	Lovastatin	4
Creatinine	30	Dipyrone	10
Glutathione	1	Methotrexate	450
Cimetidine	5	Nitrofurantoin	1
Oxytetracycline	4	Gentisic Acid	0.5
Lactose	100	Methyl Dopa	0.2
Cysteine	10	Sulfamethoxyzole	16
Fluvastatin	4	Pravastatin	0.8
Simvastatin	4	Lactate	100
Triglycerides	450	Ditaurobilirubin	5
Glucose	1200	Pyruvic Acid	0.2

- Hematocrits between 30% and 50% do not affect results.

EXPECTED VALUES

Alanine Aminotransferase:

Reference Interval, 99°F / 37°C 10–40 U/L

Values in men are slightly higher than in women.³

Performance Characteristics

Alanine Aminotransferase:

Precision: study was conducted according to NCCLS protocol EP5-A, Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (1998).³

	Commercial Control Material	Commercial Control Material	Frozen Serum Pool
	<u>Level 1</u>	<u>Level 2</u>	
\bar{X} (U/L) =	31	58	169
Within-Run CV (%) =	3.2%	3.1%	3.4%
Total CV (%) =	5.4%	4.6%	6.5%

	Whole Blood
	<u>Within-Run Precision</u>
\bar{X} (U/L) =	55
SD (U/L) =	2.3
CV (%) =	4.2%

Accuracy (Method Comparison):

The ALT cassette was compared with a validated reference method traceable to the IFCC reference method and with a bench-top point-of-care chemistry analyzer.

Results:

X = Reference Method (serum)

Sample Type, <u>Cholestech LDX</u>	No. of <u>Pairs</u>	<u>Slope</u>	<u>y-intercept</u>	<u>Correlation Coefficient</u>	<u>Range of Values</u>
Venous Whole Blood	53	1.001	−0.3	0.966	10–349
Serum	54	1.007	2.8	0.960	14–391
Fingerstick	24	1.013	3.5	0.930	15–65

Results:

X = Bench-Top Point-of-Care Chemistry Analyzer

Sample Type	No. of Pairs	Slope	y-intercept	Correlation Coefficient	Range of Values
Venous Whole Blood	53	0.916	0.3	0.975	10–349
Serum	52	0.914	0.5	0.971	19–383
Fingerstick	24	0.921	4.3	0.931	15–65

Expected Waiver Performance

A consumer accuracy study was done with 60 lay (untrained) people who reported no medical or laboratory experience. These people were asked to perform one ALT test each by following the directions in the package insert. The samples used for the testing were 60 whole blood samples that were either native samples, or samples spiked with ALT. A total of 3 professional (trained) people also assayed the 60 samples (20 samples per trained person) in order to obtain comparative data between trained and untrained populations. The results were analyzed by Deming regression statistics, as described below.

ALT CONSUMER ACCURACY STUDY (trained [x-axis] vs. untrained [y-axis])					
n	Slope	Slope 95% Confidence Interval	y-intercept	y-intercept 95% Confidence Interval	r
60	0.976	0.934 to 1.019	1.0	−1.4 to 3.3	0.996

References

- Mosbach K, ed. *Methods in Enzymology*. Vol 137. Academic Press, Inc, Harcourt Brace Jovanovich; 1988.
- Bergmeyer HU, Horder M, Rej R. Approved Recommendation (1985) on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes, Part 3. IFCC Method for Alanine Aminotransferase. *J Clin Chem Clin Biochem* 1986; 24:481–95.
- National Committee for Clinical Laboratory Standards. *Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline*. NCCLS; 1998.

IVD	<i>In vitro</i> diagnostic medical device
REF	Catalog number
⚠	Attention. See instructions for use
⊗	Single use
⊗	Do not use if package is damaged or open
LOT	Lot number
🕒	Use by:



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