

HDLC3

HDL-Cholesterol plus 3rd generation

cobas®
Substrates

Order information

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
04399803 190	HDL-Cholesterol plus 3rd generation (200 tests)	System-ID 07 6833 2 COBAS INTEGRA 400 plus COBAS INTEGRA 800
12172623 122	C.f.a.s. Lipids (3 × 1 mL)	System-ID 07 6570 8
12172623 160	C.f.a.s. Lipids (3 × 1 mL, for USA)	System-ID 07 6570 8
10781827 122	Precinorm L (4 × 3 mL)	System-ID 07 9026 5
11778552 122	Precipath HDL/LDL-C (4 × 3 mL)	System-ID 07 9028 1
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	System-ID 07 7469 3
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	System-ID 07 7469 3
05947626 160	PreciControl ClinChem Multi 1 (4 × 5 mL, for USA)	System-ID 07 7469 3
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	System-ID 07 7470 7
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	System-ID 07 7470 7
05947774 160	PreciControl ClinChem Multi 2 (4 × 5 mL, for USA)	System-ID 07 7470 7
20756350 322	NaCl Diluent 9 % (6 × 22 mL)	System-ID 07 5635 0

English

System information

Test HDLC3, test ID 0-331 on COBAS INTEGRA 400 plus systems;
test ID 0-333 on COBAS INTEGRA 800 systems

Intended use

In vitro test for the quantitative determination of HDL-cholesterol concentration in serum and plasma on COBAS INTEGRA systems.

Summary

High density lipoproteins (HDL) are responsible for the reverse transport of cholesterol from the peripheral cells to the liver. Here, cholesterol is transformed to bile acids which are excreted into the intestine via the biliary tract. Monitoring of HDL-cholesterol in serum is of clinical importance since an inverse correlation exists between serum HDL-cholesterol concentrations and the risk of atherosclerotic disease. Elevated HDL-cholesterol concentrations are protective against coronary heart disease, while reduced HDL-cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase the cardiovascular risk.¹ Strategies have emerged to increase the level of HDL-cholesterol to treat cardiovascular disease.^{2,3}

A variety of methods are available to determine HDL-cholesterol, including ultracentrifugation, electrophoresis, HPLC, precipitation-based methods and direct methods. Of these, the direct methods are used routinely. Several approaches for direct measurement of HDL-cholesterol in serum have been proposed, including the use of magnetically responsive particles as polyanion-metal combinations and the use of polyethylene glycol (PEG) with anti-apoprotein B and anti-apoprotein CIII antibodies.

This automated method for direct determination of HDL-cholesterol in serum and plasma uses PEG-modified enzymes and dextran sulfate. When cholesterol esterase and cholesterol oxidase enzymes are modified by PEG, they show selective catalytic activities toward lipoprotein fractions, with the reactivity increasing in the order:
LDL < VLDL ≈ chylomicrons < HDL.^{4,5,6,7,8,9,10,11,12,13,14,15,16}

Non-fasting sample results are slightly lower than fasting results. Comparable non-fasting results were observed with the beta quantification method.^{17,18}

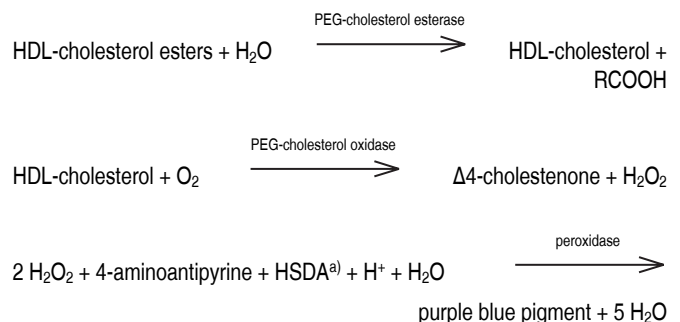
The Roche direct HDL-cholesterol assay meets the 1998 National Institutes of Health (NIH) / National Cholesterol Education Program (NCEP) goals for acceptable performance.¹⁹ The results of this method correlate with those obtained by precipitation-based methods and also by an ultracentrifugation method.

The COBAS INTEGRA HDL-Cholesterol plus 3rd generation **cobas c** pack is designed to the direct specific determination of HDL-cholesterol in the presence of LDL, VLDL, and chylomicrons. No sample pretreatment step is required.

Test principle^{4,5}

Homogeneous enzymatic colorimetric assay

In the presence of magnesium ions and dextran sulfate, water-soluble complexes with LDL, VLDL, and chylomicrons are formed which are resistant to PEG-modified enzymes. The cholesterol concentration of HDL-cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approximately 40 %). Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide.



a) Sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline

The color intensity of the blue quinoneimine dye formed is directly proportional to the HDL-cholesterol concentration. It is determined by measuring the increase in absorbance at 583 nm.

Reagents - working solutions

- R1** HEPES buffer: 10.07 mmol/L; CHES: 96.95 mmol/L, pH 7.4; dextran sulfate: 1.5 g/L; magnesium nitrate hexahydrate: > 11.7 mmol/L; HSDA: 0.96 mmol/L; ascorbate oxidase (Eupenicillium sp., recombinant): > 50 µkat/L; peroxidase (horseradish): > 16.7 µkat/L; preservative
- SR** HEPES buffer: 10.07 mmol/L, pH 7.0; PEG-cholesterol esterase (Pseudomonas spec.): > 3.33 µkat/L; PEG-cholesterol oxidase (Streptomyces sp., recombinant): > 127 µkat/L; peroxidase (horseradish): > 333 µkat/L; 4-amino-antipyrine: 2.46 mmol/L; preservative

R1 is in position B and SR is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: For prescription use only.

HDLC3

HDL-Cholesterol plus 3rd generation

Reagent handling

Ready for use

The intrinsic pink color of the cholesterol reagent does not interfere with the test.

Storage and stability

Shelf life at 2-8 °C See expiration date on
cobas c pack label

COBAS INTEGRA 400 plus system

On-board in use at 10-15 °C 12 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C 12 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum

Plasma: K₃-EDTA; Li-, NH₄⁺- and Na-heparin plasma

EDTA plasma causes decreased results.²⁰ (See note in NCEP guidelines section.)

Fasting and non-fasting samples can be used.¹⁸

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:²¹ 7 days at 2-8 °C
30 days at -70 °C

It is reported that EDTA stabilizes lipoproteins.²²

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

NaCl Diluent 9 %, Cat. No. 20756350322, system-ID 07 5635 0 for automatic postdilution. NaCl Diluent 9 % is placed in its predefined rack position and is stable for 4 weeks on-board COBAS INTEGRA 400 plus/800 analyzers.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Application for serum and plasma

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	583/659 nm
Calc. first/last	33/69
Unit	mmol/L

Pipetting parameters

		Diluent (H ₂ O)
R1	150 µL	
Sample	2.5 µL	7.0 µL
SR	50 µL	

Total volume 209.5 µL

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	583/659 nm
Calc. first/last	44/98
Unit	mmol/L

Pipetting parameters

		Diluent (H ₂ O)
R1	150 µL	
Sample	2.5 µL	7.0 µL
SR	50 µL	
Total volume	209.5 µL	

Calibration

Calibrator	C.f.a.s. Lipids
	Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures

Traceability:²¹ This method has been standardized against the designated CDC reference method (designated comparison method).¹⁹ The standardization meets the requirements of the "HDL Cholesterol Method Evaluation Protocol for Manufacturers" of the US National Reference System for Cholesterol, CRMLN (Cholesterol Reference Method Laboratory Network), November 1994.

Quality control

Reference range	Precinorm L or PreciControl ClinChem Multi 1
Pathological range	Precipath HDL/LDL-C or PreciControl ClinChem Multi 2
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Quality control materials are intended for use only as monitors of accuracy and precision. The Laboratory Standardization Panel (LSP) of the National Cholesterol Education Program in the United States recommends two levels of controls, one in the normal range (0.9-1.7 mmol/L or 35-66 mg/dL) and one near the concentration for decision making (< 0.9 mmol/L or < 35 mg/dL).

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

HDLC3

HDL-Cholesterol plus 3rd generation

Conversion factors: mmol/L \times 38.66 = mg/dL
mg/dL \times 0.0259 = mmol/L

Limitations - interference²³

Criterion: Recovery within \pm 10 % of initial value.

Serum, plasma

Icterus:²⁴ No significant interference up to an I index of 47 for conjugated bilirubin^{b)} and 60 for unconjugated bilirubin^{c)} (approximate conjugated bilirubin concentration: 804 μ mol/L or 47 mg/dL; approximate unconjugated bilirubin concentration: 1026 μ mol/L or 60 mg/dL).

These claims are based on the Glick model. Please refer to further comments below (abnormal liver function).

Hemolysis:²⁴ No significant interference up to an H index of 1200^{d)} (approximate hemoglobin concentration: 745 μ mol/L or 1200 mg/dL).

Lipemia (Intralipid):²⁴ No significant interference up to an L index of 2000^{e)}. No significant interference from native triglycerides up to 1200 mg/dL (13.7 mmol/L). There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

The claim for lipemia interference is based on the Glick model²⁴, which uses Intralipid as an artificial substrate. To date, there is no model available which can mimic interference by triglycerides, as triglyceride levels in patient specimens behave unpredictably, depending on the nature of the esterified fatty acids in the samples. Patient specimens with elevated triglyceride levels are very often lipemic. Therefore customers cannot verify interference by triglycerides in patient specimens.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{25,26}

Elevated concentrations of free fatty acids and denatured proteins may cause falsely elevated HDL-cholesterol results.

Ascorbic acid up to 50 mg/dL (2.84 mmol/L) does not interfere.

Acetaminophen intoxications are frequently treated with N-Acetylcysteine. N-Acetylcysteine at the therapeutic concentration when used as an antidote and the Acetaminophen metabolite N-acetyl-p-benzoquinone imine (NAPQI) independently may cause falsely low results.

Venipuncture should be performed prior to the administration of Metamizole. Venipuncture immediately after or during the administration of Metamizole may lead to falsely low results.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.²⁷

Abnormal liver function affects lipid metabolism; consequently, HDL and LDL results are of limited diagnostic value. In some patients with abnormal liver function, the HDL-C plus 3rd generation results may significantly differ from those obtained using acknowledged reference methods such as ultracentrifugation or the DCM (designated comparison method).

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

b) measured at HDL-C concentrations up to 1.09 mmol/L (42.1 mg/dL)

c) measured at HDL-C concentrations up to 1.14 mmol/L (44.1 mg/dL)

d) measured at HDL-C concentrations up to 1.42 mmol/L (54.9 mg/dL)

e) measured at HDL-C concentrations up to 0.90 mmol/L (34.8 mg/dL)

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

0.08-3.10 mmol/L (3-120 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:4 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 4.¹⁾

Lower limits of measurement

Lower detection limit of the test:
0.08 mmol/L (3 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the zero sample (zero sample + 3 SD, repeatability, n = 21).

f) The use of a dilution factor < 4 is not allowed.

Expected values

	No risk	Moderate risk	High risk
Men ^{28,29,30}			
mg/dL	> 55	35-55	< 35
mmol/L	> 1.45	0.90-1.45	< 0.90
Women ^{28,29,30}			
mg/dL	> 65	45-65	< 45
mmol/L	> 1.68	1.15-1.68	< 1.15

National Cholesterol Education Program (NCEP) guidelines³¹

< 40 mg/dL (1.04 mmol/L): Low HDL-cholesterol (major risk factor for CHD)
≥ 60 mg/dL (1.55 mmol/L): High HDL-cholesterol ("negative" risk factor for CHD)

HDL-cholesterol is affected by a number of factors, e.g. smoking, exercise, hormones, sex and age.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

National Cholesterol Education Program (NCEP) guidelines are based on serum values, and when classifying patients the serum or serum equivalent values should be used. Therefore the NCEP recommends a factor of 1.03 to convert EDTA-plasma values to serum values. However, our own investigations revealed that a factor of 1.06 should be used for the HDL-C plus 3rd generation reagent. To comply with the 1998 NCEP goal of a < 5 % bias we recommend that each laboratory validate this conversion factor and enter it into the test parameters for HDL-C plus 3rd generation.

Specific performance data

Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

This procedure has been certified by the Cholesterol Reference Method Laboratory Network.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (1 aliquot per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	Level 1	Level 2
Mean	0.90 mmol/L (34.8 mg/dL)	2.8 mmol/L (108 mg/dL)
CV	1.13 %	0.44 %

Intermediate precision	Level 1	Level 2
Mean	0.80 mmol/L (30.9 mg/dL)	1.6 mmol/L (61.9 mg/dL)
CV	1.0 %	0.7 %

Method comparison

HDL-cholesterol values for human serum and plasma samples obtained on a COBAS INTEGRA 800 analyzer using the COBAS INTEGRA HDL-Cholesterol plus 3rd generation reagent (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Roche/Hitachi 917 analyzer	Sample size (n) = 55
Passing/Bablok ³²	Linear regression
y = 0.9836x - 0.0475 mmol/L	y = 0.9859x - 0.0463 mmol/L

HDLC3

HDL-Cholesterol plus 3rd generation

 $\tau = 0.9710$
 $r = 0.9985$

SD (md 95) = 0.038

Sy.x = 0.019

The sample concentrations were between 0.19 and 2.48 mmol/L (7.35 and 95.9 mg/dL).

References

- Dominiczak M, McNamara J. The system of Cardiovascular prevention. 103-125; Nauk M, Wiebe D, Warnick G. Measurement of High-Density-Lipoprotein Cholesterol. 221-244. In: Handbook of Lipoprotein Testing (eds. Rifai, Warnick and Dominiczak), 2nd edition.
- Linsel-Nitschke P, Tall AR. HDL as a target in the treatment of atherosclerotic cardiovascular disease. Nature Reviews 2005;4:193-205.
- Ng DS. Treating low HDL - From bench to bedside. Clinical Biochemistry 2004;37:649-659.
- Sugiuchi H, Uji Y, Okabe H, et al. Direct Measurement of High-Density Lipoprotein Cholesterol in Serum with Polyethylene Glycol-Modified Enzymes and Sulfated α -Cyclodextrin. Clin Chem 1995;41(5):717-723.
- Matsuzaki Y, Kawaguchi E, Morita Y, et al. Evaluation of Two Kinds of Reagents for Direct Determination of HDL-Cholesterol. J Anal Bio-Sc 1996;19:419-427.
- Nauck M, März W, Jarausch J, et al. Multicenter evaluation of a homogeneous assay for HDL-cholesterol without sample pretreatment. Clin Chem 1997;43:1622-1629.
- Zawta B, Klüber J. Brochure "Wissenswertes zu Apolipoproteinen". Fragen/Antworten (Boehringer Mannheim 1991). In: Henry JB, ed. Clinical Diagnosis and Management by Laboratory Methods. 17th ed. Philadelphia: WB Saunders 1984;251-282.
- AVP Fettstoffwechselstörungen. Therapieempfehlungen 1, 1st ed. 1996;2-16.
- Hatch FT, Lees RS. Practical methods for plasma lipoprotein analysis. Adv Lipid Res 1968;6:1-68.
- Narayan KA, Kummerow FA. Disk electrophoresis of human serum lipoprotein. Nature 1965;205:246-248.
- Okazaki M, Shiraishi K, Ohno Y, et al. Heterogeneity of human high density lipoproteins on high performance liquid chromatography. J Biochem 1982;92:517-524.
- Burstein M, Scholnick HR, Morfix R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res 1970;11:583-595.
- Musto J, Lawlor JF. HDL-cholesterol: online separation and analysis utilizing an automated chemistry analyzer [Abstract]. Clin Chem 1993;39:1125.
- Kakuyama T, Kimura S, Hashiguchi Y. Fully automated determination of HDL-cholesterol from human serum with Hitachi 911 [Abstract]. Clin Chem 1994;40:1104.
- Harris N, Galpichian V, Rifai N. Three routine methods for measuring high-density lipoprotein cholesterol compared with the Reference method. Clin Chem 1996;42:738-743.
- Sugiuchi H. History of development and technical details of the homogeneous assay for HDL and LDL cholesterol. The Fats of Life 2005;IX No. 1:4-11.
- Cohn JS, McNamara JR, Schaefer EJ. Lipoprotein Cholesterol Concentrations in the Plasma of Human Subjects as Measured in the Fed and Fasted States. Clin Chem 1988;34:2456-2459.
- Pisani T, Gebiski CP, Leary ET, et al. Accurate Direct Determination of Low-density Lipoprotein Cholesterol Using an Immunoseparation Reagent and Enzymatic Cholesterol Assay. Arch Pathol Lab Med 1995 Dec;119(12):1127-1135.
- Kimberly M, Leary E, Cole T, et al. Selection, Validation, Standardization and Performance of a Designated Comparison Method for HDL-Cholesterol for Use in the Cholesterol Reference Method Laboratory Network. Clin Chem 1999;45:1803-1812.
- Tietz NW. Textbook of Clinical Chemistry, 3rd Edition 1999;842-843.
- Data on file at Roche Diagnostics.
- Cooper GR, Myers GL, Smith SJ, et al. Standardization of Lipid, Lipoprotein, and Apolipoprotein Measurements. Clin Chem 1988;34(8B):B95-B105.
- Kadri N, Douville P, Lachance P. Letter to editor. Clin Chem 2002;48:964.
- Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.
- Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.
- Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.
- Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.
- Thomas L, ed. Labor und Diagnose, 4th ed. Marburg: Die Medizinische Verlagsgesellschaft 1992;208.
- Assmann G. At what levels of total low- or high-density lipoprotein cholesterol should diet/drug therapy be initiated? European guidelines. Amer J Cardiol 1990;65:11F.
- Assmann G, Schriewer H, Schmitz G, et al. Quantification of high-density-lipoprotein cholesterol by precipitation with phosphotungstic acid/MgCl₂. Clin Chem 1983;29(12):2026-2030.
- Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). NIH Publication No 01-3670; May 2001.
- Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT

Contents of kit



Volume after reconstitution or mixing

GTIN

Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

COBAS, COBAS C, COBAS INTEGRA, PRECINORM, PRECIPATH and PRECICONTROL are trademarks of Roche.

All other product names and trademarks are the property of their respective owners.

Additions, deletions or changes are indicated by a change bar in the margin.

© 2016, Roche Diagnostics



Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336

