

English

Intended use

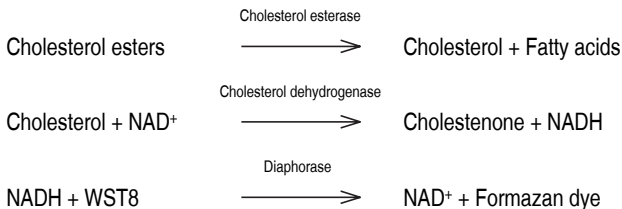
The **cobas b 101** is an in vitro diagnostic test system designed to quantitatively determine total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and triglycerides (TG) in human capillary and venous whole blood or plasma by photometric transmission measurement. A calculated value for low-density lipoprotein (LDL), non-HDL and a TC/HDL ratio is provided by the **cobas b 101** system. The system is intended for professional use in a clinical laboratory setting, or point of care (PoC) locations.

Summary

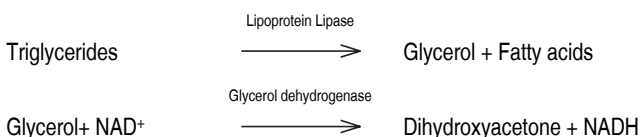
Cholesterol determinations are used in characterizing an individual's risk of developing atherosclerotic disease and aid in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders.¹ Determination of HDL-cholesterol is of clinical importance since an inverse correlation exists between HDL-cholesterol concentrations and the risk of atherosclerotic disease. Elevated HDL-cholesterol concentrations reduce the risk for coronary heart disease, while reduced HDL-cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase the cardiovascular risk.² Strategies have emerged to increase the level of HDL-cholesterol to treat cardiovascular disease.^{3,4} The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, metabolic syndrome, lipid metabolism disorders, liver obstruction, and numerous other endocrine diseases.⁵ The determination of a full lipid panel and the calculation of the LDL-cholesterol concentration according to Friedewald's formula is commonly practiced.⁶ Low Density Lipoproteins (LDL) play a key role in causing and influencing the progression of atherosclerosis, and in particular, coronary artery disease. The majority of cholesterol stored in atherosclerotic plaques originates from LDL. The LDL-cholesterol value is the most powerful clinical predictor among all of the single parameters with respect to coronary atherosclerosis. Therefore, therapies focusing on lipid reduction primarily target the reduction of LDL-cholesterol.⁷

Test principle

The erythrocytes of the capillary or venous blood sample are separated from the plasma by centrifugation. In the next step, the plasma sample is diluted with phosphate buffer. The HDL test uses a precipitation method with Mg^{2+} and phosphotungstic acid as a precipitant reagent. The components except for HDL-cholesterol are precipitated and removed. The **cobas b 101** system determines total cholesterol and HDL-cholesterol by an enzymatic method. Cholesterol esters in the sample are hydrolyzed to cholesterol and fatty acids. Cholesterol and NAD^+ generate cholestenone and NADH in the presence of cholesterol dehydrogenase. WST8 is reduced to formazan dye by diaphorase and NADH through oxidation-reduction reaction. The color intensity of formazan is measured at a specific wave length of 460 nm and is directly proportional to the concentration of HDL-cholesterol and total cholesterol in the sample.



The triglycerides test is an enzymatic method. Triglycerides in the sample are hydrolyzed to glycerol and fatty acids by lipoprotein lipase. Glycerol and NAD^+ generate dihydroxyacetone and NADH in the presence of glycerol dehydrogenase. WST8 is reduced to formazan dye by diaphorase and NADH through oxidation-reduction reaction. The color intensity of the formazan is proportional to triglyceride concentration and calculated by measuring at a wavelength of 460 nm.



Diaphorase



Low density lipoprotein (calculated)

Where the concentration of triglycerides is < 400 mg/dL (4.52 mmol/L), the LDL cholesterol is calculated using the Friedewald formula. $LDL = TC - HDL - TG/5$ (measured in mg/dL).⁸ Where the concentration of triglycerides is ≥ 400 mg/dL (4.52 mmol/L), the calculated LDL-cholesterol is not reported. The formula is also not valid for non-fasting patients and patients with Type III hyperlipoproteinemia (dysbetalipoproteinemia).

Total Cholesterol/HDL ratio and Non-high density lipoprotein

The **cobas b 101** instrument calculates the TC/HDL ratio as well as the non-HDL cholesterol (TC – HDL) from the measured values. Where the measured values data are not available, the TC/HDL ratio or non-HDL-cholesterol values are not reported.

Reagents

One test contains:

Dilution buffer: potassium dihydrogenphosphate 57 µg, dipotassium hydrogenphosphate 0.3 mg, potassium chloride 2.2 mg, sodium azide 42 µg ($\leq 0.02\%$)

Precipitant: magnesium sulfate heptahydrate 48 µg, sodium phosphotungstate n-hydrate 24 µg

Lipoprotein-lipase 0.096 U, cholesterol esterase 0.5 U, diaphorase 0.77 U, nicotinamide adenine dinucleotide 51 µg, tetrazolium salt 38 µg, glycerol dehydrogenase 0.75 U, cholesterol dehydrogenase 0.84 U

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

Reagent handling

Carefully tear open the foil pouch at the tear notch until one side is open.

Discard the disc if the foil pouch is found open or damaged, or if the disc is damaged, or the desiccant is missing, or loose desiccant particles or any other dirt or particles especially at the blood application zone are found.

Use **cobas Lipid Control** in the same way as a blood sample.

Storage and stability

Store at 2-30 °C until the expiration date printed on the pouch. Do not freeze. If stored in a refrigerator, allow the test to warm up in the closed pouch for at least 20 minutes before use. Once the pouch is opened, use the test within 20 minutes. Protect the disc from direct sunlight. Do not store opened pouches in a refrigerator.

Note: Open the pouch just before use when using control materials.

Specimen collection and preparation

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

Use fresh capillary blood, K_2^- or K_3^- -EDTA venous whole blood or plasma. Do not use other anticoagulants or other additives. Do not freeze samples. We recommend to use EDTA samples within 2 hours to comply with the NCEP goal of bias < 3 % for total cholesterol and bias < 5 % for high density lipoprotein cholesterol. Assure that the lanced site is clean and dry, and free from fatty substances. The marking on the disc clearly shows where to apply the sample. If samples are used from a venipuncture or control material, use a standard pipette or dropper to form a drop. The disc is self-filling. Do not push the sample into the disc. Do not use syringes. Assure that the disc is free from blood outside the sample application zone and the hinge cover.

Sample volume: 19 µL

Sample stability on disc

After applying blood to the disc, insert it into the instrument within 8 minutes. Please follow instructions in the operator's manual.

Assay

Instructions for use

cobas Lipid Panel

CHOL-TRIGL-HDL-LDL

cobas®

- Wash hands with soap. Warm water helps to stimulate the blood flow. Rinse the fingers extensively. Dry hands.
- Disinfect the fingertip by wiping three times the area to be lanced with a cotton swab or sterile gauze pad impregnated with 70 %-100 % isopropanol emollient free or 70 %-100 % ethanol emollient free; repeat the procedure with a second cotton swab or sterile gauze pad impregnated with 70 %-100 % isopropanol emollient free or 70 %-100 % ethanol emollient free, then dry with a cotton swab or sterile gauze pad.
- Prick the patient's finger by applying a single-use disposable lancing device (e.g. Accu-Chek Safe-T-Pro Plus). Make sure to follow the according lancing device instructions for obtaining a blood sample.
- Wipe off the first drop of blood with a swab.
- With the imprinted side of the disc facing upwards, position the disc's suction point above the drop of blood. The disc is self-filling.
- Apply blood and observe that it has filled the marked area. Check the sample volume: turn the disc on its backside. The area marked in blue has to be filled completely with blood. Do not push the blood into the disc.
- Press hinge cover down firmly to close the disc.
- Assure that the disc is free from blood outside the sample application zone and the hinge cover.
- Insert the disc into the **cobas b 101** instrument. Close the lid.
- The measurement starts automatically.

For more details, please refer to the **cobas b 101** Quick Reference Guide or **cobas b 101** Operator's Manual.

Materials provided

[REF] 06380115190, **cobas** Lipid Panel, 10 tests

Materials required (but not provided)

- Single use disposable lancing device (e.g. Accu-Chek Safe-T-Pro Plus)
- [REF] 06380182190, **cobas** Lipid Control
- [REF] 06378668190, **cobas b 101** instrument
- Optical check disc
- General laboratory equipment (e.g., sample transfer pipette for venous blood or alcohol wipes for the fingerstick)
- Timer

Calibration

Traceability: Total cholesterol and HDL-cholesterol are traceable to the designated CDC reference methods (Abell/Kendall as reference method for total cholesterol).⁹ Triglycerides are traceable to the ID/MS method.¹⁰

The instrument automatically reads in the lot-specific calibration data from the barcode information printed on the disc, eliminating the need for calibration by the user.

Quality control

For quality control, use **cobas** Lipid Control.

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.

QC info disc

Every **cobas** Lipid Control kit contains a lot-specific QC info disc for quality control. This QC info disc contains the target values and ranges for the **cobas** Lipid Panel.

The instrument display prompts the user to insert the QC info disc. The **cobas b 101** instrument reads the disc providing the lot specific target ranges.

Display of results

At the end of the automatic determination, the **cobas b 101** instrument displays the result in approximately 6 minutes. The measured total cholesterol, HDL-cholesterol, triglycerides and calculated LDL-cholesterol result will be displayed in mg/dL or mmol/L depending on the setting. Please refer to the operator's manual.

Limitations - interference

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{11,12}

Hematocrit levels between 30 % and 55 % do not affect results.

Total cholesterol

Icterus:¹³ No significant interference up to 15 mg/dL for conjugated bilirubin and up to 30 mg/dL for unconjugated bilirubin.

Hemolysis: No significant interference up to a hemoglobin concentration of 200 mg/dL.

Ascorbic acid: no significant interference up to 5 mg/dL.

Lipemia (Intralipid): No significant interference up to 500 mg/dL.

Criterion: Recovery within ± 10 % of initial values at a cholesterol concentration of 200 mg/dL (5.2 mmol/L).

Triglycerides

To test triglycerides or calculate LDL using the **cobas** Lipid Panel test ensure that the subject fasts for 9-12 hours before the sample is collected.

Icterus: No significant interference up to a conjugated/unconjugated bilirubin concentration of 15 mg/dL.

Hemolysis: No significant interference up to a hemoglobin concentration of 200 mg/dL.

Ascorbic acid: no significant interference up to 5 mg/dL.

Criterion: Recovery within ± 10 % of initial values at triglyceride levels of 203 mg/dL (2.3 mmol/L).

Fatty substances such as hand creams or soaps may contain glycerol which leads to false high triglyceride results and also false negative results for the calculated LDL.

High density lipoprotein

Icterus: No significant interference up to 15 mg/dL for conjugated bilirubin and up to 30 mg/dL for unconjugated bilirubin.

Hemolysis: No significant interference up to a hemoglobin concentration of 200 mg/dL.

Lipemia (Intralipid): No significant interference up to 500 mg/dL.

Ascorbic acid: no significant interference up to 5 mg/dL.

Criterion: Recovery ≤ 10 % of initial value at a HDL concentration of 50 mg/dL (1.3 mmol/L).

Abnormal liver function affects lipid metabolism; consequently, HDL-cholesterol and LDL-cholesterol results are of limited diagnostic value. In some patients with abnormal liver function, the HDL-cholesterol result may significantly differ from the designated comparison method (DCM) result.

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

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

Limits and ranges

Measuring range

Cholesterol: 50-500 mg/dL or 1.28-12.95 mmol/L

Triglycerides: 45-650 mg/dL or 0.50-7.35 mmol/L

HDL-cholesterol: 15-100 mg/dL or 0.38-2.60 mmol/L

Expected values

The European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS) jointly published 2011 ESC/EAS guidelines for the management of dyslipidemia.¹⁴ The new guideline recommends the assessment of total cardiovascular (CV) risk. Those with known Cardiovascular disease (CVD), type 2 diabetes or type 1 diabetes with microalbuminuria, very high levels of individual risk factors, chronic kidney disease (CKD) are at high or very high total CV risk and need active management of all risk factors. For all other patient groups, the use of a risk estimation system such as SCORE¹⁵ is recommended to estimate total CV risk because many people have several risk factors which, in combination, may result in unexpectedly high levels of total CV risk. Recommendations for lipid analyses for characterization of dyslipidemia before treatment: TC and LDL-C are recommended to be used for the primary lipid analysis for

cobas Lipid Panel

CHOL-TRIGL-HDL-LDL

cobas®

risk estimation of total CV risk; TG gives additional information on risk and is indicated for risk estimation; HDL-C contributes substantially to risk estimation.

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

Recommendations for lipid analyses as treatment target in the prevention of CVD

Concluding from the available data, an absolute reduction to an LDL-C level < 70 mg/dL (< 1.8 mmol/L) or at least 50 % relative reduction in LDL-C provides the best benefits in terms of CVD reduction. Clinical judgement is required before a final treatment plan is implemented.

National Cholesterol Education Program (NCEP) guidelines

The Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III, or ATP III) presents the National Cholesterol Education Program's (NCEP's) updated recommendations for cholesterol testing and management. The ATP III classifications are as follows:

Analyte	Concentration mg/dL (mmol/L)	Classification
LDL-cholesterol	< 100 (< 2.59)	Optimal
	100-129 (2.59-3.34)	Near optimal/above optimal
	130-159 (3.37-4.12)	Borderline high
	160-189 (4.14-4.90)	High
	≥ 190 (≥ 4.92)	Very high
HDL-cholesterol	< 40 (< 1.04)	Low
	≥ 60 (≥ 1.55)	High
Total cholesterol	< 200 (< 5.18)	Desirable
	200-239 (5.18-6.19)	Borderline high
	≥ 240 (≥ 6.20)	High
Triglycerides	< 150 (< 1.70)	Normal
	150-199 (1.70-2.25)	Borderline high
	200-499 (2.26-5.64)	High
	≥ 500 (≥ 5.65)	Very high

The NCEP guidelines are based on serum values, and when classifying patients, 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. Roche recommends that each laboratory validates its own conversion factor.

Non-high density lipoprotein

The NCEP ATP III gave the following recommendation: In persons with high triglycerides > 200 mg/dL (> 2.26 mmol/L), VLDL cholesterol should be combined with LDL-cholesterol, yielding non-HDL-cholesterol. The latter constitutes "atherogenic cholesterol" and should be a secondary target of therapy. After LDL goal is reached, secondary goal for non-HDL shall be set at 30 mg/dL (0.78 mmol/L) higher than LDL goal.

Total cholesterol/High density lipoprotein ratio

Many studies show that the TC/HDL ratio is a powerful predictor of CHD risk.^{16,17} The TC/HDL ratio predicts the risk of coronary heart disease regardless of the absolute LDL- and HDL-cholesterol. Even so, ATP III does not consider the total cholesterol/HDL-cholesterol ratio as a specified lipid target of therapy. Instead, LDL-cholesterol is retained as the primary target of lipid-lowering therapy. The total cholesterol/HDL-cholesterol ratio is also not recommended as a secondary target of therapy. Treatment according to ratios will divert priority from specific lipoprotein fractions as targets of therapy.

Specific performance data

Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using controls in a CLSI EP5-A2 protocol. Whole blood samples were measured using a modified CLSI protocol in 5 series of 4 replicates in one day. The following results were obtained:

Sample		Mean value mg/dL (mmol/L)	Repeatability		Intermediate precision	
			SD mg/dL (mmol/L)	CV %	SD mg/dL (mmol/L)	CV %
Control/Level 1 (n ^a = 84)	TC	145 (3.76)	2.7 (0.069)	1.8	3.0 (0.078)	2.1
	TG	97 (1.10)	1.3 (0.015)	1.3	1.4 (0.016)	1.4
	HDL	42 (1.08)	1.4 (0.037)	3.5	1.4 (0.037)	3.5
Control/Level 2 (n = 84)	TC	269 (6.96)	4.8 (0.123)	1.8	5.1 (0.131)	1.9
	TG	395 (4.46)	4.2 (0.048)	1.1	4.3 (0.049)	1.1
	HDL	69 (1.78)	1.9 (0.048)	2.7	2.1 (0.055)	3.1
Whole blood 1 (n = 20)	TC	166 (4.29)	3.1 (0.080)	1.9	3.7 (0.095)	2.2
	TG	336 (3.80)	4.0 (0.045)	1.2	4.4 (0.050)	1.3
	HDL	38 (0.97)	0.9 (0.023)	2.4	1.4 (0.035)	3.6
Whole blood 2 (n = 20)	TC	228 (5.89)	3.8 (0.099)	1.7	4.5 (0.117)	2.0
	TG	346 (3.91)	7.1 (0.080)	2.0	11.8 (0.133)	3.4
	HDL	46 (1.19)	1.5 (0.038)	3.2	1.7 (0.043)	3.6
Whole blood 3 (n = 20)	TC	184 (4.75)	2.9 (0.076)	1.6	3.0 (0.077)	1.6
	TG	135 (1.52)	1.6 (0.018)	1.2	5.0 (0.057)	3.7
	HDL	48 (1.24)	1.2 (0.031)	2.5	1.3 (0.033)	2.7

a) n = no. of samples

Method comparison

A comparison study with EDTA whole blood samples measured with **cobas Lipid Panel** (y) on the **cobas b 101** instrument with respective methods on the **cobas c 501** analyzer (x) gave the following correlations: Passing/Bablok¹⁸

Cholesterol:

$$y = 1.000x - 0.110 \text{ mmol/L}$$

$$r = 0.991, n = 69$$

Sample range: 2.88-7.72 mmol/L

Mean bias (%) = -2.39 %

Bias at medical decision ranges:

low: 200 mg/dL (5.2 mmol/L): -2.1 %

high: 240 mg/dL (6.2 mmol/L): -1.8 %

Triglycerides:

$$y = 1.020x - 0.009 \text{ mmol/L}$$

$$r = 0.996, n = 68$$

Sample range: 0.52-4.57 mmol/L

Mean bias (%) = 0.45 %

Bias at medical decision ranges:

low: 150 mg/dL (1.7 mmol/L): 1.5 %

high: 200 mg/dL (2.26 mmol/L): 1.6 %

High density lipoprotein:

$$y = 1.056x - 0.080 \text{ mmol/L}$$

$$r = 0.981, n = 67$$

Sample range: 0.78-2.42 mmol/L

Mean bias (%) = 0.58 %

Bias at medical decision ranges:

cobas Lipid Panel

CHOL-TRIGL-HDL-LDL

cobas®

low: 40 mg/dL (1.04 mmol/L): -2.1 %

high: 60 mg/dL (1.55 mmol/L): 0.4 %

References







- Executive Summary of The 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). JAMA. 2001;285:2486-97.
- Alberti G, Shaw J, Zimmet P, et al. The IDF consensus worldwide definition of the metabolic syndrome. International Diabetes Federation 2006. http://www.idf.org/webdata/docs/MetSyndrome_FINAL.pdf, visited 15-11-2011.
- 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.
- Rader DJ, Hobbs HH. Disorders of Lipoprotein Metabolism. In: Fauci AS, Braunwald E, Kasper DL et al. (eds.) Harrison's principles of internal medicine. New York, Mc Graw Hill, 2008; p. 2424.
- Fukuyama N, Homma K, Wakana N, et al. Validation of the Friedewald Equation for Evaluation of Plasma LDL-Cholesterol. J Clin Biochem Nutr 2008;43:1-5.
- Final Report. National Heart Lung and Blood Institute (NHLBI) 2002. <http://www.nhlbi.nih.gov/guidelines/cholesterol/atp3full.pdf> (p. 12), visited 15-11-2011.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
- Recommendations for Improving Cholesterol Measurement: A Report from the Laboratory Standardization Panel of the National Cholesterol Education Program. NIH Publication No 90-2964. February 1990.
- Bernert JT, Jr, Bell CJ, McGuffey JE. Determination of free glycerol in human serum reference materials by isotope-dilution gas chromatography-mass spectrometry. J Chromatography 1992;578:1-7.
- 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.
- Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.
- Alberico L. Catapano (EAS Chairperson), Italy, et al. ESC/EAS Guidelines for the management of dyslipidaemias The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). Atherosclerosis 217 (2011) 3-46.
- Cooney M, Dudina A, Bacquer DD, et al. How much does HDL cholesterol add to risk estimation? A report from the SCORE investigators. Eur J Cardiovasc Prev Rehabil 2009;16:304-14.
- Lemieux I, Lamarche B, Couillard C, et al. Total cholesterol/HDL cholesterol ratio vs LDL cholesterol/HDL cholesterol ratio as indices of ischemic heart disease risk in men: the Quebec Cardiovascular Study. Arch Intern Med 2001;161:2685-92.
- Bersot TP, Pépin GM, Mahley RW. Risk determination of dyslipidemia in populations characterized by low levels of high-density lipoprotein cholesterol. Am Heart J 2003;146:1052-9.
- 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.

For further information, please refer to the appropriate Operator's Manual for the instrument concerned, and the Method Sheets of all necessary components.

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 (for USA: see dialog.roche.com for definition of symbols used):

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume after reconstitution or mixing
	Global Trade Item Number

COBAS, COBAS B, and COBAS C are trademarks of Roche. INTRALIPID is a trademark of Fresenius Kabi AB.

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.

© 2019, Roche Diagnostics



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

