1. Purpose

This procedure outlines the protocol to be used to perform calibration verification of test methods employed on the Roche cobas c501.

2. Summary and explanation

Calibration verification is performed using calibration materials appropriate for the methodology in use and also can be used to verify the reportable range of a method if materials span the reportable range. At a minimum, three samples containing analyte concentrations of a zero or minimal value, a midpoint, and a maximum value should be used for each analyte.

Calibration verification must be performed in accordance with the manufacturer's calibration verification instructions if provided or in accordance with the criteria established by the laboratory at least every six months or whenever any of the following occur:

- Major preventative maintenance or replacement of critical parts is performed which may influence test performance.
- A complete change of reagents affects assay performance (i.e. reportable ranges and/or control values are adversely affected by reagent lot changes).
- Controls reflect an unusual shift or trend or are outside the acceptable limits and other means of correcting the problem have not been effective.

3. Special Safety Notes/Warnings/Precautions

Follow all procedures for handling and disposal of potentially biohazardous materials described in the Wadsworth Center Safety & Security Policy & Procedures Manual. Disposable gloves and protective clothing (lab coat) must be worn at all times when operating the Roche cobas c501 clinical analyzer. Gloves should be changed as necessary but always if torn or soiled. Wash hands thoroughly after removing gloves. A splash barrier capable of protecting the face should be used whenever performing manipulations that might produce splashes of potentially biohazardous materials. Note: The following are considered "clean areas": instrument and cobasLink keyboard, monitor, printer, and all bench areas not covered by blue absorbent pads. Contact with these areas while wearing disposable gloves should be avoided in order to prevent contamination**.**

4. Equipment/Materials/Reagents

Roche cobas c501 analyzer
Roche cobas c501 reagents
Calibrator(s) (see application information for appropriate material)
Controls (see application information for appropriate material)
Calibration verification materials (see CLC-09.0 Appendix A,B,C)

Procedure

4.1 Perform all required maintenance procedures on the Roche cobas c501.

4.2 Review on-board reagents to verify sufficient quantity and stability. Load fresh reagents as necessary.

4.3 Perform required calibration (see CLC-SOP 05.0) and quality control (see CLC-SOP 06.0). Review calibration and quality control results. If acceptable, proceed to calibration verification.

4.4 Follow all calibration verification material instructions for storage, thawing, reconstitution and stability. Each level of material must be run *at least* in duplicate.

4.5 Program calibration verification materials/test selections into the Roche cobas c501.

4.6 After all tests/samples have been entered into the cobas c501 test selection screen, fill labeled sample cups with the appropriate calibration verification material and load them into the corresponding rack/sample position. Check that there are no bubbles or foam visible on the liquid surface of each sample.

4.7 Click START button; then click large START button to begin analysis.

4.8 When testing is complete, review results to determine whether off-line dilutions of calibration verification materials should be made to closer approximate lower and/or upper reportable range limits. Print copy of results, reagent listing, and requisition list for filing.

4.9 Enter calibration verification data into the Microgenics/CASCO on-line data reduction software (see web address below). Alternate software products may be used if they provide similar functionality. http://www.cascodocservices.com/admin/login.aspx?ReturnUrl=%2fDefault.aspx).

4.9.1 Print results and review as outlined below under “Interpretation of Results”.

4.9.2 Analyst must sign calibration verification document and forward to supervisor and/or director for review.

4.9.3 After results are reviewed, accepted, and signed by supervisor (and/or director), file evaluation printout in Calibration Verification binder. Be certain to check off and indicate date of calibration verification on binder summary sheet.

5. Interpretation of Results

5.1 Agreement of measured results with expected values should be ≤ 10% for all levels, with exceptions at the extreme lower limits where a small bias may result in a large % difference due to the very low concentration of the analyte. Thus, the 10% criteria cannot always be used for the minimum calibration verification point, especially when the predicted value is very low. In such cases, professional judgment and consultation with supervisor and/or director must be used to determine acceptability.

5.2 Confirm that manufacturer's reportable range limits (refer to the manufacturer's test method application sheet) have been verified.

5.3 In the event calibration verification materials do not span the reportable range OR if results for the upper and/or lower point(s) fall out of range, consult supervisor and/or director. In such cases, the director and/or supervisor may determine that decreasing the reportable range to the limits verified is acceptable.

5.3.1 Should decreasing manufacturer's claimed reportable range be necessary and approved by supervisor and/or director, make certain to note adjusted ranges on:

- Roche method application sheet (obtain director's signature)
- Roche c501 method parameters (Utilities/Application screen). Be certain to note change in Parameter Update Log book.
- CLC-02.0 Appendix A, B, or C (as appropriate) Clinical Chemistry, Urine Chemistry, TSM Verified Ranges.

5.4 Should calibration verification results fall outside expected tolerances, perform corrective action as required and repeat calibration verification study for the analyte in question. Consult supervisor and/or director if repeat testing after corrective action(s) fails to result in acceptable performance.

6. References

cobas® 6000 analyzer series Operator's Manual. Roche Diagnostics GmbH. © 2001-2009. Version 4.0

New York State Department of Health, *Clinical Laboratory Standards of Practice*. January 2008.

Wadsworth Center Safety & Security Policy & Procedures Manual

7. Appendix

CLC-09.0 Appendix A Clinical Chemistry Calibration Verification Materials

CLC-09.0 Appendix B Urine Chemistry Calibration Verification Materials

CLC-09.0 Appendix C TSM Calibration Verification Materials

SOP Annual Review

Director or Designee	Signature	Date

1. Purpose

This procedure describes calibration requirements for the Roche cobas c501 clinical analyzer and details the mechanism used to determine when calibration must be performed, the method used to perform calibration, and the criteria employed to accept or reject a calibration.

2. Summary and explanation

The Roche cobas c501 is a fully automated system for photometric analysis. Calibration is the process that establishes a relationship between measurement values (such as absorbance values) and corresponding results (concentration of an analyte). The relationship between measurement values and results is subject to various environmental and reagent conditions and may drift over the course of time. It is thus necessary to repeat calibrations regularly. To keep the resulting calibration management simple and efficient, the Roche cobas c501 system automatically recommends calibrations.

This procedure provides an outline of the steps that must be performed in order to perform calibrations on the Roche cobas c501. The procedures described in this document have been obtained from the Roche cobas 6000 Operator's Manual and can be reviewed in greater detail by consulting appropriate sections of that manual and/or the cobas 6000 analyzer series Quick Reference Card for calibrations (CLC-10.0 Appendix A).

3. Equipment/Materials/Reagents

Roche cobas c501 clinical analyzer
Reagent cassettes for applicable tests
Calibrator(s) as required (see CLC-05.0 Appendix A)
Quality control materials as required (see SOP CLC-06.0)

4. Special Safety Notes/Warnings/Precautions

Follow all procedures for handling and disposal of potentially biohazardous materials described in the Wadsworth Center Safety & Security Policy & Procedures Manual. Disposable gloves and protective clothing (lab coat) must be worn at all times when operating the Roche cobas c501 clinical analyzer. Gloves should be changed as necessary but always if torn or soiled. Wash hands thoroughly after removing gloves. A splash barrier capable of protecting the face should be used whenever performing manipulations that might produce splashes of potentially biohazardous materials. Note: The following are considered "clean areas": instrument and cobasLink keyboard, monitor, printer, and all bench areas not covered by blue absorbent pads. Contact with these areas while wearing disposable gloves should be avoided in order to prevent contamination**.**

5. Procedure

The number and type of calibrators varies and is dependent upon the test to be calibrated. Calibration data are automatically downloaded via the cobasLink and then downloaded by the operator into the cobas c501 control unit. This data may also be entered manually.

Calibrators do not contain sample ID bar codes. However, each calibrator has been assigned specific rack and position numbers. Each calibrator rack has been pre-labeled with the calibrator names to aid in correct calibrator placement.

5.1 ISE Calibration (Na, K, and Cl)

ISE calibration is required every 24 hours, after ISE cleaning/maintenance, after changing the ISE reagent solutions or cartridges, and as required following quality control troubleshooting protocols.

5.1.1 From the System Overview screen click CALIBRATION

5.1.2 Click the STATUS tab to display the CALIBRATION STATUS SCREEN.

5.1.3 Select ISE-A-IS1 and ISE-B-IS1. The selected line is highlighted in blue.

5.1.4 Select the appropriate button in the METHOD area for a FULL calibration. The selected option appears in the METHOD column highlighted in green, the CAUSE column indicates MANUAL, and the SAVE button turns yellow.

5.1.5 Click SAVE to save the changes.

5.1.6 Click the PRINT button and then select CALIBRATOR LOAD LIST, then click PRINT to print a detailed list of the calibrators and their corresponding rack positions to assist in loading those on the analyzer.

5.1.7 Calibrators for sodium, potassium, and chloride are supplied in as ready-to-use liquids. Consult Appendix APP CLC-00.0) for a list of tests and their associated calibrators. Current calibrator value sheets/inserts are filed in the “Calibrator Inserts” binder.

5.1.8 Fill labeled sample cups with the calibrators and place those into the appropriate rack/rack positions. Check that there are no bubbles or foam visible on the liquid surface.

5.1.9 Load the calibrator rack and onto the Roche cobas c501. Select START, and then click the large START button to begin the calibration.

5.2 Non-ISE Calibrations

Calibration is generally required after reagent lot change, at specific test-specific time intervals after initial lot calibration, and as required following quality control troubleshooting protocols. For specific calibration requirements, refer to the individual analyte application sheets.

5.2.1 From the main screen select CALIBRATION.

5.2.2 Click STATUS tab to display the CALIBRATION STATUS SCREEN.

5.2.3 Select the reagent and test to be calibrated from the list. The selected line is highlighted in blue.

5.2.4 Select the appropriate button in the METHOD area for a 2-point, full, or blank calibration. The selected option appears in the METHOD column highlighted in green, the CAUSE column indicates MANUAL, and the SAVE button turns yellow.

5.2.5 Click SAVE to save the changes.

5.2.6 Click the PRINT button and then select CALIBRATOR LOAD LIST, then click PRINT to print a detailed list of the calibrators and their corresponding rack positions. This list will assist the operator in loading the appropriate calibrators on the Roche c501.

5.2.7 Prepare calibrator(s) as required for the test to be calibrated. Calibrators are supplied in either lyophilized form or as ready-to-use liquids. Consult Appendix CLC-05.0 Appendix A) for a list of tests and their associated calibrators. Current calibrator value sheets/inserts are filed in the “Calibrator Inserts” binder.

5.2.8 Fill labeled sample cups with the appropriate calibrators and place them into the corresponding rack(s). Check that there are no bubbles or foam visible on the liquid surface.

5.2.9 Once loaded on the instrument, click START, and then click the large START button to begin the calibration.

6. Interpretation of Results

6.1 Calibration results will automatically be assessed and printed by the analyzer. Make certain to review the results, checking for calibration alarms and/or unusual results, and date/initial calibration printout. In general, quality control samples are analyzed immediately after a calibration is performed, and should be used as an additional check of the validity of the calibration performed.

6.2 Should an unacceptable calibration occur, proceed as follows before commencing patient testing:

6.2.1 Check that appropriate calibrator(s) were loaded in the correct rack/position.

6.2.2 Check that calibrator(s) dating is within specified expiration date and that reconstitution (if required) was performed correctly.

6.2.3 Check reagent expiration and on-board stability.

6.2.4 Check that all maintenance is up-to-date and acceptable.

6.2.5 Repeat calibration and quality control samples.

6.2.6 If calibration continues to fail, install fresh reagent and/or reagent cassette and repeat calibration and quality control.

6.2.7 Consult supervisor and/or Roche Technical Support if the above remedies fail to correct calibration problems.

7. References

cobas® 6000 analyzer series Operator's Manual. Roche Diagnostics GmbH. © 2001-2009. Version 4.0

Wadsworth Center Safety & Security Policy & Procedures Manual

8. Appendix

SOP CLC-05.0A Roche cobas c501 Calibrator/Test list

SOP Annual Review

Director or Designee	Signature	Date

1. Purpose

This procedure provides a description of the protocol to be followed for validation of any new method and/or instrumentation.

2. Summary and explanation

Method validation is performed in order to verify the manufacturer's claims for precision, accuracy, and reportable range of all new methods and/or instrumentation. Validation results must be reviewed and accepted by the laboratory director before patient testing can commence. Records from validation studies must be retained while a procedure is in use and for two years after the procedure has been discontinued.

3. Equipment/Materials/Reagents

Roche cobas c501 clinical analyzer
Reagent cassettes for applicable tests
Calibrator(s)
Quality control materials
Patient and/or proficiency test specimens

4. Special Safety Notes/Warnings/Precautions

Follow all procedures for handling and disposal of potentially biohazardous materials described in the Wadsworth Center Safety & Security Policy & Procedures Manual. Disposable gloves and protective clothing (lab coat) must be worn at all times when operating the Roche cobas c501 clinical analyzer. Gloves should be changed as necessary but always if torn or soiled. Wash hands thoroughly after removing gloves. A splash barrier capable of protecting the face should be used whenever performing manipulations that might produce splashes of potentially biohazardous materials. Note: The following are considered "clean areas": instrument and cobasLink keyboard, monitor, printer, and all bench areas not covered by blue absorbent pads. Contact with these areas while wearing disposable gloves should be avoided in order to prevent contamination**.**

5. Procedure

5.1 Assessment of precision

5.1.1 Refer to SOP CLC-12.0 Precision Assessment-Within Run. Values for standard deviation/coefficient of variation should fall within manufacturer's specifications.

5.2 Assessment of accuracy

5.2.1 Analyze assayed quality control materials provided by the manufacturer. If results do not fall within allowable limits, consult Calibration and Quality Control procedures (SOP CLC-05.0 and SOP CLC-06.0) for troubleshooting advice.

5.2.2 Analyze proficiency test specimens (eg. NYS, CAP) and compare measured values with target values and allowable ranges for similar reagent/instrument systems. Results must fall within acceptable limits.

5.3 Confirmation of reportable range/calibration verification

5.3.1 Refer to SOP CLC-09.0 Calibration Verification. Results should confirm manufacturer's reportable range claims for all methods tested. If calibration verification materials do not span the manufacturer's stated reportable ranges, adjust in-house reporting limits accordingly.

5.4 Director review and sign-off

5.4.1 Assemble all precision, accuracy, and reportable range data and summaries for director review.

5.4.2 Obtain a printed copy of the Roche cobas 6000 application sheet for the test undergoing validation and include with validation data for director review and signature.

5.4.3 After all documents/data have been reviewed and signed by the director, file validation summaries in the cobas c501 installation binder. File signed Roche cobas application sheet in the Method Manual (SOP CLC-02.0)

6. References

Wadsworth Center Safety & Security Policy & Procedures Manual

SOP Annual Review

Director or Designee	Signature	Date

Application Sheet

cobas®

Laboratory Name

Test Name: Cholesterol Gen.2

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
Cholesterol Gen.2			**cobas c** 311	**cobas c** 501/502
400 tests	Cat. No. **03039773** 190	System-ID 07 6726 3	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Precinorm L (4 x 3 mL)	Cat. No. **10781827** 122	Code 304		
Precipath L (4 x 3 mL)	Cat. No. **11285874** 122	Code 305		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

Effective date

Effective date for this procedure: _____

Author

Source documentation compiled by Roche Diagnostics
Revised by: _____

Schedule for review

Last date revised: _____
Date Reviewed: _____ Approved: _____
Date Reviewed: _____ Approved: _____
Date Reviewed: _____ Approved: _____
Date Reviewed: _____ Approved: _____

System information

For **cobas c** 311/501 analyzers:
CHO2I: ACN 798: ID/MS Standardization
CHO2A: ACN 433: Abell/Kendall Standardization
For **cobas c** 502 analyzer:
CHO2I: ACN 8798: ID/MS Standardization
CHO2A: ACN 8433: Abell/Kendall Standardization

Intended use

In vitro test for the quantitative determination of cholesterol in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary

Cholesterol is a steroid with a secondary hydroxyl group in the C3 position. It is synthesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately three quarters of cholesterol is newly synthesized and a quarter originates from dietary intake. Cholesterol assays are used for screening for atherosclerotic risk and in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders.
Cholesterol analysis was first reported by Liebermann in 1885 followed by Burchard in 1889. In the Liebermann-Burchard reaction, cholesterol forms a blue-green dye from polymeric unsaturated carbohydrates in an acetic acid/acetic anhydride/concentrated sulfuric acid medium. The Abell and Kendall method is specific for cholesterol, but is technically complex and requires the use of corrosive reagents. In 1974, Roeschlau and Allain described the first fully enzymatic method. This method is based on the determination of Δ4-cholestenone after enzymatic cleavage of the cholesterol ester by cholesterol esterase, conversion of cholesterol by cholesterol oxidase, and subsequent measurement by the Trinder reaction of the hydrogen peroxide formed. Optimization of ester cleavage (> 99.5 %) allows standardization using primary and secondary standards and a direct comparison with the CDC and NIST reference methods.[1,2,3,4,5,6,7,8,9]
Nonfasting sample results may be slightly lower than fasting results.[10,11,12]
The Roche cholesterol assay meets the 1992 National Institutes of Health (NIH) goal of less than or equal to 3 % for both precision and bias.[12]
The assay is optionally standardized against Abell/Kendall and isotope dilution/mass spectrometry. The performance claims and data presented here are independent of the standardization.

Test principle

Enzymatic, colorimetric method.
Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminophenazone to form a red quinone-imine dye.

$$\text{Cholesterol esters} + H_2O \xrightarrow{CE} \text{cholesterol} + \text{RCOOH}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{cholest-4-en-3-one} + H_2O_2$$

$$2\ H_2O_2 + \text{4-AAP} + \text{phenol} \xrightarrow{POD} \text{quinone-imine dye} + 4\ H_2O$$

The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance.

Reagents – working solutions

R1 PIPES buffer: 225 mmol/L, pH 6.8; Mg^{2+}: 10 mmol/L; sodium cholate: 0.6 mmol/L; 4-aminophenazone: ≥ 0.45 mmol/L; phenol: ≥ 12.6 mmol/L; fatty alcohol polyglycol ether: 3 %; cholesterol esterase (Pseudomonas spec.): ≥ 25 µkat/L (≥ 1.5 U/mL); cholesterol oxidase (E. coli): ≥ 7.5 µkat/L (≥ 0.45 U/mL); peroxidase (horseradish): ≥ 12.5 µkat/L (≥ 0.75 U/mL); stabilizers; preservative

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Reagent handling

Ready for use.

Storage and stability

CHOL2

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	4 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	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: Li-heparin and K_2-EDTA plasma
Do not use citrate, oxalate or fluoride.[13]
Fasting and nonfasting samples can be used.[11]
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:[14,15] 7 days at 15-25 °C
7 days at 2-8 °C
3 months at (-15)-(-25) °C

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section.
General laboratory equipment
Other suitable control material can be used in addition.

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.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 311 **test definition**

Assay type	1 Point		
Reaction time / Assay points	10 / 57		
Wavelength (sub/main)	700/505 nm		
Reaction direction	Increase		
Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	47 µL	93 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	2 µL	15 µL	135 µL
Increased	4 µL	–	–

cobas c 501/502 **test definition**

Assay type	1 Point		
Reaction time / Assay points	10 / 70		
Wavelength (sub/main)	700/505 nm		
Reaction direction	Increase		
Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	47 µL	93 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	2 µL	15 µL	135 µL
Increased	4 µL	–	–

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized according to Abell/Kendall[12] and also by isotope dilution/mass spectrometry.[16]

Quality Control

For quality control, use control materials as listed in the “Order information” section.
Other suitable control material can be used in addition.
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 limits.
Follow the applicable government regulations and local guidelines for quality control.
If controls do not recover within the specified limits, take the following corrective action:

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	mmol/L x 38.66 = mg/dL
	mmol/L x 0.3866 = g/L
	mg/dL x 0.0259 = mmol/L

Limitations – interference[17]

Criterion: Recovery within ± 10 % of initial values at a cholesterol concentration of 5.2 mmol/L (200 mg/dL).
Icterus: No significant interference up to an I index of 16 for conjugated bilirubin and 14 for unconjugated bilirubin (approximate conjugated bilirubin concentration 274 µmol/L (16 mg/dL) and approximate unconjugated bilirubin concentration 239 µmol/L (14 mg/dL)).
Hemolysis: No significant interference up to an H index of 700 (approximate hemoglobin concentration: 435 µmol/L (700 mg/dL)).
Lipemia (Intralipid): No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
Drugs: No interference was found at therapeutic concentrations using common drug panels.[18,19]
In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the Carry over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1 + 2/SCCS Method Sheets. For further instructions refer to the operator manual.
cobas c 502 analyzer: All special wash programming necessary for avoiding carry over is available via the **cobas** link, manual input is not required.
Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range
0.1-20.7 mmol/L (3.86-800 mg/dL)
Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 10.
Lower limits of measurement
Lower detection limit of the test
0.1 mmol/L (3.86 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 three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

Clinical interpretation according to the recommendations of the European Atherosclerosis Society:[20]

	mmol/L	*mg/dL*	*Lipid metabolic disorder*
Cholesterol	< 5.2	(< 200)	No
Triglycerides	< 2.3	(< 200)	
Cholesterol	5.2-7.8	(200-300)	Yes, if HDL-cholesterol < 0.9 mmol/L (< 35 mg/dL)
Cholesterol	> 7.8	(> 300)	Yes
Triglycerides	> 2.3	(> 200)	

Recommendations of the NCEP Adult Treatment Panel for the following risk-cutoff thresholds for the US American population:[21]

Desirable cholesterol level	< 5.2 mmol/L	(< 200 mg/dL)
Borderline high cholesterol	5.2-6.2 mmol/L	(200-240 mg/dL)
High cholesterol	≥ 6.2 mmol/L	(≥ 240 mg/dL)

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

Specific performance data

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

Precision

Precision was determined using human samples and controls in an internal protocol. Repeatability* (n = 21), intermediate precision** (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

*Repeatability **	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	2.29 (88.5)	0.02 (0.8)	1.1
Precipath U	4.74 (183)	0.04 (2)	0.9
Human serum 1	2.85 (110)	0.03 (1)	1.1
Human serum 2	7.39 (286)	0.05 (2)	0.7

*Intermediate precision ***	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	2.31 (89.3)	0.04 (1.6)	1.6
Precipath U	4.85 (188)	0.08 (3)	1.6
Human serum 3	1.97 (76.2)	0.03 (1.2)	1.6
Human serum 4	7.13 (276)	0.10 (4)	1.4

* repeatability = within-run precision

** intermediate precision = total precision / between run precision / between day precision

Method comparison

Cholesterol values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).
Sample size (n) = 266

Passing/Bablok[22]	Linear regression
y = 1.002x + 0.045 mmol/L	y = 1.012x - 0.015 mmol/L
τ = 0.953	r = 0.997

The sample concentrations were between 1.53 and 18.5 mmol/L (59.1 and 715 mg/dL).

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Alternative method

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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.

Version 7
2010-07

Reagent manufacturer

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

Source document

Reagent Name: CHOL2
Package Insert Version: 2010-03, V7 English

Application Sheet

cobas®

Laboratory Name

Test Name: Creatinine Jaffé Gen.2

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
			cobas c 311	**cobas c** 501/502
Creatinine Jaffé Gen.2				
700 tests	Cat. No. **04810716** 190	System-ID 07 6928 2	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Precinorm PUC (4 x 3 mL)	Cat. No. **03121313** 122	Code 240		
Precipath PUC (4 x 3 mL)	Cat. No. **03121291** 122	Code 241		
PreciControl ClinChem Multi 1 (20 x 5 mL)	Cat. No. **05117003** 190	Code 391		
PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Cat. No. **05947626** 160	Code 391		
PreciControl ClinChem Multi 2 (20 x 5 mL)	Cat. No. **05117216** 190	Code 392		
PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Cat. No. **05947774** 160	Code 392		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

Effective date

Effective date for this procedure: _____

Author

Source documentation compiled by Roche Diagnostics
Revised by: _____

Schedule for review

Last date revised: _____
Date Reviewed: _____ Approved: _____
Date Reviewed: _____ Approved: _____
Date Reviewed: _____ Approved: _____
Date Reviewed: _____ Approved: _____

System information

For **cobas c** 311/501 analyzers:
CREJ2: ACN 690 (Rate blanked, compensated, serum and plasma)
CRJ2U: ACN 691 (Rate blanked, urine)
SCRE2: ACN 773 (STAT, compensated, serum and plasma, reaction time: 4)
SCR2U: ACN 774 (STAT, urine, reaction time: 4)
For **cobas c** 502 analyzer:
CREJ2: ACN 8690 (Rate blanked, compensated, serum and plasma)
CRJ2U: ACN 8691 (Rate blanked, urine)
SCRE2: ACN 8773 (STAT, compensated, serum and plasma, reaction time: 4)
SCR2U: ACN 8774 (STAT, urine, reaction time: 4)

Intended use

In vitro test for the quantitative determination of creatinine in human serum, plasma and urine on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5]

Chronic kidney disease is a worldwide problem that carries a substantial risk for cardiovascular morbidity and death. Current guidelines define chronic kidney disease as kidney damage or glomerular filtration rate (GFR) less than 60 mL/min per 1.73 m^2 for three months or more, regardless of cause.
The assay of creatinine in serum or plasma is the most commonly used test to assess renal function. Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). It is freely filtered by the glomeruli and, under normal conditions, is not re-absorbed by the tubules to any appreciable extent. A small but significant amount is also actively secreted.
Since a rise in blood creatinine is observed only with marked damage of the nephrons, it is not suited to detect early stage kidney disease. A considerably more sensitive test and better estimation of glomerular filtration rate (GFR) is given by the creatinine clearance test based on creatinine's concentration in urine and serum or plasma, and urine flow rate. For this test a precisely timed urine collection (usually 24 hours) and a blood sample are needed. However, since this test is prone to error due to the inconvenient collection of timed urine, mathematical attempts to estimate GFR based only on the creatinine concentration in serum or plasma have been made. Among the various approaches suggested, two have found wide recognition: that of Cockroft and Gault and that based on the results of the MDRD trial. While the first equation was derived from data obtained with the conventional Jaffé method, a newer version of the second is usable for IDMS-traceable creatinine methods. Both are applicable for adults. In children, the Bedside Schwartz formula should be used.[6,7,8,9]

In addition to the diagnosis and treatment of renal disease, the monitoring of renal dialysis, creatinine measurements are used for the calculation of the fractional excretion of other urine analytes (e. g., albumin, α-amylase). Numerous methods were described for determining creatinine. Automated assays established in the routine laboratory include the Jaffé alkaline picrate method in various modifications, as well as enzymatic tests.

Test principle[10,11,12]

This kinetic colorimetric assay is based on the Jaffé method. In alkaline solution, creatinine forms a yellow-orange complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen. The assay uses “rate-blanking” to minimize interference by bilirubin. To correct for non-specific reaction caused by serum/plasma pseudo-creatinine chromogens, including proteins and ketones, the results for serum or plasma are corrected by -26 µmol/L (-0.3 mg/dL).

Creatinine + picric acid $\xrightarrow{\text{Alkaline pH}}$ yellow-orange complex

Reagents - working solutions

R1	Potassium hydroxide: 900 mmol/L; phosphate: 135 mmol/L; pH ≥ 13.5; preservative; stabilizer
R2/R3	Picric acid: 38 mmol/L; pH 6.5; non reactive buffer

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.
This kit contains components classified as follows according to the European directive 1999/45/EC.

 C – Corrosive. R1 contains potassium hydroxide.

R 1: Explosive when dry. R 4: Forms very sensitive, explosive metallic compounds. R 34: Causes burns.
S 24-25: Avoid contact with skin and eyes. S 26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S 35: This material and its container must be disposed of in a safe way.
S 36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
S 45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
Contact phone: all countries: +49-621-7590, USA: +1-800-428-2336

Reagent handling

Ready for use.

Storage and stability

CREJ2

Shelf life at 15-25 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Specimen collection and preparation[13]

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
Serum.
Plasma: Li-heparin and K_2-EDTA plasma.
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.
Urine.
Collect urine without using additives. If urine must be collected with a preservative for other analytes, only hydrochloric acid (14 to 47 mmol/L urine, e.g. 5 mL 10 % HCl or 5 mL 30 % HCl per liter urine) or boric acid (81 mmol/L, e.g. 5 g per liter urine) may be used.

Stability in *serum/plasma:*[14]	7 days at 15-25 °C 7 days at 2-8 °C 3 months at (-15)-(-25) °C
Stability in *urine* (without preservative):[14]	2 days at 15-25 °C 6 days at 2-8 °C 6 months at (-15)-(-25) °C
Stability in *urine* (with preservative):[15]	3 days at 15-25 °C 8 days at 2-8 °C 3 weeks at (-15)-(-25) °C

Centrifuge samples containing precipitates before performing the assay.

Materials provided

See “Reagents - working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section.
General laboratory equipment
Other suitable control material can be used in addition.

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.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 311 **test definition**

Assay type	Rate A	
Reaction time / Assay points	10 / 27-37 - 15-23	
	(STAT 4 / 12-19)	
Wavelength (sub/main)	570/505 nm	
Reaction direction	Increase	
Units	µmol/L (mg/dL, mmol/L)	
Reagent pipetting		Diluent (H_2O)
R1	13 µL	77 µL
R3	17 µL	30 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	10 µL	–	–
Decreased	10 µL	20 µL	80 µL
Increased	10 µL	–	–

Enter the correction value for the non-specific protein reaction as the instrument factor **y = ax + b** for mg/dL or for µmol/L, where **a = 1.0** and **b = -0.3** (mg/dL) or **a = 1.0** and **b = -26** (µmol/L).

cobas c 501/502 **test definition**

Assay type	Rate A	
Reaction time / Assay points	10 / 42-52 - 24-34	
	(STAT 4 / 17-27)	
Wavelength (sub/main)	570/505 nm	
Reaction direction	Increase	
Units	µmol/L (mg/dL, mmol/L)	
Reagent pipetting		Diluent (H_2O)
R1	13 µL	77 µL
R3	17 µL	30 µL

Sample volumes	*Sample*		*Sample dilution*
		Sample	*Diluent (NaCl)*
Normal	10 µL	–	–
Decreased	10 µL	20 µL	80 µL
Increased	10 µL	–	–

Enter the correction value for the non-specific protein reaction as the instrument factor **y = ax + b** for mg/dL or for µmol/L, where **a = 1.0** and **b = -0.3** (mg/dL) or **a = 1.0** and **b = -26** (µmol/L).

Application for urine

cobas c 311 **test definition**

Assay type	Rate A	
Reaction time / Assay points	10 / 27-37 - 15-23	
	(STAT 4 / 12-19)	
Wavelength (sub/main)	570/505 nm	
Reaction direction	Increase	
Units	µmol/L (mg/dL, mmol/L)	
Reagent pipetting		Diluent (H_2O)
R1	13 µL	77 µL
R3	17 µL	30 µL

Sample volumes	*Sample*		*Sample dilution*
		Sample	*Diluent (NaCl)*
Normal	10 µL	6 µL	144 µL
Decreased	10 µL	2 µL	180 µL
Increased	10 µL	10 µL	115 µL

cobas c 501/502 **test definition**

Assay type	Rate A	
Reaction time / Assay points	10 / 42-52 - 24-34	
	(STAT 4 / 17-27)	
Wavelength (sub/main)	570/505 nm	
Reaction direction	Increase	
Units	µmol/L (mg/dL, mmol/L)	
Reagent pipetting		Diluent (H_2O)
R1	13 µL	77 µL
R3	17 µL	30 µL

Sample volumes	*Sample*		*Sample dilution*
		Sample	*Diluent (NaCl)*
Normal	10 µL	6 µL	144 µL
Decreased	10 µL	2 µL	180 µL
Increased	10 µL	10 µL	115 µL

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration
	• after reagent lot change
	• as required following quality control procedures

Traceability: This method has been standardized against ID/MS.
For the USA, this method has been standardized against a primary reference material (SRM 914 and SRM 967 (ID/MS)).

Quality control

For quality control, use control materials as listed in the “Order information” section.
Other suitable control material can be used in addition.
Serum/plasma
For quality control use undiluted serum control material as listed above. Other suitable control material can be used in addition.
Urine
For quality control use Precinorm PUC and Precipath PUC as listed above. Other suitable control material can be used in addition.
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 limits.
Follow the applicable government regulations and local guidelines for quality control.
If controls do not recover within the specified limits, take the following corrective action:

_____

_____

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	µmol/L x 0.0113 = mg/dL
	µmol/L x 0.001 = mmol/L

Limitations – interference

Criterion: Recovery within ± 10 % of initial value at a creatinine concentration of 80 µmol/L (0.90 mg/dL) in serum/plasma and 2500 µmol/L (28.3 mg/dL) in urine.
Serum/plasma
Icterus (*CREJ2*):[16] No significant interference up to an I index of 5 for conjugated bilirubin and 10 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 86 µmol/L (5 mg/dL) and approximate unconjugated bilirubin concentration: 171 µmol/L (10 mg/dL)).
Icterus (*SCRE2*):[16] No significant interference up to an I index of 2 for conjugated bilirubin and 3 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 34 µmol/L (2 mg/dL) and approximate unconjugated bilirubin concentration: 51 µmol/L (3 mg/dL)).

Hemolysis:[16] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).
Lipemia (Intralipid):[16] No significant interference up to an L index of 800. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
Drugs: No interference was found at therapeutic levels using common drug panels.[17,18]
Exception: Cefoxitin causes artificially high creatinine results.
Exception: Cyanokit (Hydroxocobalamin) may cause interference with results.
Values < 15 µmol/L (< 0.17 mg/dL) or negative results are reported in rare cases in children < 3 years and in elderly patients. In such cases use the Creatinine plus test to assay the sample.
Do not use Creatinine Jaffé for the testing of creatinine in hemolyzed samples from neonates, infants or adults with HbF levels ≥ 60 mg/dL for *CREJ2* applications (≥ 30 mg/dL for *SCRE2* applications).[19] In such cases, use the Creatinine plus test (≤ 600 mg/dL HbF) to assay the sample.
Estimation of the Glomerular Filtration Rate (GFR) on the basis of the Schwartz Formula can lead to an overestimation.[20]
In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.
The presence of ketone bodies can cause artificially high results in serum and plasma.
Urine
Icterus: No significant interference up to a conjugated bilirubin concentration of 855 µmol/L (50 mg/dL).
Hemolysis: No significant interference up to a hemoglobin concentration of 621 µmol/L (1000 mg/dL).
Glucose < 120 mmol/L (< 2162 mg/dL) and urobilinogen < 676 µmol/L (< 40 mg/dL) do not interfere.
Drugs: No interference was found at therapeutic levels using common drug panels.[18]
Exception: Cyanokit (Hydroxocobalamin) may cause interference with results.
High homogentisic acid concentrations in urine samples lead to false results.
The presence of ketone bodies can cause artificially high results in urine.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the Carry over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1 + 2/SCCS Method Sheets. For further instructions refer to the operator manual.
cobas c 502 analyzer: All special wash programming necessary for avoiding carry over is available via the **cobas** link, manual input is not required.
Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range
Serum/plasma
15-2200 µmol/L (0.17-24.9 mg/dL)
The technical limit in the instrument setting is defined as 0.47-25.2 mg/dL due to the compensation factor of 0.3.
Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 5.

Urine
375-55000 µmol/L (4.2-622 mg/dL)
Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:3.6 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 3.6.
Lower limits of measurement
Lower detection limit of the test
Serum/plasma
15 µmol/L (0.17 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 three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).
Urine
375 µmol/L (4.2 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 three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

Serum/plasma

Adults[21]		
Females	44-80 µmol/L	(0.50-0.90 mg/dL)
Males	62-106 µmol/L	(0.70-1.20 mg/dL)
Children[22]		
Neonates (premature)	25-91 µmol/L	(0.29-1.04 mg/dL)
Neonates (full term)	21-75 µmol/L	(0.24-0.85 mg/dL)
2-12 m	15-37 µmol/L	(0.17-0.42 mg/dL)
1- < 3 y	21-36 µmol/L	(0.24-0.41 mg/dL)
3- < 5 y	27-42 µmol/L	(0.31-0.47 mg/dL)
5- < 7 y	28-52 µmol/L	(0.32-0.59 mg/dL)
7- < 9 y	35-53 µmol/L	(0.40-0.60 mg/dL)
9- < 11 y	34-65 µmol/L	(0.39-0.73 mg/dL)
11- < 13 y	46-70 µmol/L	(0.53-0.79 mg/dL)
13- < 15 y	50-77 µmol/L	(0.57-0.87 mg/dL)

Urine

1st morning urine[21]		
Females	2470-19200 µmol/L	(28-217 mg/dL)
Males	3450-22900 µmol/L	(39-259 mg/dL)
24-hour urine[23]		
Females	7000-14000 µmol/24 h	(740-1570 mg/24 h)
Males	9000-21000 µmol/24 h	(1040-2350 mg/24 h)
Creatinine clearance[23,24]	71-151 mL/min	

Refer to reference 25 for a prospective study on creatinine clearance in children.[25]
Roche has not evaluated reference ranges in a pediatric population.
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

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

Precision

Precision was determined using human samples and controls in an internal protocol. *Serum/plasma:* repeatability* (n = 21), intermediate precision** (3 aliquots per run, 1 run per day, 21 days); *Urine:* repeatability* (n = 21), intermediate precision** (3 aliquots per run, 1 run per day, 10 days). The following results were obtained:

Serum/plasma (CREJ2)

*Repeatability**	*Mean*	*SD*	*CV*
	µmol/L (mg/dL)	*µmol/L (mg/dL)*	*%*
Precinorm U	105 (1.19)	2 (0.03)	2.1
Precipath U	360 (4.07)	4 (0.05)	1.1
Human serum 1	206 (2.33)	3 (0.03)	1.2
Human serum 2	422 (4.77)	5 (0.06)	1.3

*Intermediate precision***	*Mean*	*SD*	*CV*
	µmol/L (mg/dL)	*µmol/L (mg/dL)*	*%*
Precinorm U	101 (1.14)	4 (0.05)	3.5
Precipath U	351 (3.97)	8 (0.09)	2.2
Human serum 3	201 (2.27)	5 (0.06)	2.5
Human serum 4	411 (4.64)	9 (0.10)	2.2

Urine (CRJ2U)

*Repeatability**	*Mean*	*SD*	*CV*
	µmol/L (mg/dL)	*µmol/L (mg/dL)*	*%*
Control Level 1	8083 (91.3)	115 (1.3)	1.4
Control Level 2	15618 (177)	213 (2)	1.4
Human urine 1	19318 (218)	234 (3)	1.2
Human urine 2	7958 (89.9)	130 (1.5)	1.6

*Intermediate precision***	*Mean*	*SD*	*CV*
	µmol/L (mg/dL)	*µmol/L (mg/dL)*	*%*
Control Level 1	8130 (91.9)	164 (1.9)	2.0
Control Level 2	15533 (176)	251 (3)	1.6
Human urine 3	19353 (219)	385 (4)	2.0
Human urine 4	7932 (89.6)	166 (1.9)	2.1

Serum/plasma (SCRE2)

*Repeatability**	*Mean*	*SD*	*CV*
	µmol/L (mg/dL)	*µmol/L (mg/dL)*	*%*
Precinorm U	106 (1.20)	2 (0.02)	2.2
Precipath U	346 (3.91)	5 (0.06)	1.5
Human serum 1	543 (6.14)	6 (0.07)	1.1
Human serum 2	69 (0.78)	2 (0.02)	3.1

*Intermediate precision***	*Mean*	*SD*	*CV*
	µmol/L (mg/dL)	*µmol/L (mg/dL)*	*%*
Precinorm U	100 (1.13)	4 (0.05)	4.0
Precipath U	334 (3.77)	10 (0.11)	3.0
Human serum 3	522 (5.90)	12 (0.14)	2.4
Human serum 4	64 (0.72)	3 (0.03)	5.0

Urine (SCR2U)

*Repeatability**	*Mean*	*SD*	*CV*
	µmol/L (mg/dL)	*µmol/L (mg/dL)*	*%*
Control Level 1	6287 (71.0)	82 (0.9)	1.2
Control Level 2	15252 (172)	182 (2)	1.2
Human urine 1	24174 (273)	212 (2)	0.9
Human urine 2	2146 (24.2)	48 (0.5)	2.2

*Intermediate precision***	*Mean*	*SD*	*CV*
	µmol/L (mg/dL)	*µmol/L (mg/dL)*	*%*
Control Level 1	6943 (78.5)	114 (1.3)	1.6
Control Level 2	15394 (174)	229 (3)	1.5
Human urine 3	24230 (274)	354 (4)	1.5
Human urine 4	2184 (24.7)	54 (0.6)	2.5

* repeatability = within-run precision

** intermediate precision = total precision / between run precision / between day precision

Method comparison

Creatinine values for human serum, plasma and urine samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined on Roche/Hitachi 917/MODULAR P analyzers (x), using the corresponding Roche/Hitachi reagent.

Serum/plasma (CREJ2)

Sample size (n) = 273

Passing/Bablok[26]	Linear regression
y = 1.000x - 0.653 µmol/L	y = 1.002x - 0.978 µmol/L
τ = 0.973	r = 0.999

The sample concentrations were between 38 and 2178 µmol/L (0.429 and 24.6 mg/dL).

Urine (CRJ2U)
Sample size (n) = 223

Passing/Bablok[26]	Linear regression
y = 0.999x + 20.7 µmol/L	y = 0.999x + 41.5 µmol/L
τ = 0.969	r = 0.999

The sample concentrations were between 934 and 50228 µmol/L (10.6 and 568 mg/dL).

Serum/plasma (SCRE2)
Sample size (n) = 224

Passing/Bablok[26]	Linear regression
y = 1.000x - 14.4 µmol/L	y = 0.996x - 12.2 µmol/L
τ = 0.964	r = 0.999

The sample concentrations were between 66 and 1775 µmol/L (0.746 and 20.1 mg/dL).

Urine (SCR2U)
Sample size (n) = 223

Passing/Bablok[26]	Linear regression
y = 0.999x + 67.8 µmol/L	y = 0.998x + 113 µmol/L
τ = 0.973	r = 0.999

The sample concentrations were between 931 and 48729 µmol/L (10.5 and 551 mg/dL).

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Version 13
2011-08

Reagent manufacturer

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

Source document

Reagent Name: CREJ2
Package Insert Version: 2011-07, V13 English

Application Sheet

cobas®

Laboratory Name

Test Name: Triglycerides

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
			cobas c 311	**cobas c** 501/502
Triglycerides				
250 tests	Cat. No. **20767107** 322	System-ID 07 6710 7	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Precinorm L (4 x 3 mL)	Cat. No. **10781827** 122	Code 304		
Precipath L (4 x 3 mL)	Cat. No. **11285874** 122	Code 305		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

Effective date

Effective date for this procedure: _____

Author

Source documentation compiled by Roche Diagnostics
Revised by: _____

Schedule for review

Last date revised: _____
Date Reviewed: _____ Approved: _____
Date Reviewed: _____ Approved: _____
Date Reviewed: _____ Approved: _____
Date Reviewed: _____ Approved: _____

System information

For **cobas c** 311/501 analyzers:
TRIGL: ACN 781
For **cobas c** 502 analyzer:
TRIGL: ACN 8781

Intended use

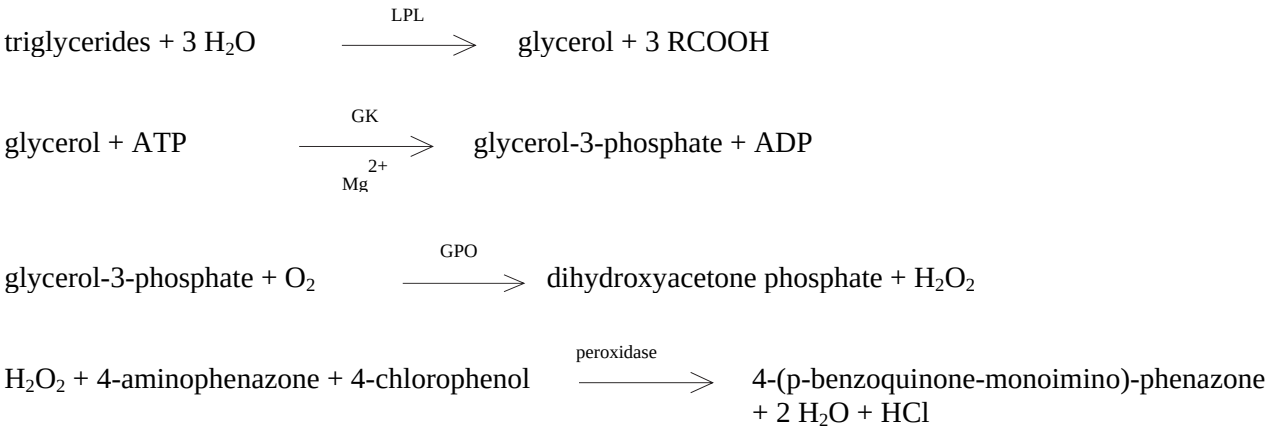
In vitro test for the quantitative determination of triglycerides in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5,6]

Triglycerides are esters of the trihydric alcohol glycerol with 3 long-chain fatty acids. They are partly synthesized in the liver and partly ingested in food.
The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases.
The enzymatic triglycerides assay as described by Eggstein and Kreutz still required saponification with potassium hydroxide. Numerous attempts were subsequently made to replace alkaline saponification by enzymatic hydrolysis with lipase. Bucolo and David tested a lipase/protease mixture; Wahlefeld used an esterase from the liver in combination with a particularly effective lipase from Rhizopus arrhizus for hydrolysis.
This method is based on the work by Wahlefeld using a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder endpoint reaction). The color intensity of the red dyestuff formed is directly proportional to the triglyceride concentration and can be measured photometrically.

Test principle[6]

Enzymatic colorimetric test.



Reagents - working solutions

R1 PIPES buffer: 50 mmol/L, pH 6.8; Mg^{2+}: 40 mmol/L; sodium cholate: 0.20 mmol/L; ATP: ≥ 1.4 mmol/L; 4-aminophenazone: ≥ 0.13 mmol/L; 4-chlorophenol: 4.7 mmol/L; lipoprotein lipase (Pseudomonas spec.): ≥ 83 µkat/L; glycerokinase (Bacillus stearothermophilus): ≥ 3 µkat/L; glycerol phosphate oxidase (E. coli): ≥ 41 µkat/L; peroxidase (horseradish): ≥ 1.6 µkat/L; preservative

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Reagent handling

Ready for use.

Storage and stability

TRIGL

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	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: Li-heparin and K_2-EDTA plasma
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:[7] 5-7 days at 2-8 °C
3 months at (-15)-(-25) °C
several years at (-60)-(-80) °C

Materials provided

See “Reagents - working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section.
General laboratory equipment
Other suitable control material can be used in addition.

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.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 311 **test definition**

Assay type	1 Point		
Reaction time / Assay points	10 / 57		
Wavelength (sub/main)	700/505 nm		
Reaction direction	Increase		
Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	28 µL	

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	4 µL	–	–

cobas c 501/502 **test definition**

Assay type	1 Point		
Reaction time / Assay points	10 / 70		
Wavelength (sub/main)	700/505 nm		
Reaction direction	Increase		
Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	28 µL	

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	4 µL	–	–

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against the ID/MS method.

Quality control

For quality control, use control materials as listed in the “Order information” section.
Other suitable control material can be used in addition.
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 limits.
Follow the applicable government regulations and local guidelines for quality control.
If controls do not recover within the specified limits, take the following corrective action:

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	mmol/L x 88.5 = mg/dL
	mg/dL x 0.0113 = mmol/L

Limitations - interference[8]

Criterion: Recovery within ± 10 % of initial values at triglyceride levels of 2.3 mmol/L (203 mg/dL).
Icterus: No significant interference up to an I index of 10 for conjugated and 35 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 171 µmol/L (10 mg/dL) and approximate unconjugated bilirubin concentration: 599 µmol/L (35 mg/dL)).
Hemolysis: No significant interference up to an H index of 700 (approximate hemoglobin concentration: 434 µmol/L (700 mg/dL)).
Lipemia: The L index correlates with sample turbidity but not with triglycerides level. Extremely lipemic samples (triglycerides greater than 3000 mg/dL) can produce normal results.[9]
Prozone Check: The flag > Kin is an indicator for extremely high triglyceride concentrations in the sample. False normal results are due to oxygen depletion during assay reaction.
Endogenous unesterified glycerol in the sample will falsely elevate serum triglycerides.
Drugs: No interference was found at therapeutic concentrations using common drug panels.[10,11]
Exception: Ascorbic acid and calcium dobesilate cause artificially low triglyceride results. Intralipid is directly measured as analyte in this assay and leads to high triglyceride results.
In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the Carry over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1 + 2/SCCS Method Sheets. For further instructions refer to the operator manual.
cobas c 502 analyzer: All special wash programming necessary for avoiding carry over is available via the **cobas** link, manual input is not required.
Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range
0.1-10.0 mmol/L (8.85-885 mg/dL)
Determine samples having higher concentrations via the rerun function. Recommended dilution of samples via the rerun functions is a 1:5 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 5.
Lower limits of measurement
Lower detection limit of the test
0.1 mmol/L (8.85 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 three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values according to NCEP[12]

Normal range: < 2.26 mmol/L (< 200 mg/dL)
Clinical interpretation according to the recommendations of the European Atherosclerosis Society:[13]

	mmol/L	mg/dL	Lipid metabolism disorder
Cholesterol Triglycerides	< 5.18 < 2.26	< 200 < 200	No
Cholesterol	5.18-7.77	200-300	Yes if HDL-cholesterol < 0.9 mmol/L (< 35 mg/dL)
Cholesterol Triglycerides	> 7.77 > 2.26	> 300 > 200	Yes

Note: If the free glycerol is to be taken into account, then 0.11 mmol/L (10 mg/dL) must be subtracted from the triglycerides value obtained.[7]
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

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

Precision

Precision was determined using human samples and controls in an internal protocol. Repeatability* (n = 21), intermediate precision** (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

*Repeatability**	*Mean* *mmol/L (mg/dL)*	*SD* *mmol/L (mg/dL)*	*CV* *%*
Precinorm U	1.41 (125)	0.01 (1)	0.9
Precipath U	2.40 (212)	0.02 (2)	0.8
Human serum 1	1.67 (148)	0.02 (2)	1.1
Human serum 2	2.72 (241)	0.02 (2)	0.7

*Intermediate precision***	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	1.39 (123)	0.03 (3)	2.0
Precipath U	2.33 (206)	0.04 (4)	1.6
Human serum 3	1.18 (104)	0.02 (2)	1.9
Human serum 4	2.95 (261)	0.05 (4)	1.8

* repeatability = within-run precision

** intermediate precision = total precision / between run precision / between day precision

Method comparison

Triglycerides values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 71

Passing/Bablok[14]	Linear regression
y = 1.015x - 0.005 mmol/L	y = 1.001x + 0.018 mmol/L
τ = 0.976	r = 0.999

The sample concentrations were between 0.560 and 9.13 mmol/L (49.6 and 808 mg/dL).

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Version 6
2010-06

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Source document

Reagent Name: TRIGL
Package Insert Version: 2010-03, V6 English