



## Application Note Guideline for Determination of Lambda Light Chain in Urine on Roche Cobas c501

### General information

<b>Intended use</b>	The Application Note is intended for the quantitative determination of Lambda light chain in human urine sample material by turbidimetry on Roche Cobas c501 (1, 2).																				
<b>Measuring range</b>	Approximately 3-150 mg/L depending on the specific lot of the calibrator.																				
<b>Reference interval</b>	It is recommended to determine the reference interval for the local population.																				
<b>Instrument settings</b>	Instrument programming is performed according to "Instrument Settings" on page 2.																				
<b>Reagents</b>	<table border="0"> <thead> <tr> <th></th> <th><b>Code</b></th> <th><b>Name</b></th> </tr> </thead> <tbody> <tr> <td><u>Antibody</u></td> <td>Q0499</td> <td>Polyclonal Rabbit Anti-Human Lambda Light Chains</td> </tr> <tr> <td><u>Reaction buffer</u></td> <td>S2007</td> <td>Dako Turbidimetry/Nephelometry Reaction Buffer 1</td> </tr> <tr> <td><u>Diluent</u></td> <td>S2005</td> <td>Dako Turbidimetry/Nephelometry Dilution Buffer 1</td> </tr> <tr> <td><u>Calibrator</u></td> <td>X0908</td> <td>Human Serum Protein Calibrator</td> </tr> <tr> <td><u>Control</u></td> <td>X0940</td> <td>Human Serum Protein High Control</td> </tr> </tbody> </table>		<b>Code</b>	<b>Name</b>	<u>Antibody</u>	Q0499	Polyclonal Rabbit Anti-Human Lambda Light Chains	<u>Reaction buffer</u>	S2007	Dako Turbidimetry/Nephelometry Reaction Buffer 1	<u>Diluent</u>	S2005	Dako Turbidimetry/Nephelometry Dilution Buffer 1	<u>Calibrator</u>	X0908	Human Serum Protein Calibrator	<u>Control</u>	X0940	Human Serum Protein High Control		
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<u>Calibrator</u>	X0908	Human Serum Protein Calibrator																			
<u>Control</u>	X0940	Human Serum Protein High Control																			
<b>Samples</b>	Centrifuged human urine. Stable for 10 days at 2-8 °C. It is not recommended to freeze the samples before analysis.																				
<b>Calibrator</b>	Predilute the Dako Human Serum Protein Calibrator, Code X0908, 1:3 (e.g. 200 µL calibrator + 400 µL diluent). The final dilution of standards is performed automatically by the instrument.																				
<b>Control</b>	Predilute the Dako Human Serum Protein High Control, Code X0940 1:40 (e.g. 50 µL control + 1950 µL diluents) respectively 1:400 (e.g. 25 µL control + 9975 µL diluents) as control of the calibration curve.																				
<b>Reaction buffer (R1)</b>	The reaction buffer is ready for use. On board stability is 30 days.																				
<b>Antibody (R2)</b>	<p>The antibody is ready for use. If in rare cases the antibody appears slightly turbid, filtration through a 0.22 µm membrane filter is recommended. Stability at 2-8 °C: See specification sheet and expiry on the label. On board stability is 30 days.</p> <p><u>Capacity:</u> 1 mL of Q0499 is equivalent to approximately 50 cuvette readings of standards or samples. The dead volume of the reagent bottle should be added when calculating the required amount of reagent.</p>																				
<b>Calibration stability</b>	It is recommended to recalibrate every 30th day or when reagent lots change, when the antibody is filtered, or quality control results fall outside the range as established by the individual laboratory.																				
<b>Trouble shooting</b>	If performance is unacceptable, try to recalibrate. Check reagents and procedure. If the problem persists, please contact instrument supplier or Dako Technical Support.																				

**Performance Data**

**Sensitivity** An OD value of approximately 0.65 on Roche Cobas c501 corresponds to a concentration around 150 mg/L Lambda Light Chain.

**Detection limit** The detection limit is estimated to 2.4 mg/L.

**Precision** The precision was estimated by using three urine Lambda Light Chain levels, analysis of 5 runs and 3 determinations in each run. (CLSI guidelines EP15-A2).

Samples	Lambda Light Chain Mean value (mg/L)	Standard deviation (mg/L)			Total CV (%)	n
		Within run	Between run	Total		
Low human urine sample	24	0.45	0.38	0.53	2.2	15
Medium human urine sample	77	0.86	0.94	1.17	1.5	15
High human urine sample	133	1.32	2.32	2.55	1.9	15

**Accuracy** A recovery of Lambda Light Chain of 85–115% can be expected for Dako Human Serum Protein High Control, Code X0940, diluted 1:40 and 1:400.

**Linearity** The assay is linear in the range 5-150 mg/L.

**Security range** No antigen excess is found for Lambda Light Chain concentrations below 14960 mg/L (the highest concentration tested).

**Interference** No interference is found at concentrations up to 2.0 g/L of hemoglobin.

**Method comparison** Determinations of Lambda Light Chain according to this Application Note was compared with other commercial turbidimetric assays.

- References**
1. Bliup-Jensen S. Protein Standardization III: Method optimization: Basic principles for quantitative determination of human serum proteins on automated instruments based on turbidimetry or nephelometry. Clin Chem Lab Med 2001; 39:1098-1109.
  2. Roche Cobas c501 manual(s).

### Instrument Settings

<b>Analyze</b>	Assay/Time/Point	2 Point End 10 9 70 0 0					
	Wavelength (2nd/Primary)	Cancel 415					
	Sample Volume	Cassette configuration					
	Normal	15	0	0	Code XXXX*		
	Decrease	16	15	104	Expiration date 99		
	Increase	15	0	0	Reagent volumen		
	Diluent	<input checked="" type="radio"/> Water <input checked="" type="radio"/> Diluent 952* 1					
	Linearity Limit	0%	0%	0	0	R1 180 0 Inactive	
	Prozone Limit	0	0	0	0	0	inside 0 0
	Abs. Limit	32000		Increase		R2 20 20	
Cell Detergent	Detergent 1		Stirring level 1		R3 0 0		
Stirring settings	UP <input type="text" value="stirring"/> LOW <input type="text" value="stirring"/> <input type="text" value="stirring"/> <input type="text" value="stirring"/>						
<b>Calib.</b>	Calibration Type	Spline				Auto Calibration	
	Point	6				Timeout	
	Span	4				Cassette	
	Weight	0				Cancel 0 Day	
	Update Type	None 0 0				Change over cassette	
	SD Limit	999				QC Violation	
	Duplicate Limit	10	100	Abs		Cancel	
	Sensitivity Limit	-99999		99999		Method	
	S1 Abs. Limit	-32000		32000		Blank	
						Rule 1s	
					Control 1 None		
					Control 2 None		
					Control3 None		
<b>Range</b>	Application Code	901*				Expected Value	
	Unit	mg/L				Male	
	Report Name	U-Lam				99 Year -99999 999999	
	Dato Mode	Active				100 Year -99999 999999	
	Automatic Rerun					-99999 999999	
	Technical Limit	3	150	Female			
	Repeat Limit	-99999	99999	99 Year -99999 999999			
	Control interval Time	0				100 Year -99999 999999	
	Automatic Qc on board Dtability	1				-99999 999999	
	Qualitative	<input type="checkbox"/> 1 <input type="text" value="0"/> <input type="checkbox"/> 2 <input type="text" value="0"/> <input type="checkbox"/> 3 <input type="text" value="0"/> <input type="checkbox"/> 4 <input type="text" value="0"/> <input type="checkbox"/> 5 <input type="text" value="0"/> <input type="checkbox"/> 6 <input type="text" value="0"/>				Default	
	<input type="checkbox"/> L <input type="text" value="0"/> <input type="checkbox"/> H <input type="text" value="0"/> <input type="checkbox"/> I <input type="text" value="0"/>				Sex		
					<input type="radio"/> Male <input type="radio"/> Female		
					Range		
					<input type="radio"/> Range 1 <input type="radio"/> Range 2 <input type="radio"/> Range 3		
<b>Others</b>	Standard						
	Calibration Code*	801	803	803	803	803	803
	Concentration**	Cal mg/L x 0	Cal mg/L x 0,0071	Cal mg/L x 0,0180	Cal mg/L x 0,0357	Cal mg/L x 0,0714	Cal mg/L x 0,1061
	Rack No. - Pos.*	S00009-1	S0009-3	S0009-3	S0009-3	S0009-3	S0009-3
	Sample volume	15	3	8	15	30	35
	Diluted S. Volume	0	15	15	15	15	15
	Diluent Volume	0	138	140	125	110	75

Diluent – Code S2005 R1 – Code S2007 R2 – Code Q0499.

[1] Defined by the customer.

[\*\*] Calibrator, Code X0908. The calibrator is prediluted 1:3. The concentration is calculated as the factor times the calibrator value in mg/L for the specific lot (Cal mg/L), stated in the X0908 Analytical Value Sheet.

