PLAC® Test Reagent Kit

Roche Cobas C 501 Chemistry Analyzer Application Sheet

PLAC® Test for Lp-PLA$_2$

This procedure is to be used in conjunction with the package insert for the PLAC Test Reagent Kit. Read and follow the package insert instructions and the Specimen Handling Best Practices carefully. Failure to follow the instructions may result in inaccurate results. Reagent barcode labels are not provided. For ordering information, contact Customer Service at 1-877-752-2837 or visit www.plactest.com. For further information, see the analyzer operator's manual.

PROCEDURE:

1. Program a user defined test with the parameters listed below. For more detailed instructions refer to the Roche Cobas C 501 User Instructions.
2. Transfer PLAC Test reagents into the appropriate reagent cartridge. Use Roche supplied evaporation caps to provide best on-board stability.
3. Load the cartridge as described in the User Operating Instructions.
4. Calibrate with PLAC calibrator set (REF 90108 or REF 10-0108).

NOTE:

- Do not report samples with results greater than 360 ng/mL.
- Do not dilute and retest.
- See PLAC Test turbidimetric immunoassay method package insert for further information. Refer to the sections: *Warnings & Precautions, Procedural Notes and Limitations.*

USER DEFINED PARAMETERS:

Analyze | **Calib.** | **Range** | **Other**

Assay / Time / Point	2Point End	10	38	70	0	0
Wavelength (2nd / Pril.)	800	570				

Sample Volume

Norm.	4.8	0.0	0
Dec.	1.6	0.0	0
Inc.	4.8	0.0	0

Cassette Configuration

Code	########
Expiration Days	*1

Reagent Volume

R1	148	0	Inactive
R2	0	0	Inactive
R3	48	0	Inactive

Dilution

- Water
- Diluent

Linearity Limit		%		%					
Prozone Limit								Inside		
Abs. Limit	32000		Increase						
Cell Detergent	*2			Stirring Level	8				

Stirring Setting		M1	M2	M3
UP	Stirring	LOW	Stirring	Stirring	Stirring

*1: User Defined
*2: Alkaline detergent

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Analyze | **Calib.** | **Range** | **Other**

Calibration Type	Spline		
Point	5		
Span	5		
Weight	0		
Update Type	None	0	0

SD Limit	50			
Duplicate Limit	99	%	32000	Abs.
Sensitivity Limit	-99999		99999	
S1 Abs. Limit	-32000		32000	

☐ Auto Masking

Auto Calibration

○ Timeout

Cassette: Cancel; 0 Day

Changeover

Cassette: Cancel

○ QC Violation

Method	Blank
Rule	1s
Control 1	None
Control 2	None
Control 3	None

Analyze | **Calib.** | **Range** | **Other**

Application Code	###
Unit	ng/mL
Report Name	PLAC
Data Mode	Active
☐ Automatic Return	
Technical Limit	-9999 / 500
Report Limit	-9999 / 99999
☐ Control Interval Time	0
☐ Automatic QC On Board Stability	1

Expected Values

Male

		-99999	999999
99	Year	-99999	999999
100	Year	-99999	999999

Female

		-99999	999999
99	Year	-99999	999999
100	Year	-99999	999999

☐ Qualitative

(1)	0	L	0
(2)	0	H	0
(3)	0	I	0
(4)	0		
(5)	0		
(6)			

Default

Sex: ☐ Male ☐ Female

Range: ☐ Range 1 ☐ Range 2 ☐ Range 3

Analyze | **Calib.** | **Range** | **Other**

Standards

	(1)	(2)	(3)	(4)	(5)	(6)
Calibrator Code	*1	*1	*1	*1	*1	
Concentration	0.0	50.0	100.0	250.0	500.0	
Rack No.-Pos	*1	*1	*1	*1	*1	
Sample Volume	4.8	4.8	4.8	4.8	4.8	
Diluted S. Volume	0.0	0.0	0.0	0.0	0.0	0.0
Diluent Volume	0	0	0	0	0	0

*1: User Defined. Initial default parameters have been entered where appropriate.

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PERFORMANCE CHARACTERISTICS

Performance characteristics were established using the Roche Cobas C 501 chemistry analyzer.

On-board Analyzer Reagent Storage

Open bottles of reagents stored in the refrigerated compartment on the Cobas C501 analyzer should be stable up to 2 weeks. Use the reagent evaporation caps provided by Roche to give the best on-board stability. Laboratories should verify on-board stability on their own analyzers, under typical laboratory conditions.

Sensitivity

The clinical sensitivity of the assay is 12 ng/mL as determined by the limit of quantitation (the lowest concentration with acceptable precision).

The analytical sensitivity of the assay is 4 ng/mL, as calculated by interpolation of the mean plus two standard deviations of 20 replicates of the 0 ng/mL Lp-PLA$_2$ calibrator from the standard curve.

Assay Precision

Intra-assay and inter-assay variability were determined by testing two human serum samples with Lp-PLA$_2$ concentrations distributed throughout the calibration range of the assay. The data are summarized below:

Sample	Intra-assay		Inter-assay	
	Mean Concentration Lp-PLA$_2$ (ng/mL)	% CV (n=20)	Mean Concentration Lp-PLA$_2$ (ng/mL)	% CV (n=20)
Low Serum	189	3.1	150	6.0
High Serum	398	1.5	327	2.4

Linearity

From three pairs of serum samples with known high or low Lp-PLA$_2$ levels, a dilution series was prepared for each pair combination to assess linearity. Percent recoveries of the combined samples were determined as the measured value divided by the expected value, multiplied by 100. The average recovery was 100%, demonstrating linearity of the diluted samples over a range of 87 to 473 ng/mL Lp-PLA$_2$.

Interfering Substances

Endogenous substances found in blood were evaluated for interference in the assay. Four individual serum samples with Lp-PLA$_2$ values ranging from 276 to 441 ng/mL were spiked with potential interferents. No appreciable interference was observed for the following substances at the spiked levels tested.

Endogenous	
Potential Interferent	Test Concentration
Bilirubin	20 mg/dL
Cholesterol	500 mg/dL
Hemoglobin	500 mg/dL
Triglycerides	3000 mg/dL
Total Albumin*	~9000 mg/dL
* 5 g/dL albumin added to plasma pool of presumptively 4 g/dL albumin	

Method Comparison

Correlation studies were performed comparing the PLAC Test turbidimetric immunoassay to the values established on the Hitachi 917 and Olympus AU-400. Human serum samples with Lp-PLA$_2$ concentrations ranging from 117 to 481 ng/mL were tested. Results from linear regression analysis are shown below.

Linear Regression

Slope = 1.03
y-intercept = 18.5
Correlation coefficient r = 0.98
Number of samples = 30

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