TPLA2

SEKISUI

Mediace TPLA Gen.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
07404182 190	Mediace TPLA Gen.2 (250 tests)	System-ID 07 7590 8	Roche/Hitachi **cobas c** 501/502
07404085 190	Mediace TPLA Gen.2 Calibrator Set (5 × 2 mL)	Codes 770-774	
07404077 190	Mediace TPLA Gen.2 Control Set Control Level A (1 × 3 mL) Control Level B (1 × 3 mL)	 Code 133 Code 134	
04489357 190	Diluent NaCl 9 % (50 mL)*	System-ID 07 6869 3	

*provided by Roche Diagnostics

English

System information

For **cobas c** 501 analyzer:

TPLA2: ACN 507

For **cobas c** 502 analyzer:

TPLA2: ACN 8507

Intended use

Mediace TPLA Gen.2 is an immunoturbidimetric assay for the quantitative in vitro determination of anti-Treponema pallidum antibodies in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4]

The presence of anti Treponemal antibodies in serum or plasma can be used together with results from other methods and clinical findings to help the clinician in the diagnosis of syphilis infections.

This automated assay is based on the immunological agglutination test principle using latex as reaction enhancer.

Test principle

Immunoturbidimetric assay.

- Sample and addition of R1 (buffer)
- Addition of R3 (antigen-coated latex) and start of reaction:

  Polystyrene latex particles coated with antigen derived from Treponema pallidum (Nichols strain) react to anti-Treponemal antibodies in serum or plasma to form an agglutinate. This agglutination results in an increase in turbidity of the reactant mixture which can be measured as absorbance at 700 nm using a photometer. The titre of the anti-Treponema antibodies in the sample can be determined by measuring the turbidity at two different intervals after commencing the reaction.

Reagents - working solutions

R1 Phosphate buffer: 100 mmol/L, pH 7.2-7.4; BSA; stabilizers and preservatives

R3 Phosphate buffer: 100 mmol/L, pH 7.2-7.5; latex particles coated with Treponema pallidum-derived antigen: 1.5-3.5 mg/mL; BSA; preservatives

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Both reagents contain < 0.1 % sodium azide as a preservative. Sodium azide may react with lead and copper plumbing to form potentially explosive metal azide buildup. Flush with copious amounts of water when discarding material.
Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed. Avoid the formation of foam.

Storage and stability

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

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: Fresh, clear serum and serum from gel separation tubes.
Plasma: Fresh clear Li-heparin and K_2-EDTA plasma from gel separation tubes.

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 for serum and plasma:	7 days at 2-8 °C[4,5]
	1 day at 15-25 °C[5]
	4 weeks at (-15)-(-25) °C[5]

Thawed specimens may not be refrozen.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

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 Sekisui is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 501/502 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10/42-70	
Wavelength (sub/main)	– /700 nm	
Reaction direction	Increase	
Units	T.U. (titre units)	
Reagent pipetting		Diluent (H_2O)
R1	140 µL	–
R3	20 µL	–
Sample volumes	*Sample*	*Sample dilution*

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		Sample	*Diluent (NaCl)*
Normal	13 µL	–	–
Decreased	13 µL	15 µL	135 µL
Increased	13 µL	–	–

Note

For technical reasons it is necessary to specify a dummy unit (mg/dL) on **cobas c** *analyzers. Values can be converted to the correct unit (T.U.) via the host computer.*

Calibration

Calibrators	S1: TPLA2 Calibrator 1 (negative) S2-5: TPLA2 Calibrators 2-5 (positive)
Calibration mode	Spline
Calibration frequency	Full calibration ▪ after 2 weeks ▪ after **cobas c** pack change ▪ as required following quality control procedures

Recovery from TPLA2 controls having known values will be within ± 20 %.

Traceability: This method has been standardized against an in-house standard.

Quality control

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

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

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

Calculation

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

Limitations - interference

Criterion: Recovery within ± 15 % of initial value.

Icterus:[5,6] No significant interference up to an I index of 19 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 325 µmol/L or 19 mg/dL).

Hemolysis:[5,6] No significant interference up to an H index of 490 (approximate hemoglobin concentration: 304 µmol/L or 490 mg/dL).

Lipemia:[5,6] No significant interference up to an L index of 100. Samples exceeding an L index of 100 should be centrifuged at 15000 g for 10 minutes before measurement. There is poor correlation between the L-index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: no significant interference up to 500 IU/mL.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[7]

A high-dose hook effect may occur at analyte levels above 725 T.U.

A negative result that does not match the clinical signs may occur when there is a very high antibody level in the serum (Prozone effect). In such cases the sample may be diluted with 0.9 % NaCl solution and rerun to permit correct measurement. False-positive results can be caused by a non-specific reaction to the reagent. This may be seen in some patients with auto-immune diseases. Similar false-positive results can occur in patients who have received blood products containing immunoglobulins.

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

Carry-over

Samples which are to be analyzed for other infectious diseases should first be measured on the Elecsys system.

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-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's 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

4.6-250 T.U.

For the quantitative application, samples over 150 T.U. should be rerun after dilution because of a possible high-dose hook effect.

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 using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Lower detection limit of the test:
4.6 T.U.

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero.

When run on the Roche/Hitachi **cobas c** 501/502 analyzers, a negative serum will result in an optical density change of less than 0.01 absorbance units. A positive serum containing 450 T.U. will cause an optical density change within the range 0.10-0.70 absorbance units.

Expected values[2]

Results are expressed in titre units (T.U.). Please note that T.U. is based on the Treponema pallidum haemagglutination assay (TPHA): 1280 T.U. is equivalent to titre of 1:1280 TPHA.

A result of 10 T.U. or more is considered positive. Such results should be judged in relation to other clinical signs. Determinations yielding a positive result should be repeated on a fresh sample at a later date. A positive result with Mediace TPLA assay should be confirmed by further serological tests, including for example, Tp immunoblot or FTA-ABS test, and a quantitative cardiolipin test should also be performed. If any further serological tests show positive results and early infection is suspected, the serum should be tested for Treponema-specific IgM antibodies. Some patients who are infected may test negative, especially in the early stage of infection or where there is an immunodeficiency.

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 with repeatability (n = 10) and intermediate precision (duplicate analysis per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	*Mean*	*SD*	*CV*
	T.U.	*T.U.*	*%*
Human serum 1	0.0	0.0	-
Human serum 2	30.1	0.7	2.4
Human serum 3	87.1	1.3	1.5
Human serum 4	192	5.5	2.7

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Intermediate precision	*Mean*	*SD*	*CV*
	T.U.	*T.U.*	*%*
Human serum	0.0	0.0	-
TPLA2 Control Level A	27.1	0.9	3.4
TPLA2 Control Level B	80.3	3.6	4.5

A positive control measured 42 times gave a CV of ≤ 15 %.

Method comparison

TPLA values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer using the Mediace TPLA2 reagent (y) were compared with those determined using the Mediace TPLA reagent on a Roche/Hitachi **cobas c** 501 analyzer (x).

Sample size (n) = 54

y = 1.05x - 2.59

r = 0.997

Values were between 6.9 and 191.7 T.U.

Analytical specificity

When control serum of a known concentration is measured, all of the measured values of the positive control serum will be equal to or above 10 T.U. In addition, all the measured values of the negative control serum will be less than 10 T.U.

229 samples containing potentially interfering substances were tested with the Mediace TPLA assay, comprising specimens:

- containing antinuclear antibody (ANA), elevated titres of rheumatoid factor[5]
- from patients with collagenosis, patients undergoing dialysis[4]
- from pregnant women[4]

No false-positive results were found.

Clinical sensitivity[1,2,3]

A total of 268 selected confirmed syphilis-positive samples in various stages of the disease were tested. The sensitivity with these samples was 100 %.

Syphilis-confirmed positive samples, n = 268

Mediace TPLA	Positive	268
	Negative	0

Clinical specificity[1,2,3,4,5]

A total of 3427 syphilis-negative samples were tested. The specificity with these samples was 99.6 %.

Syphilis-negative samples, n = 3427

Mediace TPLA	Positive	13
	Negative	3414

References

1 Osato K, Nagao T, Inuzumi K, et al. Clinical Evaluation of Latex Agglutination Test Kits for Detecting Anti-syphilitic Lipoidal Antibodies and Anti-treponemal Antibodies. Japanese Journal of Sexually Transmitted Diseases 2002:13(1):124-130.

2 Osato K, Matsubayashi T, Nagao T, et al. Clinical Evaluation of Automated Latex Agglutination Test Kits (TPLA) for Syphilis Diagnosis. The Journal of Clinical Laboratory Instruments and Reagents 1991;14(4):739-743.

3 Kataniwa Y, Nakano M. Clinical Evaluation of Latex Reagent Semedia TPLA for Diagnosis of Syphilis. The Journal of Clinical Laboratory Instruments and Reagents 1991;14(4):735-740.

4 Shibazaki M, Ieiri T. An Automated Measurement of Anti_Treponema Antibody Titer by MEDIACE TPLA, a Latex Agglutination Test using Hitachi 7170 Automatic Analyzer. The Journal of Clinical Laboratory Instruments and Reagents 1996;19(4):635-641.

5 Data on file at Sekisui Medical Co., Ltd.

6 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

7 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

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

Symbols

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

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

Sekisui Marketing Approval No. (Japan): 20900AMZ00373000.

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