RPR2

SEKISUI

Mediace RPR Gen.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
07404174 190	Mediace RPR Gen.2 (250 tests)	System-ID 07 7588 6	Roche/Hitachi **cobas c** 501/502
04955170 190	RPR Calibrator Set (5 × 1 mL)	Codes 931-935	
04955196 190	RPR Control Set Positive control (2 × 1 mL) Negative control (2 × 1 mL)	 Code 136 Code 135	
04489357 190	Diluent NaCl 9 % (50 mL)*	System-ID 07 6869 3	

*provided by Roche Diagnostics

English

System information

For **cobas c** 501 analyzer:

RPR2: ACN 453

For **cobas c** 502 analyzer:

RPR2: ACN 8453

Intended use

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

Summary[1,2]

The presence of syphilitic anti-lipid antibodies in serum and plasma can be used together with results from other methods and clinical findings to help the clinician in the diagnosis and follow-up 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 lipid antigens (cardiolipin and lecithin) react to syphilitic anti-lipid 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-lipid 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: 28.8 mmol/L, pH 7.1-7.5; BSA; stabilizers and preservatives

R3 Phosphate buffer: 100 mmol/L, pH 7.1-7.5; latex particles coated with coated with cardiolipin and lecithin antigens: 1.0-4.0 mg/mL; stabilizers; 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[3]	7 days at 2-8 °C
	1 day at 15-25 °C
	4 weeks at (–15)-(–25) °C
Stability for plasma[3]	7 days at 2-8 °C
	1 day at 15-25 °C
	7 days at (–15)-(–25) °C

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	R.U. (RPR units)
Reagent pipetting	Diluent (H_2O)

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R1	118 µL	20 µL	
R3	43 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	14 µL	–	–
Decreased	14 µL	10 µL	90 µL
Increased	14 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 (R.U.) via the host computer.*

Calibration

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

Recovery from negative RPR control will be ≤ 0.4 R.U.

Recovery from positive RPR 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:[3,4] 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:[3,4] No significant interference up to an H index of 490 (approximate hemoglobin concentration: 304 µmol/L or 490 mg/dL).

Lipemia:[3,4] No significant interference up to an L index of 50. Samples exceeding an L index of 50 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.[5]

A high-dose hook effect may occur at analyte levels above 14 R.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 and pregnant women. 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.

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

0.5-8.0 R.U.

For the quantitative application, samples over 4.0 R.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:
0.5 R.U.

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 2.6 standard deviations above that of the lowest calibrator (calibrator 1, n = 10).

When run on the Roche/Hitachi **cobas c** 501/502 analyzers, the difference between the turbidity change for 1 R.U. and the turbidity change for the reagent blank will be equal to or more than 0.0100 absorbance units.

Expected values

Results are expressed in RPR units (R.U.). Please note that R.U. is based on the WHO IU (International Standard for Syphilitic Human Serum): 1 R.U. = 0.4 IU. 1 R.U. is equivalent to a 1-fold RPR card test titre. A result of 1 R.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. 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* *R.U.*	*SD* *R.U.*	*CV* *%*
Human serum 1	0.0	0.0	-
Human serum 2	1.2	0.05	4.2
Human serum 3	4.1	0.04	1.0
Human serum 4	5.8	0.09	1.6
Intermediate precision	*Mean* *R.U.*	*SD* *R.U.*	*CV* *%*
PRP Control negative	0.0	0.0	-
RPR Control positive	2.3	0.1	3.5

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

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Method comparison

RPR values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer using the Mediace RPR2 reagent (y) were compared with those determined using the Mediace RPR reagent on a Roche/Hitachi **cobas c** 501 analyzer (x).

Sample size (n) = 50

y = 0.95x + 0.30

r = 0.997

Values were between 0.6 and 8.0 R.U.

Analytical specificity

Recovery from RPR control having known values will be within ± 20 %.

185 samples containing potentially interfering substances were tested with the Mediace RPR assay, comprising specimens:

- containing autoantibodies (antinuclear antibody - ANA, anti-dsDNA antibody, anti-ssDNA antibody, elevated titres of rheumatoid factor)[6], from patients with collagenosis[3]
- from patients with collagenosis, patients undergoing dialysis[3]
- from pregnant women[3]

Biological false-positive (BFP) results were found with 5/109 specimens containing autoantibodies and from collagenosis patients.

False-positive values were found with 3/150 specimens from patients undergoing dialysis. An influence of fibrinogen on Mediace RPR was reported.[7]

Clinical sensitivity[1,2]

A total of 187 selected confirmed syphilis-positive samples in various stages of the disease were tested. The sensitivity with these samples was 99.5 %.

Syphilis-confirmed positive samples, n = 187

Mediace RPR	Positive	186
	Negative	1

Clinical specificity[6]

A total of 2639 syphilis-negative samples were tested. The specificity with these samples was 99.5 %.

Syphilis-negative samples, n = 2639

Mediace RPR	Positive	12
	Negative	2627

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 Kawai K, Osato K. The possibility of assessing the stage of infection by using Sekisui's Automated TPLA and RPR. The Journal of Clinical Laboratory Instruments and Reagents. 2003;26(4):301-304.

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

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

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

6 Kinyo T, Nago T, Sakiyama K, et al. Laboratory-based evaluation of Latex Agglutination Turbidimetric Assay by Mediace RPR on P Module of Hitachi Autoanalyzer 7600 to Quantitatively Determine Serum RPR Antibody. Japanese Journal of Clinical Laboratory Automation (JJCLA) 2005;30(3):257-262.

7 Noda M, Abe Y, Kasai C, et al. Evaluation of reagents for measurement of Anti-Treponema Pallidum and Anti-Lipoidal antibodies in serum using Latex agglutination immunoassay on an automated analyzer. Japanese Journal of Medical Technology 2003;52(10):1279-1282.

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): 21200AMZ00424000.

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