

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
04340892 190	Cyclosporine II (100 tests)	System-ID 07 6822 7 COBAS INTEGRA 400 plus COBAS INTEGRA 800
20766305 122	Cyclosporine Calibrators 0 ng/mL (1 × 2.5 mL) 50, 100, 200, 350, 500 ng/mL (5 × 2.0 mL)	System-ID 07 6630 5
20766399 122	Cyclosporine Calibrator 0 ng/mL (3 × 2.5 mL)	System-ID 07 6639 9
20766364 122	COBAS INTEGRA Cyclosporine Sample Pretreatment Reagent (4 × 40 mL)	System-ID 07 6636 4

English

System information

Test CSAII, test ID 0-007

Intended use

In vitro diagnostic test for the quantitative determination of cyclosporine A in human whole blood as an aid in the management of cyclosporine therapy in transplant patients.

Summary

Cyclosporine is a cyclic undecapeptide of fungal origin and a potent immunosuppressive agent. Since its introduction in 1983, cyclosporine has substantially improved patient and graft survival in patients receiving heart, kidney, liver, pancreas, or lung transplants. Many studies have documented the effect of cyclosporine in combating organ rejection. Inadequate cyclosporine doses and levels may result in rejection of the transplanted organ. Toxic levels of cyclosporine are associated with many serious side effects, including nephrotoxicity, hepatotoxicity, and a range of other complications. Physicians are particularly concerned with the nephrotoxic effects of the drug in renal transplantation because of the difficulty in distinguishing between organ rejection and cyclosporine toxicity.^{1,2}

Monitoring parent drug cyclosporine concentrations in whole blood and interpreting these concentrations in conjunction with other laboratory data and clinical considerations is the most effective means of ensuring adequate immunosuppressant therapy for recipients of solid-organ transplants. Whole blood, rather than plasma, is the matrix of choice for the measurement of cyclosporine since the drug is rapidly distributed into the red blood cells. Cyclosporine concentrations should be measured using a method that is specific for the parent drug because the contribution of the more than 30 cyclosporine metabolites to immunosuppression or toxicity remains uncertain.

The methods historically used to monitor cyclosporine concentrations in blood include high performance liquid chromatography (HPLC), radioimmunoassay (RIA), and fluorescence polarization immunoassays (FPIA).

Test principle

Enzyme Multiplied Immunoassay Technique (EMIT)

The COBAS INTEGRA Cyclosporine II reagent is a homogeneous enzyme immunoassay technique used for the analysis of cyclosporine in whole blood. The assay contains mouse monoclonal antibodies with a high specificity for cyclosporine.

The COBAS INTEGRA Cyclosporine II reagent is based on competition for cyclosporine antibody binding sites.

Cyclosporine in the sample competes with cyclosporine in the Enzyme reagent that is labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH). Active (unbound) enzyme converts oxidized nicotinamide adenine dinucleotide (NAD) in the Antibody reagent to NADH, resulting in a kinetic absorbance change that can be measured spectrophotometrically. Enzyme activity decreases upon binding to the antibody, allowing the cyclosporine concentration in the sample to be measured in terms of enzyme activity. Endogenous serum G6PDH does not interfere because the co-enzyme NAD functions only with the bacterial (*Leuconostoc mesenteroides*) enzyme employed in the assay.

Before testing with the COBAS INTEGRA Cyclosporine II assay, samples, calibrators, and controls must be pretreated with the COBAS INTEGRA Cyclosporine Sample Pretreatment Reagent. The reagent lyses the cells, extracts the cyclosporine, and precipitates blood proteins. The pretreated samples are centrifuged, and an aliquot of the resulting supernatant

containing cyclosporine is then assayed using the COBAS INTEGRA Cyclosporine II assay.

Reagents - working solutions

R1 Antibody reagent

Anti-cyclosporine monoclonal antibodies (mouse), nicotinamide adenine dinucleotide, glucose-6-phosphate, sodium chloride, bulking agent, surfactant, and preservatives including 0.1 % sodium azide and 0.005 % streptomycin sulfate.

SR Enzyme reagent

Cyclosporine labeled with bacterial (*Leuconostoc mesenteroides*) glucose-6-phosphate dehydrogenase, TRIS buffer, bulking agents, stabilizers, and preservatives including 0.1 % sodium azide and 0.005 % streptomycin sulfate.

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

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: For prescription use only.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C See expiration date on **cobas c** pack label

COBAS INTEGRA 400 plus system

On-board in use at 10-15 °C 4 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C 4 weeks

Do not freeze reagents or expose them to temperatures above 27 °C. The cyclosporine calibrators are transported on dry ice and must be stored frozen. Prior to initial use, allow calibrators to thaw to room temperature for at least one hour.

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:
Whole blood

The only acceptable anticoagulant is EDTA. Sample being tested should not exceed 54 % hematocrit.

Pharmacokinetic factors influence the correct time of sample collection after the last drug dose. These factors include dosage, mode of administration, concomitant drug therapy, and biological variations affecting drug disposition. A trough sample is recommended for measurement of cyclosporine. Heparinized samples are not recommended because they may form clots during storage. Use fresh samples. If samples are to be tested within 8 hours of collection, they may be stored at a room temperature of 18-25 °C. They may be stored refrigerated at 2-8 °C for up to one week. If longer storage is necessary, samples may be frozen at -20 °C. Cyclosporine has been shown to be stable in whole blood samples for at least 3 months when stored at -20 °C.^{3,4}

Thaw and thoroughly mix frozen samples before testing. Repeated freeze-thaw cycles should be avoided. Insoluble materials that may form when samples are frozen should be avoided when pipetting.

ACTION REQUIRED

Calibrators, controls, and/or samples must be pretreated with COBAS INTEGRA Cyclosporine Sample Pretreatment Reagent, Cat. No. 20766364122, system-ID 07 6636 4, before analysis. Follow the steps described in the instructions for use of the COBAS INTEGRA Cyclosporine Sample Pretreatment Reagent to pretreat calibrators, controls, and/or samples. The technical notes are an essential part of the instructions and must be read thoroughly before completing each step.

Diluting high samples

High samples (> 500 ng/mL) should be diluted with either the zero calibrator or cyclosporine negative EDTA whole blood.

1. Invert or rock the high whole blood sample and the diluent (zero calibrator or cyclosporine negative EDTA whole blood) gently, but thoroughly just before use.
2. Combine one part high whole blood sample with two parts diluent.
3. Mix the diluted sample gently but thoroughly by repeated inversion.
4. Pretreat the diluted sample following the steps described in the instructions for use of the COBAS INTEGRA Cyclosporine Sample Pretreatment Reagent.
5. Assay the sample and multiply the result by 3 to obtain an estimate of the cyclosporine concentration.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

For pretreatment of calibrators, controls, and/or samples use COBAS INTEGRA Cyclosporine Sample Pretreatment Reagent, Cat. No. 20766364122, system-ID 07 6636 4.

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.

Application for whole blood

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	340/378 nm
Calc. first/last	50/102
Postdilution factor	No
Unit	ng/mL

Cyclosporine is measured as long analysis test (duration approximately 17 minutes).

Pipetting parameters

		Diluent (H ₂ O)
R1	65 µL	2 µL
Sample	9 µL	11 µL
SR	26 µL	14 µL
Total volume	127 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR

Reaction direction	Increase
Wavelength A/B	340/378 nm
Calc. first/last	74/170
Postdilution factor	No
Unit	ng/mL

Cyclosporine is measured as long analysis test (duration approximately 17 minutes).

Pipetting parameters

		Diluent (H ₂ O)
R1	65 µL	2 µL
Sample	9 µL	11 µL
SR	26 µL	14 µL
Total volume	127 µL	

Calibration

Calibrator	Cyclosporine Calibrators
Cyclosporine conc.	0, 50, 100, 200, 350, 500 ng/mL (0, 41.6, 83.3, 167, 291, 416 nmol/L)
Calibration mode	Logit/log 4
Calibration replicate	Duplicate recommended
Deviation low/high	< 10 % at ≥ 50 ng/mL (41.6 nmol/L)
Calibration interval	Each cobas c pack and as required following quality control procedures

A calibration curve must be prepared using the Cyclosporine Calibrators. Calibrators must be placed from the highest concentration (F) first, to the lowest (A) last, on the CAL/QC rack. This curve is retained in memory by the COBAS INTEGRA systems and recalled for later use.

Traceability: The Cyclosporine Calibrators are prepared to contain known quantities of cyclosporine in normal human serum and are traceable to USP reference standards.

Quality control

Quality Control	BIO-RAD Lyphochek Whole Blood Immunosuppressant Controls
Control interval	With each patient sample run
Control sequence	User defined
Control after calibration	Recommended

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

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factor: ng/mL × 0.833 = nmol/L

Limitations - interference

See the "Specific performance data" section of this document for information on substances tested with this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).

The COBAS INTEGRA Cyclosporine II **cobas c** pack is for in vitro diagnostic use in the measurement of cyclosporine in whole blood. This assay is not intended to be used for measuring cyclosporine in serum or plasma. The effect of carry-over should be considered when evaluating a low concentration sample that follows a sample with a cyclosporine concentration of 500 ng/mL or higher. The amount of carry-over varies from instrument to instrument. To minimize carry-over, properly maintain your

instrument and sample handling equipment, and carefully follow the assay procedures.

Criterion: Recovery within $\pm 10\%$ of initial value at a cyclosporine concentration of 85 ng/mL (70.8 nmol/L).

Icterus: No significant interference up to a bilirubin concentration of 855 $\mu\text{mol/L}$ or 50 mg/dL.

Hemolysis: No significant interference over a hematocrit range from 15-54 %.

Lipemia: No significant interference up to a triglycerides concentration of 1500 mg/dL.

Uric acid: No significant interference up to an uric acid concentration of 20 mg/dL.

Cholesterol: No significant interference up to a cholesterol concentration of 500 mg/dL.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.⁵

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 COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

15-500 ng/mL (12.5-416 nmol/L)

Lower limits of measurement

Lower detection limit of the test:

15 ng/mL (12.5 nmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from the zero calibrator at a 95 % confidence level. It was determined using the zero calibrator. The result was obtained on all the COBAS INTEGRA analyzers.

Expected values

No firm therapeutic range exists for cyclosporine in whole blood. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of cyclosporine, co-administration of other immunosuppressants, type of transplant, time post transplant, and a number of other factors contribute to different requirements for optimal blood levels of cyclosporine. Individual cyclosporine values cannot be used as the sole indicator for making changes in the treatment regimen. Each patient should be thoroughly evaluated clinically before treatment adjustments are made, and each assay user must establish his or her ranges based on clinical experience.

These ranges will vary according to the commercial in vitro diagnostic test used. Ranges must be established for each commercial test used.

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 COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Bio-Rad Lyphochek Whole Blood Controls in accordance with the CLSI guidelines EP05-A2⁶ requirements with repeatability ($n = 84$) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days).

The following results were obtained on a COBAS INTEGRA 400 analyzer:

Repeatability	Mean ng/mL (nmol/L)	SD ng/mL (nmol/L)	CV %
Level 1	90.8 (75.5)	6.1 (5.1)	6.7
Level 2	185 (154)	8 (6.7)	4.6

Repeatability	Mean ng/mL (nmol/L)	SD ng/mL (nmol/L)	CV %
Level 3	346 (288)	18 (15.0)	5.1

Intermediate precision	Mean ng/mL (nmol/L)	SD ng/mL (nmol/L)	CV %
Level 1	90.8 (75.5)	9.0 (7.5)	9.9
Level 2	185 (154)	13 (10.8)	6.9
Level 3	346 (288)	24 (20.0)	6.9

The following results were obtained on the COBAS INTEGRA 800 analyzer:

Repeatability	Mean ng/mL (nmol/L)	SD ng/mL (nmol/L)	CV %
Level 1	92.8 (77.2)	6.9 (5.7)	7.5
Level 2	191 (159)	10 (8.3)	5.2
Level 3	358 (298)	19 (15.8)	5.2

Intermediate precision	Mean ng/mL (nmol/L)	SD ng/mL (nmol/L)	CV %
Level 1	92.8 (77.2)	10 (8.3)	11.1
Level 2	191 (159)	14 (11.7)	7.5
Level 3	358 (298)	21 (17.5)	6.0

Method comparison

The COBAS INTEGRA Cyclosporine and COBAS INTEGRA Cyclosporine II assays with COBAS INTEGRA Cyclosporine Pretreatment Reagent were compared to a commercially available EMIT assay using both a methanol (MeOH) extraction method and a commercially available EMIT cyclosporine sample pretreatment reagent on the COBAS MIRA analyzer.

COBAS INTEGRA Cyclosporine and COBAS INTEGRA Cyclosporine II assays showed equivalent performance compared to EMIT Cyclosporine assay. The data shown below is representative of the comparisons. Samples used for method comparisons came from 277 whole blood samples collected from post-transplant patients. In some cases, more than one sample may have been drawn from the same patient at different post-transplant times. Samples were frozen at $-20\text{ }^{\circ}\text{C}$ and analyzed within a month of being drawn. Of these 277, 14 samples from the MeOH comparison, and 15 samples from the new pretreatment comparison, were shown to contain cyclosporine concentrations of $< 40\text{ ng/mL}$ (EMIT sensitivity) or $> 500\text{ ng/mL}$ (test limit), and were omitted from the statistical analysis. The remaining sample population is comprised of three transplant types, 24 liver, 93 heart, and 145 kidney (for COBAS INTEGRA 700 analyzer Cyclosporine vs. EMIT with pretreatment reagent comparison) or 146 kidney (for COBAS INTEGRA 700 analyzer Cyclosporine vs. EMIT with methanol extraction comparison).

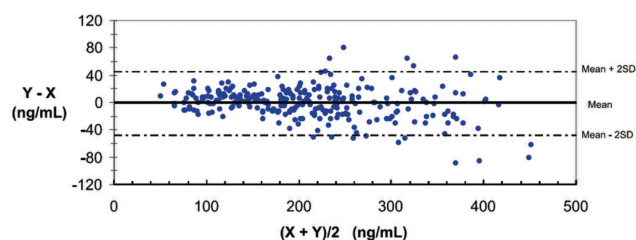
Statistical analysis using the Deming regression method is provided below.

		EMIT MeOH	EMIT Sample Pretreatment Reagent
Number of samples		263	262
Range of values	min.	41 ng/mL	43 ng/mL
	max.	489 ng/mL	485 ng/mL
Slope		0.937	0.939
95 % confidence interval	lower limit	0.915	0.917
	upper limit	0.959	0.961
Intercept (ng/mL)		12.9	13.3
95 % confidence interval			

lower limit	8.0	8.1
upper limit	17.8	18.6
Correlation coefficient	0.968	0.963

The same data sets are shown below in Bland-Altman difference plots.

COBAS INTEGRA 700 analyzer Cyclosporine vs. EMIT with Methanol Extraction



N = 263

Mean (Y-X) = -0.3

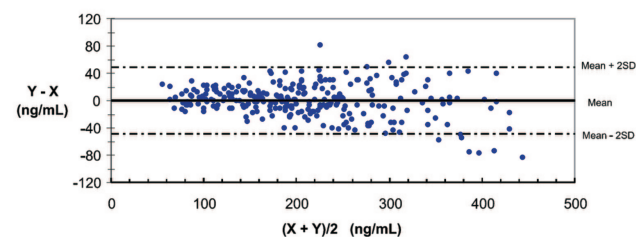
SD (Y-X) = 23.0

2 SD = 46.0

Mean + 2 SD = 45.7

Mean - 2 SD = -46.4

COBAS INTEGRA 700 analyzer Cyclosporine vs. EMIT with Pretreatment Reagent



N = 262

Mean (Y-X) = 0.4

SD (Y-X) = 24.3

2 SD = 48.5

Mean + 2 SD = 48.9

Mean - 2 SD = -48.1

Analytical specificity³

The following metabolites, cross-reactive substances, and structurally related or potentially co-administered compounds were evaluated on the COBAS INTEGRA systems in a normal human EDTA whole-blood pool spiked with cyclosporine at 200 ng/mL (166.5 nmol/L). Each substance was tested at 10 times the highest concentration for its therapeutic or normal range, as per the protocol described by NCCLS.⁷ The imprecision of the assay was taken into account when determining cross-reactivity. Cross-reactivity was designated as "not detectable" (ND) if the obtained value was less than the sensitivity of the assay.

$$\text{Cross-reactivity (\%)} = \frac{100 \times (\text{analytical result} - \text{analyte concentration})}{\text{concentration of interferent}}$$

Drug	Level tested ng/mL	Cross-reactivity %
(AM9) M1	667	13
(AM19) M8	500	3.2
(AM1) M17	500	ND
(AM4N) M21	500	5.1
ND = Not Detectable		

Drug	Level tested ng/mL	Cross-reactivity %
Acetaminophen	200	ND
Albuterol	0.18	ND
Allopurinol	600	ND
Alprazolam	0.37	ND
Amphotericin B	20	ND
Atenolol	40	ND
Azathioprine	10	ND
Captopril	50	ND
Carbamazepine	120	ND
Ceflacor	230	ND
Chloramphenicol	250	ND
Cimetidine	100	ND
Cyclophosphamide	250	ND
Digoxin	0.02	ND
Dipyridamole	25	ND
Disopyramidine	30	ND
Erythromycin	200	ND
Furosemide	20	ND
Gancyclovir	400	ND
Gentamicin	120	ND
Heparin	800 U/L	ND
Hydralazine	32	ND
Hydrochlorothiazide	40	ND
Isoniazid	70	ND
Isoproterenol hydrochloride	0.06	ND
Lidocaine	600	ND
Methylprednisolone	12	ND
Metoclopramide	4	ND
Mycophenolic acid	100	ND
Naproxen	1000	1.5
Phenobarbital	150	ND
Phenytoin	100	11.8
Piperacillin	8	ND
Prazosin	3	ND
Prednisolone	12	ND
Prednisone	12	ND
Promethazine	10	ND
Salicylic acid	500	ND
Sulfamethoxazole	400	ND
Theophylline	250	ND

Drug	Level tested ng/mL	Cross-reactivity %
Triamterene	2.8	ND
Trimethoprim	20	ND
Vancomycin	630	ND

ND = Not Detectable

Linearity

To assess the linearity of the assay, a 7-level dilution series was prepared using a whole blood sample from a patient taking cyclosporine diluted with negative human whole blood. Results were evaluated by Passing/Bablok regression.⁸

$$y = 0.998x + 1.07$$

$$r = 1.0$$

Slope 95 % CI (0.971, 1.02)

Intercept 95 % CI (-0.610, 11.1)

Lower limit of the range = 28.1

Any modification of the instrument as set forth in this labeling requires validation by the laboratory.

References

- 1 McMillan MA. Clinical pharmacokinetics of cyclosporine. *Pharmac Ther* 1989;42:135-156.
- 2 Kahan BD. Cyclosporine. *N Engl J Med* 1989;321:1725-1738.
- 3 Schran HF, Rosano TG, Hassell AE, et al. Determination of cyclosporine concentrations with monoclonal antibodies. *Clin Chem* 1987;33:2225-2229.
- 4 Wong PY, Mee AV, Glenn J, et al. Quality assessment of cyclosporine monitoring Canadian validations. *Transplant Proc* 1990;22:1216-1217.
- 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 Clinical and Laboratory Standards Institute. Evaluation of Precision of Quantitative Measurement Methods: Approved Guideline, EP05-A2 2004.
- 7 National Committee for Clinical Laboratory Standards. Interference Testing in Clinical Chemistry; Proposed Guideline. Villanova, PA.: NCCLS; 1986;6(13). NCCLS Publication EP7-P.
- 8 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. *J Clin Chem Clin Biochem* 1988 Nov;26(11):783-790.

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

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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.

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