Acid/Prostatic Phosphatase

Order information

COBAS INTEGRA Acid/Prostatic Phosphatase	100 Tests	Cat. No. **20737321** 322 System-ID 07 3732 1
Precinorm U	20 × 5 mL	Cat. No. **10171743** 122 System-ID 07 7997 0
Precipath U	20 × 5 mL	Cat. No. **10171778** 122 System-ID 07 7998 7
Precinorm U plus	10 × 3 mL	Cat. No. **12149435** 122
Precinorm U plus (for USA)	10 × 3 mL	Cat. No. **12149435** 160 System-ID 07 7999 7
Precipath U plus	10 × 3 mL	Cat. No. **12149443** 122
Precipath U plus (for USA)	10 × 3 mL	Cat. No. **12149443** 160 System-ID 07 8000 6

● Indicates analyzer(s) on which cassette can be used

COBAS INTEGRA 400/400 plus	COBAS INTEGRA 700	COBAS INTEGRA 800
●	●	●

One cassette provides reagent for a maximum of 50 prostatic acid phosphatase determinations.

System information
COBAS INTEGRA Acid/Prostatic Phosphatase (ACPP).
Test ACP, test ID 0-032; test NPACP, test ID 0-132.

Intended use
In vitro test for the quantitative determination of the catalytic activity of total and prostatic acid phosphatase (EC 3.1.3.2; orthophosphoric-monoester phosphohydrolase, acid optimum) in human serum on COBAS INTEGRA systems.

Summary[1,2,3]
The group of phosphatases with optimal activity below a pH of 7.0 is summarized under the name of acid phosphatases. These enzymes are found in the liver, spleen, milk, erythrocytes, platelets, bone marrow, and also the prostate gland in males. The total acid phosphatase activity in serum of males is derived from disintegrating platelets and erythrocytes, approximately half of which come from the prostate gland and half from the liver. In females, the serum activity is presumably derived from erythrocytes and platelets solely from the liver.

Prostatic acid phosphatase levels are greatly increased in cases of prostatic cancer (especially, but not always, with metastases). Total acid phosphatase activity can be elevated in Paget's disease, in hyperparathyroidism with skeletal involvement, in the presence of malignant invasion of the bones by cancer, in Gaucher's disease, in Niemann-Pick disease, and in myelocytic leukemia.

Test principle
Method according to Hillmann with 1-naphthylphosphate and 1,5-pentanediol. Inhibition of prostatic acid phosphatase by tartrate.[4,5,6]
Acid phosphatase (ACP) catalyzes the hydrolysis of 1-naphthylphosphate to 1-naphthol and phosphate. 1-Naphthol released by the enzyme couples with Fast Red TR and forms a red diazo dye. 1,5-Pentanediol accelerates the reaction by acting as phosphate acceptor.

$$\text{1-Naphthylphosphate} + H_2O \xrightarrow{ACP} \text{1-naphthol} + \text{phosphate}$$

$$\text{1-Naphthol} + \text{Fast Red TR} \longrightarrow \text{red diazo dye}$$

The rate of the color formation is directly proportional to the catalytic acid phosphatase activity. It is determined by measuring the increase in absorbance at 409 nm.
The prostatic acid phosphatase is selectively inhibited by tartrate. The difference between the total acid phosphatase (ACP) and the tartrate resistant form (nonprostatic acid phosphatase, NPACP) corresponds to the prostatic acid phosphatase (ACPP):
Prostatic ACP = total ACP - nonprostatic ACP

Reagents - working solutions

Components	Concentrations (reconstituted)					
				NPACP	ACP	
	R1	R2	R3=SR	Test	Test	
Citric acid	115	115		28	28	mmol/L
1,5-Pentanediol	830	830		200	200	mmol/L
Tartrate		415		100		mmol/L
1-Naphthylphosphate			15	3	3	mmol/L
Fast Red TR salt[a]			5	1	1	mmol/L
pH	5.5	5.5	3.5	5.4	5.4	

a) Diazotized 2-amino-5-chlorotoluene 1,5-naphthalene disulfonate salt

Reagent R3 (SR) contains nonreactive stabilizers. Reagents R1 and R2 contain nonreactive surfactants.

Precautions and warnings
Pay attention to all precautions and warnings listed in this Method Manual, Chapter 1, Introduction.

Reagent handling
COBAS INTEGRA 400/400 plus systems
Before insertion of the cassette pierce the aluminium foil of the reagent bottles using the tip of the unlock rack tool. After insertion of the cassette, granulate SR is automatically reconstituted with the appropriate volume of water. Place the reconstituted cassette on the Cassette Mixer and mix for 10 minutes.
COBAS INTEGRA 700/800 systems
Granulate SR is automatically reconstituted and mixed within approx. 4 minutes with the appropriate volume of water.

Storage and stability

Shelf life at 2 to 8°C	See expiration date on cassette
COBAS INTEGRA 400/400 plus systems	
On-board in use at 10 to 15°C	6 weeks
COBAS INTEGRA 700/800 systems	
On-board in use at 8°C	6 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: free from hemolysis, icterus, and lipemia.
Serum is the only acceptable specimen.

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.

Note
Do not use plasma or samples from patients undergoing heparin treatment. The enzyme is subject to circadian rhythms. Consecutive sample collections should be done at the same time of day. Acid phosphatase activity in serum is known to be unstable. Prostatic acid phosphatase is particularly labile and over 50% of the activity may be lost in 1 hour at room temperature.[1] Therefore serum must be separated immediately from the clot and stabilized by the addition of 20 µL of a 1.5 mol/L solution of acetic acid per mL of serum. A 1.5 mol/L solution of acetic acid can be prepared by diluting commercially available glacial acetic acid 1 : 12 with water (e.g. by adding 1 mL of glacial acetic acid to 11 mL of water).
Avoid repeated freezing and thawing.[3]

Stability: several hours at 15-25°C
up to 7 days at 2-8°C

Centrifuge samples containing precipitates before performing the assay.

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

Acetic acid 1.5 mol/L for sample stabilization.
See "Specimen collection and preparation" section for details on the sample stabilization procedure.

Assay

For optimal performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

Applications for total and nonprostatic acid phosphatase

COBAS INTEGRA 400/400 plus test definition

Measuring mode		Absorbance
Abs. calculation mode		Kinetic
Reaction mode	*ACP*	R1-S-SR
	NPACP	R2-S-SR
Reaction direction		Increase
Wavelength A/B		409/659 nm
Calc. first/last		57/66
Unit		U/L

Pipetting parameters

ACP		Diluent (H_2O)
R1	30 µL	20 µL
Sample	10 µL	20 µL
SR	25 µL	20 µL
Total volume	125 µL	

NPACP		Diluent (H_2O)
R2	30 µL	20 µL
Sample	10 µL	20 µL
SR	25 µL	20 µL
Total volume	125 µL	

COBAS INTEGRA 700/800 test definition

Measuring mode		Absorbance
Abs. calculation mode		Kinetic
Reaction mode	*ACP*	R1-S-SR
	NPACP	R2-S-SR
Reaction direction		Increase
Wavelength A/B		409/659 nm
Calc. first/last		83/98
Unit		U/L

Pipetting parameters

ACP		Diluent (H_2O)
R1	30 µL	20 µL
Sample	10 µL	20 µL
SR	25 µL	20 µL
Total volume	125 µL	

NPACP		Diluent (H_2O)
R2	30 µL	20 µL
Sample	10 µL	20 µL
SR	25 µL	20 µL
Total volume	125 µL	

Ratio definition for prostatic acid phosphatase

Abbreviated ratio name	ACPPR (0-332)
Equation	ACP - NPACP
Unit	U/L

Use the predefined profile (ACPP, 0-232) for simultaneous order entry of total (ACP) and nonprostatic (NPACP) acid phosphatase tests from the same sample. The result for prostatic acid phosphatase will automatically be calculated after result output of both tests.

Calibration

Calibrator	Water is used as blank calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures
Blank correction	

Both tests are calibrated using a water blank and fixed calibration factors. For each reagent lot a one-point calibration (blank) is performed.

COBAS INTEGRA 400/400 plus analyzers: Calibration definition is ACPP. Calibrator ACPP is water. Enter zero as the STD 1 value.

COBAS INTEGRA 700/800 analyzers: Calibration definition is Calibrator f.a.s.but only system water is used. Enter zero for both calibrator concentrations. Both Calibrator f.a.s. and water must be presented to the instrument.

Tracebility: This method has been standardized manually against Roche reagent.

Quality control

Reference range	Precinorm U or Precinorm U plus
Pathological range	Precipath U or Precipath U plus
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

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

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 limits.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte activity of each sample. For more details please refer to Chapter 7, Data Analysis, User Manual (COBAS INTEGRA 700 analyzer), or to Data analysis in the online Help (COBAS INTEGRA 400/400 plus/800 analyzers).

Conversion factor: U/L × 0.0167 = µkat/L

Limitations - interference[7]

Criterion: Recovery within ±10% of initial value.

Icterus	Avoid icteric specimens. Unconjugated (direct) bilirubin levels higher than 12 µmol/L (0.7 mg/dL) decrease the apparent ACP activity significantly. It is reported that unconjugated bilirubin interferes with the indicator reaction by forming a colored azo-compound with the Fast Red TR salt.[8]
Hemolysis	Avoid hemolyzed specimens. Erythrocyte contamination interferes with the results, since ACP activities in erythrocytes are about 70 times higher than those in normal serum.[9]
Lipemia	Avoid lipemic specimens. Even slight lipemia interferes with the test.
Drugs	*ACP* Of the drugs tested in vitro, ascorbic acid, methyldopa, oxytetracycline, and sulfamethoxazole cause artificially high ACP values at the tested drug level. Theophylline causes artificially low ACP values at the tested drug level. Refer to Chapter 1, Introduction, for a list of tested drugs and their concentration. *NPACP* Ascorbic acid, methyldopa, nikethamide, oxytetracycline, and sulfamethoxazole cause artifically high NPACP values at the tested drug level. *ACPP* Due to the fact that the ACPP result is calculated by the difference of ACP minus NPACP drug interferences on either ACP or NPACP may cause a drastic increase or decrease in the calculated ACPP value even if the interferences for ACP and NPACP are within specification. Discrepant ACPP values should be checked for possible drug interference.
Other	In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

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

Measuring range

1.0-100 U/L (0.02-1.7 µkat/L)

Extended measuring range (calculated)
Postdilution factor : 10 recommended
1.0-1000 U/L (0.02-17 µkat/L)

Lower detection limit
1.0 U/L (0.02 µkat/L)
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of a zero sample (zero sample + 3 SD, within-run precision, n = 30).

Expected values

37°C	Total acid phosphatase	<7.3 U/L (<0.12 µkat/L)
37°C	Prostatic acid phosphatase	<1.9 U/L (<0.03 µkat/L)

These reference values are the central 95th percentile of results from a study of 53 healthy male individuals.
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 for total acid phosphatase

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 20, between-run n = 20). The following results were obtained:

	Level 1	Level 2
Mean	4 U/L (0.07 µkat/L)	11 U/L (0.18 µkat/L)
CV within-run	4.5%	2.5%
CV between-run	5.7%	4.6%

Method comparison

Total acid phosphatase values for human serum samples obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA Acid/Prostatic Phosphatase reagent were compared to those determined with reagents for total acid phosphatase on a COBAS MIRA analyzer and a commercially available alternative clinical chemistry system. Samples were measured in duplicate. Sample size (n) represents all replicates. Values ranged from:

COBAS MIRA analyzer	1.5 to 53 U/L (0.03 to 0.89 µkat/L)
Alternative system	0.72 to 97 U/L (0.01 to 1.6 µkat/L)

		COBAS MIRA analyzer	Alternative system
Method		1-Naphthyl-phosphate	1-Naphthyl-phosphate
Pentanediol activation		No	Yes
Sample size	(n)	260	150
Corr. coefficient	(r)	0.978	0.997
	(r_s)	0.979	0.770
Lin. regression		$y = 1.54x$ - 0.1 U/L	$y = 1.01x$ + 3.3 U/L
Passing/Bablok[10]		$y = 1.60x$ + 0.2 U/L	$y = 1.05x$ + 2.7 U/L

Specific performance data for prostatic acid phosphatase

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 20, between-run n = 20). The following results were obtained:

	Level 1	Level 2
Mean	1 U/L (0.02 µkat/L)	3 U/L (0.05 µkat/L)
CV within-run	23%	9.4%
CV between-run	25%	15%

Method comparison

Prostatic acid phosphatase values for human serum samples obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA Acid/Prostatic Phosphatase reagent were compared to those determined with reagents for prostatic acid phosphatase on a COBAS MIRA analyzer and a commercially available alternative clinical chemistry system. Samples were measured in duplicate. Sample size (n) represents all replicates. Values ranged from:

COBAS MIRA analyzer	0.9 to 42 U/L (0.02 to 0.70 µkat/L)
Alternative system	1.9 to 88 U/L (0.03 to 1.5 µkat/L)

		COBAS MIRA analyzer	Alternative system
Method		1-Naphthyl-phosphate	1-Naphthyl-phosphate
Pentanediol activation		No	Yes
Sample size	(n)	264	150
Corr. coefficient	(r)	0.996	0.997
	(r_s)	0.988	0.770
Lin. regression		$y = 1.83x + 0.5$ U/L	$y = 1.10x + 0.8$ U/L
Passing/Bablok[10]		$y = 1.85x + 0.4$ U/L	$y = 1.12x + 0.5$ U/L

References

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