MEDICA

IVD CE

REF 10221-4 4 x 29 mL / 6 mL

PHOSPHORUS (PHOS)

Wedges each contain usable volumes of 29 mL of R1 reagent and 6 mL of R2 reagent.

INTENDED USE

The EasyRA PHOS reagent is intended for the quantitative measurement of phosphorus in human serum and plasma (with lithium heparin as anticoagulant), using the MEDICA "EasyRA® Clinical Chemistry Analyzer." Phosphorus measurements are used in the diagnosis and treatment of parathyroid gland, kidney diseases, and vitamin D imbalance.
For *in vitro* diagnostic use only. For professional use only.

SUMMARY AND EXPLANATION

The majority of Phosphorus (PHOS; 80%) found in the human body is bound to calcium and located in the bones and teeth. The remaining inorganic phosphate is in combination with carbohydrates, proteins, lipids and distributed throughout the body. An inverse relationship exists between calcium and phosphorus concentrations within the body. An increase in one usually leads to a decrease of the other and vice versa. An increase in serum inorganic phosphate is usually the result of Vitamin D overdose, hypoparathyroidism or renal failure. A decrease in serum phosphate may be a result of rickets, hyperparathyroidism or an indicator of a decrease in the absorption of phosphorus by the patient.[1]

PRINCIPLE OF THE PROCEDURE

The method described by Daly and Ertingshausen,[2] involves the reaction of inorganic phosphorus with ammonium molybdate in an acidic medium to form a phosphomolybdate complex with a yellow color.

$$\text{Phosphorus (Inorganic)} + \text{Ammonium molybdate} \xrightarrow{H_2S0_4} \text{Phosphomolybdate complex}$$

The increase in absorbance at 340 nm is directly proportional to the concentration of inorganic phosphorus in the sample.

REAGENTS

Phosphorus Reagent (R1):

Sulfuric Acid (H_2S0_4), pH <1	170 mmol/L
surfactant	

Phosphorus Reagent (R2):

Ammonium Molybdate	2.8 mmol/L
Sulfuric Acid (H_2S0_4), pH <1	170 mmol/L

PRECAUTIONS

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A2).
2. The reagents contain sulfuric acid, which is corrosive and causes burns. Do NOT inhale or swallow, and avoid any contact with skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Any contact with the skin, should be washed immediately with water for 10 minutes. If swallowed, seek medical advice immediately. Refer to the Safety Data Sheet for risk, hazard and safety information.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 18 – 25°C. The reagent is stable on-board in the refrigerated reagent area of the EasyRA Analyzer for the number of days programmed on the RFID chip on the reagent wedge if the reagent is removed at the end of each day and stored overnight at 18 – 25°C. Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values.

SPECIMEN COLLECTION AND STORAGE / STABILITY

Clear unhemolyzed serum and plasma should be used. Lithium heparin coated tubes may be used for plasma collection. Centrifuge and remove the serum or plasma as soon as possible after collection, because erythrocytes can release inorganic phosphate by hydrolysis or inherent phosphatase activity.[3] Serum phosphorus is stable for 7 days at 2 – 8°C [4] or 6 months at -20°C.

PROCEDURE

Materials Provided
Medica PHOS Reagent Wedge, REF 10214

Additional materials required
Medica EasyCal Chemistry, REF 10651
Medica EasyQC® Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC Chemistry/Electrolytes – Level B, REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use
The reagent is ready to use as supplied. Remove the cap and place the reagent in the EasyRA Analyzer reagent tray located in the reagent area. The on-board stability (30 days maximum) is programmed on the RFID chip on the reagent wedge. The PHOS wedge must be recapped and removed from the analyzer at the end of the day and left at 18 – 25°C.

Note: Check inside the necks of the wedge for foam after removing the caps and placing the wedge on the EasyRA Analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2.

Calibration
Medica EasyCal Chemistry (REF 10651) is recommended for the calibration of the assay. The calibration interval (30 days maximum) is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a change in reagent lot number or if a shift in quality control values occurs.

Quality Control
It is recommended that two levels of human serum based controls (normal and abnormal) be run with the assay daily whenever patient testing is performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction or procedural errors. The laboratory should follow local, state and federal quality control guidelines when using quality control materials.

Results
After completion of the assay, the EasyRA Analyzer calculates the phosphorus concentration from the ratio of the corrected absorbance (subtracting the absorbance of the reagent blank and sample blank) of the sample to the similarly corrected absorbance of the calibrator (after subtracting the absorbance of the reagent blank and sample blank) multiplied by the concentration of the calibrator.

$$\text{PHOS (mg/dL)} = \frac{[(A_{U_{340}} - A_{RBlk_{340}})] - [(A_{U_{340}} - A_{RBlk_{340}})]_{SBlk} \times dF}{[(A_{C_{340}} - A_{RBlk_{340}})] - [(A_{C_{340}} - A_{RBlk_{340}})]_{SBlk} \times dF} \times \text{CalValue}$$

Where A_U is the absorbance of the unknown, A_{RBlk} is the absorbance of the reagent blank associated with the unknown sample, and SBlk is the sample blank associated with the unknown sample. All absorbances with a subscript “C” are associated with the calibrator. As a consequence of the delayed addition of the R2 reagent, there is a dilution correction factor (dF) included in the calculation.

Expected Values[5]
The reference range for PHOS in serum and plasma is as follows:

Adult: 2.5 – 4.8 mg/dL

These values are guidelines. It is recommended that each laboratory establish its own range of expected values since differences exist among instruments, laboratories and local populations.

Procedural Limitations (e.g. if sample is above assay range)

The EasyRA Analyzer flags any result above 20 mg/dL as Linearity High “LH”. If the “Re-run” icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will extend the reportable range of the PHOS test to 40 mg/dL.

PERFORMANCE CHARACTERISTICS[6]

Reportable Range
The reportable range is 0.11 to 20 mg/dL. Extended range is 0.11 to 40 mg/dL when half of the sample is used (1:1 dilution).

Inaccuracy / Correlation (CLSI, EP9-A2)
The following table lists the data obtained in a comparison of the Medica Reagent for PHOS (y) on the EasyRA Analyzer to the performance of a similar PHOS reagent (x) on the Roche COBAS MIRA* Analyzer. The data shown below represents single determinations obtained on the EasyRA Analyzer vs. the average of two replicate values obtained on the COBAS MIRA Analyzer.

Number of samples	46	Range of Samples	0.20 to 19.67 mg/dL
Slope	0.9802	y Intercept	0.0058
Correlation Coefficient	0.9927	Regression Equation:	Y = 0.9802*X + 0.0058

The following table lists the data obtained in a comparison of matched serum (x) and Li-Heparinized plasma (y) samples using the Medica Reagent for PHOS on the EasyRA Analyzer. The data below represents a single plasma determination vs. the average of two replicate serum values.

Number of Samples	77	Range of Samples	0.75 to 18.32 mg/dL
Slope	1.0070	y Intercept	-0.2462
Correlation	0.9966	Regression Equation	Y = 1.0070*X – 0.2462

**Cobas Mira is a registered trademark of Roche Diagnostics, INC., Indianapolis, IN.*

NOTE: Plasma PHOS results may be approximately 4% lower than serum PHOS results.[7] Medica recommends that clinical laboratories establish their own reference ranges for the ALP test depending on sample type.

Imprecision (CLSI, EP5-A2)
Duplicate measurements of each of three levels of QC material were tested twice a day for 20 days. Both within-run precision and total precision were determined from these data.

Within run imprecision:

QC Level mg/dL	Within Run SD mg/dL	Within Run CV %
7.39	0.05	0.68
4.54	0.05	1.01
1.83	0.02	1.03

Total Imprecision:

QC Level mg/dL	Total Imprecision SD mg/dL	Total Imprecision CV %
7.39	0.15	2.10
4.54	0.16	3.55
1.83	0.04	1.94

Linearity (CLSI, EP6-A)
Linear from 0.11 to 20 mg/dL, based on the linear regression Y = 1.0567*X – 0.2576.

Limit of Blank (LOB):	0.04mg/dL	(CLSI, EP17-A)
Limit of Detection (LOD):	0.09mg/dL	(CLSI, EP17-A)

Interfering Substances (CLSI, EP7-A)
Less than 10% interference was classified as “no significant interference.”
No significant interference was found in levels of up to 500 mg/dL of hemoglobin.
No significant interference was found in levels up to 25 mg/dL of bilirubin.
No significant interference was found in levels up to 1740 mg/dL of triglycerides (using Intralipid*).
Samples from patients with Waldenstrom’s Macroglobulinemia has a high potential for interference and may produce unreliable results.
Samples containing Eltrombopag should not be tested for Phosphorus.

**Intralipid is a registered trademark of Pharmacia AB, Clayton, NC.*

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.[8,9]

REFERENCES

1. Endres, D.B., Rude, R.K. *Mineral and Bone Metabolism.* Tietz Fundamentals of Clinical Chemistry, Burtis, C.A. et Ashwood, E.R. (W.B. Saunders, eds. Philadelphia USA) 2001: 795.
2. Daly, J.A. and Ertingshausen, G. *Direct Method for Determining Inorganic Phosphorus in Serum with the Centrifichem*, Clin.Chem. 18:263, (1972)
3. Kaplan, L., Pesce, A.J. *Clinical Chemistry, Theory, Analysis, Correlation*, Third Edition, Mosby, P. 1996:552
4. Henry, RJ. *Clinical Chemistry – Principles and Technics*, New York, NY: Harper and Row; 1974.
5. Burtis, C.A. and Ashwood, E.R. (Eds), Tietz Textbook or Clinical Chemistry, 2nd edition, W.B. Saunders C0., Philadelphia (1994).
6. Data on file at Medica.
7. Ciuti, R., Rinaldi, G. *Serum and Plasma Compared for Use in 19 Common Chemical Tests Performed in the Hitachi 737 Analyzer,Clin. Chem*. 35:1562. (1989).
8. Young DS. *Effects of Drugs on Clinical Laboratory Tests* 4th ed. Washington, DC: AACC Press; 1995.
9. Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 2nd ed. Washington, DC. AACC Press; 1997.

EASYRA ASSAY PARAMETERS (PHOS)

Wavelength (nm)	340
Reaction Type	Endpoint (2)
Reaction Direction	Increase
Reagent Blank	Yes (with each calibration)
Sample Blank	Yes
Reaction Time	2.4 min
Calibration interval (maximum)	30 days
Reagent on-board stability	30 days

Serum/Plasma

Sample volume (µl)	4.0
Diluent 1 volume (µl)	10
Diluent 2 volume (µl)	10
Reagent volume R1 (µl)	166
Reagent volume R2 (µl)	33
Decimal Places (default)	2
Units (default values)	mg/dL
Dilution Factor	1:1 (to extend measuring range)
Linearity	0.11 to 20 mg/dL

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