MEDICA

IVD CE

REF 10217-4 4 x 39 mL

Amylase (AMY)

Wedges each contain a usable volume of 39 mL of reagent.

INTENDED USE

The EasyRA AMY reagent is intended for the quantitative determination of α-Amylase activity in human serum and plasma (with lithium heparin as anticoagulant), using the MEDICA "EasyRA® Clinical Chemistry Analyzer." Amylase measurements are used primarily for the diagnosis and treatment of pancreatitis.
For *in vitro* diagnostic use only. For professional use only.

SUMMARY AND EXPLANATION

Amylase is an enzyme produced by the exocrine glands with ability to cleave 1,4-glucose linkages. Amylase is secreted via the pancreatic ducts and then common bile ducts into the duodenum where it plays an important role in digestion of complex carbohydrates. In normal serum or plasma, about 40% of circulating amylase is of pancreatic origin, the rest coming from the salivary glands. Elevated levels of serum α-amylase are important in diagnosing pancreatitis and other pancreatic disorders.[1-3]

PRINCIPLE OF THE PROCEDURE

This test measures the activity of serum AMY by the kinetic method using the chromogenic substrate, 2-chloro-4-nitrophenol linked with maltotriose (CNPG3).[4] AMY hydrolyses the CNPG3 substrate to form 2-chloro-4-nitrophenol (CNP), 2-chloro-4-nitrophenol-α-D-maltoside ($CNPG_2$), maltotriose (G_3) and glucose (GLU).

$$10\ CNPG_3 \xrightarrow{\text{α-amylase}} 9\ CNP + 1\ CNPG_2 + G_3 + GLU$$

The CNP component is measured spectrophotometrically at an absorbance peak of 405 nm. The rate of the formation of the CNP is directly proportional to the α-amylase activity in the sample.

REAGENT

2-chloro-4-nitrophenol-α-D-maltotrioside, (CNPG3)	2.25 mmol/L
Sodium Chloride	350 mmol/L
Calcium Acetate	6.0 mmol/L
Potassium Thiocyanate	900 mmol/L
Sodium Azide	0.1%
MES buffer	pH 6.0

PRECAUTIONS

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A2).
2. Avoid inhalation or contact with skin and eyes. Wash skin or eyes with water and consult a physician if contact occurs. This reagent is not compatible with strong acids.
3. The reagent contains 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Safety Data Sheet for risk, hazard and safety information.
4. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
5. 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 2 – 8°C and protected from light. The reagent is stable on-board in the refrigerated reagent chamber of the EasyRA Analyzer for the number of days programmed on the RFID chip on the reagent wedge. Do not use the reagent it if is turbid or cloudy or if it fails to recover known serum control values.

SPECIMEN COLLECTION AND STORAGE / STABILITY

Clear unhemolyzed serum or plasma should be used. Centrifuge and remove the serum or plasma as soon as possible after collection. Serum AMY is stable for 7 days at 18 – 25°C and for several months at 2 – 8°C.[5] Lithium heparin coated tubes may be used for plasma collection.

PROCEDURE

Materials Provided
Medica Amylase Reagent Wedge, REF 10217

Additional materials required

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.

Note: Check inside the neck of the wedge for foam after removing the cap 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.

Calibration
Not applicable.

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 AMY concentration based on the change in absorbance per minute, sample volume, total volume, pathlength (cm) of 0.6 and molar absorptivity of 12.9.

$$\text{AMY (U/L)} = (\Delta\text{A/Min}) \times \frac{(\text{Total Volume}(\mu l) \times 1000)}{(\text{Molar absorptivity} \times \text{Pathlength(cm)} \times \text{Sample Volume}(\mu l))}$$

Expected Values[6]
The reference range for AMY in serum and plasma is as follows:

Normal Range: 25 – 94 U/L (37°C)

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)
Only unhemolyzed serum samples should be used.

The AMY test is linear to 1200 U/L. If the Absorbance Change per Minute (ΔA/Min) is greater than 0.281, which corresponds to approximately 1200U/L, results will be flagged with "SD" (substrate depletion) by the analyzer. 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 AMY test to 2400 U/L.

PERFORMANCE CHARACTERISTICS[7]

Reportable Range
The reportable range is 2 to 1200 U/L. Extended range is 2 to 2400 U/L 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 AMY (y) on the EasyRA Analyzer to the performance of a similar AMY reagent (x) on the Roche COBAS MIRA* analyzer. The data presented below represents single determinations on the EasyRA Analyzer vs. the average of two replicates obtained on the COBAS MIRA Analyzer.

Number of samples	87	Range of Samples	2 to 1150 U/L
Slope	1.0433	y Intercept	5.5881
Correlation Coefficient	0.9981	Regression Equation:	Y = 1.0433*X + 5.5881

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

Number of Samples	78	Range of Samples	4 to 1061 U/L
Slope	1.0247	y Intercept	-5.1770
Correlation Coefficient	0.9985	Regression Equation	Y = 1.0247*X – 5.1770

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

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 U/L	Within Run SD U/L	Within Run CV %
296	1.9	0.6
85	0.7	0.8
45	0.8	1.8

Total Imprecision:

QC Level U/L	Total Imprecision SD U/L	Total Imprecision CV %
296	2.9	1.0
85	0.9	1.1
45	0.9	1.9

Linearity (CLSI, EP6-A)

Linear from 2 to 1200 U/L based on the linear regression Y = 1.0007*X + 1.2297.

Limit of Blank (LOB):	0.78 U/L	(CLSI, EP17-A)
Limit of Detection (LOD):	1.03 U/L	(CLSI, EP17-A)

Interfering Substances (CLSI, EP7-A)
Less than 10% interference was classified as “no significant interference.”
Significant negative interference at hemoglobin levels above 125 mg/dL. Do not use hemolyzed samples.
No significant interference was found in levels up to 25 mg/dL of bilirubin.
No significant interference was found at levels of up to 1350 mg/dL of triglycerides (using Intralipid*).

**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

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2. Salt, W.B. II and Schenker, S., *Medicine* 55:269 (1976)
3. Stefanini, P., Ermini, M., and Carboni, M., *J. Am. Surg.*, 110:866 (1965).
4. Chavez, R.G., et. al. U.S. Patent 4,963,479
5. Demetriou, J. et al., Clinical Chemistry: Principles and Techniques, 2nd Ed., Harper & Row (1974).
6. Tietz NW. Textbook of Clinical Chemistry, 2nd ed. WB Saunders and Co., Philadelphia, PA, p. 831-832 (1994).
7. Data on file at Medica.
8. Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 2nd ed. Washington, DC. AACC Press; 1997.
9. Young DS. *Effects of Drugs on Clinical Laboratory Tests*. 4th ed. Washington, DC: AACC Press; 1995

EasyRA Assay Parameters (AMY)

Wavelength (nm)	405
Reaction Type	Enzyme (0)
Reaction Direction	Increase
Reagent Blank	No
Sample Blank	No
Max. first interval Abs. change	0.11
Reaction Time	4 min
Calibration interval (maximum)	N/A

Serum/Plasma

Sample volume (µl)	5.0
Diluent volume (µl)	0
Reagent volume (µl)	160
Reagent on-board stability	30 days
Decimal Places (default)	0
Units (default values)	U/L
Dilution Factor	1:1 (to extend measuring range)
Linearity	2 to 1200 U/L
Molar Absorptivity	12.9

Medica Corporation, 5 Oak Park Drive
Bedford, MA 01730-1413 USA

EC REP Emergo Europe, Prinsessegracht 20
2514 AP The Hague, The Netherlands