

Amylase (serum, plasma)

1 Name and description of analyte

1.1 Name of analyte

Amylase (serum, see 10.2)

1.2 Alternative names

α -Amylase, 1,4- α -D-Glucan glucanohydrolase (EC 3.2.1.1)

Abbreviation: AMY

Isoenzymes: AMY1 (ptyalin, S-type, S-AMY); AMY2 (P-type, P-AMY)

1.3 NLMC code

1.4 Description of analyte

Amylase is a heterogeneous calcium-dependent metalloenzyme of MW 54-62 kDa. It exists as two isoenzymes: pancreatic (P-type) and non-pancreatic (S-type). These isoenzymes are products of two closely linked loci on chromosome 1. Additional amylase heterogeneity is due to allelic variation (S-type, 12 alleles; P-type, 6 alleles). Both also undergo post-translational modification by deamidation, glycosylation, and deglycosylation to form various isoforms.

Amylase has a wide tissue distribution with the highest activities of the P- and S-types being found in the exocrine pancreas and salivary glands, respectively. P-type amylase is synthesized by pancreatic acinar cells and secreted into the intestinal tract via the pancreatic duct system. Its action is favoured by the mildly alkaline conditions in the duodenum. The greatest S-type amylase activity is in salivary glands, where it initiates the hydrolysis of starches while food is in the mouth and oesophagus. Its action is terminated by acid in the stomach. S-type amylase is also found in extracts of testes, ovaries, fallopian tubes, mullerian ducts, striated muscle, lungs and adipose tissue, as well as in semen, colostrum, tears and milk.

About 25% of plasma amylase is excreted by the kidneys but the majority is reabsorbed in the proximal tubules. The liver is suspected to be the major organ for amylase removal, resulting in a half-life of about 10 h.

1.4 Function of analyte

Amylase is an endoglycosidase enzyme of the hydrolase class that catalyses the hydrolysis of 1,4- α -glucosidic linkages between adjacent glucose units in complex carbohydrates. Both straight-chain (linear) polyglucans, such as amylose, and branched polyglucans, such as amylopectin and glycogen, are hydrolysed, but at different rates. In the case of amylose, the enzyme splits the chains at alternate α -1,4-hemiacetal (-C-O-C-) links, forming maltose and some residual glucose. In the case of branched polyglucans, maltose, glucose and a residue of limit dextrans are formed. The enzyme does not attack the α -1,6-linkages at the branch points.

The functional integrity of amylase is absolutely dependent on the presence of calcium. However, full integrity is displayed only in the presence of any one of various anions, including chloride, bromide,

nitrate, cholate or monohydrogen phosphate. Chloride and bromide are the most effective activators. The pH optimum is 6.9 to 7.0.

2 Sample requirements and precautions

2.1 Medium in which measured

Amylase is mainly measured in serum. Measurements in urine are sometimes valuable.

Amylase is occasionally measured in ascitic, peritoneal or pleural fluid where its presence can indicate pancreatitis or the presence of a tumour.

2.2 Precautions re sampling, handling etc.

With the exception of heparin, all common anticoagulants inhibit amylase activity because they chelate calcium: citrate, ethylenediaminetetraacetic acid and oxalate inhibit it by as much as 15%. Therefore, amylase assays should be performed only on serum or heparinised plasma.

Amylase is stable: activity is fully retained during storage for 4 days at room temperature, 2 weeks at +4 °C, 1 year at -25 °C, and 5 years at -75 °C.

3 Summary of clinical uses and limitations of measurements

3.1 Uses

1. The main application for measuring total amylase or P-type amylase is to support a diagnosis of acute pancreatitis: serum amylase activity typically increases within 2–12 h of onset of symptoms, has a 24 h peak (often more than five times the upper limit of normal) and remains elevated for 3–7 days.

2. Amylase isoenzyme fractionation may be valuable in determining whether hyperamylasaemia (see 6.2) in HIV patients receiving highly active anti-retroviral therapy including nucleoside analogues (ddI, ddC etc) is due to drug-induced pancreatitis or an increase in the non-pancreatic fraction.

3.2 Limitations

A significant proportion of subjects of African and Asian origin have an S-type amylase activity above the reference interval derived from Caucasian populations. This can result in an apparently elevated total amylase that is non-pathological. The presence of macroamylasaemia (see 6.2) may also result in a non-pathological increase in total amylase activity.

Measurement of total serum amylase lacks specificity for acute pancreatitis since it can also be raised in appendicitis, renal disease, pregnancy, diabetic ketoacidosis and gastro-intestinal disease, among many other causes (see 6.2).

4 Analytical considerations

4.1 Analytical methods

A. Total amylase

There are more approaches to measuring amylase than almost any other common clinical analyte. Historically, saccharogenic, amyloclastic and chromolytic starch methods were the assays of choice, but these are now obsolete. They have been replaced by methods with well-defined

substrates. This, and the use of auxiliary and indicator enzymes, has improved the reaction stoichiometry and led to more controlled and consistent hydrolysis conditions. Substrates used include small oligosaccharides and 4-nitrophenyl (4-NP)-glycoside substrates.

1. α -Glucosidase (maltase: E.C.3.2.1.20) used in combination with hexokinase (E.C.2.7.1.1) coupled with glucose 6-phosphate dehydrogenase (β D-glucose 6-phosphate: NAD(P)⁺ 1-oxidoreductase, EC.1.1.1.49). The reactions are
 - a. Maltopentose \rightarrow (α -amylase) \rightarrow maltotriose + maltose
 - b. Maltotriose + maltose \rightarrow (α -glucosidase) \rightarrow 5 glucose
 - c. Glucose + ATP \rightarrow (HK) \rightarrow glucose 6-phosphate (G6P) + ADP
 - d. G6P + NAD⁺ \rightarrow (G6P dehydrogenase) \rightarrow 6-phosphogluconolactone + NADH

The formation of NADH is measured spectrophotometrically at 340 nm.

2. Maltose phosphorylase (maltose: phosphate 1- β D-glucosyltransferase: E.C.2.4.1.8) used in combination with β -phosphoglucomutase (β D-glucose 1,6-phosphomutase, EC.5.4.2.6) coupled with glucose 6-phosphate dehydrogenase (β D-glucose 6-phosphate: NAD(P)⁺ 1-oxidoreductase, EC.1.1.1.49) to avoid interference from endogenous glucose. The reactions are:
 - a. Maltotetraose \rightarrow (α -amylase) \rightarrow 2 maltose
 - b. Maltose + inorganic phosphate \rightarrow (maltose phosphorylase) \rightarrow glucose + glucose 1-phosphate (G1P)
 - c. G1P \rightarrow (β -phosphoglucomutase) \rightarrow G6P
 - d. G6P + NAD⁺ \rightarrow (G6P dehydrogenase) \rightarrow 6-phosphogluconolactone + NADH

For each bond hydrolysed by amylase, two molecules of NADH are produced, but only if neither glucose nor maltotriose is produced by the action of amylase. In practice, these products are present in significant quantities in the reaction mixture; therefore, this method underestimates amylase.

3. α -Glucosidase (maltase: E.C.3.2.1.20) in combination with 4-nitrophenol-glycoside substrates. The reactions are:
 - a. 4-NP-(glucose)₇ \rightarrow (α -amylase) \rightarrow 4-NP-(glucose)_{4,3,2}
 - b. 4-NP-(glucose)_{4,3,2} \rightarrow (α -glucosidase) \rightarrow 4-NP-(glucose)₄ + glucose + 4-NP

The result of the combined hydrolysis by amylase in the specimen and by the reagent α -glucosidase is that >30% of the product is free 4-NP. The formation of free 4-NP is measured spectrophotometrically at 405 nm.

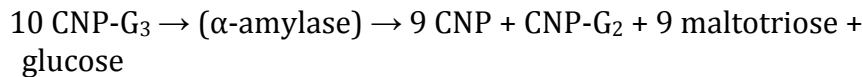
α -Glucosidase does not react with oligosaccharides containing more than four glucose molecules. Originally, there were problems with the poor stability of the reconstituted assay mixture because of the slow hydrolysis of the 4-NP-glycoside by α -glucosidase. This has been reduced by covalently linking a 'blocking' group, e.g. a 4,6-ethylidene group, to the non-reducing end of the molecule (EPS, ethylidene protected substrate). The blocked substrate also shows a different and more advantageous hydrolysis pattern. The ethylidene-4-NP-(glucose)₇ substrate fragments approximately as 4-NP-(glucose)₂ (40%), 4-NP-(glucose)₃ (40%) and 4-NP-(glucose)₄ (20%).

4. α -Glucosidase (maltase: E.C.3.2.1.20) in combination with blocked 4-nitrophenol-glycoside substrates. The reactions are:

- a. $\text{EPS-4-NP-(glucose)}_7 + \text{H}_2\text{O} \rightarrow (\alpha\text{-amylase}) \rightarrow \text{EPS-4-NP-(glucose)}_x + 4\text{-NP-(glucose)}_{7-x}$
- b. $4\text{-NP-(glucose)}_{7-x} + (7-x) \text{H}_2\text{O} \rightarrow (\alpha\text{-glucosidase}) \rightarrow 4\text{-NP} + (7-x) \text{glucose}$

IFCC has optimised this method at 37°C, and this is the reference procedure.

5. An alternative method based on the 2-chloro-*p*-nitrophenol (CNP) indicator uses 2-chloro-*p*-nitrophenyl- α D-maltotriose (CNP-G₃) as a substrate. The reaction is:



This assay does not require glucosidases and is considered a 'direct' assay. Its disadvantages include low substrate conversion rate and the variation in molar absorptivity of CNP associated with changes in pH, temperature and protein content, as well as the presence of the activator, potassium thiocyanate, causing allosteric changes to amylase and precluding the use of antibodies for P-type amylase determination.

B. Amylase isoenzyme differentiation

P-type amylase can be differentiated from the S-type amylase by selective inhibition of S-type by a wheat germ inhibitor, temperature inhibition, immunoprecipitation or immunoinhibition by a monoclonal antibody. However, only the methods based on selective inhibition by monoclonal antibodies have shown sufficient precision, reliability, practicability, and analytical speed to allow reliable measurement of P-type amylase. A commercially available assay that uses the synergistic action of two immunoinhibitory monoclonal antibodies to S-type amylase is available. The uninhibited P-type amylase activity is measured using EPS-4-NP-(glucose)₇ as a substrate. False positive P-type amylase results have been reported in subjects with macroamylasaemia, as the complex diminishes the ability of monoclonal antibodies to efficiently inhibit S-type amylase.

C. Amylase isoform separation:

The amylase isoforms can be separated by isoelectric focusing, ion-exchange chromatography or gel/capillary electrophoresis by electrophoretic endosmosis.

D. Determination of the amylase macrocomplex:

1. Electrophoresis: macroamylase forms a broad migrating band different from the homogeneous bands produced by the amylase isoenzymes present in serum.
2. Precipitation: using a polyethylene glycol (PEG) 6000 solution (240 g/L); residual amylase activity of <30% in the supernatant is indicative of significant macroamylase.

4.2 Reference method

The reference method is an optimised version (at 37 °C) of the method shown in 4.1(4).

4.3 Reference materials

Primary reference material: α -amylase from human pancreas (IRMM/IFCC-456).

4.4 Interfering substances
High serum [triglyceride] interferes with some α -amylase assays.

4.5 Sources of error
Amylase is inhibited by most non-heparin anticoagulants including citrate, ethylenediaminetetraacetic acid and oxalate.

5 Reference intervals and variance

5.1.1 Reference interval (adults)
Serum amylase is method-dependent. Using the IFCC recommended method at 37 °C, the serum reference interval in adults is 28–100 U/L (0.48–1.70 μ kat/L). P-type amylase represents 35–50% of the normal serum amylase, with S-type amylase forming the remainder.

5.1.2 Reference intervals (others)
After birth, S-type amylase activity increases in serum steadily with age and reaches normal adult values at 2 years of age. P-type amylase is not demonstrable in serum in most children less than 6 months old, but activity rises slowly thereafter to reach adult levels at 5 years of age, reflecting post-natal development of exocrine pancreatic function.
The reference interval does not differ between males and females.

5.1.3 Extent of variation

5.1.3.1 Interindividual CV: 32.1%

5.1.3.2 Intraindividual CV: 10.8%

5.1.3.3 Index of individuality: 0.34

5.1.3.4 CV of method: typically <3%

5.1.3.5 Critical difference: 30.3%

5.1.4 Sources of variation

The S-type amylase gene has undergone duplication during evolution, and many individuals have multiple tandem repeats of the gene. The gene copy number is associated with apparent evolutionary exposure to high-starch diets. Subjects of African and Asian origin tend to have a higher total amylase activity (due to increased S-type amylase activity) compared with those of Caucasian descent.

6 Clinical uses of measurement and interpretation of results

6.1 Uses and interpretation

Diagnosis of acute pancreatitis

A diagnosis of acute pancreatitis requires two of the following three features: abdominal pain characteristic of acute pancreatitis; serum amylase (and/or lipase) ≥ 3 times the upper limit of normal (ULN), and characteristic findings of acute pancreatitis on CT scan, MRI or MRCP (magnetic resonance cholangiopancreatography). This allows for the possibility that amylase might be <3 times the ULN in acute pancreatitis.

In acute pancreatitis, serum amylase activity typically increases within 2–12 h of onset of symptoms, has a 24 h peak and remains elevated for 3–7 days. The magnitude of elevation is not related to severity. However, the greater the rise, the higher the probability of acute pancreatitis. Serial measurements of serum amylase have little if any value in assessing prognosis or in altering patient management. However, if serum amylase remains elevated for several weeks, this may indicate persisting

pancreatic/peripancreatic inflammation, blockage of the pancreatic duct, or development of a pseudocyst.

6.2. Confounding factors

Diagnostic specificity is low ($\leq 70\%$), since P-type amylase is increased in a number of other acute intra-abdominal disorders and S-type amylase in other conditions, e.g. salivary disease, ovarian and lung tumours, and ruptured ectopic pregnancy. Both may be increased in diabetic ketoacidosis, renal failure, HIV and macroamylasaemia.

The peak of serum amylase activity does not correlate with the severity of acute pancreatitis.

Increased amylase activity in serum can result from a condition known as macroamylasaemia – a state in which amylase forms macromolecular complexes, usually with immunoglobulin (IgA or IgG in most cases), but also as self-polymerisation or association with other proteins. These complexes generally retain enzymatic activity but cannot be filtered by renal glomeruli; this leads to delayed clearance and increased serum amylase activity. This benign condition has been reported in as many as 1.5% of hospitalised patients, accounting for as much as 28% of chronic, otherwise unexplained hyperamylasaemia. Macroamylasaemia has a disease association with autoimmunity, malignancy, cardiovascular disease, diabetes mellitus and malabsorptive disorders.

7. Causes and investigation of abnormal results

7.1 High values

7.1.1 Causes

- Pancreatic disease: causing raised P-type amylase e.g. pancreatitis, trauma, tumours etc.
- Any other intra-abdominal condition: e.g. biliary tract disease, obstruction, liver disease, mesenteric infarction, acute appendicitis, tumours etc.
- Salivary gland disease: causing raised S-type amylase e.g. infection, trauma, irradiation.
- Many other conditions: e.g. tumours of the testes, ovary, prostate, oesophagus, thymus, thyroid or lung, ruptured ectopic pregnancy, and renal disease including renal insufficiency
- Miscellaneous conditions: including HIV, diabetic ketoacidosis, macroamylasaemia, and various drugs (e.g. opiates, diuretics, steroids).

7.1.2 Investigation

Numerous investigations can be carried out including imaging and second-line blood tests depending on the clinical features of the patient. No other laboratory investigations contribute to the diagnosis of acute pancreatitis (see 6.1 (1)) but in acute pancreatitis, biochemical abnormalities can include uraemia, hypoalbuminaemia, hypocalcaemia, hyperglycaemia, metabolic acidosis, hypoxaemia, and abnormal liver function tests. In non-acute situations, macroamylasaemia can be excluded by serum electrophoresis or precipitation methods.

7.2 Low values

7.2.1 Causes

A decreased serum P-type amylase activity (below the lower limit of normal) is highly specific for exocrine pancreatic insufficiency.

7.2.2 Investigation

Measurement of immunoreactive trypsinogen or faecal elastase, as well as imaging of the pancreas and ducts can contribute to the diagnosis of exocrine pancreatic insufficiency.

7.3 Notes

Substantial elevations in serum amylase activity (more than two times the upper limit of normal) may, for reasons that are not well understood, be less common in patients with hypertriglyceridaemia-associated pancreatitis.

8 Performance

8.1 Sensitivity, specificity etc. for individual conditions

For acute pancreatitis, total amylase activity above the upper limit of normal has a sensitivity of 95–100%, a specificity of 70%, positive predictive value of 15–72% and negative predictive value of 97–100%. If a cut-off level of 1000 U/L is chosen, the specificity of total amylase approaches 95% but the sensitivity is as low as 61%. Sensitivity is also reduced by late presentation, hypertriglyceridaemia and chronic alcoholism.

The lack of specificity of total amylase activity for acute pancreatitis has led to interest in the direct measurement of P-type amylase for the differential diagnosis of acute abdominal pain. An elevated P-type amylase has a sensitivity of 84–100%, a specificity of 40–97%, positive predictive value of 50–96% and negative predictive value of 70–100%. If a cut-off of three times the upper limit of normal is used, the specificity of P-type amylase is >90%. Measurement of P-type amylase also improves sensitivity in the late detection of acute pancreatitis since P-type amylase remains elevated for longer than total amylase activity.

9. Systematic reviews and guidelines

9.1 Systematic reviews

None identified

9.2 Guidelines

Banks PA, Freeman ML. Practice Parameters Committee of the American College of Gastroenterology (2006) Practice Guidelines in Acute Pancreatitis. *Am J Gastroenterol* 2006; 101: 2379-2400.

UK Working Party on Acute Pancreatitis. UK guidelines for the management of acute pancreatitis. *Gut* 2005; 54(III): iii1-iii9.

Toouli J, Brooke-Smith M, Bassi C *et al.* Guidelines for the management of acute pancreatitis. *J Gastroenterol Hepatol* 2002; 17 Suppl: S15.

9.3 Recommendations

Carroll JK, Herrick B, Gipson T, Lee SP. Acute Pancreatitis: Diagnosis, Prognosis, and Treatment. *Am Fam Physician* 2007; 75:1513-1520.

Kiryama S, Gabata T, Takada T *et al.* New diagnostic criteria of acute pancreatitis. *J Hepatobiliary Pancreat Sci* 2010; 17: 24–36.

10 Links

10.1 Related analytes

1. Lipase

Serum lipase is derived mainly from pancreatic acinar cells. Measurement of serum lipase activity can be used as an alternative to amylase to support a diagnosis of acute pancreatitis. Serum lipase usually rises 4–8 h after the onset of symptoms, peaks at 24 h and remains elevated for 8–14 days. Since the pancreas is the main source of lipase, it also has superior sensitivity, specificity and greater overall accuracy compared to total amylase. The sensitivity of serum lipase for the diagnosis of acute pancreatitis ranges from 85–100%. However, lipase is technically more difficult to measure and nonspecific elevations of lipase have been reported in almost as many diseases as amylase. In addition, the magnitude of increase in serum lipase can vary widely depending on the method used for measurement. Recent technical improvements such as inclusion of co-lipase in commercially available kits has improved the diagnostic accuracy.

Serum lipase measurement can be useful in suspected acute pancreatitis where another condition that causes an increase in S-type amylase is also present. It also possesses a higher sensitivity in alcohol-induced acute pancreatitis.

Most laboratories continue to measure serum amylase activity owing to the increased costs and limited additional benefits in the majority of patients with acute pancreatitis.

10.2 Related tests

Urinary amylase: hyperamylasuria occurs in acute pancreatitis, because the normal 3% amylase:creatinine clearance ratio (ACCR), indicating that approximately 3% of filtered amylase is excreted, increases to about 10%.

Urinary amylase activity often reaches very high levels in such cases and persists for longer than a raised serum amylase. However, measurement of amylase in urine is not sufficiently accurate to distinguish acute pancreatitis from other intra-abdominal conditions that are associated with a high serum amylase. Other than to diagnose macroamylasaemia, urinary amylase offers no advantage over routine serum amylase measurement.

Author: Sophie Hepburn

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