Alkaline phosphatase (serum, plasma)

1 Name and description of analyte

1.1 Alkaline phosphatase (ALP)

1.2 Alternative names
Orthophosphoric-monoester phosphohydrolase (alkaline optimal), EC 3.1.3.1)

1.3 NMLC code
To follow

1.4 Description of analyte
ALP is a zinc-containing metalloenzyme; it is activated by Mg^{2+} and other divalent ions.
It has various isoforms, some being true isoenzymes, that is, encoded by different genes. It is widely distributed in the body, but is particularly associated with:

- bone (osteoblasts)
- small intestine (mucosal cells)
- liver (cells of the biliary system)
- placenta
- kidney (proximal convoluted tubules).

The bone, liver and kidney isoforms have the same amino acid sequence (coded on chromosome 1) but differ in their carbohydrate content; the intestinal and placental forms also have their primary structures in common (coded on chromosome 2).
Variants of the placental isoenzyme are sometimes expressed in patients with malignancy (e.g. Regan isoenzyme).
ALP present in normal plasma is approximately equally of hepatobiliary and bone origin.

1.5 Function of analyte
In bone, ALP is involved in mineralization, possibly by catalyzing the formation of phosphate from pyrophosphate. In the gut, there is some evidence of its having a role in lipid transport.

2 Sample requirements and precautions

2.1 Medium in which measured
Alkaline phosphatase activity is measured in serum or heparinised plasma; plasma from blood anticoagulated with citrate, oxalate or EDTA should not be used as these substances bind activating cations.

2.1 Precautions re sampling, handling etc.
No special precautions are required. Activity is stable in serum for 4 h at room temperature; a slight increase in activity (~2%/24 h) may occur at 4 ^{o}C.

3. Summary of clinical uses and limitations of measurements

3.1 Uses

1. Jaundice and suspected hepatobiliary disease

ALP activity in plasma increases in hepatobiliary disease with cholestasis as a result of increased enzyme synthesis (enzyme induction). The highest levels are seen with complete or near-complete biliary obstruction, but the level itself does not contribute to the diagnosis of the *cause* of the obstruction (whether intra- or extra-hepatic). In hepatocellular disease with no cholestasis, there is either no or only a slight rise in activity.

2. Bone disease

ALP activity in plasma increases in bone disease in which there is increased osteoblastic activity and reflects the extent of that activity. Thus ALP is increased in Paget's disease, osteomalacia, rickets and some patients with renal osteodystrophy, but not in osteoporosis unless complicated by fracture (activity is increased with healing fractures).

3.2 Limitations

Increases in ALP activity reflect pathological processes: they may contribute to making a specific diagnosis, but cannot establish one in isolation. It should be noted that the upper reference limit is higher in children (see 5.1.2).

4 Analytical considerations

4.1 Analytical methods

1. Measurement of ALP activity

ALP is measured in a reaction in which it catalyzes the cleavage of phosphate from 4-nitrophenyl phosphate (4-NPP, colourless) to form 4-nitrophenoxide (benzenoid form), also colourless, which undergoes spontaneous rearrangement at alkaline pH to the quinonoid form (yellow). The reaction is followed by measuring absorbance at 405 nm. AMP is usually included in the reaction mixture as a phosphate acceptor; the reaction proceeds in the absence of a specific acceptor (phosphate being transferred to water) but the rate is increased by such an acceptor.

2. Measurement of isoforms

Measurement of specific isoforms of ALP has been used in the past to identify the source of an increased activity when this has not been clear on the basis of other considerations (clinical and other investigations). Techniques that have been employed include electrophoresis, differential deactivation e.g. by heat, differential response to inhibitors, affinity for lectins and immunoassay (for the bone isoform). The techniques are demanding and the separations achieved not always clear-cut. In practice, and in particular with the availability of modern imaging techniques, they are now much less frequently used than was once the case.

4.2 Reference method

A candidate reference method is based on the 4-NPP method (4.1).

4.3 Reference materials

The standard reference material has been BCR371 (derived from porcine kidney) (Institute for Reference Materials and Methods, IFMM), but a new reference material is currently (2013) being investigated by the IRMM and International Federation for Clinical Chemistry (IFCC).

4.4 Interfering substances
The presence of any substance that can chelate magnesium or zinc will reduce measured activity.

4.5 Sources of error
For measurement of activity: the pH range over which maximum catalytic activity is observed is narrow but provided that optimum conditions are maintained, the method is robust.

5 Reference intervals and variance

5.1.1 Reference interval (adults): 50–120 U/L; a slightly higher upper limit is sometimes considered appropriate in older people (>60 y) but this may reflect the relatively high prevalence of sub-clinical Paget's disease in this age group.
5.1.2 Reference intervals (others): values are typically higher in pregnancy (up to ~3x in the last trimester) and in children (usually up to ~2x, but more during the pubertal growth spurt).
5.1.3 Extent of variation
5.1.3.1 Interindividual CV: 6.7%
5.1.3.2 Intraindividual CV: 25.4%
5.1.3.3 Index of individuality 0.24
5.1.3.4 CV of method: 3%
5.1.3.5 Critical difference: 37%
5.1.4 Sources of variation
Because alkaline phosphatase is a zinc-containing enzyme, low activities may occur in patients with zinc deficiency (but see 9.1(1)); see also 5.1.1 and 5.1.2. Alkaline phosphtase activity in Wilson disease may be miseadlingly low because of competition between copper and zinc.

6 Clinical uses of measurement and interpretation of results

6.1 Uses and interpretation
1. Jaundice
In a jaundiced patient, a high serum ALP suggests predominant cholestasis (as opposed to hepatocellular damage). This may, however, be intrahepatic (e.g. cirrhosis) or extrahepatic (e.g. carcinoma of the pancreas obstructing the common bile duct); the ALP does not help to distinguish between these causes. ALP may exceed five times the upper reference limit (5 x ULN) in cholestatic jaundice. ALP is often elevated in hepatocellular causes of jaundice (because there is frequently an element of cholestasis) but typically to less than 3 x ULN. ALP activity is of prognostic value in primary biliary cirrhosis and primary sclerosing cholangitis.
2. Bone disease and disordered calcium homeostasis
Serum ALP activity is typically increased in rickets, osteomalacia and Paget's disease. In the first two of these, serial measurements can be used to monitor the efficacy of treatment. The same is true for Paget's disease although treatment decisions are more usually based on the effect of treatment on the associated pain.

6.2 Confounding factors

The reference range for ALP is higher in children than in adults.

7 Causes of abnormal results

7.1 High values

7.1.1 Causes

1. Hepatobiliary disease (with or without jaundice)
- cirrhosis (primary and secondary)
- infiltrative conditions
- intrahepatic tumours
- hepatitis (but see 6.1 (1)
- biliary atresia
- biliary obstruction
  - impacted gall stone
  - cholangitis
  - carcinoma of pancreas

2. Bone disease
- Paget's disease
- rickets
- osteomalacia
- renal osteodystrophy (not adynamic disease)
- tumours (especially lytic metastases)
- primary hyperparathyroidism (severe)

3. Other
- transiently elevated ALP (up to10 x ULN) can occur as a benign condition in children and adults.
- ALP is frequently increased in malignancy as a result of metastasis to bone (particularly in prostatic cancer) or liver, but can be increased as a result of secretion of an isoform of the enzyme by tumour tissue (e.g. Regan isoenzyme).
- ALP activity is increased in pregnancy (mainly last trimester), owing to a contribution from the placental isoenzyme.
- The intestinal isoform is increased in non-fasting secretors and some diabetic subjects and decreases to normal after an overnight fast.

7.1.2 Investigation

If increased serum ALP activity is found in association with clinical or other features of any of the conditions listed above, further investigation will be determined by the suspected diagnosis.

If an isolated increase in ALP is found, the action to be taken should depend on the value:
- ≤1.5 x ULN – recheck in 1–3 months to confirm the finding
- >1.5 x ULN on two separate occasions, investigate further
- >3.0 x ULN on single measurement, investigate further

Measurement of serum gamma-glutamyltransferase is the appropriate next investigation: if elevated, the source of the increased ALP is hepatobiliary; if not, it is likely to be bone. Isoenzyme (strictly, isoform) studies are feasible but in practice, imaging (e.g. plain X-ray of bones, ultrasound of the liver) will usually confirm the origin.

7.2 Low values

7.2.1 Causes. Low values are uncommon. Causes include
- zinc deficiency

- hypothyroidism
- hypophosphatasia.

The latter is a rare familial disorder (autosomal recessive) of varying severity characterised by orthopaedic abnormalities and a low serum ALP activity. It usually presents after six months of age but there a lethal perinatal and a late presenting form.

7.2.2 Investigation

Measurement of serum zinc concentration will indicate zinc deficiency; measurement of alkaline phosphatase is of no value for this purpose (see 9.1(1)). The characteristic finding in hypophosphatasia is an increased plasma concentration of pyridoxal 5'-phosphate.

7.3 Notes

1. It should be noted that hepatobiliary disease is not always associated with an increase in ALP or, indeed, in abnormalities of other liver function tests.
2. Measurement of ALP (and/or serum [calcium] and [phosphate] concentrations) should not be used to screen for vitamin D deficiency: they may be normal, particularly if deficiency is mild.

8 Performance

8.1 Sensitivity, specificity etc. for individual conditions

The association of increased serum alkaline phosphatase with both hepatobiliary and bone disease limits its diagnostic sensitivity and specificity. Measurement of bone alkaline phosphatase has been demonstrated to be of potential value in particular clinical circumstances, examples of which follow.

1. Withold W, Georgescu G, Khakzad H *et al.* Efficacy of simultaneous determination of bone alkaline phosphatase mass concentration in serum and urinary excretion of pyridinium cross-links for detection of bone metastases. Clin Biochem 1995; 28:511-519. *Serum bone specific alkaline phosphatase activity was found to be more frequently elevated than total alkaline phosphatase in patients with bone metasases.*
2. Bodlaj G, Hubmann R, Saleh K *et al.* Alkaline phosphatase predicts relapse in chronic hepatitis C patients with end-of-treatment response. World J Gastroenterol. 2010;16:2407-2410. *Low serum alkaline phosphatase activity may predict relapse in patients initially responding to treatment of hepatitis C infection.*
3. KDOQI Clinical Practice Guidelines for Bone Metabolism and Disease in Children With Chronic Kidney Disease. http://www.kidney.org/professionals/kdoqi/guidelines_pedbone/guide1.htm. (accessed 16.v.2012)

The predictive power of PTH measurement to detect renal osteodystrophy is enhanced by simultaneous measurement of alkaline phosphatase activity

9 Systematic reviews and guidelines

9.1 Systematic reviews

No systematic reviews identified in relation to the use of measurements of alkaline phosphatase in the more frequently occurring types of hepatobiliary and bone disease.

1. Lowe M, Fekete K, Decsi, T. .Methods of assessment of zinc status in humans: a systematic review. Am J Clin Nutr 2009; 89:2040S-2051S. *Measurement of serum alkaline phosphatase is not a useful tool in the assessment of zinc status.*

9.2 Guidelines

1. The National Academy of Clinical Biochemistry. Laboratory Guidelines for Screening, Diagnosis and Monitoring of Hepatic Injury. 2000. http://www.aasld.org/practiceguidelines/Documents/Practice%20Guidelines/Hepatic.pdf
2. Minuk GY. Canadian Association of Gastroenterology practice guidelines: evaluation of abnormal liver enzyme tests. Can J Gastroenterol 1998;12:417–421.
3. Reust CE. Hall L. What is the differential diagnosis of an elevated serum alkaline phosphatase (AP) level in an otherwise asymptomatic patient? J Fam Pract 2001;50:496-497.

9.3 Recommendations

1. Shipman KE, Holt DA, Gama R. Interpreting an isolated raised serum alkaline phosphatase level in an asymptomatic patient. BMJ 2013;346:f976.
2. Smellie S. McNulty C, Galloway M. Liver Function Tests, Primary Care and Laboratory Medicine frequently asked questions. London ACB Venture Publications 2001, pp. 211-222.

10 Links

10.1 Related analytes

Measurement of bone-specific ALP has been advocated as a marker of bone formation in metabolic bone disease, but is not widely used for this purpose.

10.2 Related tests

ALP is typically measured as a component of a panel of 'liver function' tests (or 'liver profile') (including serum albumin and bilirubin concentrations and an aminotransferase alanine aminotransferase, ALT, or aspartate aminotransferase, ASTactivity, or together with serum calcium and phosphate concentrations ('bone profile').

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