Scotland Hypophosphatasia Audit Results 2025

Audit Title: Laboratory Screening for Hypophosphatasia in Scotland	
Lead Auditors: Dr Jane McNeilly Dr Fiona Stratford	**Audit date(s):** June 2025- August 2025
Please indicate if **Regional** Please indicate which hospital & location or region **Scotland**	**Report Authors:** Name: Jane McNeilly and Fiona Stratford Email: jane.mcneilly@nhs.scot Fiona.stratford@nhs.scot

Background:

Serum alkaline phosphatases are a group of enzymes that catalyse the hydrolysis of phosphoesters to release inorganic phosphate. Reduced serum alkaline phosphatases are associated with a variety of conditions including severe nutritional deficiencies (protein, zinc, folic acid and magnesium as well as vitamins B6, B12 and C), vitamin D excess, hypothyroidism, hypoparathyroidism and coeliac disease.

Hypophosphatasia is a rare inherited bone disorder caused by inactivating mutations in the ALPL gene which encodes the tissue nonspecific form of ALP. Patients with hypophosphatasia have low ALP activity which results in accumulation of the enzyme substrates inorganic pyrophosphate (PPi), pyridoxal 5'phosphate (PLP) and the biochemical marker phosphoethanolamine. PPi inhibits bone mineralisation leading to skeletal abnormalities such as rickets, osteomalacia/osteoporosis, and fractures. Based on age of presentation and severity of symptoms hypophosphatasia can be classified into perinatal lethal, prenatal benign, infantile, childhood, adult and odontohypophosphatasia (dental manifestations only). The recessive perinatal and infantile forms are usually detected early because patients present with severe symptoms, however the dominantly inherited forms that present in childhood and adulthood may only have mild symptoms such as early tooth loss or chronic pain [1-5].

The diagnosis of hypophosphatasia is made on the basis of clinical, biochemical and radiologic features. Biochemically, patients will have a low ALP which is typically associated with elevated serum phosphate, PLP and urinary PEA levels[1].

Enzyme replacement therapy is available for children with hypophosphatasia and early diagnosis can be life-saving, in adults it may help avoid years of undiagnosed morbidity[5]. The laboratory can play an important role in identifying patients at risk of hypophosphatasia and in guiding clinicians to appropriate biochemical investigations. This audit aims to review current practices across Scotland in the laboratory screening for hypophosphatasia.

Aim:

To evaluate the current practice across Scotland in the laboratory screening for hypophosphatasia.

Standards / Guidelines:

1) Diagnostic algorithm for children with low alkaline phosphatase activities (Saraff et al, 2016 *JPEDS*)

2) ALP Decision Algorithm (Fraser WD in Tietz Clinical Chemistry and Molecular Diagnostics 6th Ed. Ch. 64 p 1468)

References

1. Whyte, M.P. (2017). Hypophosphatasia: An overview for 2017. *Bone*, 102, pp.15–25. doi:https://doi.org/10.1016/j.bone.2017.02.011.
2. Shapiro, J.R. and Lewiecki, E.M. (2017). Hypophosphatasia in Adults: Clinical Assessment and Treatment Considerations. *Journal of Bone and Mineral Research*, 32(10), pp.1977–1980. doi:https://doi.org/10.1002/jbmr.3226.
3. Linglart, A. and Biosse-Duplan, M. (2016). Hypophosphatasia. *Current Osteoporosis Reports*, 14(3), pp.95–105. doi:https://doi.org/10.1007/s11914-016-0309-0.
4. Saraff, V., Narayanan, V.K., Lawson, A.J., Shaw, N.J., Preece, M.A. and Högler, W. (2016). A Diagnostic Algorithm for Children with Low Alkaline Phosphatase Activities: Lessons Learned from Laboratory Screening for Hypophosphatasia. The Journal of Pediatrics, 172, pp.181-186.e1. doi:https://doi.org/10.1016/j.jpeds.2016.01.045.
5. Millán, J.L. and Whyte, M.P. (2015). Alkaline Phosphatase and Hypophosphatasia. Calcified Tissue International, 98(4), pp.398–416. doi:https://doi.org/10.1007/s00223-015-0079-1.
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Audit Method:

A 14-question survey was designed by the Scotland Clinical Audit Group and was sent to 14 Scottish health boards. The questionnaire remained open from 9th June 2025 to the 29th August 2025.

Summary of Results:

Nine health boards completed the survey. A summary of responses to each question is shown below.

Question 1. What platform does your laboratory currently use to measure ALP?

Three different analytical platforms are used to measure ALP, in the health boards that completed the survey. Roche Cobas was used by four health boards, three health boards used the Abbott Alinity and two health boards used the Siemens Atellica platform.

Question 2. Which method does your laboratory use to measure ALP?

All laboratories use a method based on the transphosphorylation of para-nitrophenyl phosphate to para-nitrophenol in 2-amino-2-methyl-1-propanol buffer (AMP). It is assumed that all laboratories are using an IFCC method but this requires further clarification.

Questions 3-7. Does your laboratory use gender and age specific reference ranges for ALP?

Seven out of nine laboratories use a universal reference range for male and female adults, derived by the Pathology Harmony project in 2011. One laboratory uses an in-house range for adults derived from local population (200 normal blood donor samples). Only one out of the nine health boards that completed the survey had gender specific reference ranges for adults, the source of these reference ranges was the Scottish Clinical Biochemistry Network (SCBN).

Source of Reference Range	Reference range (U/L)
Pathology Harmony (2011)	30-130 (All)
In-house	4-125 (All)

SCBN	40-129 (Males) 35-104 (Females)

Table 1: Reference ranges in use for adults in Scottish Health Boards

For paediatric patients, five laboratories were using age, gender and method specific reference ranges derived from the CALIPER study, two laboratories were using Pathology Harmony reference ranges for children and one laboratory was using in-house paediatric reference ranges. The pathology Harmony ranges are for both male and female patients and are divided into two age categories, less than 4 weeks and 4 weeks to 16 years old, these ranges are not method specific. The in-house derived ranges were for both genders and were divided into four age groups: 0-4 weeks, 1-6 months, 6 months -1 year and 1- 16 years.

Questions 8 & 9. Do you have an action limit for low ALP results in adults/children and if so what is the action limit and where does it come from?

Six health boards have a process for capturing low ALP results in a validation queue for clinical review. Two health boards hold all adult and paediatric ALP results <20 U/L in a validation queue, two health boards hold all adult and paediatric results below the lower limit of the reference interval in a validation queue and one health board holds adult results <20U/L and any paediatric ALP results in a validation queue.

Three health boards do not have a specific action limit for low ALP results. Two of the laboratories that do not have a formal action limit would still take action on observing a low ALP in an adult or a child. One laboratory had no formal action limits for low ALP results and would not do anything further with these results.

Questions 10 & 11. What action would you take on finding a persistently low serum ALP in a patient

In responding to what action would be taken on finding a low ALP result, all health boards stated that they would take the same action regardless of the age of the patient. One laboratory stated that they would have a higher index of suspicion on finding a low ALP in a child. The actions taken on finding a low ALP result in a patient were as follows:

1) Check the sample for EDTA/Citrate/Oxalate contamination (5 health boards)

2) Add a comment (5 health boards)

3) Add/recommend further tests (4 health boards)

4) No action (1 health board)

5) Other (1 health board)

The health board that took other action stated that there was no standardised process or SOP for dealing with low ALP results; however the significance of low ALP, investigations required and clinical presentation of hypophosphatasia was discussed locally at case meetings and via NEQAS cases. Commenting on low ALP results was encouraged, but there was no coded comment and the content of free text comments was at the discretion of the individual duty biochemist.

Question 12. If you add a comment to persistently low ALP results what information does that comment include?

For those laboratories that added a comment to low ALP results, the most commonly included information was to query whether there were clinical symptoms of hypophosphatasia, e.g. fractures and bone pain, premature loss of deciduous teeth and whether there were any other obvious causes of the low ALP activity e.g. zinc/magnesium deficiency.

Other information that HBs included in their comments is shown in the table below.

Information included in report comment	Number of HBs including the information
Clinical symptoms	
Fractures and bone pain	4
Premature loss of deciduous teeth	4
Short Stature failure to thrive	3
Other causes of low ALP activity	
Zinc/Magnesium deficiency	4
Hypothyroidism	3
Severe anaemia	1
Vitamin C, B12, B6 or folate deficiency	1
Coeliac disease	1
Chronic illness or protein calorie malnutrition	2
Queries about medication patient might be taking?	
Oestrogen	2
Clofibrinate	1
Other information	2

Table 2: Information included in report comment

Other information included suggesting consider genetic causes such as hypophosphatasia and referral to endocrinology.

Question 13. If you add/ recommend further routine tests to samples that have persistently low ALP results what tests do you include?

The most common routine biochemistry tests that were either recommended or added on to the sample with low ALP were zinc and magnesium (three health boards). Two health boards stated that they did not recommend any routine biochemistry investigations, two health boards stated that further tests although not explicitly recommended were implied by asking the requesting clinician to exclude secondary causes of low ALP. One health board would add on TFTs if not recently requested and one health board stated that adding on/recommending tests was biochemist dependent.

Question 14. If you provide advice on the further investigation of hypophosphatasia which specialised tests would you recommend?

Six health boards gave advice about specialised tests for the further investigation of hypophosphatasia. Four recommended measuring the plasma PLP:PA ratio, two recommended measuring urine amino acids to look for phosphoethanolamine and two recommended genetic testing for mutations in the ALPL gene. One health board stated that this was also biochemist

dependent but that specialist advice was available from the STEMDRL in GGC. The remaining 3 health boards would not provide advice about further investigation of hypophosphatasia.

Outcome: Audit Recommendations / Standards:

This audit survey has highlighted that there is variation across Scottish health boards in the way laboratories approach the finding of a persistently low ALP in a patient. It has indicated that further work may be required to determine best practice for the investigation of hypophosphatasia in adult and paediatric patients.

Particular areas for further consideration/ discussion are described below.

1. **Methodology for measurement of ALP**
   All laboratories use a method based on the transphosphorylation of para-nitrophenyl phosphate to para-nitrophenol in 2-amino-2-methyl-1-propanol buffer (AMP). It is assumed that all laboratories are using an IFCC method but this requires further clarification.

   Recommendation 1: All laboratories should be using an IFCC method.

2. **The use of different analytical platforms but common reference ranges**
   There are three different analytical platforms in use amongst the nine health boards who responded to the survey. Whilst all laboratories will be monitoring EQA performance closely, how do the results from different methods compare in EQA schemes? A brief review of recent EQA shows that while all methods have acceptable analytical performance there are variations within method groups. Laboratories using Alinity methods have slightly higher ALP results compared to laboratories using Roche and Siemen methods. The question arises is it still appropriate to use pathology harmonised reference ranges or should all laboratories consider adopting method specific ranges or even deriving in-house ranges based on local populations? Further questions around reference ranges include:
   1) Should laboratories be using gender specific ranges?
   2) In the paediatric population are CALIPER ranges more appropriate than pathology harmony ranges?
   3) If we adopt method, gender, age and population specific ranges what impact might this have on the identification of patients with a persistently low ALP?

   Recommendation 2 Laboratories should consider using reference ranges that are method-specific, age-specific and gender-specific because this is crucial for diagnosis especially in children where ALP levels are substantially higher during growth, and puberty occurs earlier in girls than in boys[1].

3. **Action limits for persistently low ALP results in adults and children**

There are variations across health boards in the action limits used to prompt further investigation of persistently low ALP results. Can we standardise practice and have a common action limit? If so, what action limit should we be using for low ALP results in adults and in children? Should all laboratories use the lower limit of reference range? Would that be appropriate for all health boards (when one health board has an in house adult range with a lower limit of normal for ALP of 4 U/L)? Several health boards use an action limit of < 20 U/L, where does this action limit come from and is it more appropriate than using an action limit below the lower limit of the reference interval?

Recommendation 3: Due to the variation in ALP concentrations with age and gender, the most appropriate action limit should be the lower limit of the appropriate age and gender specific

reference range. This should only be applied in patients who demonstrate persistently low ALP results[3,4].

4. What is the most appropriate action to take on finding a persistently low ALP?

There is variation across health boards in the approach taken on finding a persistently low ALP, ranging from taking no action at all to excluding EDTA/Citrate contamination, adding a comment, adding further routine tests and recommending further specialist investigations. Should there be a standard comment that includes common secondary causes of hypophosphatasia and provides information to the requestor about when to consider inherited hypophosphatasia as a cause of persistently low ALP? Should we recommend or add on routine tests? If so which routine tests are appropriate? Should we provide information on specialist tests to investigate hypophosphatasia and if so what specialist tests should we include? Should the approach we take be different for children and adults?

Standardised comment:

Recommendation 4: A standardised comment should highlight the most common secondary causes of a persistently low ALP including medications (e.g. glucocorticoids, oestrogens, and fibrates) and systemic illness (e.g. zinc/magnesium deficiency, coeliac disease, malnutrition, and hypothyroidism) [1-3].

Recommendation 5: A standardised comment should highlight the most common features of inherited hypophosphatasia i.e. a history premature loss of deciduous teeth, poorly healing stress fractures and osteomalacia or rickets[1- 3].

Routine tests:

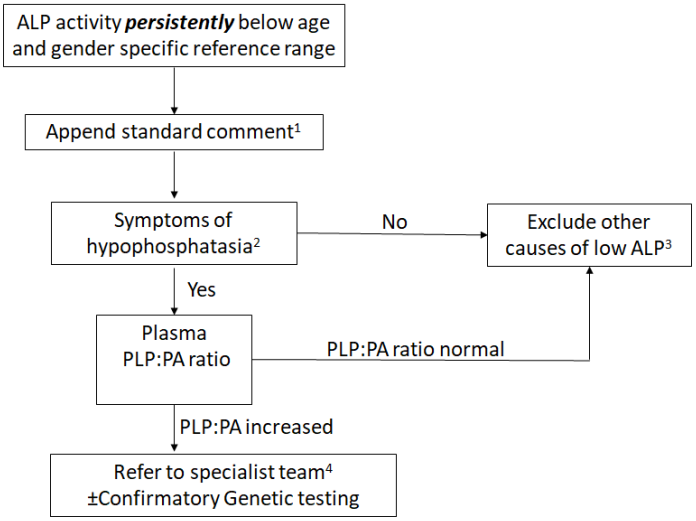
Recommendation 6: Routine tests should exclude secondary causes of hypophosphatasia and should be recommended based on clinical details patient history. These test might include: zinc, magnesium, thyroid function tests, coeliac screen.

Specialist tests: Elevated circulating PLP is a sensitive and specific biochemical marker for hypophosphatasia[1]. Phosphoethanolamine (PEA) is usually elevated in serum and urine in patients with hypophosphatasia but has been shown to be less reliable for diagnosis. This is because PEA excretion is conditioned by age, diet, and circadian rhythm, and can be unremarkable in mild hypophosphatasia and elevated in other metabolic bone diseases[1]. Hypophosphatasia can usually be diagnosed without mutation analysis however this may be necessary if clinical features and biochemistry results are equivocal or if required to determine inheritance patterns.

Recommendation 7: Specialist tests should include plasma PLP:PA ratio first line with genetic testing for confirmation in equivocal cases.

Recommendation 8: Develop a national algorithm for the investigation of hypophosphatasia.

1. Define standard comment
2. Decide as a group which symptoms to include
3. Decide which secondary causes to include and which routine tests to recommend
4. Which specialist team should patients be referred to?



Proposed All Scotland Algorithm for the Investigation of Hypophosphatasia

Please indicate to whom and when audit presented &/or circulated &/or published:

Circulated to LabMed Scotland Audit Group 17th November 2025.

Audit recommendations / standards ratified by … and when:

Date of audit report: 12th November 2025

Audit documents for upload to http://www.acb.org.uk/whatwedo/science/audit.aspx
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