



The Laboratory Services Reform Programme

ADVICE NOTE

Testing for B12 and Folate Guidance

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Clinical Practice Guidance Document Cover Sheet

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The Laboratory Services Reform Programme offers the following advice:

1.1 Advice for Laboratory Users

1. Testing for serum B12 and folate are generally requested together.
2. You should expect that laboratories will require clinical details on requests for testing for serum B12 and folate
3. You should expect that laboratories will generally consider short (legible) statements similar to the following as valid clinical indications for testing for serum B12 and folate:
 - abnormal full blood count
 - suspect dietary deficiency
 - suspect GI (gastrointestinal) malabsorption
 - CNS (central nervous) disease
 - neuropathy
 - neuropsychiatric condition
 - monitoring deficiency

Other valid indications for testing may be stated briefly on the request such as glossitis, pregnancy, alcoholism or hypothyroidism

Elaborate where necessary eg pernicious anaemic, post gastric resection or metformin therapy.

4. You may expect that HSE laboratories will not process samples requesting measurement of serum B12 and folate if no valid clinical indication for testing is provided.
5. If parenteral B12 has been administered, there is little value to retesting serum B12 within 3-6 months as levels will be high/normal.
6. If oral B12 has been prescribed, it is advised to repeat B12 measurements after 3 months to ensure that it is being absorbed.
Unnecessary testing for B12 and folate involves avoidable discomfort, risk of needle exposure and generates unnecessary clinical and laboratory waste.
7. The analysis for B12 and folate significantly slows the throughput of other tests on many large automated analysers so that unnecessary testing may contribute to delays in reporting of other tests

1.2 Advice for Laboratories

1. Measurement of folic acid and B12 levels should be performed when relevant and legible clinical details and requestor identification are provided on the request (electronic or paper) accompanying the sample and the sample received is suitable for analysis.
2. Repeat testing at intervals of less than 6 months should be limited to specific circumstances such as the assessment of response to oral B12 therapy (based on clinical details provided).
3. Laboratories should communicate to laboratory users the specific indications for testing for folic acid and B12 accepted by the laboratory and the minimum accepted interval between tests routinely accepted (based on the advice to laboratory users outlined earlier in this document).

4. To the greatest extent practical requests for testing for folic acid and B12 that do not conform to the laboratory requirements for testing should not be processed.
5. There are significant practical challenges in implementing a process to manage requests in the absence of electronic ordering. Providing users with a specific list of terms such as that indicated above in advice to users that must be legible on a request form for acceptance of the sample for testing has been used effectively in some laboratories.
6. If samples are not processed a report should issue to the effect that testing for folic acid and B12 was not performed because the criteria for testing were not met.

2. Background

Adequate levels of B12 and folate are essential for health. Deficiency is associated with serious illness including haematological and neurological disease. Appropriate laboratory testing is important. Diseases related to deficiency are preventable by adequate dietary intake or appropriate supplementation.

In women, deficiency of folate is associated with increased risk of neural tube defects in their children. The Department of Health recommends that

“all women who may possibly become pregnant within the next three months, whether intentionally or not, are advised to take oral Folic Acid (FA) 400 micrograms daily to prevent Neural Tube Defects (NTDs)”

“women who intend to become pregnant are advised to start FA at least 6 weeks before they start trying to conceive”

“after the first trimester and during breastfeeding, all women are advised to take oral Folic Acid 400 micrograms to meet the World Health Organization’s recommended daily intake for pregnancy and breastfeeding”.

This guidance is not contingent on laboratory test results therefore testing is not required before giving this advice. Note that higher folic acid intake than that stated above is recommended for certain at risk people.

Summary brief indications for testing of B12 and folate suitable for documenting on request forms are outlined above in the advice for users. Abnormalities on full blood count that point to B12 and folate deficiency include

- anaemia, in particular, macrocytic anaemia,
- hypersegmented neutrophils on blood film or
- cytopaenia.

Macrocytosis (raised MCV) may also point to B12 and folate deficiency but note that some increase in MCV may be an artefact in samples where there is a long interval between sample collection and analysis.

B12 deficiency is also associated with neuropsychiatric clinical features including cognitive impairment and neuropathy. Neurological features may present in people with no abnormality on full blood count. B12 deficiency is associated with restrictive diet, vegan diet and with malabsorption of dietary B12.

Malabsorption of B12 is classically caused by autoimmune pernicious anaemia but is also associated with bariatric surgery, small bowel surgery, Crohn’s disease and atrophic gastritis. It should be

considered in those with a family history of pernicious anaemia. Certain medications are associated with B12 deficiency including long term use of metformin or proton pump inhibitors.

The interpretation of B12 and folate levels with respect to the reference intervals can be challenging (see note below regarding reference intervals). Levels of B12 in pregnancy and in women on hormonal contraception or HRT can be low even in the absence of true B12 deficiency. Specialist advice and guidance is recommended. Measurement of Active B12, the metabolically active form of Vitamin B12, may assist if available.

Recreational use of Nitrous Oxide (N₂O) can interfere with B12 pathways causing cellular deficiency in the presence of normal serum B12 levels. If recreational N₂O use is a concern, specialist advice and guidance is recommended.

A history of vitamin supplement use including tablets, sprays, patches etc. is important in the overall assessment of B12 and folate status, as partially treated B12 deficiency can give rise to levels within the reference interval in people with an underlying condition that compromises B12 absorption.

Elevated levels of B12 may be associated with liver disease, chronic renal failure, auto immune and inflammatory disease. It has been described in myeloid blood malignancies and solid tumours. ^(3,4) This is relevant to interpretation of results above the upper limit of the reference range but is not intended to suggest that testing for B12 is a useful for diagnosis when these conditions are suspected.

2.1 Note on terminology

A reference interval (sometimes called a reference range) is quoted by laboratories on reports of many test results. In the past this was often referred incorrectly as a “normal range”. In general, the reference interval is defined in relation to the values observed in a readily accessible group of healthy people who provide samples. In some cases different reference intervals may be specified for those who have identified as men and women and for children. A reference interval is a guide to interpretation.

A value outside of the reference interval is not always “abnormal” for that person and may not be a cause for concern. A value outside of the reference interval may be physiological for some people. It may be expected in relation to age, medication or known medical condition. Equally, a value within the reference interval does not exclude illness. In each case clinical judgement is required in applying the result of a diagnostic test result to the individual's clinical circumstances

3. Relevant Materials

1. Vitamin B12 deficiency in over 16s: diagnosis and management. NICE guideline [NG239] Published: 06 March 2024
2. Folic acid supplementation. Department of Health. 2019
3. Andr  s E, Serraj K, Zhu J, Vermorken AJ. The pathophysiology of elevated vitamin B12 in clinical practice. QJM. 2013 Jun;106(6):505-15. doi: 10.1093/qjmed/hct051. Epub 2013 Feb 27. PMID: 23447660.
4. Lacombe, V., Chabrun, F., Lacout, C. *et al.* Persistent elevation of plasma vitamin B12 is strongly associated with solid cancer. *Sci Rep* **11**, 13361 (2021). <https://doi.org/10.1038/s41598-021-92945->



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