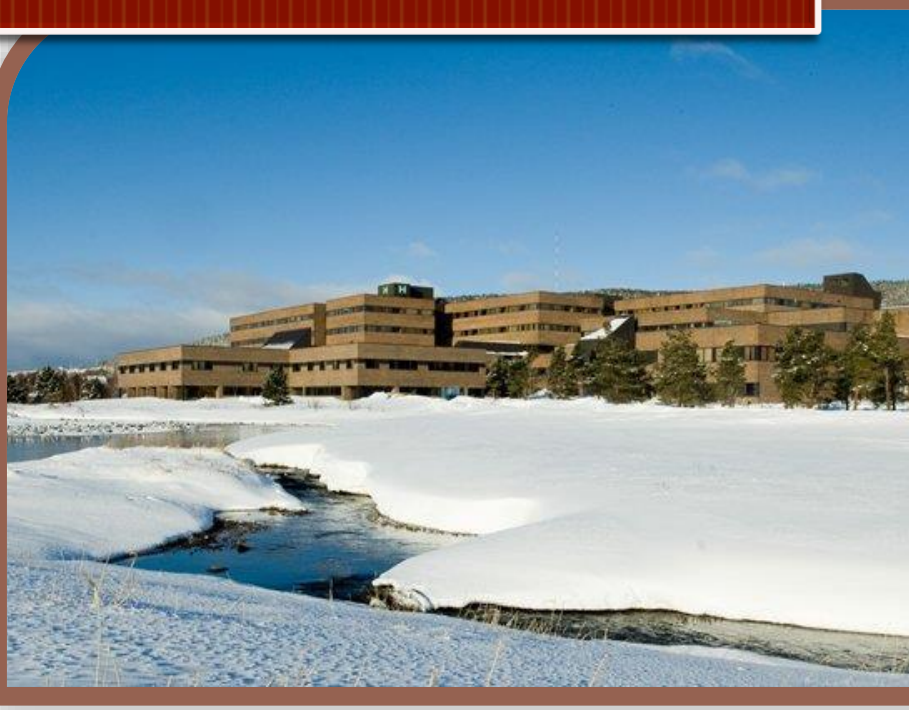
Vitamin B12 Status Testing



Clinical Biochemistry Laboratory Formulary Working Group

Laboratory Medicine Program
Eastern Health
Rm. 1J442, Health Sciences Centre, 300 Prince Philip Drive,
St. John's, NL A1B 3V6

Office: 709-777-6375
Fax: 709-777-2442
Email: Ed.Randell@easternhealth.ca
Website: www.easternhealth.ca/Professionals.aspx?d=2&id=2010&p=1507

Executive Summary

All health professionals with test ordering privileges can order serum vitamin B12 tests and related investigations. Use of this test is restricted to patients at high risk for vitamin B12 deficiency. Use of other tests of vitamin B12 status including methylmalonic acid (MMA), anti-intrinsic factor antibodies, and anti-parietal cell antibodies are available for order on consultation with the laboratory. Hence, vitamin B12 status testing is based on the following:

1. Orders for serum vitamin B12 testing is restricted to patients at high risk for vitamin B12 deficiency including those:
    a. With unexplained hematologic abnormalities (unexplained anemia/other cytopenias, unexplained macrocytosis).
    b. With unexplained neurologic abnormalities (peripheral neuropathy, dementia, unexplained neuropathy, paresthesia, numbness, and gait problems).
    c. With existing malabsorption condition (due to GI disease or surgery) or diet poor in vitamin B12 (long term strict vegans, and the elderly especially if consuming a "toast and tea" diet).
    d. On long term drug therapy (>6 months) that affect vitamin B12 absorption (like Metformin and proton pump inhibitors).
    e. In other clinical scenarios with completion of a laboratory test special authorization (LTSA) form by the ordering physician following consultation with a laboratory physician/clinical biochemist.
2. Test orders for serum vitamin B12 levels will be completed if one or more of the following is indicated on the requisition, or if a LTSA form is completed.
    a. High risk for nutritional B12 deficiency.
    b. High risk for drug-related B12 deficiency.
    c. GI disease/surgery.
    d. Unexplained hematologic abnormalities.
    e. Unexplained neurologic abnormalities.
3. Follow-up testing for MMA by a laboratory-directed algorithm (**Appendix 1**) will be done to help improve diagnosis of vitamin B12 deficiency.
4. Other follow-up and confirmatory investigations for pernicious anemia (involving MMA, anti-intrinsic factor and/or anti-parietal cell antibody levels) are restricted to patients with confirmed clinical vitamin B12 deficiency and with completion of a LTSA form following consultation with a laboratory physician/clinical biochemist.

Disclaimer

Contents

Scope

These recommendations apply to:

- Investigation of patients at high risk for vitamin B12 deficiency and monitoring patients being treated for vitamin B12 deficiency.

Rationale

There is no "gold standard" test for vitamin B12 deficiency. Serum vitamin B12 levels show high sensitivity for deficiency but have low specificity. Relatively common situations like pregnancy and oral contraceptive use falsely lower serum vitamin B12 levels. This means that without careful patient selection there is increased risk for over diagnosis and mistreatment for vitamin B12 deficiency. Treatment of vitamin B12 deficiency is fairly inexpensive and innocuous, but misdiagnosis can divert attention away from serious underlying conditions, delaying diagnosis and appropriate management. Use of other highly sensitive tests for functional vitamin B12 deficiency, like methylmalonic acid (MMA), can increase specificity for vitamin B12 deficiency, and improve diagnostic accuracy.

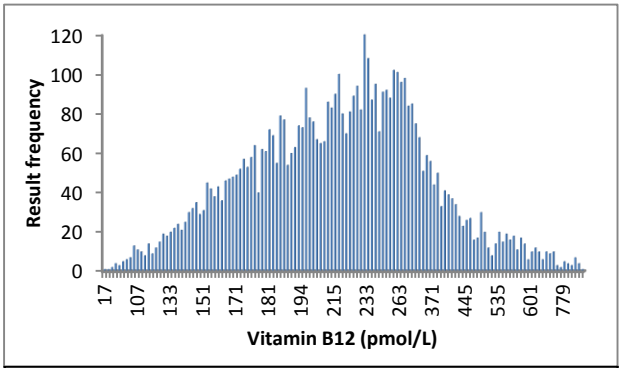


Figure 1. Distribution of vitamin B12 results based on ~24,000 consecutive analyses (Eastern Health Authority, 2014)

Review of laboratory workload statistics from our Eastern Health Authority laboratories shows evidence that much of the vitamin B12 testing done is on asymptomatic individuals at low risk for clinical deficiency. This ultimately leads to excessive laboratory testing costs, and increased potential for misdiagnosis of vitamin B12 deficiency. The distribution of vitamin B12 test results shows a result profile similar to the general population rather than a high risk group (**Figure 1**) and also show:

- The median age for screening falls well below 60 years.
- The lower limit of normal identifies only 2.9% as having low vitamin B12 levels.
- The proportion with extremely low vitamin B12 levels (<74 pmol/L) is only 0.2%.
- Only 20-25% of patients with abnormally low vitamin B12 (<138 pmol/L) have abnormally high MMA levels (>0.4 µmol/L), and only 10-15% have MMA levels >0.8 µmol/L, indicating strong evidence of true vitamin B12 deficiency. Less than 2% of those screened have anemia with increased MCV.
- Over 75% have no hematologic evidence of either anemia or increased MCV.

These findings suggest excessive misuse of the test.

Background

Clinical vitamin B12 deficiency is a relatively common condition that if left untreated leads to serious clinical consequences such as bone marrow failure and demyelinating neurological abnormalities. Clinical findings in vitamin B12 deficiency include anemia (megaloblastic in 70%), neurological symptoms (~50%), cognitive symptoms, and malabsorption (1, 6). Treatment involves use of vitamin B12 (cobalamin) given orally or by injection. There is no clear evidence that either form of cobalamin delivery is superior, but supplementation with oral high dose cobalamin is much less costly than injections (1, 2).

Vitamin B12 is an essential and water soluble vitamin present in foods of animal origin, such as meat, poultry, fish, seafood, eggs and milk products (8). It is required by the human body for normal DNA synthesis and peripheral nervous system functioning. It exists in two main active forms which are cofactors for: Methylmalonyl CoA mutase to catalyze the conversion of MMA to succinic acid; and methionine synthase, which catalyzes the conversion of homocysteine to methionine. High levels of MMA indicate functional insufficiency of vitamin B12, but it can also be affected by thyroid dysfunction, renal disease and possibly other conditions. Additionally, MMA levels increase in the elderly without obvious clinical evidence of vitamin B12 deficiency and are not necessarily predictive for developing vitamin B12 deficiency in the future.

The best known causes of vitamin B12 deficiency include nutritional deficiency, pernicious anemia (PA), and malabsorption of the vitamin (10). Pernicious anemia is caused by autoimmune mediated destruction of gastric parietal cells leading to low intrinsic factor production and impaired absorption of dietary vitamin B12. The characteristic hematological abnormalities of PA include megaloblastic anemia (macrocytosis and hyper-segmentation of granulocytes in peripheral blood, and characteristic bone marrow abnormalities). Impaired DNA production during vitamin B12 deficiency leads to anemia and production of red blood cells (RBCs) with increased MCV (indicating macrocytosis). This occurs as blood cell progenitors cannot produce DNA quickly enough to keep up with rapid cell growth and thus grow too large before division. Hence, increased MCV and decreased hemoglobin are common hematologic manifestations of inadequate vitamin B12 status. The prevalence of PA depends on the diagnostic criteria used but can be as high as 4% among elderly adults. The median age of onset is 60 years (3). As PA is an autoimmune disease, positivity for anti-intrinsic factor and/or anti-parietal cell antibodies supports the diagnosis. Also, autoimmune thyroid disease is common among patients with PA. Chronic atrophic gastritis associated with PA can be diagnosed by elevated fasting gastrin levels.

Milder forms of vitamin B12 deficiency, including sub-clinical B12 deficiency, may affect up to 20% of the elderly. This includes individuals with compromised cellular cobalamin metabolism and unexplained low vitamin B12 levels, and who are asymptomatic and without anemia. Autoimmune-mediated loss of intrinsic factor or defective cobalamin absorption is rarely responsible for these cases. The likelihood of progression in individual cases is unknown, but cases variably progress slowly, transiently, or not at all (4). Following high risk patients by vitamin B12 measurement at a biannual interval is adequate for early identification of cases prior to clinical deficiency. It typically takes two to five years to exhaust the body's supply of

vitamin B12, and under normal conditions a diet that includes meats, eggs, seafood, and dairy products is sufficient to maintain body stores.

This guideline involves use of a diagnostic algorithm (**Appendix 1**) for vitamin B12 deficiency diagnosis. The traditional approach to vitamin B12 deficiency diagnosis relied mainly on vitamin B12 testing alone, and lacks sensitivity for cases presenting with neurologic sequel, and lacks specificity for clinical vitamin B12 deficiency. Specific metabolic markers, like MMA, are considerably more sensitive and specific for clinical vitamin B12 deficiency than serum vitamin B12 measurement alone. However, MMA is more difficult to measure and its use for high test workloads is not feasible. There is no recognized "gold standard" test for defining vitamin B12 deficiency; however, high MMA levels are accepted as the best biochemical marker for functional vitamin B12 deficiency. **In presence of symptoms of vitamin B12 deficiency, high serum MMA levels indicate clinical vitamin B12 deficiency. In asymptomatic individuals at high risk for developing vitamin B12 deficiency, high serum MMA levels indicate sub-clinical vitamin B12 deficiency.**

Assessment of Vitamin B12 Status

There is no known clinical benefit of population or age-based screening for vitamin B12 deficiency in asymptomatic individuals. Most evidence based guidelines focus on selective screening of high-risk and symptomatic individuals, and treatment of identified cases with cobalamin (1, 13-16). Vitamin B12 deficiency is typically diagnosed by finding low serum B12 levels (1). Furthermore, the more sensitive biomarkers of vitamin B12 deficiency are not necessarily cost-effective replacements when screening asymptomatic individuals (6, 21). However, a number of studies support a testing cascade approach, using serum vitamin B12 and MMA, to improve vitamin B12 deficiency diagnosis (9-11, 20), similar to that proposed in **Appendix 1**.

Serum vitamin B12 testing has several limitations. The commonly measured form is metabolically inactive and serum levels of vitamin B12 show poor correlation with symptoms of deficiency. Low levels of serum vitamin B12 levels occur when there are decreased carrier protein levels (transcobalamin) such as in women taking oral contraceptives, but without clinical symptoms of deficiency. Pregnancy also results in falsely low levels of serum vitamin B12, making serum vitamin B12 levels an unreliable marker of vitamin B12 deficiency. Cases like this represent false positives especially when levels of MMA are within its reference range and where there is no obvious evidence of hematological or neurological abnormality (1, 20). Nevertheless, the lower limit of normal is believed to perform reasonably well at identifying clinical deficiency (with sensitivity ranging from 65-95%) (1, 5, 6). Elevated levels of MMA are found in ≥95% of patients with neurological manifestations with or without anemia, and significant numbers of these patients with clinical B12 deficiency may have vitamin B12 levels within the normal range (1, 6). About 90% of patients with elevated MMA have vitamin B12 levels <221 pmol/L and vitamin B12 levels above this are rarely associated with B12 deficiency induced hematological or neurological disease (5, 8). Extremely low vitamin B12 levels (<74 pmol/L) are usually accompanied by clinical evidence of vitamin B12 deficiency (5). Hence, very

low levels of vitamin B12 < 74 pmol/L, or moderately low levels of vitamin B12 ranging from 75 to 220 pmol/L but with elevated MMA, are suggestive of clinical vitamin B12 deficiency in symptomatic individuals.

Rationalized Use of Vitamin B12 Status Tests

Implementation of new strategies to improve diagnostic processes is part of the mandate of the laboratory program to provide useful and cost-effective diagnostic services. Results from interventional studies show that only a small minority of patients, diagnosed with vitamin B12 deficiency on the basis of serum levels, show clinical improvement in response to vitamin B12 therapy (9, 19). Patients showing benefit are typically those with the most profound decreases in vitamin B12 levels and/or elevation in MMA. An optimized vitamin B12 deficiency diagnostic strategy, and making greater use of MMA, is required to direct appropriate test utilization and improve diagnosis. The testing rules are based on recognized clinical parameters associated with higher risk for vitamin B12 deficiency, and focus vitamin B12 ordering toward these situations. Vitamin B12 testing is therefore restricted to situations where one or more of the following conditions are met:

1. Patient has unexplained hematologic abnormalities (unexplained anemia/other cytopenias, unexplained macrocytosis).
2. Patient has unexplained neurologic abnormalities (peripheral neuropathy, dementia, unexplained neuropathy, paresthesia, numbness, and gait problems).
3. Patient is at high risk for nutritional deficiency.
4. Patient has malabsorptive state that impairs vitamin B12 absorption.
5. Patient is receiving drug therapy known to impair vitamin B12 absorption.

To facilitate appropriate test selection in rarer conditions where vitamin B12 testing may be required, vitamin B12 status testing is also permitted on consultation with a laboratory physician or clinical biochemist and with completion of a laboratory test special authorization (LTSA) form. The minimum re-order interval is set at 6 months, and tests failing to meet the above criteria are canceled. Retesting cobalamin after 6 months of therapy is sufficient to confirm normalization of serum vitamin B12 levels in patients who do not respond clinically.

A laboratory directed algorithmic (testing cascade) approach can improve diagnostic sensitivity and specificity for vitamin B12 deficiency. All samples meeting the criteria will be analyzed according to the algorithm indicated in **Appendix 1** with MMA being done when vitamin B12 levels fall between 74 and 221 pmol/L. McHugh et al. (7) used a similar strategy to show a 70% decrease in utilization of vitamin B12 testing, but with no compromise to diagnostic yield by the strategy. The McHugh strategy did not utilize the superior sensitivity and specificity of a second tier test like MMA. Others recommend second tier testing using MMA as a strategy to improve diagnostic sensitivity and specificity (9-11, 17, 18, 20, 21).

Risk Factors for Vitamin B12 Deficiency

Under normal conditions consuming a diet that includes meats, eggs, seafood, and dairy products is sufficient to maintain body stores. Dietary deficiency is therefore rare, but becomes more likely in long-term vegans and in the elderly consuming low protein diets ("toast and tea" diets). Risk of deficiency also increases in the elderly due to age related decrease in gastric acid production (and due to chronic atrophic gastritis). Long term use of certain drugs like histamine blockers, proton pump inhibitors, or metformin reduce vitamin B12 absorption and increase the risk for deficiency. Testing patients about 6 to 12 months into long term drug therapy is warranted for early identification of vitamin B12 deficiency. Risk for deficiency is also increased in patients with GI disease, pancreatic insufficiency, or having had gastrectomy, gastric bypass, or ileal resection (20).

Hematologic Abnormalities and Vitamin B12 Testing

Vitamin B12 deficiency leads to megaloblastic anemia. Accompanying laboratory findings include an increased MCV, and/or decreased hemoglobin, with presence of oval macrocytes, hypersegmented neutrophils, or pancytopenia. These findings are not specific for vitamin B12 deficiency and true deficiency is present in less than 30% of all macrocytosis cases. Furthermore, abnormal hematologic findings are absent in a high proportion of patients with clinical vitamin B12 deficiency and with neurologic manifestations. Caution is therefore warranted in attributing low vitamin B12 levels in the context of abnormal hematological findings alone to vitamin B12 deficiency or normal values of vitamin B12 as indicating adequacy if characteristic neurologic findings are present. Second tier testing using highly sensitive biomarkers may improve diagnostic accuracy of vitamin B12 testing in these cases.

Neurologic Abnormalities and Vitamin B12 Testing

Patients with unexplained neurologic symptoms, including memory loss or cognitive and personality changes, paresthesia, numbness, and poor motor coordination, should be tested for vitamin B12 deficiency. Up to 30% of patients with vitamin B12 deficiency associated neurologic symptoms have no hematologic abnormalities. Second tier testing using more sensitive biomarkers like MMA may improve identification of functional vitamin B12 deficiency in patients with neurological manifestations of vitamin B12 deficiency (9, 10, 20). Evidence supporting significant reversal of cognitive function loss following supplementation is weak, but supplementation with vitamin B12 may slow progression (12, 13).

Pernicious Anemia Diagnosis in an Algorithmic and Tiered Approach

There exists a considerable grey zone of vitamin B12 levels as it relates to PA. However, risk progressively increases as vitamin B12 levels decrease below about 221 pmol/L, and specificity for PA increases to about 90% in patients with clinical manifestations and serum vitamin B12 levels below 74 pmol/L. Serum MMA levels ≥0.4 µmol/L generally confirms vitamin B12 deficiency in symptomatic patients with low vitamin B12 levels but levels of MMA below 0.4

µmol/L essentially rules out PA in an untreated individual. Increase in MMA with age and decreasing renal function must also be taken into consideration when interpreting serum MMA levels. MMA also identifies more subclinical vitamin B12 deficiency, especially when levels are borderline high (<2x ULN).

Confirmation of PA in samples where MMA is ≥ 0.4 µmol/L is based on demonstrating positivity for anti-Intrinsic Factor antibody, anti-Parietal Cell antibody, or elevated fasting gastrin levels (See **Table 1**). A cascade approach is recommended when using these tests, beginning with anti-Intrinsic factor antibody and progressing to anti-parietal cell antibody and finally gastrin measurements before PA is ruled out.

Table 1. Recommended Usage of Tests.

Serum Vitamin B12	•First tier test for assessment of vitamin B12 status for high risk and symptomatic patients. •High sensitivity (>90%) in patients with hematology abnormalies.
Serum MMA	•Second tier test for assessing functional vitamin B12 status for high risk and symptomatic patients with low/low normal vitamin B12 levels. •High sensitivity (>95%) in patients with functional vitamin B12 deficiency.
Anti-Intrinsic Factor Antibody	•Third tier test for use in patients with anemia and/or neuropathy with confirmed vitamin B12 deficiency to help diagnose pernicious anemia. •Low sensitivity (~50%) but high specificity (~100%).
Anti-Parietal Cell Antibody	•Third tier test for use in patients with anemia and/or neuropathy with confirmed vitamin B12 deficiency to help determine cause. •High sensitivity (~80%) and acceptable specificity (50 to 100%).
Fasting Gastrin	•Third tier test for use in patients with anemia and/or neuropathy with confirmed vitamin B12 deficiency to help determine cause. •High levels indicate gastric atrophy.
Homocysteine	•Third tier test for assessment of functional vitamin B12 status for high risk and symptomatic patients with low/low normal vitamin B12 levels and equivocal MMA results. •Redundant and inferior to MMA for functional vitamin B12 deficiency.

Conclusions

Rationalized use of MMA and vitamin B12, by test utilization rules and a laboratory initiated testing cascade have the potential to:

1. Improve diagnosis of vitamin B12 deficiency.
2. Decrease misuse of vitamin B12 tests.
3. Reduce testing costs.

Another potential benefit is a reduction in the number of patients receiving unnecessary vitamin B12 injections due to diagnoses based on low vitamin B12 levels alone.

Clinical Biochemistry Laboratory Formulary Committee

The Clinical Biochemistry Laboratory Formulary Committee is a multidisciplinary group involved in improving the usage of laboratory services within Eastern Health.

The main purposes of the committee include:

1. Assisting in decision making on in-house testing menus including retiring of redundant tests and adding of new tests.
2. Reviewing and advising on issues related to laboratory utilization to promote evidence-based usage of laboratory services and best practices guidelines.
3. Advising on development of a tiered formulary for all laboratory tests available through the Clinical Biochemistry laboratory.

We acknowledge the following as committee members in development of this guideline:

- Dr. Brendan Barrett
- Dr. Paul Bonisteel
- Barry Dyer
- Dr. Christopher Kovacs
- Natasha Lee
- Peggy Manning
- Colleen Mercer
- Dr. David Parry
- Dr. Edward Randell
- Dr. Robert Woodland
- Dr. Gabe Woollam

Acknowledgements

The Clinical Biochemistry Laboratory Formulary Committee acknowledges contributions of the following in preparing this guideline: Wael Demian for statistical analysis of laboratory test workloads; Drs. Kuljit Grewal, Sergei Likhodi, Lucinda Whitman, Jerry McGrath, Dejun Xu, and Amy Tong for thoughtful review and preliminary feedback on this guideline; and Zoë Moores for proofreading and formatting this guideline.

References

1. Stabler SP. Vitamin B12 Deficiency. N Engl J Med 2013; 368:149-60.
2. Lin J, Kelsberg G, & Safranek S Is high-dose oral B12 a safe and effective alternative to a B12 injection? J. Family Practice 2012; 61:162-3.
3. Annibale B, Lahner E, & Fave GD. Diagnosis and Management of Pernicious Anemia. Curr Gastroenterol Rep 2011; 13:518–524.
4. Carmen R. Subclinical cobalamin deficiency. Curr Opin Gastroenterol 2012, 28:151–158.
5. Snow CF. Laboratory diagnosis of vitamin B12 and folate deficiency: A guide to the primary care physician. Arch. Intern. Med. (1999) 159:1289-1298.
6. Carmel R. Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. Am J Clin Nutr (2011) doi: 10.3945/ajcn.111.013441.
7. McHugh J, Afghan R, O'Brien E, Kennedy P, Leahy M, & O'Keeffe D. Impact of the introduction of guidelines on vitamin B12 Testing. Clin. Chem. (2012) 58: 471–475.
8. Watanabe F. Vitamin B12 sources and bioavailability. *Exp Biol Med*. **2007**,232,1266-1274.
9. Berg RL, Shaw GR. Laboratory evaluation for vitamin B12 deficiency: the case for cascade testing. Clin Med Res 2013; 11:7–15.
10. Toprak, B., Yalcın, H. Z., Colak, A. Vitamin B12 and folate deficiency: should we use a different cutoff value for hematologic disorders? Int. J. Lab. Hem. 2014:36: 409-414.
11. Rice L. Laboratory diagnosis of vitamin B12 and folate deficiency. Arch Intern Med 1999;159:2746–7.
12. O'Leary F, Allman-Farinelli M, Samman S. Vitamin B12 status, cognitive decline and dementia: a systematic review of prospective cohort studies. British Journal of Nutrition 2012; 108(11): 1948-1961
13. Health Quality Ontario. Vitamin B12 and cognitive function: an evidence-based analysis. Ont Health Tech Assess Ser [Internet]. 2013 November;13(23):1–45. Available from http://www.hqontario.ca/evidence/publications-and-ohtac-recommendations/ontario-health-technology-assessment-series/B12-cognitive-function
14. British Columbia Ministry of Health (BCMH). Cobalamin (vitamin B12) deficiency - Investigation & management. Victoria, BC: Guidelines and Protocols Advisory Committee 2013. Available from: http://www.bcguidelines.ca/pdf/cobalamin.pdf.
15. Health Quality Ontario. Serum vitamin B12 testing: a rapid review. Toronto, ON: Health Quality Ontario; 2012 Dec. 16 p. Available from: www.hqontario.ca/evidence/publications-and-ohtac-recommendations/rapid-reviews.
16. College of Physicians & Surgeons of Saskatchewan (CPSS) and Laboratory Quality Assurance Program (LQAP). Guidelines for laboratory practice. Saskatoon, SK: CPSS; 2014. Available from: http://www.quadrant.net/cpss/pdf/2014-Laboratory-Guidelines.pdf.

17. Mayo Medical Laboratories. Pernicious Anemia Testing Cascade (Testing Algoritm). August 2013. Available from: http://www.mayomedicallaboratories.com/it-mmfiles/Pernicious_Anemia_2013_FINAL.pdf
18. ARUP Laboratories. Megaloblastic Anemia Testing (Testing Algorithm). 2010 Available from: http://www.arupconsult.com/Topics/MegaloblasticAnemia.html#tabs=3
19. Hvas, A. M., Ellegaard, J., & Nexø, E. (2001). Vitamin B12 treatment normalizes metabolic markers but has limited clinical effect: a randomized placebo-controlled study. *Clinical chemistry*, *47*(8), 1396-1404.
20. Devalia, V., Hamilton, M. S., & Molloy, A. M. (2014). Guidelines for the diagnosis and treatment of cobalamin and folate disorders. *British journal of haematology*, *166*(4), 496-513.
21. Canadian Agency for Drugs and Technologies in Health. Vitamin B12 Testing in the General Population: Clinical and Cost-Effectiveness and Guidelines. Rapid Response Report. January 21, 2015. Available from www.cadth.ca

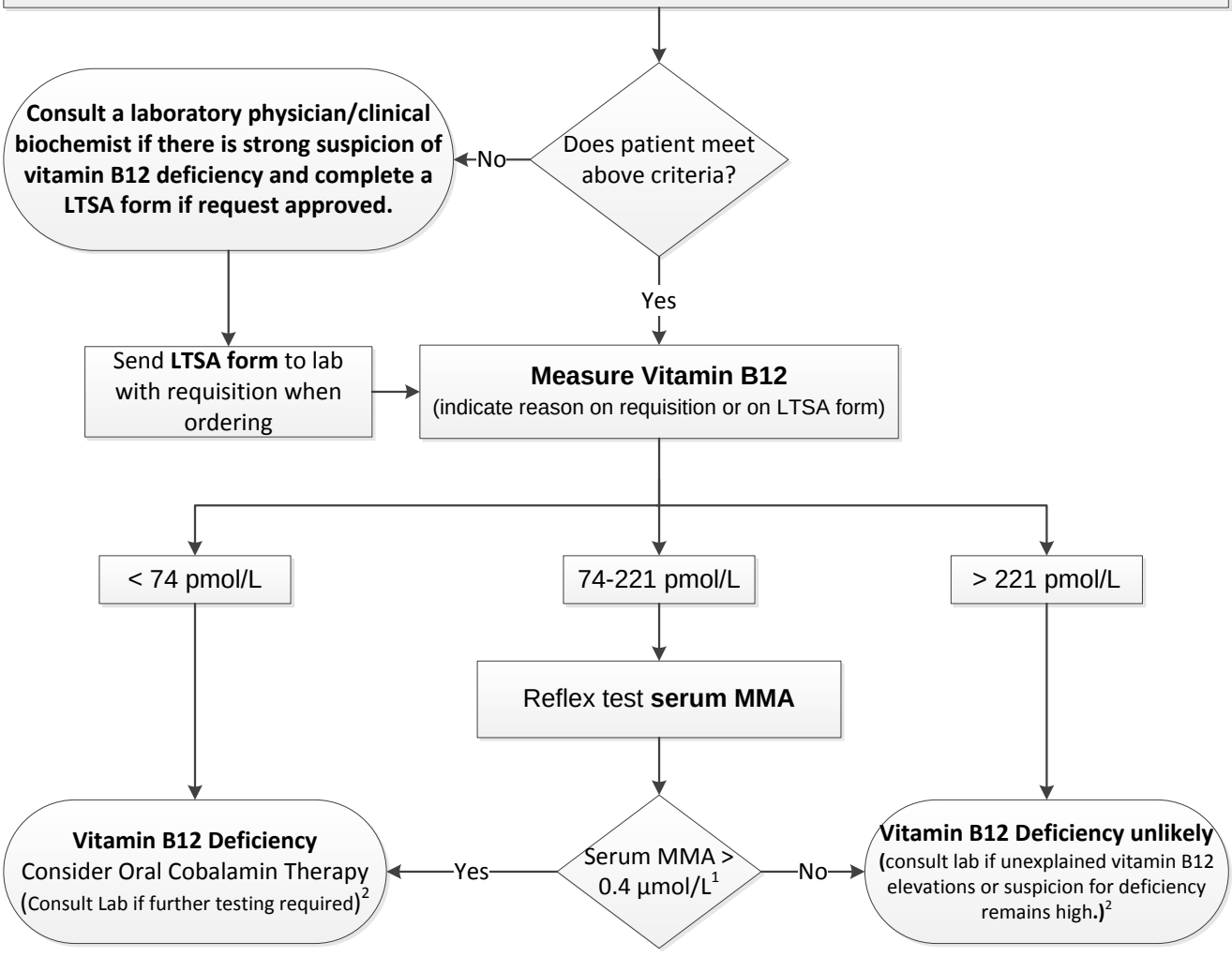
Appendix 1

Vitamin B_{12} testing is restricted to investigating patients with clear clinical indication.

Accepted reasons for vitamin B_{12} Status testing include:

1. High risk for nutritional B12 deficiency (*Long term strict vegan; Elderly > 75yrs or "toast and tea diet"*)
2. High risk for drug-related B12 deficiency. (*Long term Metformin, H_2 receptor antagonists, or proton pump inhibitors*)
3. GI disease/surgery (*Gastrectomy, gastric bypass, ileal resection, pancreatic insufficiency*)
4. Unexplained hematologic abnormalities (*Unexplained anemia/cytopenia, unexplained increased MCV*)
5. Unexplained neurologic abnormalities (*peripheral neuropathy, dementia, paresthesias, numbness, and gait problems*)

(Indicate which one(s) apply on requisition when ordering Vitamin B12. Failure to do so will result in delay or cancellation of the request.)



Note:

[1]Mild elevations in MMA occur in renal failure. Exercise caution when interpreting borderline elevations in MMA <0.8 µmol/L especially when serum creatinine >140 µmol/L .

[2]Repeat testing of vitamin B12 at a frequency greater than once per year is rarely indicated. Other follow-up and confirmatory investigations for pernicious anemia (involving MMA, anti-intrinsic factor and/or anti-parietal cell antibody levels) are restricted to patients with confirmed clinical vitamin B12 deficiency and with completion of a LTSA form following consultation with a laboratory physician/clinical biochemist.