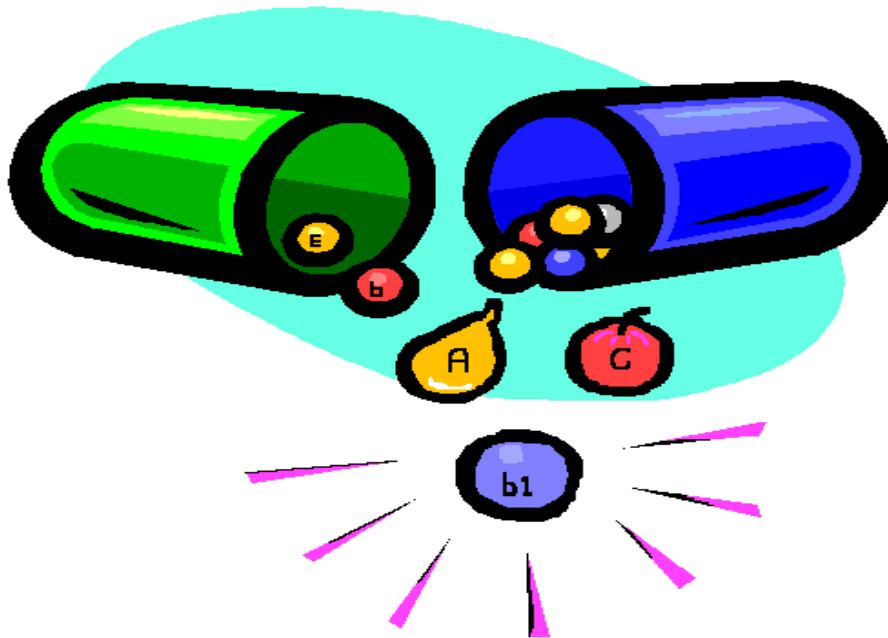
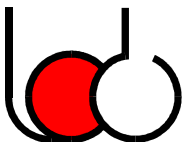


Handbook of Vitamin Analyses for Health



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Canterbury Health Laboratories
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Handbook of Vitamin Analyses for Health

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Table of Contents

1. Introduction	5
2. Specimen Preparation Protocols	6
3. Requesting Analyses	8
4. External Quality Control	8
5. Vitamin & Trace Mineral Deficiency & Excess	8
6. Scope of Laboratory Accreditation	9
7. Vitamin A	10
8. Vitamin B₁	11
9. Vitamin B₂	13
10. Vitamin B₆	14
11. Vitamin B₁₂	16
12. Vitamin C	18
13. Vitamin D	20
14. Vitamin E	22
15. Vitamin K	24
16. Carotene	26
17. Coenzyme Q₁₀	28
18. Folate	30
19. Homocysteine	32
20. Methylmalonic Acid	34



1. Introduction

Canterbury Health Laboratories provides complete laboratory services to the Canterbury District Health Board from its laboratory at Christchurch Hospital. It also provides a specialist testing services to hospital and community laboratories throughout New Zealand. Canterbury Health Laboratories operates as an independent business unit of Canterbury District Health Board and employs 300 staff, including 20 pathologists, 16 research scientists and 100 laboratory scientists.

As New Zealand's premier specialist medical testing laboratory, Canterbury Health Laboratories performs more than 3 million tests annually from a repertoire of over 1200 different procedures. Canterbury Health Laboratories provides one of the most comprehensive testing services in New Zealand, offering an extensive range of analyses on modern state of the art equipment. Samples for vitamin assays are currently received from hospital specialists, general practitioners and veterinary surgeons.

Please contact the Laboratory for further details or visit www.cdhb.govt.nz/chlabs

2. Specimen Preparation Protocols

The following tests are available from Canterbury Health Laboratories.

Table of Analytes for Vitamin Analysis

Blood collected for light sensitive analytes must be wrapped in aluminium foil.

Vitamin and Synonyms	Specimen	Sample aliquot	Turn around time
<u>Vitamin A</u> -Retinol -Retinoic acid	5 mL of plain, heparinised or EDTA blood. Protect from light.	500 µL plasma or serum. Minimum 200 µL. Freeze aliquot. Protect from light.	Performed weekly
<u>Vitamin B1</u> -Thiamine Pyrophosphate -Transketolase -Thiamine	5 mL of heparinised blood. Refer to laboratory for specimen processing protocol	2 mL whole blood washed with NaCl, dilute 1:1 with distilled H ₂ O. Minimum 500 µL prepared red cells. Freeze aliquot.	Performed weekly
<u>Vitamin B2</u> -FAD -Flavine Adenine Dinucleotide -Riboflavin	5 mL of EDTA blood . Protect from light.	5 mL of whole blood. Minimum 500 µL. Freeze aliquot. Protect from light.	Performed fortnightly
<u>Vitamin B6</u> -Pyridoxal 5 Phosphate -PLP -Pyridoxine	5 mL of EDTA blood. Protect from light.	5 mL of whole blood. Minimum 500 µL. Freeze aliquot. Protect from light.	Performed fortnightly
<u>Vitamin B12</u> -Cobalamin	5 mL of plain blood.	500 µL of serum.	Performed daily



Vitamin and Synonyms	Specimen	Sample aliquot	Turn around time
<u>Vitamin C</u> <i>-Ascorbic Acid</i> -Leucocyte Ascorbic Acid	Two 5 mL of heparinised tubes of blood on ice, processed within 1 hour.	Contact laboratory for protocol. Reagents can be supplied to labs wishing to prepare and send stable WBC filtrates. Contact laboratory for protocol.	Performed weekly
<u>Vitamin D</u> <i>-25 Hydroxy Cholecalciferol</i> -25 Hydroxy Vitamin D	2 mL of EDTA, heparinised or plain blood. Protect from light.	500 µL of plasma or serum. Minimum 200 µL. Protect from light.	Twice weekly
<u>Vitamin E</u> <i>-Tocopherol</i>	5 mL of plain, heparinised or EDTA blood. Protect from light.	500 µL of plasma or serum. Minimum 200 µL. Freeze aliquot. Protect from light.	Performed weekly
<u>Vitamin K</u>	5 mL of plain blood. Protect from light.	2 mL of serum. Minimum 1.0 mL. Freeze aliquot. Protect from light.	Performed monthly
<u>Carotene</u> <i>-Beta carotene</i>	5 mL of heparinised, plain or EDTA blood. Protect from light.	1 mL of plasma or serum. Minimum 600 µL for paediatrics. Protect from light.	Performed weekly
<u>Coenzyme Q₁₀</u>	5 mL of lithium heparinised blood. Protect from light.	500 µL of plasma. Freeze aliquot. Protect from light.	Performed fortnightly
<u>Folate-Serum</u> <i>-Folic acid</i>	5 mL of plain blood	1 mL of serum.	Performed daily
<u>Folate-Red Cell</u> <i>-Erythrocyte folate</i>	5 mL of EDTA blood.	1 mL of whole blood.	Performed daily
<u>Homocysteine</u>	5 mL of EDTA blood collected on ice, separate within 4 hours. Patient must be fasting.	500µL of EDTA plasma. Minimum 250 µL. Freeze aliquot.	Performed daily
<u>Methylmalonic Acid</u>	5 mL of plain, heparinised or EDTA blood.	2 mL of serum or plasma. Minimum 500 µL.	Performed weekly

3. Requesting Analyses

Specimens should be accompanied by a request form showing:

- Patient's name, sex and date of birth
- Patient's location
- Requesting doctor and contact details
- Charge to location
- Date and time of specimen collection
- Test(s) requested
- Any special sampling requirements
 - patient fasting
 - sample protected from light
 - sample collected on ice
- Clinical history including vitamin supplementation and list of medication

4. External Quality Control

The laboratory actively participates in the following external quality control programs.

- **Murex External Quality Assurance Services**
Consists of 2 cycles per year containing 12 samples in each cycle which are sent fortnightly for vitamin B₁₂ and folate.
- **ERNDIM European Resources Network**
Eight samples per year for homocysteine
- **RCPA Quality Assurance Programs**
Two samples sent four times a year for vitamin B₁₂ and folate.
Two samples sent monthly for vitamin A, vitamin E and carotene
- **NEQAS Vitamin K Quality Assurance Program**
Three samples per year are sent for vitamin K

5. Vitamin & Trace Mineral Deficiency & Excess



Introduction

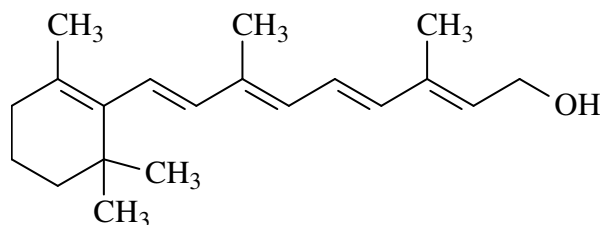
Vitamins and trace minerals are essential constituents of the human diet since they are either inadequately synthesized or not synthesized in the human body. Vitamins are involved in most metabolic pathways as enzyme cofactors. Overt vitamin or trace mineral deficiencies are now rare in western countries due to a plentiful, varied, and inexpensive food supply; however, multiple combined nutrient deficiencies may present in persons with malabsorption or chronic malnutrition, due to alcohol abuse or cancer cachexia. Moreover, subclinical vitamin and trace mineral deficiencies, as diagnosed by laboratory testing, are quite common in the normal population, especially those with special needs such as the very young, the older population and during pregnancy.

Body stores of vitamins and minerals vary tremendously. For example, the stores of vitamin B₁₂ and vitamin A are generally large, and an adult may not become deficient for one or more years after being on a depleted diet. However, folate and thiamine stores may become depleted within weeks of dietary deficiencies. Therapeutic modalities can deplete nutrients from the body; for example, haemodialysis removes water-soluble vitamins, which must be supplemented.

6. Scope of Laboratory Accreditation

Canterbury Health Laboratories is accredited with International Accreditation New Zealand (IANZ) for Medical Testing, refer to www.cdhb.govt.nz/chalbs for details.

7. Vitamin A



Vitamin A (Retinol)

Introduction

Vitamin A is an important nutritional fat-soluble vitamin. It is essential to normal vision and to prevent night blindness. It is also necessary for normal cell division and growth, the development of bones and teeth, and for the health of skin, mucous membranes and epithelial tissue.

In vivo, vitamin A is transported by retinol binding protein and pre-albumin.

Sample Requirements

500 μL of plasma or serum (minimum 200 μL) is required for this assay. Samples should be protected from light and frozen before being sent to the laboratory.

Interpretation

Vitamin A 200 – 800 $\mu\text{g/L}$ (recommended level)

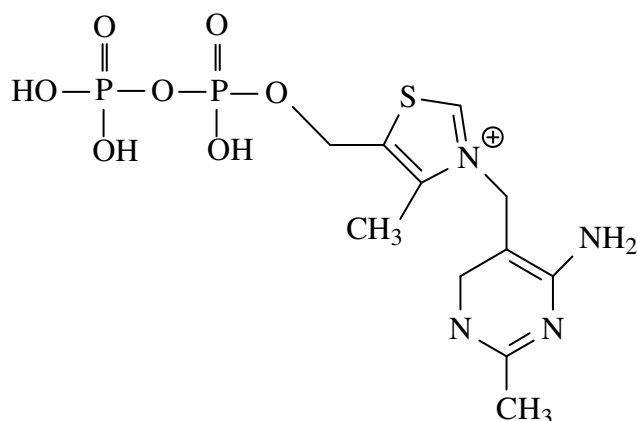
Vitamin A Deficiency

Primary vitamin A deficiency is usually caused by prolonged dietary deprivation. It is endemic in areas such as southern and eastern Asia where rice, devoid of carotene, is the staple diet. Secondary deficiency may be due to inadequate conversion of carotene to vitamin A, interference with absorption, storage, or transport of vitamin A. Interference with absorption is likely in coeliac disease, cystic fibrosis, pancreatic disorders, duodenal bypass, giardiasis and cirrhosis of the liver. Vitamin A deficiency is common in protein-energy malnutrition, not only because the diet is deficient but also because vitamin A storage and transport are defective. Liver stores are depleted in deficiency before plasma levels begin to fall, followed by retinal dysfunction, and finally by epithelial structural changes.

Vitamin A Toxicity

Pharmacological doses of vitamin A can produce toxic symptoms of nausea, headache, fatigue, loss of appetite, dizziness, and dry skin, and ultimately in liver damage, osteoporosis, and haemorrhage. Because of harmful effects on the foetus, vitamin A intake should not be increased during pregnancy. Synthetic retinoids are used in treating psoriasis and troublesome acne.

8. Vitamin B₁



Thiamine pyrophosphate

Introduction

Thiamine is a water-soluble B-complex vitamin, which is stored in the body for only a few weeks. Thiamine serves as a cofactor for several enzymes involved primarily in carbohydrate catabolism. Sources of thiamine include yeast, wheat, whole grain, cereals, nuts, peas, potatoes and most vegetables. A state of severe depletion is seen in patients on a strict thiamine-deficient diet in 18 days, but the most common cause of thiamine deficiency in affluent countries is alcoholism. Dietary deficiency is further suspected in persons with anorexia, vomiting, diarrhoea or post-operative states. Thiamine deficiency is common in the elderly.

Sample requirements

Sample required - 5mL heparinised blood (minimum 2 mL). The red blood cells must be washed, haemolysed and frozen within 4 hours of collection.

Method

1. Centrifuge the sample at 3000 rpm for 5 minutes.
2. After centrifugation use a pasteur pipette to remove and discard the plasma and buffy coat layer.
3. The red cells are washed by adding an equivalent volume of isotonic saline (9 g/L NaCl) and mixing thoroughly.
4. Centrifuge the sample at 3000 rpm for 5 min.
5. Label a plastic tube suitable for transport, with the patient surname and laboratory number.
6. After centrifugation use a pasteur pipette to remove the top saline layer and 1 mm of red cells. Discard, leaving behind the washed red cells.
7. To the labelled tube add 1 mL of washed packed red blood cells (RBC) from the middle of the washed cells.
8. Using the same pipette tip, draw up 1 mL of distilled water and add this to the cells in the labelled tube.
9. Stopper, mix thoroughly by rolling the haemolysate around the inside of the tube.
10. Place the tube inside a plastic bag and freeze.
11. Send frozen to the laboratory.

Interpretation

Red Cell Thiamine Pyrophosphate > 140 nmol/L (recommended level)

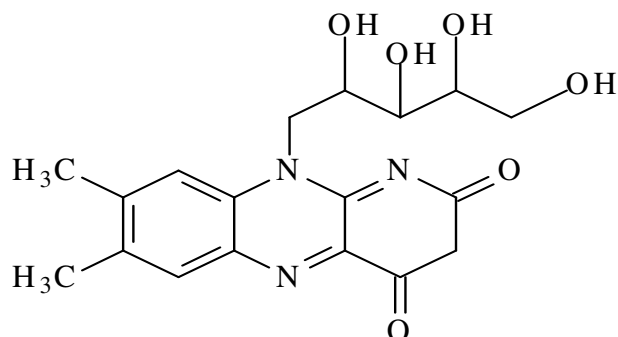
Thiamine Deficiency

Thiamine deficiency in its early stage induces anorexia, irritability, apathy, and generalized weakness. The major manifestations of prolonged thiamine deficiency in humans involve the cardiovascular (wet beriberi) and nervous system (dry beriberi) as well as Wernicke encephalopathy in patients with alcohol abuse.

Thiamine Toxicity

There is no documented toxicity associated with extremely high levels of thiamine. Very high levels of thiamine usually indicate that the patient has been receiving thiamine supplements.

9. Vitamin B₂



Vitamin B₂

Introduction

Vitamin B₂ is a water-soluble B-complex vitamin, also known as riboflavin. In the body, riboflavin is primarily found as an integral component of the coenzymes, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). Flavin coenzymes participate in redox reactions in numerous metabolic pathways. Flavins are critical for the metabolism of carbohydrates, fats, and proteins. FAD is part of the electron transport chain, which is central to energy production. In conjunction with cytochrome P-450, flavins also participate in the metabolism of drugs and toxins.

Sample requirements

5 mL of whole blood EDTA (minimum 500 µL) is required for this assay. Samples should be protected from light and the whole blood frozen and sent to the laboratory.

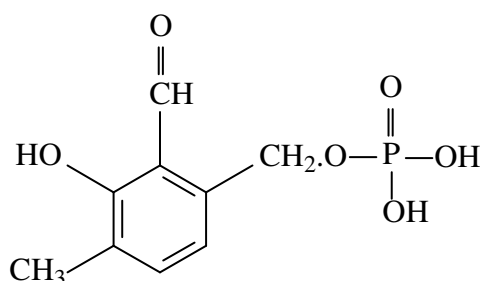
Interpretation.

Vitamin B₂ as FAD 155 – 380 nmol/L in whole blood (reference range)

Vitamin B₂ Deficiency Primary riboflavin deficiency is rarely found in isolation; it occurs frequently in combination with deficiencies of other water-soluble vitamins. Symptoms of deficiency include normochromic/normocytic anaemia, pharyngitis, angular stomatitis, glossitis and dermatitis.

Vitamin B₂ Toxicity Because of its low solubility and limited absorption from the GI tract, riboflavin has no significant toxicity. At extremely high doses there may be crystallisation of riboflavin in the kidney because of its low solubility.

10. Vitamin B₆



Pyridoxal 5'-phosphate

Introduction

Vitamin B₆ comprises a group of closely related compounds: pyridoxine, pyridoxal, and pyridoxamine. They are metabolized and phosphorylated in the body to Pyridoxal 5'-phosphate (PLP), which functions as a coenzyme in many reactions, including decarboxylation and transamination of amino acids, deamination of hydroxyamino acids and cysteine, conversion of tryptophan to niacin, and metabolism of fatty acids. Consequently, the vitamin B₆ group is important in blood, CNS, and skin metabolism. Vitamin B₆ is important in erythropoiesis because pyridoxal phosphate is needed in the formation of δ -aminolevulinic acid, the rate-limiting step in heme biosynthesis.

Sample requirements

5 mL of whole blood EDTA (minimum 500 μ L) is required for this assay. Samples should be protected from light and the whole blood frozen and sent to the laboratory.

Interpretation

Vitamin B₆ (PLP) 35 – 107 nmol/L (reference range)



Vitamin B₆ Deficiency

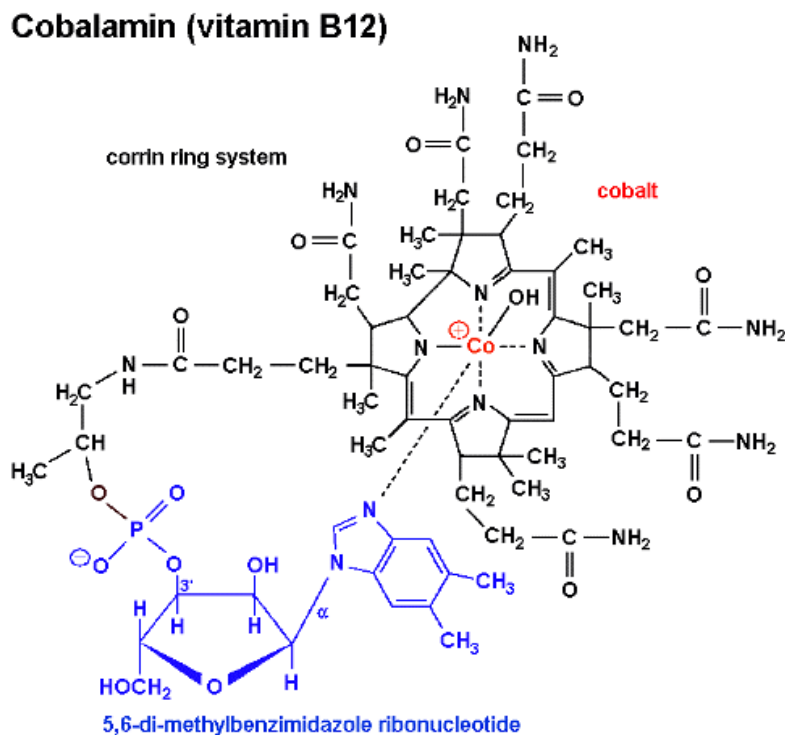
Primary deficiency is rare, because most foods contain vitamin B₆. Nonetheless, an outbreak of convulsions in infants did follow the inadvertent destruction of vitamin B₆ in infant formulas.

Secondary deficiency may result from malabsorption, alcoholism, oral contraceptive use, chemical inactivation by drugs (eg, isonicotinic acid hydrazide, cycloserine, hydralazine, penicillamine), excessive loss, and increased metabolic activity

Vitamin B₆ Toxicity

The ingestion of large amounts of pyridoxine may cause progressive sensory ataxia and profound lower limb impairment of position and vibration sense. Senses of touch, temperature, and pain are less affected. The motor and central nervous systems are impaired. Recovery is slow and, in some patients, is only partial after pyridoxine ingestion is stopped.

11. Vitamin B₁₂



Introduction

Vitamin B₁₂ is the largest and most complex of all the vitamins. It is unique among vitamins in that it contains a metal ion, cobalt (Co). For this reason cobalamin is the term used to refer to compounds having B₁₂ activity.

Function

Methylcobalamin is a co-factor of the folate-dependent enzyme methionine synthase. This enzyme is required for the remethylation of homocysteine to methionine. Methionine is required for the synthesis of S-adenosylmethionine, a methyl group donor used in many biological methylation reactions, including the methylation of a number of sites within DNA and RNA. Methylation of DNA may be important in cancer prevention. Inadequate function of methionine synthase can lead to an accumulation of homocysteine, which has been associated with increased risk of cardiovascular disease.

Sample requirements

1 mL of serum (minimum 500 µL) is required for this assay.

Interpretation

Vitamin B₁₂ 120 – 450 pmol/L (local reference range 2002)

B12 deficiency is not excluded in patients within the range 120 - 250 pmol/L, especially in the elderly. Vitamin B₁₂ is transported in the plasma in 2 forms the active form (halo-transcobalamin) bound to transcobalamin and the inactive form bound to haptocorrin. If the haptocorrin bound vitamin B₁₂ is raised in the presence of normal or low total vitamin B₁₂ the active halo-transcobalamin will therefore be low and the patient B₁₂ deficient.

Vitamin B₁₂ Deficiency

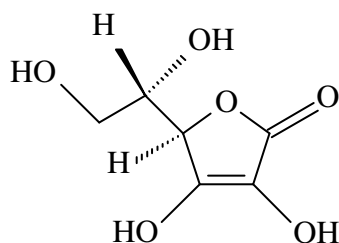
The most common causes of vitamin B₁₂ deficiency are pernicious anaemia and ileal vitamin B₁₂ malabsorption. The Schilling or Dicopak test is used to identify the underlying cause of vitamin B₁₂ deficiency. Untreated vitamin B₁₂ deficiency causes peripheral neuropathy due to damage to the posterior and lateral columns and dementia as a result of demyelination. Symptoms include 'beefy tongue' and fatigue. Treatment is recommended for any patient with clinical symptoms of vitamin B₁₂ deficiency.

Assessment of Methylmalonic acid (MMA) and homocysteine levels may be informative in some young patients. High levels are found in true vitamin B₁₂ deficiency.

Vitamin B₁₂ Toxicity

No toxic or adverse effects have been associated with large intakes of vitamin B₁₂ from food or supplements in healthy people. High levels usually reflect recent intramuscular injection or hepatocellular damage.

12. Vitamin C



Vitamin C

Introduction

Vitamin C (ascorbic acid) is a water-soluble vitamin and is required for the synthesis of collagen, and an important component of blood vessels, tendons, ligaments and bone. Unlike most mammals, humans do not have the ability to make their own vitamin C and therefore must be obtained from dietary sources.

Vitamin C status cannot be assessed on the basis of plasma levels alone as the plasma level can show a transitory rise immediately after intake of vitamin C and be lowered by recent infections. Determination of the vitamin C content of the leucocytes (including platelets) is the common procedure for accessing tissue concentrations as leucocyte levels have been shown to parallel the amount of vitamin C retained in the body.

Sample requirements

Two 5 mL heparinised blood tubes, collected on ice and processed by the laboratory within 1 hour.

Outlying referring laboratories can have tubes sent to them so they can prepare stable aliquots suitable for transportation to Canterbury Health Laboratories. Refer to www.cdhb.govt.nz/chlabs for details of specimen collection and processing protocol.



Interpretation

Plasma ascorbic acid 6 – 85 $\mu\text{mol/L}$ (reference range)

Leucocyte ascorbic acid >0.6 fmol/WBC

Low results for plasma ascorbic acid can indicate that the diet has only recently been low in ascorbic acid, but if leucocyte levels are low as well then clinical ascorbic acid deficiency is present.

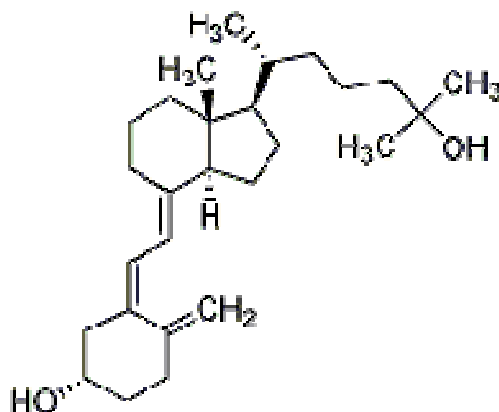
Vitamin C Deficiency

Severe vitamin C deficiency has been known for many centuries as the potentially fatal disease scurvy, which represents a failure of collagen synthesis. Symptoms of scurvy include bleeding and bruising, hair and tooth loss, joint pain and swelling. Scurvy is rare in developed countries, however, recent cases have occurred in children and the elderly on very restricted diets.

Vitamin C Toxicity

There is no reliable scientific evidence to suggest that large doses of vitamin C are toxic or detrimental to health. There is some recent literature, however, which suggests that very large chronic doses of vitamin C may facilitate the formation of renal oxalate stones.

13. Vitamin D



25-hydroxy cholecalciferol (25-hydroxyvitamin D₃)

Introduction

Vitamin D is not technically a vitamin, because it is not required in the diet. It is more correctly classified as a pro-hormone. Vitamin D (calciferol) exists in two main forms:-

Vitamin D₃ (cholecalciferol) is synthesised in the skin by the action of sunlight on 7-dehydrocholesterol. Natural dietary sources of D₃ include fish oils, egg yolk and milk fat.

Vitamin D₂ (ergocalciferol) is prepared from ergosterol that is readily available in yeast and used in food additives for humans and livestock.

Vitamin D₃ undergoes two successive hydroxylations. The first occurs in liver at carbon 25 to form 25-hydroxyvitamin D₃ - the main circulating metabolite, with little biological activity. 25-hydroxyvitamin D₃ reflects hepatic stores.

When calcium levels are low, hydroxylation occurs at carbon 1 (in renal tubules, placenta, bone), yielding the active form, 1,25-dihydroxyvitamin D₃, which stimulates osteoblasts to take up calcium. In the intestine, 1,25-dihydroxyvitamin D₃ stimulates calcium absorption into the bloodstream. When calcium levels are adequate, hydroxylation occurs instead at carbon 24, yielding the inactive 24,25-dihydroxyvitamin D₃ form. Vitamin D levels show seasonal variation in conjunction with UV exposure: levels are highest in the summer and lowest during winter months.



Sample requirements

500 μL of plasma or serum (minimum 200 μL) is required for this assay. Samples should be protected from light and frozen before sending to the laboratory.

Interpretation

25-hydroxyvitamin D₃ (nmol/L)

<25	Moderate to severe vitamin D deficiency
5 - 50	Mild vitamin D deficiency
0 - 150	Optimal target range for bone health
>150	Toxicity

Vitamin D Deficiency

Vitamin D deficiency is a common problem and its incidence is rising in New Zealand. It is highly prevalent in persons with darker skin pigmentation and who cover their skin for cultural reasons. Other causes include dietary deprivation, malabsorption (e.g. coeliac disease), renal disease and drugs, such as anticonvulsants. Sunlight deprivation is also found in institutionalised elderly people.

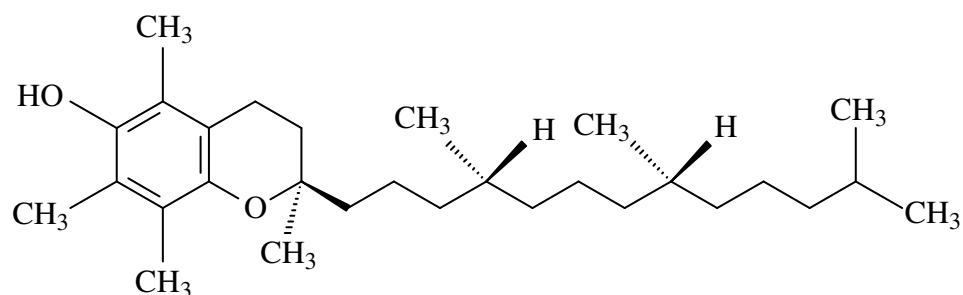
One of the earliest clinical signs of vitamin D deficiency is generalised bone pain. This should be considered as differential diagnosis in African or Asian migrants who complain of total body pain.

Biochemically, low levels of calcium and phosphorus coupled with mildly raised PTH are early indicators of hypovitaminosis D. Severe hypocalcaemia may result in muscle weakness and tetany, and this can be the first presenting symptom. Established vitamin D deficiency in adults causes osteomalacia and rickets in children, which refers to the clinical manifestation of bone demineralisation.

Vitamin D Toxicity

Vitamin D toxicity results from excessive ingestion of vitamin D, either iatrogenically or by misguided use of over-the-counter multivitamins. It causes hypercalcaemia, which is responsible for the clinical symptoms of anorexia, nausea, vomiting and weakness. Hypercalcaemia causes nephrogenic diabetes insipidus leading to polyuria and dehydration. Excess vitamin D can also cause metastatic calcifications.

14. Vitamin E



α -tocopherol

Introduction

Vitamin E is a group of compounds called tocopherols, of which α -tocopherol is the most active. It acts as an antioxidant in preventing the oxidation of lipids in cell membranes. It has been suggested that vitamin E can reduce the risk of some types of cancers and heart disease. The amount of vitamin E needed in the diet is related to the amount of polyunsaturated fatty acids consumed. Since vegetable oils are rich sources of both, deficiency is rare.

Sample requirements

500 μ L of plasma or serum (minimum 200 μ L) is required for this assay. Samples should be protected from light and frozen before being sent to the laboratory.

Interpretation

Vitamin E 23 – 70 μ mol/L (recommended level)

Infants are born in a state of relative vitamin E deficiency, with plasma α -tocopherol levels below 11.6 μ mol/L. The smaller and more premature the infant, the greater the degree of deficiency. Vitamin E deficiency in premature infants persists during the first few weeks of life and can be attributed to limited placental transfer of vitamin E, low tissue levels at birth, relative dietary deficiency in infancy, intestinal malabsorption, and rapid growth. As the digestive system matures, vitamin E absorption improves, and blood vitamin E levels rise.



In children and adults, malabsorption generally underlies vitamin E deficiency. Genetic abnormality in the transport of vitamin E can also play a role.

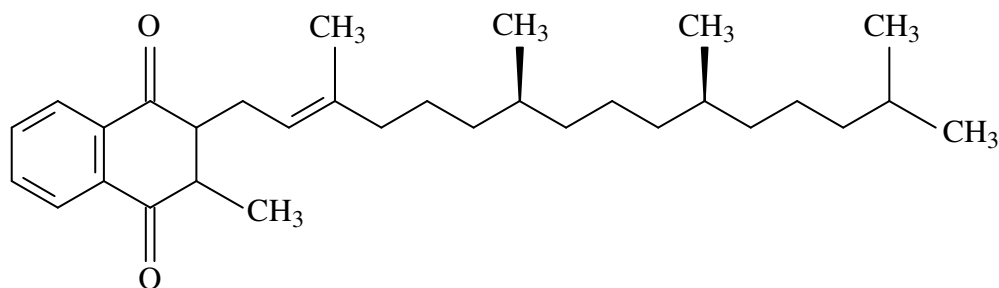
Vitamin E Deficiency

In humans, the most common manifestations of vitamin E deficiency are mild haemolytic anaemia associated with increased erythrocyte haemolysis, and spinocerebellar disease in children. Children with chronic cholestatic hepatobiliary disease or cystic fibrosis manifest the neurologic syndrome of vitamin E deficiency. Its signs are spinocerebellar ataxia with loss of deep tendon reflexes, truncal and limb ataxia, loss of vibration and position sense, ophthalmoplegia, muscle weakness, ptosis, and dysarthria. In adults with malabsorption, spinocerebellar ataxia due to vitamin E deficiency is extremely rare, no doubt because adults have large vitamin E stores in adipose tissue. Abetalipoproteinemia, due to the genetic absence of apolipoprotein B, causes serious fat malabsorption and steatorrhea, with progressive neuropathy and retinopathy in the first two decades of life. Plasma vitamin E levels are usually undetectable.

Vitamin E Toxicity

Adults can take relatively large amounts of vitamin E without any apparent harm. Occasionally, muscle weakness, fatigue, nausea and diarrhoea have occurred in persons taking excess amounts. The most significant toxic effect of vitamin E is antagonism to vitamin K action and enhancement of the effect of oral coumarin anticoagulants, which may result in overt haemorrhage.

15. Vitamin K



Vitamin K₁

Introduction

Vitamin K is a fat-soluble vitamin. Vitamin K is found naturally in two forms: K₁, also known as phylloquinone, is found in green leafy vegetables and K₂, also known as menaquinone, and is synthesised by intestinal bacteria. Vitamin K is essential because the 1,4-naphthoquinone nucleus cannot be synthesized in the body.

Vitamin K controls the formation of coagulation factors II (prothrombin), VII (proconvertin), IX (Christmas factor, plasma thromboplastin component), and X (Stuart factor) in the liver. Other coagulation factors dependent on vitamin K are protein C, protein S, and protein Z; proteins C and S are anticoagulants. Two bone matrix proteins necessary for normal bone metabolism are vitamin K-dependent. There are some indications that vitamin K may decrease the incidence or severity of osteoporosis and slow bone loss.

In the intestines it also assists in converting glucose to glycogen.

Sample requirements

2 mL of serum (minimum 1 mL) is required for this assay. Serum samples should be protected from light and frozen before sending to the laboratory.

Interpretation

Vitamin K	0.09 – 2.12 mg/ L (reference range)
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Vitamin K deficiency

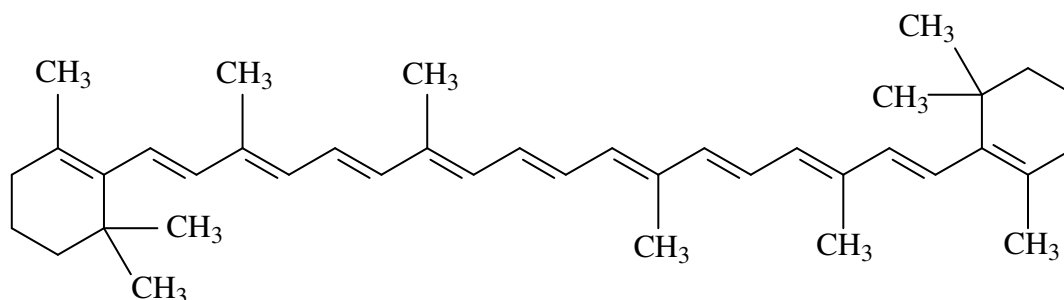
In newborns, vitamin K nutrition is at risk because (1) the placenta transmits lipids relatively poorly; (2) the neonatal liver is immature with respect to prothrombin synthesis; (3) breast milk is low in vitamin K, and (4) the neonatal gut is sterile during the first few days of life. Hemorrhagic disease of the newborn, caused by vitamin K deficiency, generally occurs 1 to 7 days postpartum and may be manifested by cutaneous, GI, intrathoracic, or, in the worst cases, intracranial bleeding. Late hemorrhagic disease, which has the same clinical manifestations, occurs 1 to 3 months postpartum. It is usually associated with malabsorption or liver disease. If the mother has ingested hydantoin anticonvulsants, cephalosporin antibiotics, or coumarin anticoagulants, the risk of both types of hemorrhagic disease is increased.

In healthy adults, primary vitamin K deficiency is uncommon. Adults are protected from a lack of vitamin K because vitamin K is widely distributed in plant and animal tissues, the vitamin K cycle conserves the vitamin, and the microbiologic flora of the normal gut synthesizes menaquinones. However, vitamin K deficiency can occur in adults with marginal dietary intake if they undergo trauma, extensive surgery, or long-term parenteral nutrition with or without treatment with broad-spectrum antibiotics. Persons with biliary obstruction, malabsorption, or parenchymal liver disease also have a higher risk of vitamin K deficiency; those who ingest certain drugs, including anticonvulsants, anticoagulants, certain antibiotics (particularly cephalosporins), salicylates, and megadoses of vitamin A or E are vulnerable to vitamin K-related hemorrhagic disease. Persons receiving warfarin should attempt to keep vitamin K intake constant to avoid fluctuating prothrombin levels.

Vitamin K toxicity

There is no known toxicity associated with high doses of vitamin K₁ (phylloquinone), a natural form of vitamin K. Vitamin K₁ is not toxic at 500 times the Recommended Daily Allowance (0.5 mg/kg/day). However, menadione, a vitamin K precursor, has a finite toxicity resulting from its reaction with sulfhydryl groups; it can cause hemolytic anemia, hyperbilirubinemia, and kernicterus in infants. Menadione should *not* be used to treat vitamin K deficiency.

16. Carotene



β Carotene

Introduction

Carotenes, a number of naturally occurring pigments, are precursors to vitamin A. They are found in the yellow, red and green parts of plants and are especially abundant in carrots.

The major carotenoids in serum comprise of alpha- and beta-carotene, lycopene and xanthophyll and are exclusively located in the lipoprotein fractions.

Beta-carotene is effectively two molecules of vitamin A joined end to end and can be converted to vitamin A in the intestinal mucosal cells.

Carotene is measured mainly as a procedure for diagnosing fat malabsorption, where carotene in serum is greatly decreased.

Sample requirements

1 mL of plasma or serum (minimum 600 μ L) is required for this assay. Samples should be protected from light before sending to the laboratory.

Interpretation

Carotene 1.5 – 3.0 $\mu\text{mol/L}$ (reference range)

The serum level is greatly influenced by the amount of carotene in the diet. An increase in carotenes may be seen if large amounts of carotene rich vegetables are consumed.

Carotene Deficiency

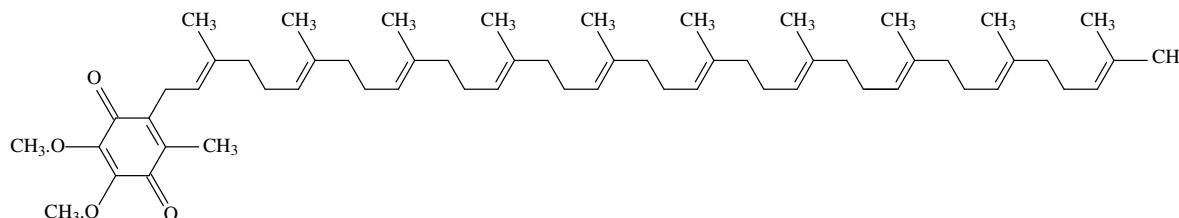
Carotenes almost disappear from the blood in a few weeks if absent in the diet. Low serum carotene in the presence of adequate dietary intake is an indicator of malabsorption. Symptoms of carotene deficiency mimic those of a vitamin A deficiency: dry skin, night blindness and susceptibility to infection. Low levels are also found in high fever and liver disease.

Carotene Toxicity and Excess

Carotene does not carry the same toxicity risk of that of vitamin A. However, excessive ingestion of carotene-rich foods (such as carrot juice) may result in xanthoderma, a benign condition leading to orange discolouration of palms and soles. This will improve if carotene intake is reduced.

Other causes of xanthoderma include hypothyroidism, liver disease, chronic nephritis and hyperlipaemia due to diabetes mellitus.

17. Coenzyme Q₁₀



Coenzyme Q₁₀

Introduction

Coenzyme Q₁₀ (CoQ₁₀) is essential to life, acting as a mobile electron carrier in the mitochondrial electron transport chain. This chain is essential for the production of adenosine triphosphate (ATP).

CoQ₁₀ is transported in the blood in low-density lipoproteins (LDL). Reduced CoQ₁₀ can also act as an antioxidant, and therefore it is accepted that CoQ₁₀ aids in protection of LDL from oxidation.

CoQ₁₀ is synthesised in the liver by the mevalonate pathway. This pathway is also used for the endogenous production of cholesterol. It is therefore not surprising that statins, drugs which inhibit the mevalonate pathway, also inhibit synthesis of CoQ₁₀. Small amounts of CoQ₁₀ are also obtained from the diet, where meats are an especially good source.

Primary deficiency of CoQ₁₀ is a rare autosomal recessive disorder, with symptoms of ataxia and myopathy.

Secondary CoQ₁₀ deficiency occurs in patients receiving statin therapy, and this is expected to be the main reason for CoQ₁₀ requests. Some statin-induced myopathies have been attributed to an induced CoQ₁₀ deficiency.



Sample requirements

500 μL of Lithium heparin plasma samples only. Serum and EDTA plasma samples are unsuitable for analysis. Patients are required to be fasting.

Plasma should be protected from light, sent and stored frozen.

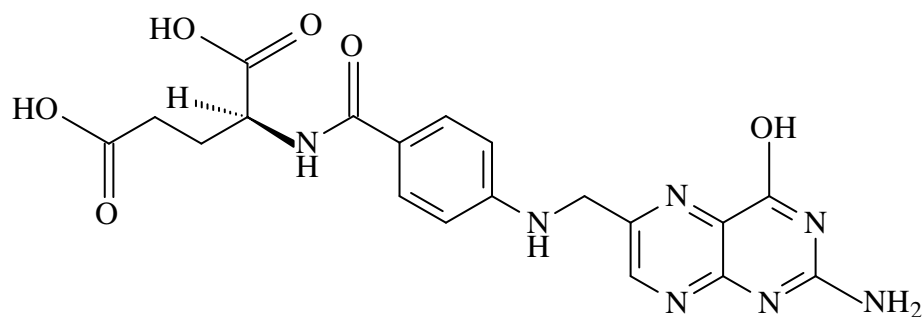
Interpretation

Coenzyme Q_{10} 0.5 - 1.8 $\mu\text{mol/L}$ (reference range)

Deficiencies in patients 1-30 years of age indicate a possible primary coenzyme Q_{10} deficiency, and supplementation may be necessary.

A deficiency in older patients may be due to statin therapy, or if this is not the case, a mitochondrial disorder. No clinical significance is associated with high coenzyme Q_{10} levels, which may be diet-related, or due to supplementation.

18. Folate



Folic Acid

Introduction

Folic acid is necessary for the synthesis of nucleic acids and the formation of haem, the pigmented, iron-carrying component of the haemoglobin in red blood cells. The requirement for folic acid is notably increased in pregnancy and other conditions where there is increased cellular turnover.

Folic acid, the most stable of the folate group, occurs rarely in foods or the human body, but is the form most often used in vitamin supplements and fortified foods.

Sample Requirements

Both red cell and serum folate levels are available.

1 mL of serum (500 μ L minimum) is required for serum folate

1 mL of whole blood EDTA (400 μ L minimum) is required for red cell folate

Interpretation

Serum folate is influenced by recent folate intake.

Serum folate 8.0 – 45.0 nmol/L (local reference range 2002)

Red cell folate levels provides an indication of total folate storage

Red cell folate 445 – 1200 nmol/L (local reference range 2002)



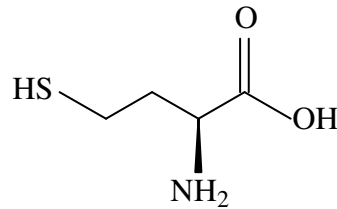
Folate Deficiency

Folic acid deficiency is one of the most common vitamin deficiencies. It can result from inadequate dietary intake, defective absorption (coeliac disease), abnormal metabolism or increased requirements. Conditions such as pregnancy or malignancy result in increased rates of cell division and metabolism, leading to an increase in the body's demand for folate. Symptoms of deficiency include mild anaemia, inflammatory lesions of the mouth, and disturbed hair formation and growth.

Folate Toxicity

Oral folic acid is well tolerated, with no documented reports of toxicity. However, folic acid replacement can mask a vitamin B₁₂ deficiency by improving haematological signs of vitamin B₁₂ deficiency, such as macrocytosis. As a result, the diagnosis of vitamin B₁₂ deficiency will be delayed until more serious neurological signs occur, such as subacute combined degeneration of the cord (SCDC). It should therefore not be used indiscriminately in patients with anaemia and vitamin B₁₂ levels should be checked before commencing folate.

19. Homocysteine

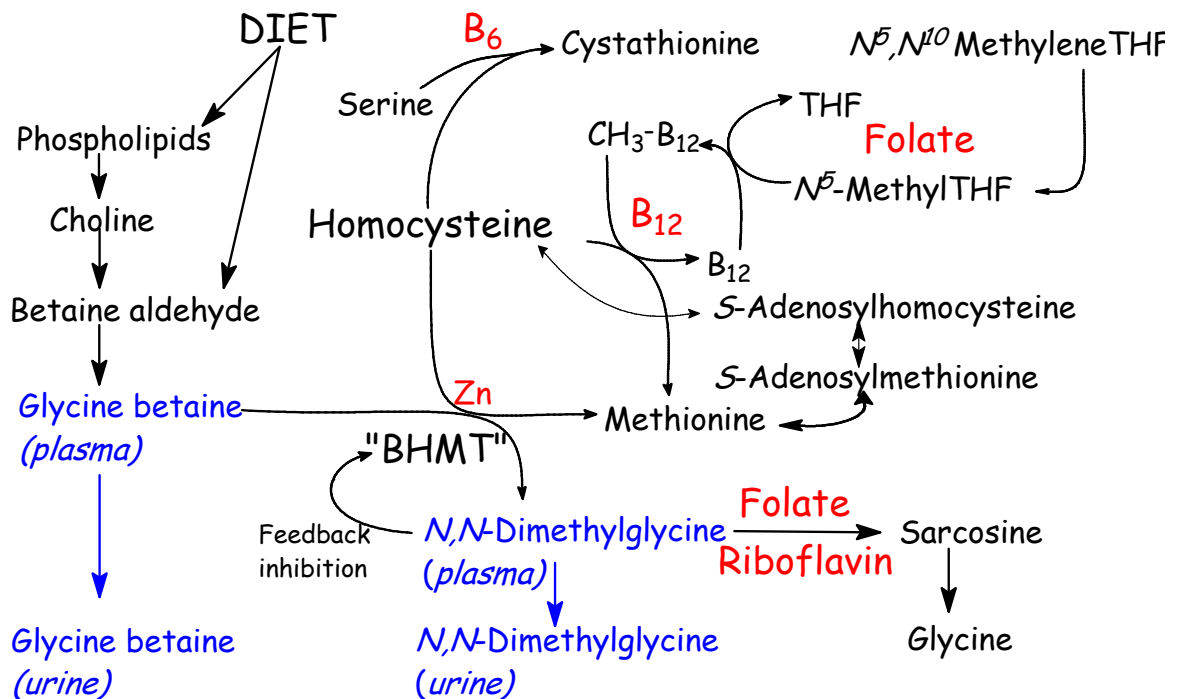


Homocysteine

Introduction

Homocysteine is a sulphur containing amino acid that does not participate in protein synthesis. It is an intermediary product in the metabolism of methionine, an essential amino acid and precursor of important methyl donors.

Hyperhomocysteinaemia has recently been identified as an independent cardiovascular risk factor and should be included in risk assessment of patients. Plasma homocysteine levels are inversely correlated with vitamin intake; in particular, vitamin B₆, vitamin B₁₂ and folate, as they are essential cofactors in homocysteine metabolising pathways.



Homocysteine Metabolic Pathway



Sample requirements

500 μL of EDTA plasma (250 μL minimum) is required for this assay. Because synthesis of homocysteine continues in red blood cells after drawing, it is important to put the EDTA sample straight into ice after venesection. The plasma should be separated from the red cells and frozen within 4 hours of collection. The patient must be fasting prior to collection.

Interpretation

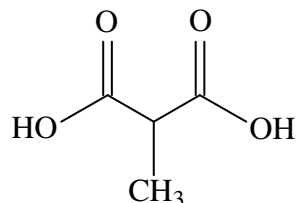
Homocysteine 5 – 15 $\mu\text{mol/L}$ (reference range)

Optimal level for the secondary protection of cardiovascular disease
< 10 $\mu\text{mol/L}$.

The total homocysteine concentration of healthy individuals will vary with age, gender, geographical area, and genetic factors (e.g. MTHFR polymorphism). Values are typically lower for women than for men, postmenopausal women show increased values for homocysteine. Metabolic causes of hyperhomocysteinaemia include impaired renal function, hypothyroidism, alcohol abuse and use of folate antagonists, such as methotrexate.

Markedly elevated levels should prompt consideration of underlying genetic causes such as MTHFR polymorphism or the rarer homocysteinemia.

20. Methylmalonic Acid



Methylmalonic Acid

Introduction

Methylmalonic acid (MMA) is an early and specific indicator of vitamin B₁₂ deficiency. It is derived from methylmalonic CoA, which is converted to succinyl CoA by methylmalonyl CoA mutase. This enzyme requires vitamin B₁₂ as a cofactor, so when B₁₂ concentrations are sub-optimal, enzyme activity slows and intracellular levels of methylmalonyl CoA increase. Consequently methylmalonic acid levels increase in the blood. Some reports suggest that 25% of people with low vitamin B₁₂ levels may not have functional deficiency. MMA measurement may assist in the identification of functional B₁₂ deficiency.

Sample requirements

2 mL of plasma or serum (500 µL minimum) is required for this assay.

Interpretation

Methylmalonic acid levels < 0.40 µmol/L excludes a diagnosis of vitamin B₁₂ deficiency.

Levels 0.4 - 1.0 µmol/L are consistent with vitamin B₁₂ deficiency or low stores, assuming normal renal function.

Levels > 1.0 µmol/L are consistent with vitamin B₁₂ deficiency.

Extreme levels > 10.0 µmol/L are seen in methylmalonic aciduria.

