Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Review on quantification vitamin B12 in blood

Priyabrata Das[1], Dr. Subodh Daronde[2]

[1]Research Scholar, Department of Biomedical Engineering, Faculty of Engineering &Technology, Datta Meghe Institute Of Higher Education And Research, Wardha, India

Email ID: [1]priyabratadas832@gmail.com

[2]Assistant Professor, Department of Biomedical Engineering, Faculty of Engineering &Technology, Datta Meghe Institute Of Higher Education And Research, Wardha, India

Email ID: Subodhd.feat@dmiher.edu.in

Cite this paper as: Priyabrata Das, Dr. Subodh Daronde, (2025) Review on quantification vitamin B12 in blood. *Journal of Neonatal Surgery*, 14 (32s), 4827-4847.

ABSTRACT

Vitamin B12 is one of the micronutrients that are required in the formation of blood cells, the functions of the nervous systems and also the synthesis of DNA. Importantly, Vitamin B12 level in blood should be measured precisely for detecting deficiencies and monitoring therapeutic interventions. Some of the quantification methods that have been discussed in this review paper include chemiluminescent immunoassays, LC-MS/MS and electrochemiluminescence immunoassays alongside their performance in terms of their efficiency, sensitivity, specificity, and feasibility. These evaluations are the primary objective of this review paper as well. Through a thorough analysis of recent studies, the technological innovations and methodological refinements that have enhanced the accuracy and reliability of vitamin B12 measurements are highlighted clearly. By bringing together all the existing research findings and identifying areas that need more investigation, the current review paper is prepared efficiently thereby providing guidance for both future research and clinical practices as well. Notably, the ongoing discussion regarding the improvement of Vitamin B12 measurement methods and its significance in clinical diagnostics is explained well in this review paper by referencing key studies from 2019 to 2024.

Keywords: *Quantification vitamin B12 in blood, vitamin B12, LC-MS/MS, B12 in human health, Physiology, metabolism.*

1. INTRODUCTION

Generally, Cobalamin is known as Vitamin B12, is a water-soluble vitamin. It is highly notable that Vitamin B12 deficiency, a very important condition that can result in a variety of clinical symptoms i.e. ranging from megaloblastic anemia to severe neurological disorders. Therefore, it is necessary to accurately measure the amount of Vitamin B12 level in blood. Various factors such as inadequate dietary intake, malabsorption syndromes, certain medications, and genetic disorders might lead to Vitamin B12 deficiency. Vitamin B12 deficiency, if not treated, might lead to several clinical implications and as a result, various bodily functions will get slightly impacted at first and then it might lead to neurological disorder.

The impact of vitamin B12 deficiency in diverse populations and contexts has been highlighted by recent studies conducted by (Azzini et al., 2021) and (Yang et al., 2024). There are traditional methods like Microbiological assays, radioimmunoassays, and enzyme-linked immunosorbent assays (ELISAs) and these methods can be utilized for measuring Vitamin B12 deficiency accurately. However, limitations exist in traditional methods regarding how well they detect and identify vitamin B12, and how practical they are for use in hospitals and clinics as well.

There has been significant progress made from the years 2019 to 2024 in developing and improving methods to measure Vitamin B12 levels in blood. All the advancements made in the vitamin B12 quantification techniques over the past four years are examined in this review paper.

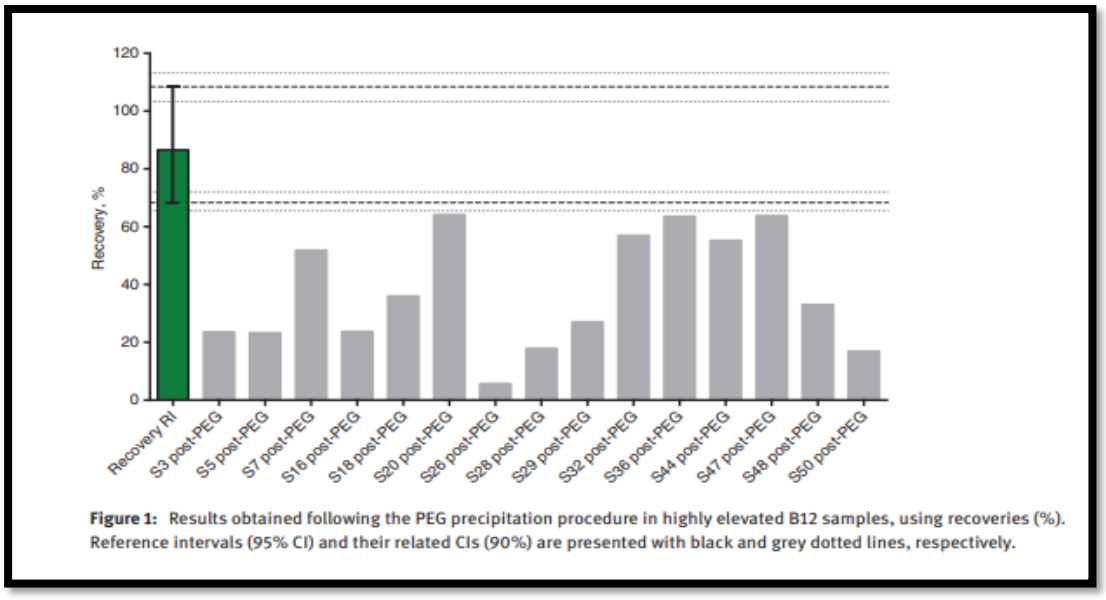


Figure 1: Results obtained following the PEG precipitation procedure in highly elevated B12 samples, using recoveries (%). Reference intervals (95% CI) and their related CIs (90%) are presented with black and grey dotted lines, respectively.

Figure 1: Vitamin B12 samples (Soleimani et al., 2019).

Innovations in technology, identification of ongoing challenges and future research directions are examined through systematic approach in order to optimize vitamin B12 quantification and improve patient outcomes.

1.1 Importance of B12 in human health:

Importantly, Vitamin B12 is effectively involved in neurological function as well. Valuable information regarding the role of B12 in the human body is provided by reviewing recent studies. A systematic review and network meta-analysis is conducted by (Abdelwahab et al., 2024). The importance of selecting accurate supplementation methods to effectively manage B12 deficiency and prevent associated health problems are highlighted in the findings conducted by (Abdelwahab et al., 2024). The interconnection among B12 deficiency in early pregnancy, maternal obesity, and dyslipidemia are examined by (Adaikalakoteswari et al., 2020).

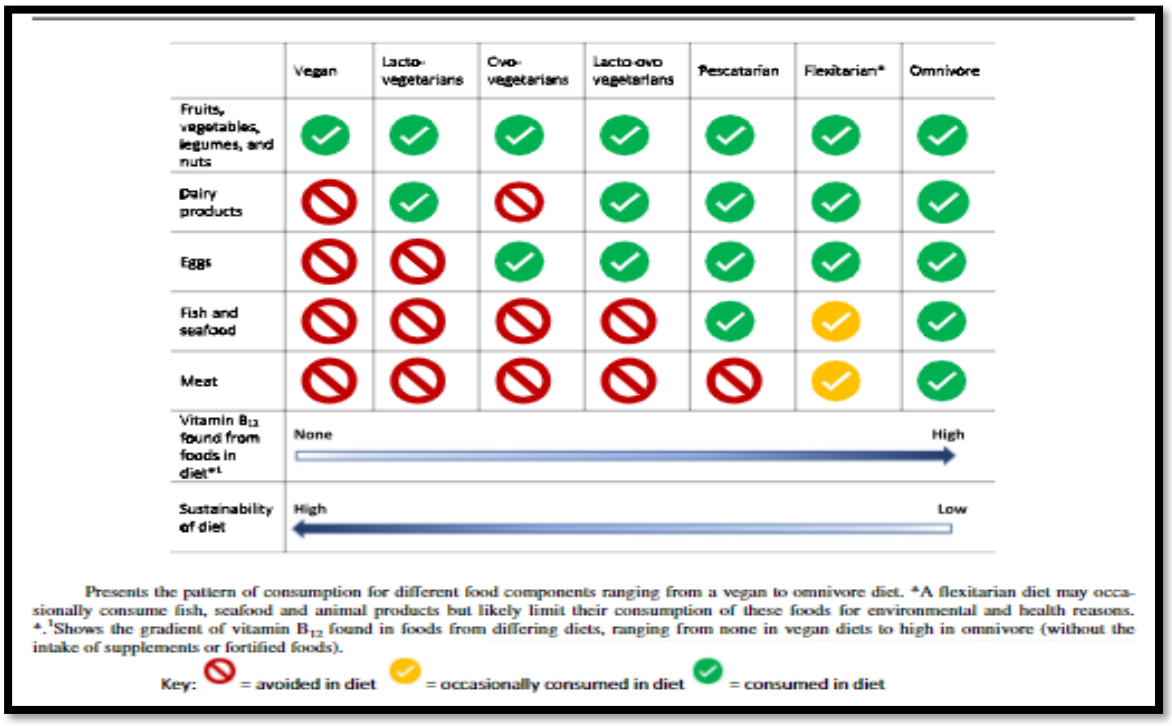


Figure 2: Pattern of consumption for different food ranging components (Niklewicz., 2022).

Through systematic review, (Behere et al., 2021) examined how a mother's vitamin B12 levels impact the pregnancy outcomes and the health of offspring in India and their review indicated that the poor maternal B12 status can drastically affect maternal and child health. The need for public health policies to address this problem is mainly focused in their review.

A systematic review and meta-analysis on B12 levels in thyroid disorders is conducted by (Benites-Zapata et al., 2023). The relationship between B12 deficiency and thyroid dysfunction is highly notable in their review. Their study explained that it is crucial to ensure that pregnant women get enough B12 and if there is any shortage with the B12 level it should be solved by taking specific supplements and public health strategies are very essential for better health outcomes.

1.2 Clinical Significance of accurate quantification:

Vitamin B12 (cobalamin) levels should be measured accurately in clinical settings. If the B12 level is not appropriate, then the person's health might get impacted in many ways. In particular, inadequate B12 levels affect the health of pregnant women, newborns and individuals with certain chronic conditions.

Vitamin B12 is important for health and it should be measured accurately as explained in the recent studies, which is highlighted in this review paper. During pregnancy, Vitamin B12 levels should be adequate because it is crucial for both maternal health and fetal development. (Behere et al., 2021) explained that low vitamin B12 level during pregnancy may lead to adverse pregnancy outcomes and poor neonatal health. Moreover, vitamin B12 deficiency may cause major neurological and cognitive problems.

The impact of Vitamin B12 deficiency on the nervous systems is explained in the research work conducted by (Mathew et al., 2024) and the need for accurate measurement for diagnosing and managing related conditions is highlighted as well.

1.3 Overview of the paper structure:

In this review paper, the importance of vitamin B12 is explained very clearly. A detailed outlook at the diverse health outcomes that are related to vitamin B12 consumption is also provided in this section. Usually, in the literature review section all the recent studies and important findings are combined together to identify areas where further investigation is needed.

Following the literature review section, details about the research design, data sources, and analytical techniques used to investigate the effects of vitamin B12 are provided in the methodology section. A comprehensive analysis of the data is presented in the result section where significant associations and potential causal pathways are revealed. For example, (Madjedi et al., 2023) investigated the link between physical activity and glaucoma.

The strength and weakness of the study are highlighted in the review paper along with other findings as well as the study includes perspectives from various studies, as per the systematic review conducted by (Markun et al., 2021).

As a whole, a detailed explanation about the role of vitamin B12 in health is provided in this review paper.

2. LITERATURE REVIEW

Many studies have revealed that, Vitamin B12 levels are very crucial during pregnancy as well as for pregnancy related outcomes and the health of offspring. The crucial role of adequate maternal vitamin B12 levels is explained in a systematic review conducted by (Behere et al., 2021), highlighting its potential in promoting positive pregnancy outcomes and optimal health for their children.

2.1 Overview of quantification vitamin B12 in blood:

The Vitamin B12 levels are crucial for diagnosing deficiencies and monitoring supplementation efficiency and so it is very essential to be measured accurately. Some innovative methods have been examined thoroughly by recent studies to improve the accuracy of B12 measurement. For example, (Dubascoux et al., 2021) used liquid chromatography with plasma-mass spectrometry to get better results in human milk analysis.

This progress shows the importance of robust analytical techniques in understanding B12 role across various biological matrices is highlighted in the research conducted by (Dubascoux et al., 2021).

(Dastidar & Sikder, 2022) in their research work highlighted the advancements in clinical diagnostics like evaluation of serum active B12 (holo-transcobalamin), which are crucial in overcoming the issues associated with traditional total B12 measurements. (Flieger et al., 2024), in their study explored how cyanocobalamin sticks to mesoporous superparamagnetic iron oxide nanoparticles and this study highlighted the new ways in improving B12 measurement techniques.

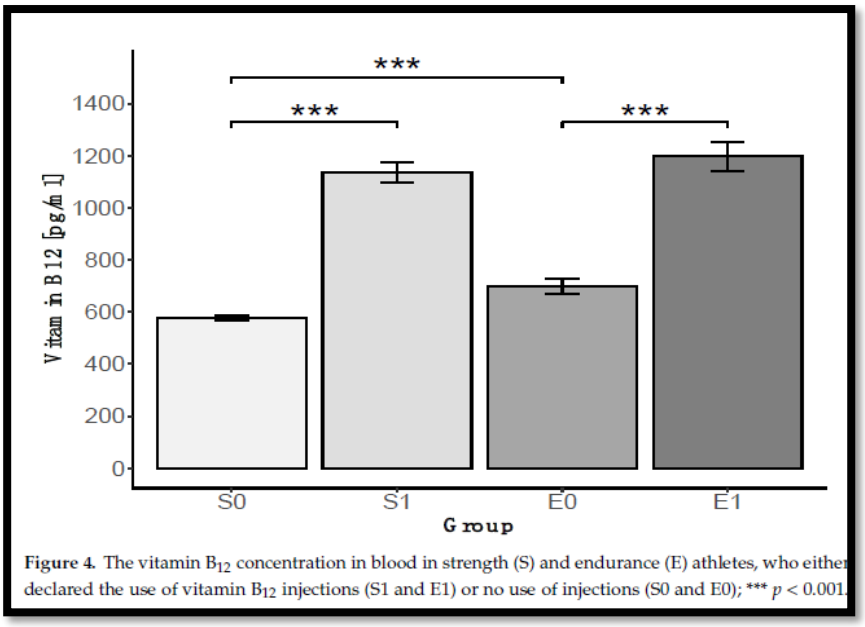


Figure 3: Vitamin B12 concentration in blood (Krzywański et al., 2020).

New innovative methods are very essential to address the complexities that are associated with vitamin B12 deficiency and it is highly notable that these methods are crucial to provide treatment for people who have inadequate vitamin B12 level in blood.

2.2 Previous studies on B12 in human health:

(Feng et al., 2023) performed combined transcriptomic and proteomic analyses, showing that BES1 helps Arabidopsis plants tolerate salt better and their findings helps to understand clearly that the molecular mechanisms are used by the plants to respond to environmental stressors. The impact of maternal vitamin B12 deficiency on perinatal outcomes in southern India is investigated by (Finkelstein et al., 2021). The key findings and focus of each referenced study are summarized in these passages as well.

Table 1: Summary of the existing works

Reference number	Year published	Methods used
[1].	2024	Systematic review, network meta-analysis
[2].	2020	Observational, cross-sectional
[3].	2021	Follow-up analysis of intervention trial
[4].	2023	Cross-sectional, observational
[5].	2021	Review article, synthesis of evidence
[6].	2021	Review article, case studies
[7].	2020	Cross-sectional, community-based survey
[8].	2023	Cross-sectional study
[9].	2021	Systematic review
[10].	2023	Systematic review, meta-analysis
[11].	2020	Review article, synthesis of evidence
[12].	2020	Diagnostic accuracy study
[13].	2023	Review article, future trends
[14].	2023	A community-based, double-blind, randomised, placebo-controlled trial
[15].	2023	Experimental, method development
[16].	2022	Cross-sectional, observational
[17].	2021	Randomized controlled trial
[18].	2024	Literature review

[19].	2022	Review article, diagnostic study
[20].	2022	Diagnostic study
[21].	2024	Bioinformatics analysis
[22].	2021	Analytical chemistry
[23].	2021	Analytical chemistry
[24].	2023	Transcriptomics
[25].	2024	Review article, critical analysis
[26].	2023	Transcriptomics, proteomics
[27].	2021	Epidemiological study
[28].	2024	Materials science
[29].	2023	Clinical trial, secondary analysis
[30].	2020	Review article, clinical approach
[31].	2023	Diagnostic study
[32].	2022	Diagnostic study
[33].	2019	Methods review
[34].	2022	Systematic review, meta-analysis
[35].	2021	Systematic review, meta-analysis
[36].	2024	Epidemiological study
[37].	2022	Analytical chemistry, pharmacological study
[38].	2021	Epidemiological study
[39].	2020	Cross-sectional study
[40].	2021	Retrospective cohort study
[41].	2024	Population-based cross-sectional study
[42].	2020	Systematic review, meta-analysis
[43].	2023	Epidemiological study
[44].	2024	Epidemiological study
[45].	2023	Epidemiological study
[46].	2021	Systematic review, meta-analysis
[47].	2023	Review article, theoretical approach
[48].	2024	Review article, biological models
[49].	2022	Review article, clinical application
[51].	2022	Review article, dietary study
[52].	2024	Expert consensus
[53].	2022	Observational study
[54].	2022	Nutritional assessment
[55].	2022	Experimental study, clinical observation
[56].	2023	In vitro study, computational modeling
[57].	2021	Review article, theoretical approach
[58].	2023	Randomized Controlled Trial
[59].	2021	Prospective Cohort Study (PRiDE study)
[60].	2023	Review
[61].	2021	Critical Review, Clinical and Laboratory Assessment
[62].	2019	Review
[63].	2023	Epidemiological Study
[64].	2023	Epidemiological Study, Ophthalmological Assessment
[65].	2021	Spectrometry
[66].	2021	Epidemiological Study, NHANES
[67].	2023	Animal Study, Neuronal Morphology Assessment
[68].	2024	Epidemiological Study, NHANES
[69].	2023	Systematic Review, Meta-analysis

[70].	2020	Epidemiological Study
[71].	2021	Assessment of Serum Vitamin B12
[72].	2022	Analyzed role of gut microbiota in vitamin B
[73].	2024	Transcriptome Analysis
[74].	2021	Analyzed role of vitamin B12 deficiency
[75].	2024	Analyzed fast quantification of vitamin B12
[76].	2022	An observational cohort study
[77].	2020	Analyzed High-concentration homocysteine
[78].	2023	A case report
[79].	2023	Analysed the effects of vitamin B12 deficiency

3. RESEARCH METHODOLOGY

Vitamin B12, known as cobalamin, is very essential for human beings because it has a crucial part in various physiological processes such as DNA synthesis, nervous system function, and red blood cell formation. A detailed research on recent studies was carried out to find ways to measure B12 level in blood accurately. Electronic databases such as PubMed, Web of Science, and Scopus were performed in the search.

Selection Criteria

The current paper reviewed articles which are based on inclusion and exclusion criteria as follows:

Inclusion Criteria: In this review paper, studies that are published in peer-reviewed journals, written in English, conducted on human subjects, and methods that are focused on vitamin B12 quantification in blood are included.

Exclusion Criteria: Studies that do not meet the inclusion criteria, reviews lacking original data and the studies published between 2020 and 2024 are excluded in this review paper.

Data Extraction

Data extraction is nothing but the systematic process of extracting information that is relevant from selected studies. The key components included in the data extraction are study design, sample size, participant characteristics (e.g., age, gender, health status), analytical methods used for vitamin B12 quantification, and reported outcomes such as sensitivity, specificity, and analytical performance metrics.

Some of the previously published studies of Vitamin B12 quantification of blood have been reviewed in this paper. As a result, the results of those reviews were used to evaluate the performance characteristics of each method especially for Vitamin B12 quantification in blood.

Discussion

From the results that were discussed in previous studies, the implications of both clinical practice and research are examined well. For future research, the methodological standardization for measuring vitamin B12 level in blood should be focused effectively and innovative technologies are required to accurately examine the vitamin B12 level in blood.

3.1 Research design:

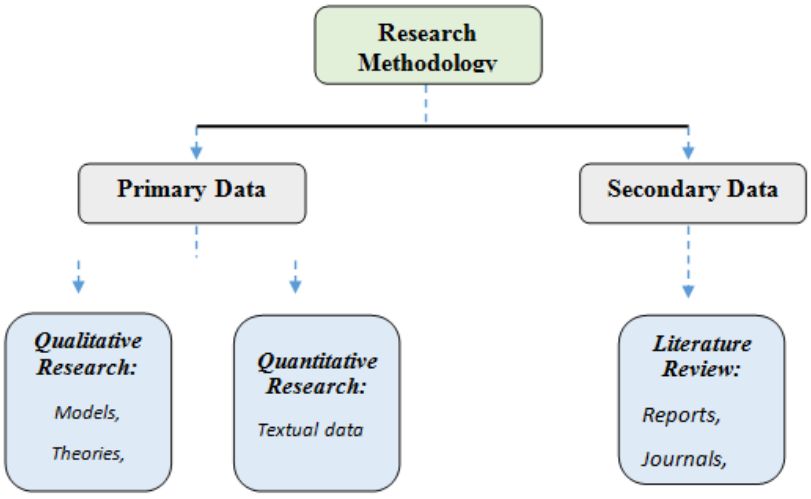


Figure 4: Research design

3.2 Research questions:

1. *What are some of the important current methods and technologies which are available for measuring vitamin B12 levels in blood?*
2. *What are the pros and cons of each quantification method for vitamin B12 in blood?*
3. *What are some of the important factors that thoroughly influence the choice of quantification method for vitamin B12 in blood?*
4. *What are the some of the important evolving trends and advancements in technologies for vitamin B12 quantification?*

4. PHYSIOLOGY AND METABOLISM OF VITAMIN B12

4.1 Sources of Vitamin B12:

Vitamin B12, or cobalamin, is one of the vital nutrients and it is highly necessary for many bodily functions in human beings. The role of Vitamin B12 in the human body can be understood better from this review paper. Valuable information regarding the role of B12 has been provided by some research and findings and this is highlighted in this review paper as well. The recent improvements in vitamin B12 testing methodologies and their importance in accurately diagnosing deficiencies is explained in the research work conducted by (Hamel and Spry, 2022) and their findings were found very useful for doctors to identify and manage vitamin B12-related health issues. The diverse roles of vitamin B12 in human health i.e. from maternal and child welfare to neurological and ocular health are highlighted in this review paper. To collect further information about the Vitamin B12 nutrient, the referenced studies are closely explored as each and every study provides some information regarding the essential nutrient.

4.2 Absorption and metabolism in the body:

Absorption and metabolism pathways of Vitamin B12 are very essential to understand for evaluating the overall impact of the nutrient on health and addressing potential deficiencies. Vitamin B12 absorption process, which is a difficult process that occurs primarily in the small intestine and several complicate steps are involved:

First, vitamin B12 is released from dietary proteins in the stomach. (Liu et al., 2023), in their research work, explained that the binding of vitamin B12 to R-proteins forms complexes that protect it from degradation in the acidic environment. Pancreatic enzymes digest R-proteins in the small intestine, allowing vitamin B12 to bind with intrinsic factor (IF).

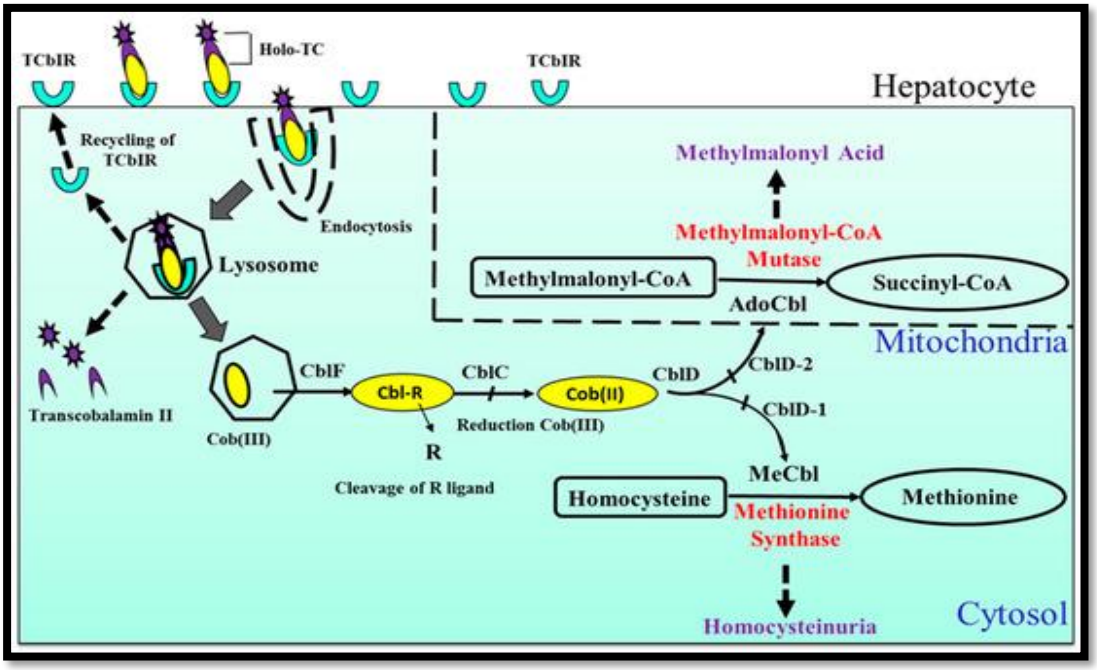


Figure 5: Metabolism of vitamin B12 (Boachie et al., 2020).

Vitamin B12, once absorbed, goes through several metabolic transformations inside cells. Vitamin B12, an essential nutrient, is converted into two active coenzymes inside cells—methylcobalamin and adenosylcobalamin. Vitamin B12 binds to transcobalamin II (TCII) in the bloodstream for transporting it to different parts of the body. (Nawaz et al., 2020; Mathew et

al., 2024), in their research work, explained that Vitamin B12 deficiency might cause neurological disorders such as peripheral neuropathy and cognitive impairment. The relationship between vitamin B12 status and eye diseases such as glaucoma is explained in the study conducted by (Liu et al., 2023; Stuart et al., 2023) and their study also highlighted the role of vitamin B12 in maintaining optic nerve health. The complex processes are involved in the absorption and metabolism of Vitamin B12 and those processes are highly essential to maintain optimal physiological function.

4.3 Cellular functions and physiological roles:

Vitamin B12 (cobalamin) is very crucial for various physiological processes that are highly essential for human health. Vitamin B12 is absolutely necessary for DNA synthesis and also has a crucial role in the production of genetic material needed for cell division (Mathew et al., 2024). Vitamin B12 is crucial for maintaining the integrity of the nervous system, as it supports the formation of the myelin sheath around nerves and this is essential for efficient nerve conduction and optimal neurological function (Mathew et al., 2024). Cobalamin, known as vitamin B12, is very crucial in energy metabolism and it helps in the breakdown of fatty acids and amino acids as well, which are essential steps in the production of ATP. (Mathew et al., 2024). B12 supports haemoglobin synthesis, ensuring adequate oxygen transport throughout the body (Mathew et al., 2024).

As a whole, vitamin B12 is very essential for DNA synthesis, nervous system function, energy metabolism, and immune modulation.

5. CLINICAL IMPLICATIONS OF VITAMIN B12 DEFICIENCY

5.1 Hematological manifestations:

Vitamin B12 (cobalamin) is essential for hematopoiesis, the process of blood cell formation, and its deficiency can result in a range of hematological manifestations. (Behere et al., 2021), in their study, highlighted that inadequate maternal B12 levels during pregnancy are associated with negative pregnancy outcomes and might adversely affect the health of offspring. The crucial role of B12 in heamtological health is thoroughly explained in their study. (Behere et al., 2021) and (Benites-Zapata et al., 2023) explained the crucial role of vitamin B12 levels in thyroid disorders, highlighting its role in maintaining thyroid function, which is crucial for erythropoiesis. (Christian et al., 2022) conducted a study on B12 and folate levels in females in Nigeria, highlighting their correlation with hematological parameters and their study is very crucial to understand clearly about the impact of Vitamin B12 deficiency. As a whole, sufficient vitamin B12 level in blood is very essential for preventing hematological complications. In this review paper, the diverse role of B12 in erythropoiesis and hematological health is highlighted well by examining recent research findings thoroughly.

5.2 Neurological complications:

A range of neurological complications i.e. from mild symptoms to severe and irreversible damage are caused by vitamin B12 deficiency. Neuropsychiatric symptoms associated with B12 deficiency are highlighted in this review paper. Early detection and treatment are crucial as these symptoms may lead to more severe neurological manifestations (Mathew et al., 2024). Optic neuropathy that occurs due to vitamin B12 deficiency can lead to optic atrophy and visual disturbances. Research done by (Medori et al., 2022), indicates that prompt B12 supplementation may help prevent or potentially reverse visual impairment associated with B12 deficiency. As a whole, vitamin B12 deficiency, if not addressed properly, might have a wide range of neurological complications. As a result, the health of human beings might get affected due to inadequate vitamin B12 levels in blood. To find out the early signs and symptoms of B12 deficiency, it is very essential for clinicians to remain vigilant in order to provide timely treatment and care.

5.3 Other health implications:

Recent studies have revealed other health effects beyond the nervous system. In this review paper, various health implications that are related to vitamin B12 status are explored very clearly. (Poole et al., 2022; Roth & Mohamadzadeh, 2021) highlighted that vitamin B12 deficiency-related changes in metabolite profiles have been observed in brain tissues and the role of B12 in cardiovascular health is highlighted in a very effective manner.

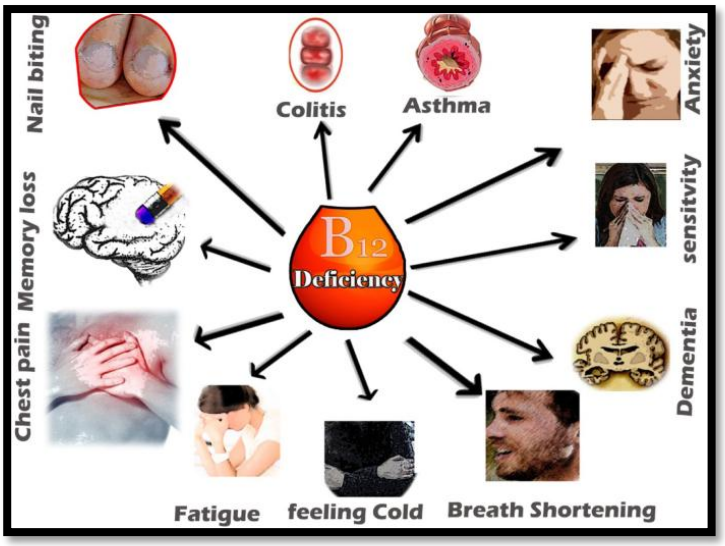


Figure 6: Deficiency of vitamin B12 (Nawaz et al., 2020).

An increased risk of gestational diabetes has also been associated with low maternal vitamin B12 levels is highlighted by (Saravanan et al., 2021) and their study also showed the importance of B12 in fetal development and metabolic health as well.

6. METHODS FOR QUANTIFYING VITAMIN B12 IN BLOOD

Vitamin B12 levels in blood should be measured accurately as it is very crucial for diagnosing deficiencies and monitoring treatment efficiency. Recent advancements in methods and technologies are explored effectively to treat vitamin B12 deficiency thereby collecting information from current literature:

6.1 Immunoassay-based Methods - Enzyme-linked immunosorbent assay (ELISA) - Radioimmunoassay (RIA) - Chemiluminescent immunoassay (CLIA):

The unique characteristics of immunoassays like sensitivity and specificity makes it important in the process of measuring B12 level. This article helps in learnings, ELISA, RIA, and CLIA, the three important immunoassay methods which offers rare advantages during clinical and research settings.

Enzyme-linked immunosorbent assay (ELISA)

ELISA is the common used method. Antibodies specific to vitamin B12 which are connected to an enzyme plays a major role in this technique.

Radioimmunoassay (RIA)

Radioisotopes are used to label vitamin B12 analogs in RIA,which helps detect antigen-antibody complexes by using radioactive decay. Highly sensitive RIA with extraordinary accuracy can detect trace amount of vitamin B12 even when there are concerns over radioactivity (Hamel & Spry, 2022).

Chemiluminescent immunoassay (CLIA)

Chemiluminescent markers are utilised by CLIA which is combined to antibodies and it emits light when antigen-antibody binds together. To find vitamin B12 in samples, the emissions are measured quantitatively (Guillerme et al., 2023). The improvement in vitamin B12 diagnostics and results of patient care is important and therefore the principles and comparative advantages of CLIA should be learned properly.

6.2 Chromatography-based Methods - High-performance liquid chromatography (HPLC) -Gas chromatography (GC) - Liquid chromatography-mass spectrometry (LC-MS):

In biological samples to precisely measure and analyse vitamin B12, chromatography-based methods are important. There are three important chromatographic methods: HPLC, GC, and LC-MS are discussed in this study, which are providing rare benefits in sensitivity, specificity and application.

High-performance liquid chromatography (HPLC)

To separate, measure and identify vitamin B12 compounds in complex mixtures a robust technique is highly used called

HPLC. The interaction of different components in the mixture with stationary phase varies which can result in their separation depending upon the molecular size, polarity and affinity (Yang et al., 2024).

Gas chromatography (GC)

To separate and check volatile compounds which includes some form of vitamin B12 as well gas is utilised as the mobile phase in GC. To interact with a stationary phase samples are injected in a column by vaporising it. Afterwards, a detector such as a mass spectrometer (MS) is used to detect separate components which further gives measured data as per the holding time of the analytes (Yang et al., 2024).

Liquid chromatography-mass spectrometry (LC-MS)

With the capabilities like detection and identification of mass spectrometry, the separating power of liquid chromatography is combined by LC-MS. To determine the molecular weights and structures vitamin B12 compounds are separated in a liquid mobile phase by a chromatographic column and then mass spectrometer ionises them (Yang et al., 2024).

6.3 Spectrophotometric Methods - UV-Visible spectrophotometry - Fluorometric methods:

The properties of sensitivity and specificity of Spectrophotometric methods, which includes UV-Visible spectrophotometry and fluorometric methods makes it very important in the measurement and analysis of vitamin B12. In clinical and research settings how these techniques and applications would work shall be learned in this article.

UV-Visible Spectrophotometry

In biological samples to analyse vitamin B12 levels UV-Visible spectrophotometry is commonly used as it is simple and accurate. In these process vitamin B12 molecules in visible solution absorbs ultraviolet or visible lights (Guillerme et al., 2023).

Fluorometric Methods

In biological samples, the fluorescent properties of vitamin B12 derivatives are studied in fluorometric methods to measure their concentration. As compared to UV-Visible spectrophotometry, vitamin B12 present in the sample is related to the intensity of fluorescence it emits which provides better sensitivity (Guillerme et al., 2023).

6.4 Electrochemical Methods - Electrochemical sensors – Voltammetry:

Electrochemical sensors and voltammetry have been a strong tool because of their properties of sensitivity, selectivity and ease of miniaturisation.

Electrochemical Sensors

Vitamin B12 is detected with the help of electrochemical sensors which consist of a transducer that converts the chemical information of the analyte into an electrical signal. In complex biological matrices with high specificity like serum and urine, these sensors can detect different forms of vitamin B12 which include cyanocobalamin and methylcobalamin (Dib et al., 2022).

Voltammetry

Voltammetry methods like cyclic as well as differential pulse voltammetry, are used in analysis of vitamin B12 because they give quick as well as precise measurements. This method gives the nature of species which are electroactive in a sample, giving the amount of Vitamin B13 through its redox behaviour. More research and new improvements in the sensors which are electrochemical will help in using these methods a lot in finding vitamin B12 deficiency, personal medicine, as well monitoring public health.

6.5 Molecular Methods - Polymerase chain reaction (PCR) - Next-generation sequencing (NGS):

Molecular methods, like PCR as well as NGS, have changed the way of Vitamin B12 research, giving tools which are helpful for detection, analysis as well as getting to know its role in health and disease.

Polymerase Chain Reaction (PCR)

PCR is very important method that increases some DNA patterns, helps in looking for and calculating the amount of vitamin B12-related genes as well as sign genes. Researchers use PCR to study genetic variations linked with vitamin B12 metabolism, like change in transcobalamin and methylenetetrahydrofolate reductase (MTHFR) genes, which affect vitamin B12 status and its downstream metabolic pathways (Abdelwahab et al., 2024).

Next-Generation Sequencing (NGS)

NGS technologies have boosted genomic studies of vitamin B12-related pathways, giving new views on molecular mechanisms underlying its physiological functions and problems which are metabolic. By sequencing all the genomes or targeted gene areas, NGS helps in overall analyses of genetic variants, epigenetic changes, and expression of gene profiles

which are with vitamin B12 metabolism (Abdelwahab et al., 2024). Also, the development of portable as well as cost-effective PCR and NGS platforms will help their widespread adoption in low resource settings, increasing access to molecular diagnostics as well as personalised vitamin B12 management worldwide (Abdelwahab et al., 2024).

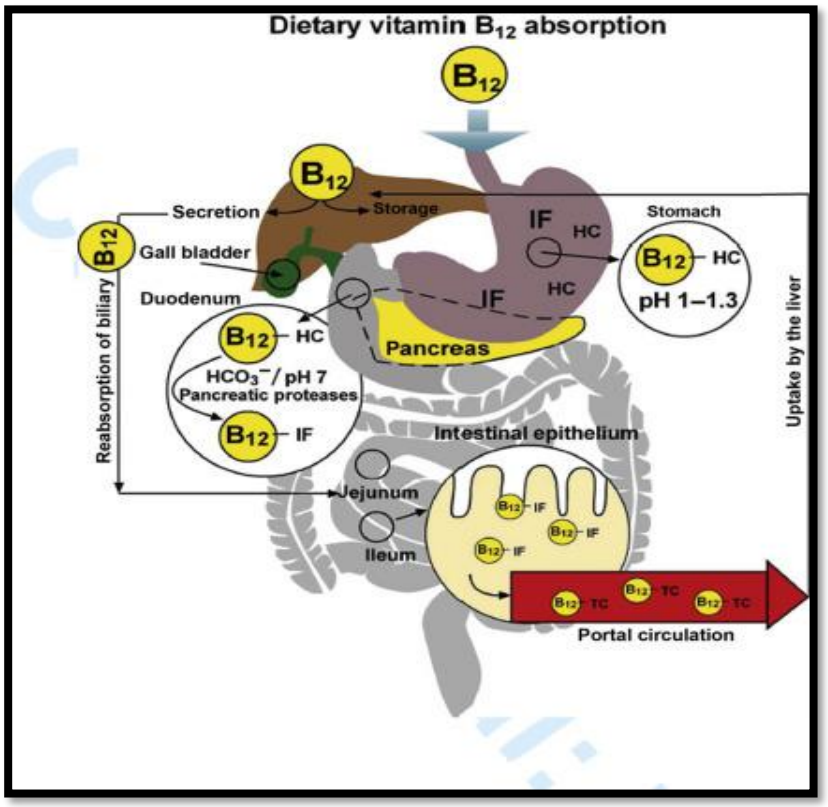


Figure 7: Methods for Quantifying Vitamin B12 in Blood (D. J. 2019).

PCR as well as NGS shows tools which cannot be disposed of in molecular biology and genetics, moving forward transformative discoveries in vitamin B12 research.

7. COMPARISON OF QUANTIFICATION METHODS

Looking carefully at vitamin B12 levels is much needed for curing deficiencies as well as checking on health outcomes. Many methods are available, each with attributes which are specific that affect their suitability for clinical and also research applications.

7.1 Sensitivity, specificity, and accuracy:

Immunoassays: Usually high sensitivity but may lack specificity due to cross-reactivity with analogues (Dib et al., 2022).

HPLC: Gives good specificity by distinguishing between B12 matches, making sure to get right quantification (Dubascoux et al., 2021).

Mass Spectrometry (MS): Gives excellent sensitivity and specificity, capable of noticing many B12 forms all at once (Ding et al., 2024).

Biomarker Combinations: e.g., CB12 improves accuracy by reflecting active B12 levels, increasing the diagnostic precision (Dib et al., 2022).

7.2 Cost-effectiveness and scalability:

Immunoassays: Cost-effective as well as scalable for daily checkups, but may require confirmatory tests (Dib et al., 2022).

HPLC and MS: Usually costs more, due to need of right equipment and technical expertise, limiting scalability in low resource settings (Dubascoux et al., 2021; Ding et al., 2024).

Point-of-Care Devices: New Technologies aims to improve cost-effectiveness as well as accessibility, though validation is going on currently (Chau et al., 2023).

7.3 Time required for analysis:

Immunoassays: Quick results within hours, good for high-throughput screenings (Dib et al., 2022).

HPLC and MS: Analysis times are long, due to sample preparation as well as chromatographic separation, varying from some minutes to hours (Dubascoux et al., 2021; Ding et al., 2024).

Point-of-Care Devices: Good for quick analysis within minutes, increasing clinical usage and patient management (Chau et al., 2023).

7.4 Sample requirements and handling considerations:

Immunoassays: Less sample volume is needed, good with many biological fluids (Dib et al., 2022).

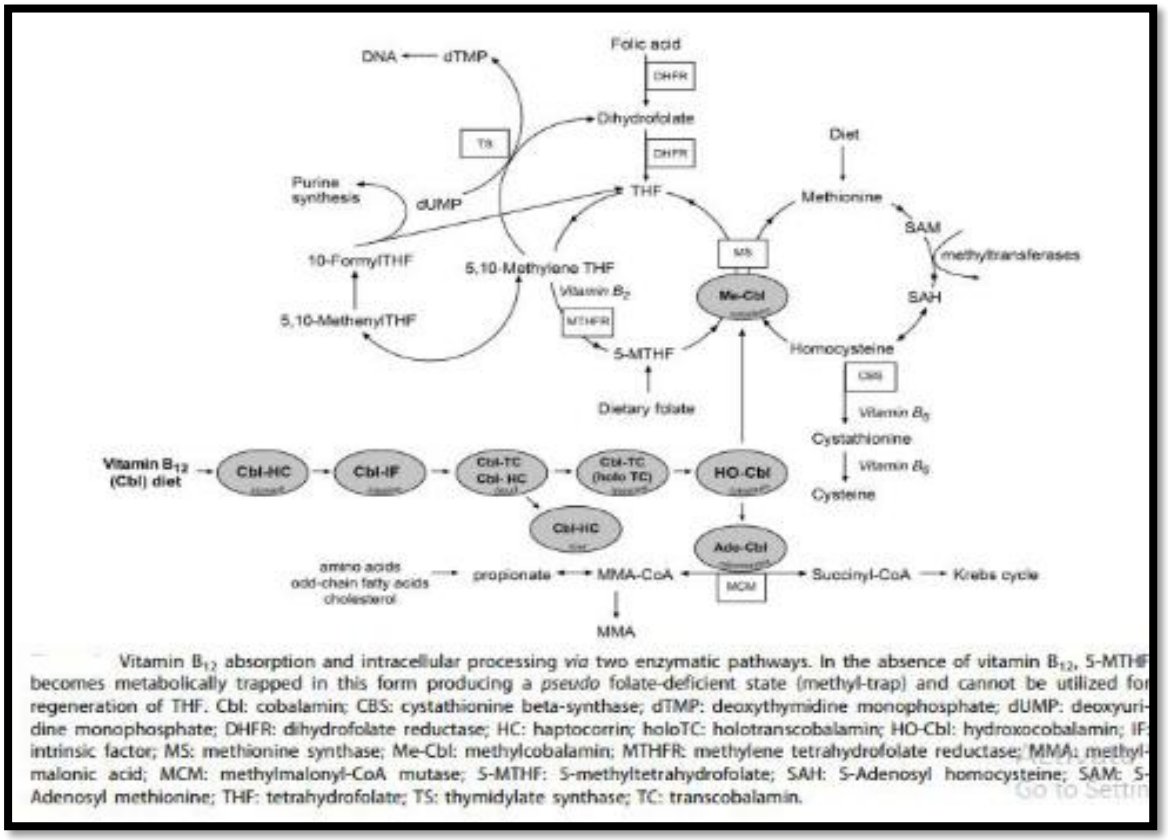
HPLC and MS: Require lots of sample volumes and rigorous sample preparation to get accurate results, limiting applicability in some cases (Dubascoux et al., 2021; Ding et al., 2024).

Point-of-Care Devices: Handles sample handling challenges looks for simple methods and reduced sample volumes (Chau et al., 2023).

8. RESULTS

8.1 Overview of vitamin B12:

New progress in nanotechnology has given new ways to deliver as well as measure vitamin B12. Improvements in the presence as well as stability of vitamin B12 by using nanoparticles are shown by this study, which could highly improve its needs in therapies. Vitamin B12 is also very important during pregnancy as well as lactation. Proper levels of this vitamin are much needed for foetal development and also maternal health. Noticing early as well as prevention of vitamin B12 deficiency will stay as a global health priority. Improvements in diagnostic ways have bought a good change in the accuracy of vitamin B12 deficiency checking. (Hamel and Spry, 2022) checked on the effectiveness of vitamin B12 testing in people, who might have its deficiency. The study found that both vitamin B12 deficiency as well as lots of folic acid intake is linked with an increased risk of GDM, showing the need for correct intake of these nutrients during pregnancy to reduce health risks. **(**Hou et al., 2024) checked on the relationship between dietary intake of B vitamins, along with vitamin B12, and glaucoma.



Vitamin B_{12} absorption and intracellular processing *via* two enzymatic pathways. In the absence of vitamin B_{12}, 5-MTHF becomes metabolically trapped in this form producing a *pseudo* folate-deficient state (methyl-trap) and cannot be utilized for regeneration of THF. Cbl: cobalamin; CBS: cystathionine beta-synthase; dTMP: deoxythymidine monophosphate; dUMP: deoxyuridine monophosphate; DHFR: dihydrofolate reductase; HC: haptocorrin; holoTC: holotranscobalamin; HO-Cbl: hydroxocobalamin; IF: intrinsic factor; MS: methionine synthase; Me-Cbl: methylcobalamin; MTHFR: methylene tetrahydrofolate reductase; MMA: methylmalonic acid; MCM: methylmalonyl-CoA mutase; 5-MTHF: 5-methyltetrahydrofolate; SAH: S-Adenosyl homocysteine; SAM: S-Adenosyl methionine; THF: tetrahydrofolate; TS: thymidylate synthase; TC: transcobalamin.

Figure 8: Vitamin B12 absorption and intracellular processing (Sobczyńska-Malefora et al.,).

In short, one of the much needed nutrients along with various roles in human health is Vitamin B12. From improving diagnostic methods to increasing therapeutic delivery methods as well as getting to know more about its health effects, research will continue to get to know the need of maintaining right levels of vitamin B12 for well-being.

8.2 Analysis of quantification vitamin B12 in blood:

Checking vitamin B12 levels in blood is much needed for handling deficiencies and to give right treatment. New ways of checking methods have improved the level as well as trust of using these kinds of measurements. (Lacombe et al., 2021) found that continuously elevated plasma vitamin B12 levels are strongly linked with solid cancer. 2 different studies have shown the link between vitamin B12 levels as well as glaucoma. (Lee et al., 2024) exposed a possible connection between folate which is elevated and the risk of developing glaucoma. (Liampas et al., 2020) made a study on folate, pyridoxine, serum homocysteine, as well as vitamin B12 levels in patients who have migraines. (Nawaz et al., 2020) studies vitamin B12 deficiency as well as neurological disorders. This shows the need for early detection as well as right treatment for vitamin B12 deficiency to manage neurological damage in the long-term.

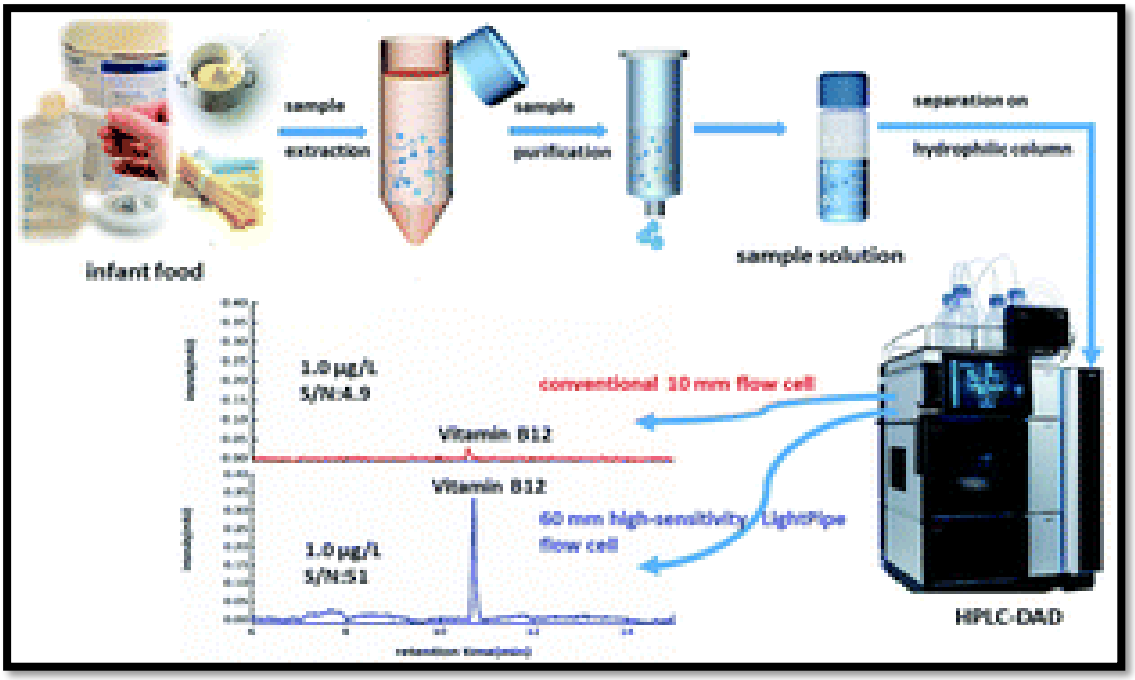


Figure 9: Vitamin B12 quantification in infant food (Fan et al., 2021).

In short, new developments in checking the amount of vitamin B12 in blood have helped the ability to cure as well understand vitamin B12 implications status in a much better way.

9. DISCUSSIONS

9.1 Review on quantification vitamin B12 in blood:

Precise digits are necessary for the essential nutrients such as vitamin B12 in blood for differentiating the normal patient with any one with low levels of the micronutrient. This paper deals with the new techniques that have been found to count the micronutrient with the reference given by (Guillerme et al., 2023; Hamel and Spry, 2022). With the vitamin B12 insufficiency in the body of the patients, (Hamel and Spry, 2022) have discussed the available testing of the micronutrient; the paper was purely about the methods and methodologies used to identify the disease and its treatment. (He et al., 2022) highlighted the relation between the micronutrient and the diabetes mellitus in women during pregnancy with meta analysis.

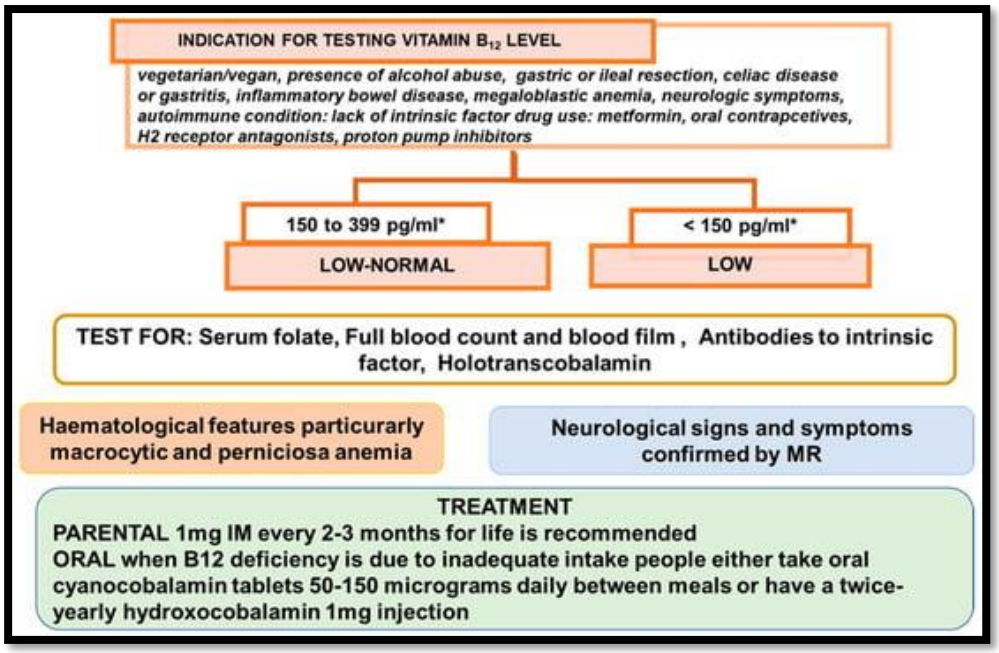


Figure 10: Brief review on vitamin B12 deficiency (Azzini et al., 2021).

In final, the Importance of error less measurement of the vitamin B12 in the body is more to understand the body. All the previous studies have only talked about the absolute values and it's methods involved for the absorption of the vitamin B12 thereby improving the health of the patient.

9.2 Challenges and Limitations:

To find the accurate values of the micronutrient in the body, the process is very difficult to cross and has many restraints. The 2 major problems are: intervention with the similar by-products and metabolites, framework structures in samples.

9.2.1 Interference from analogs and metabolites:

The main area of concern is the interaction of the similar byproducts of vitamin B12 and metabolites with vitamin B12 and the difficulty in error less measurement. The are many shapes of vitamin B12 similar looking complexes which are active in the body and thereby used as supplements instead of direct vitamin B12. (Boachie et al, 2020) discussed about the failure of the conventional methods that used for the identification of the vitamin B12 causing misinterpretation. Like an example, overinterpretation of the vitamin B12 is possible if any similar looking complex interacts with B12. This causes total difficulty in the quantification. (Dastidar and Sikder, 2022) highlighted the presence and utilization of another type of B12 which acts as marker for b12 insufficiency. The complex only shows a small part fir the micronutrient to attach and interpret.

9.2.2 Matrix effects in biological sample:

The complex present in the sample except the main component has a major setback in the measurement of vitamin B12. The body fluids has many complex mixture of components which can intervene in the process and give misinterpretation of the results while estimation. (Boachie et al., 2020) noticed and documented that fat dissolution can affect vitamin B12 in the body. Fats and other complex mixture present can interfere thereby giving false results. Methods as pre-treatment and various methods are used for the corrections. Fluorescent detection methods are also used which was identified by (Chau et al., 2023). For the accurate measurement and dependable results, the problems and setbacks faced from the analysis need to be addressed.

9.2.3 Standardization and calibration issues:

The similarity and the measurement of the vitamin B12 is another major limitation as the laboratories have to give the results the same without any error. The standardisation is difficult because of:

There are different systematic techniques as follows: immunoassays, HPLC, LC-MS/MS and LC. The calibration and standardisation of each technique is difficult and different.

Due to the no standard measurements already present it's difficult to calibrate the micronutrient. In blood, there are many components which can intervene with the main reactant and forming products can vary from each individual and resulting in further problems in standardisation. (Flieger et al., 2024) explained about the enhancement of the complex structure

cyanocobalamin(a form of micronutrient in the blood) can adsorption on the iron oxide nanoparticles complex particle to help in detecting the vitamin B12.

9.2.4 Variability in results across methods:

Various assays as well as analysis methods identify vitamin B12 using many ways, leading to different results. For example, immunoassays and vitamin B12 might cross-react, whereas LC-MS/MS can tell apart different forms of the vitamins. The absence of a standard process for evaluation across labs can result in measurements which are not consistent. Labs may use various evaluation methods which will result in different values of vitamin B12 levels.

Methods like LC-MS/MS will give more precision but are not much available because of their complex nature as well as cost. (Hamel and Spry, 2022) made a discussion on the indication of using various vitamin B12 testing methods. They strongly showed the need for people who offer healthcare to be aware of limits as well as differences which come with different assays to make timely decisions about treatment. In short, the standard process for evaluation, along with changes in results across different methods, will be a challenge to measure vitamin B12 properly. Handling these problems will need efforts in creating ways to get more standardised as well as reliable assays, improve process of evaluation, and increase the specificity and sensitivity of available analytical techniques.

10. CLINICAL APPLICATIONS AND INTERPRETATIONS

The clinical uses of vitamin B12 testing are, diagnosis of deficiency, keeping track of efficiency of treatment, and studies that are population-based. Understanding as well as addressing vitamin B12 levels is much needed because of their impacts on health.

10.1 Diagnosis of Vitamin B12 deficiency:

Diagnosing lack of vitamin B12 is an important step in avoiding and managing many health problems like, neurological as well as haematological disorders.

Many methods are used in the process of diagnosis:

1. **Serum Vitamin B12 Levels**: The most common initial test measures total serum vitamin B12. But, this method may not correctly show active B12 levels or deficiency states.

2. **Holotranscobalamin (Active B12):** This test measures the form of vitamin B12 that is biologically active, giving a correct indication of deficiency. (Dastidar and Sikder, 2022) showed the need of serum active B12 (holo-transcobalamin) in properly evaluating vitamin B12 deficiency, noting its need in current diagnostic methods.

3. **Methylmalonic Acid (MMA) and Homocysteine:** Increased levels of these metabolites can show a functional deficiency of vitamin B12, even when serum B12 levels look normal.

10.2 Monitoring treatment efficiency:

Once lack of vitamin B1 is diagnosed, keeping track of the effectiveness of treatment is much needed to get the desired outcome. Treatment involves oral or intramuscular vitamin B12 supplementation, depending on the severity of the deficiency as well as underlying absorption issues. Monitoring includes:

Regular measurement of serum vitamin B12, MMA, as well as levels of homocysteine helps in knowing the response to treatment and adjustments of guides in therapy. Improvement in clinical symptoms like anaemia, neurological function, as well as complete well-being are important indicators of treatment efficacy. (Benites-Zapata et al., 2023) carried out a review and meta-analysis on vitamin B12 levels in disorders related to thyroid.

10.3 Population-based studies and epidemiological research:

Studies that is population-based as well as research gives good insights on the commonness, causes, and after effects of vitamin B12 deficiency in various populations. The view on public health strategies as well as policy decisions aimed at improving nutritional value and avoiding deficiency-related health issues are given by these studies. (Chandyo et al., 2023) made a trial in Nepal. More research as well as new ways of curing is thoroughly needed to take care of global health effects of vitamin B12 deficiency and improve patient care and also interventions of public health as well.

11. CONCLUSION

The overall process of review shows very important ongoing condition of the research about measurement of vitamin B12 in blood, showing the need of techniques for measuring it with accuracy because of the main role of vitamin B12 in much physiological process as well. The closer look at the methods that we have today, from traditional assays to more developed chromatographic and mass spectrometric methods, shows the overall progress made so far in increasing factors like sensitivity, condition of being specific and overall reliability. New developments in tech have become good in the ways to check the levels of vitamin B12 with much better accuracy. New techniques have set new standards in performance analytics,

making the detection of vitamin B12 at much lower concentrations and with higher accuracy. Anyhow, even with these developments in tech, the difficulties with standardising these methods stays across various labs, making sure about the consistency of control measures. Changes in results of assays due to differences in preparation of sample, calibration of instrument, and gauging the data can cause variations in measurements of vitamin B12, mainly affecting the process of making decisions that are clinical. The combination of multi-omics ways shows a good path for a detailed assessment of the status of vitamin B12, this will help to understand better as well as handle related health conditions.

11.1 Future perspectives and Recommendations:

11.1.2 Standardization efforts and quality control measures:

Standardization and quality measures should be intact in research and clinical practice to ensure consistency and reliability in vitamin B12. B12 testing is done by harmonization of diagnostic criteria and the development of standardized protocols. The accuracy in diagnosing B12 deficiency can be achieved by evaluating new assays' diagnostic performance such as the Roche Elecsys® active vitamin B12 test, against traditional total vitamin B12 screening (Guillerme et al., 2023). It is important to regularise vitamin B12 supplementation guidelines for which the efforts are taking place. Studies like the B-PROOF trial explains the prolonged effects of supplementation and helps to prevent fracture risk and cardiovascular disease by improving the dosage (Stricker et al., , 2021)

11.1.3 Integration of multi-omics approaches for comprehensive assessment:

A whole understanding of the role of vitamin B12 in health and disease has been received and the detailed study by including multi-omics ways, involving genomics, transcriptomics, proteomics, and metabolomics. The important disclosure of molecular mechanisms fundamental vitamin B12's effect on cellular function and disease processes has been achieved by the inclusion of integrative transcriptomic and proteomic analyses (Feng et al., 2023).The diagnosing and treating B12-related disorders can be approached in-whole by combining multi-omics data which helps explain complex interactions between vitamin B12 and different other biological pathways. As per recent research, the wider implications of vitamin B12 status in conditions like metabolic syndrome is relevant and learned by the thorough assessment (Ashok et al., 2021).A serious understanding of vitamin B12's role in human health has been understood by accepting the latest technologies, standardization efforts, and multi-omics approaches which can help the field to grow and it paves the path for better diagnostic tools and treatment strategies.

REFERENCES

[1] Abdelwahab, O. A., Abdelaziz, A., Diab, S., Khazragy, A., Elboraay, T., Fayad, T., Diab, R. A., &Negida, A. (2024). Efficacy of different routes of vitamin B12 supplementation for the treatment of patients with vitamin B12 deficiency: A systematic review and network meta-analysis. Irish Journal of Medical Science. https://doi.org/10.1007/s11845-023-03602-4

[2] Adaikalakoteswari, A., Wood, C., Mina, T. H., Webster, C., Goljan, I., Weldeselassie, Y., Reynolds, R. M., &Saravanan, P. (2020). Vitamin B12 deficiency and altered one-carbon metabolites in early pregnancy is associated with maternal obesity and dyslipidaemia. Scientific Reports, 10(1). https://doi.org/10.1038/s41598-020-68344-0

[3] Araghi, S. O., Jong, J. C. K., Van Dijk, S. C., Swart, K. M., Ploegmakers, K. J., Zillikens, M. C., Van Schoor, N. M., De Groot, L. C., Lips, P., Stricker, B. H., Uitterlinden, A. G., & Van Der Velde, N. (2021). Long-term effects of folic acid and vitamin-B12 supplementation on fracture risk and cardiovascular disease: Extended follow-up of the B-PROOF trial. Clinical Nutrition, 40(3), 1199–1206. https://doi.org/10.1016/j.clnu.2020.07.033

[4] Arnold, T., & Johnston, C. S. (2023). An examination of relationships between vitamin B12 status and functional measures of peripheral neuropathy in young adult vegetarians. Frontiers in Nutrition, 10. https://doi.org/10.3389/fnut.2023.1304134

[5] Ashok, T., Puttam, H., Tarnate, V. C. A., Jhaveri, S., Avanthika, C., Treviño, A. G. T., Sl, S., & Ahmed, N. T. (2021). Role of vitamin B12 and folate in metabolic syndrome. Curēus. https://doi.org/10.7759/cureus.18521

[6] Azzini, E., Raguzzini, A., & Polito, A. (2021). A brief review on vitamin B12 deficiency looking at some case study reports in adults. *International Journal of Molecular Sciences*, *22*(18), 9694. https://doi.org/10.3390/ijms22189694

[7] Barney, A., Abraham, V., Danda, S., Cherian, A., &Vanitha, S. (2020). Prevalence of Vitamin B12 deficiency and its associated risk factors among pregnant women of rural South India: A community-based cross-sectional study. Indian Journal of Community Medicine/Indian Journal of Community Medicine, 45(4), 399. https://doi.org/10.4103/ijcm.ijcm_403_19

[8] Basalamah, M. A., Ibrahim, M. O., Qutob, M. S., Jazar, A. S., Bakr, E. H., Alazzeh, A. Y., Al-Slaihat, A. H.,

&Azzeh, F. S. (2023). Vitamin B12 status among asymptomatic young adult females and its association with some anthropometric and biochemical parameters: A cross-sectional study from Makkah (cobalamin deficiency in young adult females). Medicine, 102(44), e35838. https://doi.org/10.1097/md.0000000000035838

[9] Behere, R. V., Deshmukh, A. S., Otiv, S., Gupte, M. D., &Yajnik, C. S. (2021). Maternal Vitamin B12 status during pregnancy and its association with outcomes of pregnancy and health of the offspring: A Systematic Review and implications for Policy in India. Frontiers in Endocrinology, 12. https://doi.org/10.3389/fendo.2021.619176

[10] Benites-Zapata, V. A., Ignacio-Cconchoy, F. L., Ulloque-Badaracco, J. R., Hernandez-Bustamante, E. A., Alarcón-Braga, E. A., Al-Kassab-Córdova, A., & Herrera-Añazco, P. (2023). Vitamin B12 levels in thyroid disorders: A systematic review and meta-analysis. Frontiers in Endocrinology, 14. https://doi.org/10.3389/fendo.2023.1070592

[11] Boachie, J., Adaikalakoteswari, A., Samavat, J., & Saravanan, P. (2020). Low Vitamin B12 and Lipid Metabolism: Evidence from Pre-Clinical and Clinical Studies. *Nutrients*, *12*(7), 1925. https://doi.org/10.3390/nu12071925

[12] Campos, A. J., Risch, L., Nydegger, U., Wiesner, J., Van Dyck, M. V., Renz, H., Stanga, Z., & Risch, M. (2020). Diagnostic accuracy of holotranscobalamin, vitamin B12, methylmalonic acid, and homocysteine in detecting B12 deficiency in a large, mixed patient population. *Disease Markers*, *2020*, 1–11. https://doi.org/10.1155/2020/7468506

[13] Chandramahanti, K., Chiratanagandla, A., Ammula, S., &Kommineni, B. (2023). Role of Vitamin B12 and folate in metabolic syndrome – Current trends and future perspectives. BLDE University Journal of Health Sciences/BLDE University Journal of Health Sciences, 8(1), 200. https://doi.org/10.4103/bjhs.bjhs_71_22

[14] Chandyo, R. K., Kvestad, I., Ulak, M., Ranjitkar, S., Hysing, M., Shrestha, M., Schwinger, C., McCann, A., Ueland, P. M., Basnet, S., Shrestha, L., & Strand, T. A. (2023). The effect of vitamin B12 supplementation during pregnancy on infant growth and development in Nepal: a community-based, double-blind, randomised, placebo-controlled trial. Lancet, 401(10387), 1508–1517. https://doi.org/10.1016/s0140-6736(23)00346-x

[15] Chau, P. B. K., Vu, T. H., & Kim, M. I. (2023). Highly efficient fluorescent detection of vitamin B12 based on the inner filter effect of Dithiol-Functionalized silver nanoparticles. Nanomaterials, 13(17), 2444. https://doi.org/10.3390/nano13172444

[16] Christian, S. G., Elekima, I., Moore-Igwe, B., Mac-Aworu, I., &Ariolu, B. C. (2022). Assessment of Vitamin B12, Folate Levels and some Haematological Parameters among Females on De-Deon syrup in Port Harcourt, Nigeria. African Journal of Laboratory Haemotology and Transfusion Science, 1(1), 39–53. https://doi.org/10.59708/ajlhts.v1i1.2206

[17] D'souza, N., Behere, R. V., Patni, B., Deshpande, M., Bhat, D., Bhalerao, A., Sonawane, S., Shah, R., Ladkat, R., Yajnik, P., Bandyopadhyay, S. K., Kumaran, K., Fall, C., &Yajnik, C. S. (2021). Pre-conceptional maternal vitamin B12 supplementation improves offspring neurodevelopment at 2 years of age: PRIYA trial. Frontiers in Pediatrics, 9. https://doi.org/10.3389/fped.2021.755977

[18] Da Silva, T. R. (2024). Can supplementing vitamin B12 improve mental health outcomes?: a literature review. British Journal of Community Nursing, 29(3), 137–146. https://doi.org/10.12968/bjcn.2024.29.3.137

[19] Dastidar, R., &Sikder, K. (2022). Diagnostic reliability of serum active B12 (holo-transcobalamin) in true evaluation of vitamin B12 deficiency: Relevance in current perspective. BMC Research Notes, 15(1). https://doi.org/10.1186/s13104-022-06224-8

[20] Dib, M., Gumban-Marasigan, M., Yoxall, R., Andrew, T., Harrington, D. J., Sobczyńska-Malefora, A., & Ahmadi, K. R. (2022). Evaluating the diagnostic value of a combined indicator of vitamin B12 status (CB12) throughout pregnancy. Frontiers in Nutrition, 8. https://doi.org/10.3389/fnut.2021.789357

[21] Ding, L., Wang, J., Qiu, S., Ren, Z., Li, Y., & An, P. (2024). Bioinformatics approach to identify the pathogenetic link of gut Microbiota-Derived Short-Chain fatty acids and ischemic stroke. Molecular Neurobiology. https://doi.org/10.1007/s12035-024-04176-7

[22] Dubascoux, S., Payot, J. R., Sylvain, P., Nicolas, M., &Gimenez, E. C. (2021). Vitamin B12 quantification in human milk – Beyond current limitations using liquid chromatography and inductively coupled plasma – Mass spectrometry. Food Chemistry, 362, 130197. https://doi.org/10.1016/j.foodchem.2021.130197

[23] Fan, D., Zhang, Y., & Wu, H. (2021). Development of a simple and sensitive HPLC-DAD method for quantification of vitamin B12 fortified in infant food. *Analytical Methods*, *13*(41), 4920–4925. https://doi.org/10.1039/d1ay01118a

[24] Fang, X., Mo, J., Zhou, H., Shen, X., Xie, Y., Xu, J., & Yang, S. (2023). Comparative transcriptome analysis

of gene responses of salt-tolerant and salt-sensitive rice cultivars to salt stress. Scientific Reports, 13(1). https://doi.org/10.1038/s41598-023-46389-1

[25] Fedosov, S. N., &Nexo, E. (2024). Macro-B12 and unexpectedly high levels of plasma B12: A critical review. Nutrients, 16(5), 648. https://doi.org/10.3390/nu16050648

[26] Feng, L., Li, Y., Zhou, Y., Meng, G., Ji, Z., Lin, W., & He, J. (2023). Integrative transcriptomic and proteomic analyses reveal a positive role of BES1 in salt tolerance in Arabidopsis. Frontiers in Plant Science, 14. https://doi.org/10.3389/fpls.2023.1034393

[27] Finkelstein, J. L., Fothergill, A., Krisher, J. T., Thomas, T., Kurpad, A. V., &Dwarkanath, P. (2021). Maternal vitamin B12 deficiency and perinatal outcomes in southern India. PloS One, 16(4), e0248145. https://doi.org/10.1371/journal.pone.0248145

[28] Flieger, J., Żuk, N., Pasieczna-Patkowska, S., Flieger, M., Panek, R., Klepka, T., &Franus, W. (2024). Optimization of Cyanocobalamin (Vitamin B12) Sorption onto Mesoporous Superparamagnetic Iron Oxide Nanoparticles. Molecules/Molecules Online/Molecules Annual, 29(9), 2094. https://doi.org/10.3390/molecules29092094

[29] Godfrey, K. M., Titcombe, P., El-Heis, S., Albert, B. B., Tham, E. H., Barton, S. J., Kenealy, T., Chong, M. F., Nield, H., Chong, Y. S., Chan, S., &Cutfield, W. S. (2023). Maternal B-vitamin and vitamin D status before, during, and after pregnancy and the influence of supplementation preconception and during pregnancy: Prespecified secondary analysis of the NiPPeR double-blind randomized controlled trial. PLoS Medicine, 20(12), e1004260. https://doi.org/10.1371/journal.pmed.1004260

[30] Gramer, G., & Hoffmann, G. F. (2020). Vitamin B12 Deficiency in Newborns and their Mothers—Novel Approaches to Early Detection, Treatment and Prevention of a Global Health Issue. Current Medical Science, 40(5), 801–809. https://doi.org/10.1007/s11596-020-2260-7

[31] Guillerme, J., Feugray, G., Girot, H., Brunel, V., &Muraine, M. Q. (2023). Preliminary evaluation of the diagnostic performance of Roche Elecsys® active vitamin B12 versus total vitamin B12 for vitamin B12 deficiency screening. Annals of Clinical Biochemistry. https://doi.org/10.1177/00045632231194157

[32] Hamel, C., & Spry, C. (2022). Vitamin B12 testing in people with suspected vitamin B12 deficiency. Canadian Journal of Health Technologies, 2(3). https://doi.org/10.51731/cjht.2022.291

[33] Harrington, D. J. (2019). Methods for assessment of Vitamin B12. In *Elsevier eBooks* (pp. 265–299). https://doi.org/10.1016/b978-0-12-813050-6.00012-7

[34] He, J., Jiang, D., Cui, X., & Ji, C. (2022). Vitamin B12 status and folic acid/vitamin B12 related to the risk of gestational diabetes mellitus in pregnancy: a systematic review and meta-analysis of observational studies. BMC Pregnancy and Childbirth, 22(1). https://doi.org/10.1186/s12884-022-04911-9

[35] He, T., Jin, X., Koh, Y. S., Zhang, Q., Zhang, C., & Liu, F. (2021). The association of homocysteine, folate, vitamin B12, and vitamin B6 with fracture incidence in older adults: a systematic review and meta-analysis. Annals of Translational Medicine, 9(14), 1143. https://doi.org/10.21037/atm-21-2514

[36] Hou, J., Wen, Y., Gao, S., Jiang, Z., & Tao, L. (2024). Association of dietary intake of B vitamins with glaucoma. Scientific Reports, 14(1). https://doi.org/10.1038/s41598-024-58526-5

[37] Jiang, X., Wang, Y., & Liu, J. (2022). Simultaneous determination of four cobalamins in rat plasma using online solid phase extraction coupled to high performance liquid chromatography-tandem mass spectrometry: Application to pentylenetetrazole-induced seizures in Sprague-Dawley rats. PloS One, 17(6), e0269645. https://doi.org/10.1371/journal.pone.0269645

[38] Kim, J., Aschard, H., Kang, J. H., Lentjes, M. A., Do, R., Wiggs, J. L., Khawaja, A. P., & Pasquale, L. R. (2021). Intraocular pressure, glaucoma, and dietary caffeine consumption. Ophthalmology, 128(6), 866–876. https://doi.org/10.1016/j.ophtha.2020.12.009

[39] Krzywański, J., Mikulski, T., Pokrywka, A., Młyńczak, M., Krysztofiak, H., Frączek, B., & Ziemba, A. (2020). Vitamin B12 status and optimal range for hemoglobin formation in elite athletes. *Nutrients*, *12*(4), 1038. https://doi.org/10.3390/nu12041038

[40] Lacombe, V., Chabrun, F., Lacout, C., Ghali, A., Capitain, O., Patsouris, A., Lavigne, C., &Urbanski, G. (2021). Persistent elevation of plasma vitamin B12 is strongly associated with solid cancer. Scientific Reports, 11(1). https://doi.org/10.1038/s41598-021-92945-y

[41] Lee, J. Y., Choi, J. A., Park, S. P., &Jee, D. (2024). Association between high blood folate levels and glaucoma in a representative Korean population. Investigative Ophthalmology & Visual Science, 65(1), 6. https://doi.org/10.1167/iovs.65.1.6

[42] Liampas, I., Siokas, V., Mentis, A. A., Aloizou, A., Dastamani, M., Tsouris, Z., Aslanidou, P., Brotis, A., &Dardiotis, E. (2020). Serum homocysteine, pyridoxine, folate, and vitamin B12 Levels in migraine: Systematic Review and Meta-Analysis. Headache, 60(8), 1508–1534. https://doi.org/10.1111/head.13892

[43] Liu, Z., Hu, Y., Wang, Y., Xu, B., Zhao, J., & Yu, Z. (2023). Relationship between high dose intake of vitamin B12 and glaucoma: Evidence from NHANES 2005–2008 among United States adults. Frontiers in Nutrition, 10. https://doi.org/10.3389/fnut.2023.1130032

[44] Lv, J., Zhang, L., Kong, X., Zhao, Y., Li, X., Wang, J., Yang, X., Cheng, Z., Li, W., Wang, X., & Yang, C. (2024). Association between vitamin B2 intake and prostate-specific antigen in American men: 2003–2010 National Health and Nutrition Examination Survey. BMC Public Health, 24(1). https://doi.org/10.1186/s12889-024-18582-y

[45] Madjedi, K. M., Stuart, K. V., Chua, S. Y., Ramulu, P. Y., Warwick, A., Luben, R. N., Sun, Z., Chia, M. A., Aschard, H., Wiggs, J. L., Kang, J. H., Pasquale, L. R., Foster, P. J., & Khawaja, A. P. (2023). The Association of Physical Activity with Glaucoma and Related Traits in the UK Biobank. Ophthalmology, 130(10), 1024–1036. https://doi.org/10.1016/j.ophtha.2023.06.009

[46] Markun, S., Gravestock, I., Jäger, L., Rosemann, T., Pichierri, G., &Burgstaller, J. M. (2021). Effects of vitamin B12 supplementation on cognitive function, depressive symptoms, and fatigue: A Systematic Review, Meta-Analysis, and Meta-Regression. Nutrients, 13(3), 923. https://doi.org/10.3390/nu13030923

[47] Martami, F., & Holton, K. F. (2023). Targeting Glutamate Neurotoxicity through Dietary Manipulation: Potential Treatment for Migraine. Nutrients, 15(18), 3952. https://doi.org/10.3390/nu15183952

[48] Mathew, A. R., Di Matteo, G., La Rosa, P., Barbati, S. A., Mannina, L., Moreno, S., Tata, A. M., Cavallucci, V., &Fidaleo, M. (2024). Vitamin B12 Deficiency and the Nervous System: Beyond Metabolic Decompensation—Comparing Biological Models and Gaining New Insights into Molecular and Cellular Mechanisms. International Journal of Molecular Sciences, 25(1), 590. https://doi.org/10.3390/ijms25010590

[49] Medori, M. C., Naureen, Z., Dhuli, K., Placidi, G., Falsini, B., &Bertelli, M. (2022). Dietary supplements in retinal diseases, glaucoma, and other ocular conditions. PubMed, 63(2 Suppl 3), E189–E199. https://doi.org/10.15167/2421-4248/jpmh2022.63.2s3.2760

[50] Nawaz, A., Khattak, N. N., Khan, M. S., Nangyal, H., Sabri, S., &Shakir, M. (2020). Deficiency of vitamin B12 and its relation with neurological disorders: a critical review. Journal of Basic & Applied Zoology /Journal of Basic & Applied Zoology, 81(1). https://doi.org/10.1186/s41936-020-00148-0

[51] Niklewicz, A., Smith, A. D., Smith, A., Holzer, A., Klein, A., McCaddon, A., Molloy, A. M., Wolffenbuttel, B. H. R., Nexo, E., McNulty, H., Refsum, H., Gueant, J., Dib, M., Ward, M., Murphy, M., Green, R., Ahmadi, K. R., Hannibal, L., Warren, M. J., & Owen, P. J. (2022). The importance of vitamin B12 for individuals choosing plant-based diets. *European Journal of Nutrition*, *62*(3), 1551–1559. https://doi.org/10.1007/s00394-022-03025-4

[52] Obeid, R., Andrès, E., Češka, R., Hooshmand, B., Guéant-Rodriguez, R., Prada, G. I., Sławek, J., Traykov, L., Van, B. T., Várkonyi, T., &Reiners, K. (2024). Diagnosis, Treatment and Long-Term Management of Vitamin B12 Deficiency in Adults: A Delphi Expert Consensus. Journal of Clinical Medicine, 13(8), 2176. https://doi.org/10.3390/jcm13082176

[53] Özek, S. Ü. (2022). A study on the correlation between pain frequency and severity and vitamin B12 levels in episodic and chronic migraine. Arquivos De Neuro-Psiquiatria, 80(6), 586–592. https://doi.org/10.1590/0004-282x-anp-2021-0192

[54] Palchetti, C. Z., Steluti, J., Sales, C. H., Fisberg, R. M., &Marchioni, D. M. L. (2022). Folate and vitamin B12 status: temporal evaluation after mandatory fortification in Brazil. European Journal of Clinical Nutrition, 76(9), 1266–1272. https://doi.org/10.1038/s41430-022-01096-4

[55] Poole, J., Jasbi, P., Pascual, A. S., North, S., Kwatra, N., Weissig, V., Gu, H., Bottiglieri, T., &Jadavji, N. M. (2022). Ischemic stroke and dietary vitamin B12 deficiency in Old-Aged females: impaired motor function, increased ischemic damage size, and changed metabolite profiles in brain and cecum tissue. Nutrients, 14(14), 2960. https://doi.org/10.3390/nu14142960

[56] Rakić, M., Lunić, T., Bekić, M., Tomić, S., Mitić, K., Graovac, S., Božić, B., &Nedeljković, B. B. (2023). Vitamin B complex suppresses neuroinflammation in activated microglia: in vitro and in silico approach combined with dynamical modeling. International Immunopharmacology, 121, 110525. https://doi.org/10.1016/j.intimp.2023.110525

[57] Roth, W., &Mohamadzadeh, M. (2021). Vitamin B12 and gut-brain homeostasis in the pathophysiology of ischemic stroke. EBioMedicine, 73, 103676. https://doi.org/10.1016/j.ebiom.2021.103676

[58] Sadeghvand, S., Barzegar, M., Shiva, S., Tarmahi, V., Hamed, H., Khamaneh, E. R., Golchinfar, Z., &Raeisi, S. (2023). The effects of Vitamin B-Complex supplementation on serum homocysteine levels and migraine severity in children A randomized controlled trial. PubMed, 17(3), 143–155. https://doi.org/10.22037/ijcn.v17i3.40053

[59] Saravanan, P., Sukumar, N., Adaikalakoteswari, A., Goljan, I., Venkataraman, H., Gopinath, A., Bagias, C., Yajnik, C. S., Stallard, N., Ghebremichael-Weldeselassie, Y., & Fall, C. H. D. (2021). Association of maternal vitamin B12 and folate levels in early pregnancy with gestational diabetes: a prospective UK cohort study (PRiDE study). Diabetologia, 64(10), 2170–2182. https://doi.org/10.1007/s00125-021-05510-7

[60] Shukla, S., &Shrivastava, D. (2023). Unraveling the link between serum homocysteine levels and nutrient deficiency in subfertility: A comprehensive review. Curēus. https://doi.org/10.7759/cureus.49296

[61] Sobczyńska-Malefora, A., Delvin, E., McCaddon, A., Ahmadi, K. R., & Harrington, D. J. (2021). Vitamin B12status in health and disease: a critical review. Diagnosis of deficiency and insufficiency – clinical and laboratory pitfalls. *Critical Reviews in Clinical Laboratory Sciences*, *58*(6), 399–429. https://doi.org/10.1080/10408363.2021.1885339

[62] Soleimani, R., Favresse, J., Roy, T., Gruson, D., & Fillée, C. (2019). Macro vitamin B12: an underestimated threat. *Clinical Chemistry and Laboratory Medicine*, *58*(3), 408–415. https://doi.org/10.1515/cclm-2019-0999

[63] Sourander, A., Silwal, S., Surcel, H., Hinkka-Yli-Salomäki, S., Upadhyaya, S., McKeague, I. W., Cheslack-Postava, K., & Brown, A. S. (2023). Maternal Serum Vitamin B12 during Pregnancy and Offspring Autism Spectrum Disorder. Nutrients, 15(8), 2009. https://doi.org/10.3390/nu15082009

[64] Stuart, K. V., Luben, R. N., Warwick, A. N., Madjedi, K. M., Patel, P. J., Biradar, M. I., Sun, Z., Chia, M. A., Pasquale, L. R., Wiggs, J. L., Kang, J. H., Kim, J., Aschard, H., Tran, J. H., Lentjes, M. A., Foster, P. J., Khawaja, A. P., Aschard, H., Chia, M., . . . Trucco, E. (2023). The Association of Alcohol Consumption with Glaucoma and Related Traits. Ophthalmology Glaucoma, 6(4), 366–379. https://doi.org/10.1016/j.ogla.2022.11.008

[65] Sunitha, Y., & Kumar, S. (2021). An assessment of vitamin B12 through determination of cobalt by X-ray fluorescence spectrometry. Radiation Physics and Chemistry, 188, 109583. https://doi.org/10.1016/j.radphyschem.2021.109583

[66] Taechameekietichai, T., Chansangpetch, S., Peerawaranun, P., & Lin, S. C. (2021). Association between Daily Niacin Intake and Glaucoma: National Health and Nutrition Examination Survey. Nutrients, 13(12), 4263. https://doi.org/10.3390/nu13124263

[67] Tat, L., Cannizzaro, N., Schaaf, Z., Racherla, S., Bottiglieri, T., Green, R., &Zarbalis, K. S. (2023). Prenatal folic acid and vitamin B12 imbalance alter neuronal morphology and synaptic density in the mouse neocortex. Communications Biology, 6(1). https://doi.org/10.1038/s42003-023-05492-9

[68] Tian, S., Yu, X., Wu, L., Zheng, H., Zhong, X., Xie, Y., & Wu, W. (2024). Vitamin B6 and folate intake are associated with lower risk of severe headache or migraine in adults: An analysis based on NHANES 1999–2004. Nutrition Research, 121, 51–60. https://doi.org/10.1016/j.nutres.2023.11.008

[69] Ulloque-Badaracco, J. R., Hernandez-Bustamante, E. A., Alarcon-Braga, E. A., Al-Kassab-Córdova, A., Cabrera-Guzmán, J. C., Herrera-Añazco, P., &Benites-Zapata, V. A. (2023). Vitamin B12, folate, and homocysteine in metabolic syndrome: a systematic review and meta-analysis. Frontiers in Endocrinology, 14. https://doi.org/10.3389/fendo.2023.1221259

[70] Villatoro-Santos, C. R., Ramirez-Zea, M., &Villamor, E. (2020). B-vitamins and metabolic syndrome in Mesoamerican children and their adult parents. Public Health Nutrition, 24(14), 4537–4545. https://doi.org/10.1017/s1368980020003936

[71] W, W. T., S, G., G, N., G, T., F, G., D, C., Y, B., Z, G., & T, A. D. (2021). Assessment of Serum Vitamin B12 and Folate Levels and Macrocytosis in Patients with Type 2 Diabetes Mellitus on Metformin Attending TikurAnbessa Specialized Hospital, Addis Ababa, Ethiopia: A Cross-Sectional Study. DOAJ (DOAJ: Directory of Open Access Journals). https://doaj.org/article/1cae46ac27f84560a01d4a7ef491e59a

[72] Wan, Z., Zheng, J., Zhu, Z., Sang, L., Zhu, J., Luo, S., Zhao, Y., Wang, R., Zhang, Y., Hao, K., Chen, L., Du, J., Kan, J., & He, H. (2022). Intermediate role of gut microbiota in vitamin B nutrition and its influences on human health. Frontiers in Nutrition, 9. https://doi.org/10.3389/fnut.2022.1031502

[73] Wang, Q., Wang, Z., Guan, J., & Song, J. (2024). Transcriptome Analysis Reveals the Important Role of Vitamin B12 in the Response of Natronorubrumdaqingense to Salt Stress. International Journal of Molecular Sciences, 25(8), 4168. https://doi.org/10.3390/ijms25084168

[74] Yahn, G. B., Abato, J. E., &Jadavji, N. M. (2021). Role of vitamin B12 deficiency in ischemic stroke risk and

outcome. Neural Regeneration Research/Neural Regeneration Research, 16(3), 470. https://doi.org/10.4103/1673-5374.291381

[75] Yang, Y., Zhou, B., & Zheng, C. (2024). The fast quantification of vitamin B12 in milk powder by High-Performance Liquid Chromatography-Inductively coupled plasma Mass spectrometry. Molecules/Molecules Online/Molecules Annual, 29(8), 1795. https://doi.org/10.3390/molecules29081795

[76] Yuan, X., Han, X., Zhou, W., Long, W., Wang, H., Yu, B., & Zhang, B. (2022). Association of folate and vitamin B12 imbalance with adverse pregnancy outcomes among 11,549 pregnant women: An observational cohort study. Frontiers in Nutrition, 9. https://doi.org/10.3389/fnut.2022.947118

[77] Zhang, T., Huang, D., Hou, J., Li, J., Zhang, Y., Tian, M., Li, Z., Tie, T., Cheng, Y., Su, X., Man, Z., & Ma, Y. (2020). High-concentration homocysteine inhibits mitochondrial respiration function and production of reactive oxygen species in neuron cells. Journal of Stroke and Cerebrovascular Diseases, 29(10), 105109. https://doi.org/10.1016/j.jstrokecerebrovasdis.2020.105109

[78] Zheng, X., Qiu, R., Zhang, W., Chen, X., & Wang, M. (2023). Vitamin B12 deficiency presenting as psychotic symptoms in a psychiatry Department: a case report. Curēus. https://doi.org/10.7759/cureus.50492

[79] Zhou, L., Song, X., Wang, J., Tan, Y., & Yang, Q. (2023). Effects of vitamin B12 deficiency on risk and outcome of ischemic stroke. Clinical Biochemistry, 118, 110591. https://doi.org/10.1016/j.clinbiochem.2023.110591