Delgado JA, Pastor García MI, Jiménez NM, Petit GC, Pablo Cànaves JA, Robles J, et al. Challenges in the diagnosis of hypervitaminemia B12. Interference by immunocomplexes. Clin Chim Acta. 2023 Feb;541:117267. https://doi.org/10.1016/j.cca.2023.117267

# CHALLENGES IN THE DIAGNOSIS OF HYPERVITAMINEMIA B12. INTERFERENCE BY IMMUNOCOMPLEXES

**Authors:** Jose Antonio Delgado[1], María I. Pastor García[1], Nuria Márquez Jiménez[1], Gemma Costa Petit[1], Josep A. Pablo Cànaves[2], Juan Robles[1], Josep Miquel Bauça[1,3].

**Affiliations:**

1. Department of Laboratory Medicine, Hospital Universitari Son Espases, Palma, Spain.
2. Serveis Cientificotècnics. Universitat de les Illes Balears, Spain.
3. Institut d´Investigació Sanitària de les Illes Balears (IdISBa), Spain

**Corresponding author:**

Jose Antonio Delgado Rodríguez

Department of Laboratory Medicine, Hospital Universitari Son Espases

Ctra. de Valldemossa, 79, J+1

07010 Palma, Balearic Islands, Spain

Email address: joandelro@gmail.com

**Short title:** Hypervitaminemia B12: interference by immunocomplexes

| | |
|---|---|
| Word count | 2797 |
| Abstract word count | 200 |
| Tables | 1 |
| Figures | 3 |

**Conflict of interest**

All authors declare no conflicts of interest.

Delgado JA, Pastor García MI, Jiménez NM, Petit GC, Pablo Cànaves JA, Robles J, et al. Challenges in the diagnosis of hypervitaminemia B12. Interference by immunocomplexes. Clin Chim Acta. 2023 Feb;541:117267. https://doi.org/10.1016/j.cca.2023.117267

**HIGHLIGHTS**

- Hypervitaminemia B12 is a common finding in clinical practice
- PEG precipitation is a useful screening method in ruling out macromolecules
- The presence of B12-immunocomplexes is a phenomenon barely known by physicians
- A algorithm for the screening of macroB12 interferences is highly cost-effective

Delgado JA, Pastor García MI, Jiménez NM, Petit GC, Pablo Cànaves JA, Robles J, et al. Challenges in the diagnosis of hypervitaminemia B12. Interference by immunocomplexes. Clin Chim Acta. 2023 Feb;541:117267. https://doi.org/10.1016/j.cca.2023.117267

**ABSTRACT**

**BACKGROUND-AIM**

High vitamin B12 concentrations are considered a common finding in clinical practice. Thanks to immunoassay accessibility, vitamin B12 has become a usual test in routine health checkups. However, these analytical methods usually present antibody-mediated interferences.

Our aim was to propose an algorithm for the screening of antibody-mediated analytical interferences on vitamin B12 immunoassays on the Alinity platform.

**METHODS**

Observational, prospective, case-control study was performed during 12 months. Individuals with persistently elevated cobalamin concentrations [>554pmol/L] were considered as cases in the absence of supplementation or other justifying cause. Individuals under treatment with vitamin B12, or in the context of alcoholism were included as controls.

A thorough interference study by macromolecules in immunoassays was performed in serum samples: PEG precipitation, rheumatoid factor, heterophile antibodies and gel permeation chromatography (GPC). Albumin, total B12, IgG and IgM were measured in every GPC collected fraction and chromatograms were drafted.

**RESULTS**

Up to 45% of cases presented interference by B12-immunocomplexes and the precipitation for all of them was >50%. The individual with the lowest interfered vitamin B12 result was 661pmol/L.

**CONCLUSION**

The presence of antibody-mediated interferences, mainly B12-immunocomplexes, is a relatively common phenomenon. A simple algorithm for the screening of interferences is useful and reliable in ruling out healthy individuals and highly cost-effective.

**Keywords:** cobalamin; interference; macromolecules; immunoassay; macrocomplex

Delgado JA, Pastor García MI, Jiménez NM, Petit GC, Pablo Cànaves JA, Robles J, et al. Challenges in the diagnosis of hypervitaminemia B12. Interference by immunocomplexes. Clin Chim Acta. 2023 Feb;541:117267. https://doi.org/10.1016/j.cca.2023.117267


## INTRODUCTION

Vitamin B12, known as cobalamin, is an essential micronutrient that plays a fundamental role in erythropoiesis and methylation, and is required for cell metabolism and DNA synthesis.

With increasing laboratory automation, reduction of costs, and immunoassay accessibility, vitamin B12 has become a usual test performed in routine health checkups, mainly in the quest for deficiency. However, unexpected high concentrations are sometimes found in these patients [1]. In the literature, hypervitaminemia B12 (hB12) is considered a relatively common finding, between 7-20% [2], even more than its deficiency. However, despite its prevalence, hB12 is a clearly underestimated analytical abnormality [3].

Such a finding should be regarded as of special relevance, given that high vitamin B12 concentrations are strongly associated with benign conditions (chronic kidney disease, liver disease, infections, autoimmune diseases…), malignant hematological diseases (myeloproliferative syndromes, multiple myeloma, polycythemia vera…), and solid tumors (liver, pancreas, breast, colon, stomach…), after supplementation has been ruled out [4]. Moreover, vitamin B12 has also been postulated as a useful biomarker of severity (prognosis) and death in such diseases [5-7], as well as, increased vitamin B12 concentrations might paradoxically be masking a deficiency [8]. This functional deficiency is associated with alterations in the uptake and tissue processing of cobalamin, or with the presence of antibody-mediated analytical interferences such as macrocomplexes of vitamin B12 [macroB12, generally with immunoglobulins (immunocomplexes)] [9-12]. The presence of macrocomplexes is also a cause of hB12 little known by clinicians [13]. These are high molecular weight circulating macromolecules that are slowly cleared from the circulation, and have no biological activity or clinical significance [8].

When a case of hB12 appears, early screening for macroB12 interference by means of polyethylene glycol (PEG) precipitation makes it possible to rule out the need for further medical examinations. However, this practice is barely established in laboratory routine, unlike other analytes such as prolactin [14].

The aims of our study were *[1]* to characterize the type of interference in our vitamin B12 immunoassay, *[2]* to propose an algorithm for the screening of antibody-mediated analytical interferences, and *[3]* to evaluate the economic impact of the implementation of such an algorithm.

Delgado JA, Pastor García MI, Jiménez NM, Petit GC, Pablo Cànaves JA, Robles J, et al. Challenges in the diagnosis of hypervitaminemia B12. Interference by immunocomplexes. Clin Chim Acta. 2023 Feb;541:117267. https://doi.org/10.1016/j.cca.2023.117267


## MATERIAL AND METHODS

An observational, prospective, case-control study performed at Hospital Universitari Son Espases (Palma, Spain), which is a tertiary hospital attending a direct population of 325,000 individuals, and is a reference for an approximate population of 1,200,000 inhabitants. The period assessed was from November 2020 to October 2021 (12 months). Biochemical data were obtained from the laboratory information system (GestLab, Indra, Spain), and clinical data were retrieved from the hospital information system (Millennium, Cerner Corporation, USA) after approval by the Ethics Committee of the Balearic Islands (CEI-IB - 4355/20 PI).

### Subjects

By way of inclusion criteria for *cases*, we considered individuals ≥18 years of age who were requested a vitamin B12 test as part of their routine clinical checkup, with results above the upper limit of reference (554 pmol/L) in serial determinations for more than 6 months in the absence of other clinical or biochemical findings in their medical records that could explain such a concentration (supplementation, alcoholism, liver disease, chronic kidney disease, autoimmune diseases, infectious diseases (HIV, syphilis), myeloproliferative syndromes, or a previous diagnosis of cancer).

As *controls*, individuals ≥18 years of age were included if they had vitamin B12 results above the upper limit of reference and were under treatment with vitamin B12 during the months preceding the collection of blood samples, or in the context of alcoholism.

### Analytical procedure

Vitamin B12 was measured in serum by a chemiluminescent immunoassay on the Alinity i platform (Abbott Diagnostics, USA). Measurement quality was ensured by the usual laboratory procedures, with a coefficient of variation (CV) below 6% in the range of concentrations of the internal quality controls (269-674 pmol/L). The test was accredited by ISO 15189. The manufacturer describes as interferences in B12 immunoassay: rheumatoid factor and heterophile antibodies (both of which would precipitate with PEG).

*Assessment of analytical interferences*

Delgado JA, Pastor García MI, Jiménez NM, Petit GC, Pablo Cànaves JA, Robles J, et al. Challenges in the diagnosis of hypervitaminemia B12. Interference by immunocomplexes. Clin Chim Acta. 2023 Feb;541:117267. https://doi.org/10.1016/j.cca.2023.117267


All patients' serum samples (cases and controls) were stratified by vitamin B12 concentration range (554 – 886; 887 – 1181; 1182 – 1476 and >1476 pmol/L) and underwent a precipitation with PEG, determination of rheumatoid factor and interference by heterophile antibodies and gel permeation chromatography (GPC).

PEG precipitation was carried out following the classic protocol [15]: 200 µL of serum was mixed with 200µL of 25% PEG 6000 solution. Samples were mixed and centrifuged at 2200g for 15 minutes. Vitamin B12 concentration was measured in the supernatant. Percentage of B12 recovery after PEG precipitation was calculated according to the formula:

$$Recovery\ (\%) = \frac{[2 \cdot B12\ after\ PEG]}{B12\ before\ PEG} \cdot\ 100$$

Quantification of rheumatoid factor was performed by immunoturbidimetry on an Alinity ci platform (Abbott Diagnostics, USA). The linearity of the method was 20-200 IU/mL, and the CV reported by the manufacturer was 0.5% at 49.3 IU/mL.

Interference by heterophile antibodies was assessed by incubating 500 µl of the patient's serum sample in Heterophilic Blocking Tubes® (Scantobodies, USA) for 1 hour at room temperature and vitamin B12 was further assayed.

*Gel permeation chromatography*

Subsequently, samples from both cases and controls underwent a gel permeation chromatography [reference method [16]], in order to characterize the type of interference and establish a cut-off value for precipitation suggesting the presence of B12-immunocomplexes. A total of 500 µl of simple was injected into a FPLC (fast protein liquid chromatography) Äkta Purifier (GE Healthcare). For the chromatography, a Superdex 200 10/300 GL column was used (GE Healthcare, Spain) and PBS pH 7.4 as mobile phase with a flux of 0.75 mL/min. Signal was monitored at 280 nm, and 500 µL eluate fractions were collected.

Each fraction was tested for vitamin B12, immunoglobulins (IgG and IgM), and albumin, and chromatograms were drafted. Quantification of IgG, IgM, and albumin was performed on the Alinity ci platform. The manufacturer reports a CV <2% for all three analytes.

*Statistical analysis*


Data were analyzed using Excel 2010 software (Microsoft Inc, USA). For the calculation of the optimal cut-off value for B12 concentration above which the presence of macroB12 should be investigated, a ROC curve analysis was performed including all participants and considering the estimated limit of PEG precipitation (50%) as confirmatory for the presence of interference using MedCalc v.19.3 software (Belgium).

*Evaluation of the economic impact*

The economic impact of the early detection of macroB12 interferences was evaluated by considering the costs of laboratory tests and medical interventions required for the differential diagnosis of hypervitaminemia B12, according to the data published in the Official Gazette of the Balearic Islands (BOIB 2018). The panel of tests and procedures required for a complete differential diagnosis include: a complete blood count, basic metabolic panel, tumor biomarkers, serologies, autoimmunity panel, serum protein electrophoresis and immunophenotyping, bone marrow biopsy, imaging studies and different consultation with specialist physician. Performance of the complete panel would imply an estimated healthcare cost of 1,596 euro per patient with hypervitaminemia B12. The costs associated for ruling out interference is 65 euro per patient.

## RESULTS

During the period assessed, a total of 155 individuals were included as cases, and 109 as controls. The final distribution of cases and controls, along with the identification of interferences, are shown in Figure 1.

It is noteworthy that eight individuals first recruited as *controls* according to the review of the medical records were found to present an antibody-mediated interference (either by heterophile antibodies or by B12-immunocomplexes). Therefore, those 8 individuals were reclassified as Pseudo-cases. Likewise, 69 of the 155 original cases (45%) showed interference by macrocomplexes in the immunoassay (subgroup *Pseudo-cases*).

The characteristics of the participants and the results of the interference studies are presented in Table 1.

*Characterization of interferences by immunocomplexes*

GPC elution profiles were compared between cases and controls. The molecular weight (MW) of vitamin B12 is 1.4 kDa if free; 68 kDa if bound to haptocorrin (transcobalamin I); and 44 kDa if bound to transcobalamin II. All of them are lower than the MW of IgG (150 kDa) or IgM (970 kDa).

Given that larger molecules (higher MW) elute earlier in GPC, the samples whose B12 elution preceded the elution of immunoglobulins were considered to be positive for B12-immunocomplexes.

As seen in Figure 2, we found interferences due to immunocomplexes B12-IgG, B12-IgM, and one sample with both immunocomplexes B12-IgG and B12-IgM. In contrast, the samples with interference other than immunocomplexes-B12 have an elution profile similar to those of controls.

*Determination of the optimal cut-off value for the concentration of vitamin B12 for the suspicion of interference.*

The ROC curve analysis yielded an area under the curve (AUC) of 0.779 (CI 95%: 0.719 – 0.839) The optimal cut-off value for total vitamin B12 was 1061 pmol/L, with a sensitivity of 78% (CI 95%: 68 – 86) and a specificity of 65% (CI 95%: 58 – 72). The individual with the lowest result for vitamin B12 and a confirmed presence of immunocomplex by GPC was 661 pmol/L.

*Establishment of a cut-off value for the definition of interference after PEG precipitation as a screening method.*

In our study, the cut-off value that confirmed the presence of interference was 50%, given that all individuals with a PEG precipitation above this value presented interference by B12-immunocomplexes confirmed by GPC, heterophile antibodies, or rheumatoid factor. The 95$^{th}$ percentile for both controls and individuals included in the subgroup *Cases* was <40%.

The recommended algorithm for the screening of interferences by B12-macrocomplexes given in our results is presented in Figure 3.

*Evaluation of the economic impact of the detection of interferences in the measurement of serum vitamin B12*

Considering a healthcare cost of 1596 euro for the performance of the appropriate complementary tests for the differential diagnosis, and 65 euro for the performance of the


screening of interferences by macrocomplexes, detection of 77 cases with potential interferences would imply savings above 117,000 euro for the healthcare system.

## DISCUSSION

To the best of our knowledge, this is the first study suggesting an algorithm for the screening of antibody-mediated analytical interferences, especially by macroB12 and evaluating the economic impact of the implementation of such an algorithm.

Interference due to vitamin B12 macrocomplexes in Abbott's immunoassay has been previously reported elsewhere [17], as well as for other analytical platforms [13,18]. PEG precipitation is an inexpensive, rapid and simple method which is broadly available for the detection of other macroforms such as prolactin and thyrotropin [19,20], but scarcely implemented for vitamin B12. In addition, not all immunoassays show the same number of interferences due to macrocomplexes. Therefore, each laboratory needs to establish their own cut-off values for suggesting the presence of antibody-mediated interferences [21].

Gel-permeation chromatography is considered the gold standard methodology for the characterization of macrocomplexes, although it is not feasible for routine use, as it requires expertise in the field and is inaccessible for most clinical laboratories [22]. Hence, it is important to implement a reliable, cost-effective algorithm for the screening of interferences by means of PEG precipitation considering laboratory workload and personnel.

Our chromatographic findings are consistent with previous literature reporting circulating macrocomplexes of vitamin B12 in the form of immunocomplexes B12-IgG and B12-IgM [9, 23-26]. Nevertheless, literature is limited to clinical cases or small series of patients lacking a proper evaluation of the cut-off value for the concentration of vitamin B12 above which the quest for macrocomplexes should be started. In our study, by means of a ROC curve analysis, we suggest that all vitamin B12 results above 1061 pmol/L be further explored for interferences, with a sensitivity of 77% and a specificity of 65%. Although this cut-off value is far from ideal, it is important to bear in mind that any case with undetected macrocomplexes will imply a significant waste of resources compared with the low cost of screening for interferences by means of PEG precipitation. Given that the individual with the lowest vitamin B12 and confirmed presence of immunocomplexes by GPC had a result of 661 pmol/L, we recommend screening for interferences by means of PEG precipitation in any individual with


persistent vitamin B12 results above the upper limit of reference for Abbott's Alinity ci platform over 6 months, in the absence of other clinical or biochemical findings that could explain such a concentration.

In previous reports, the vitamin B12 result above which the presence of interferences should be suspected was established either arbitrarily or using the upper limit of the reference interval. Remacha et al. in a prospective study [27] only considered as cases individuals with vitamin B12 concentrations >2500 pmol/L and found a 25% prevalence of immunocomplexes. Soleimani and colleagues [17] recommended the systematic screening by PEG precipitation of all samples with B12 above the upper limit of reference, and found a lower prevalence (18%). Based on our results and from our point of view, such a recommendation is neither feasible nor cost-effective for clinical laboratories, given the great number of vitamin B12 test requests and the high prevalence of results above the upper limit. In our study, we found that 45% of cases presented an antibody-mediated interference, which underlines the high prevalence of macrocomplexes in the selected patients. According to our results, we strongly recommend precipitation with PEG of all individuals whose vitamin B12 is persistently above the upper limit of reference in serial determinations for at least 6 months, in the absence of any other justifying cause.

Given these recommendations, and considering the values from GPC, the precipitation cut-off that would confirm the presence of interference by biologically inactive macrocomplexes is 50%. This result matches the one arbitrarily established elsewhere [18], but is above the one suggested by Soleimani (35.3%) which was calculated in healthy controls and not using GPC as a confirmation methodology. In our study, the 95$^{th}$ percentile-recovery for PEG precipitation in both controls and in the subgroup of Cases was 40%. Hence, as is the case of other analytes, a "grey zone" should be defined (between 40-50% recovery, according to our data) which would require further confirmation, as described in the algorithm shown in Figure 3.

Our algorithm is not free from limitations. First, eight individuals initially recruited as controls by means of the review of medical records did show an antibody-mediated interference (either by heterophile antibodies or by the presence of immunocomplexes-B12). Therefore, such individuals were reclassified as Pseudo-cases. This finding is in accordance with previous reports, that is, the production of antibodies against vitamin B12 in individuals with prolonged intramuscular B12 supplementation [26]. The presence of macrocomplexes has also been

described in individuals excluded by our algorithm, such as patients affected with hematological conditions or chronic kidney disease [17,27].

Besides, although some controversy is seen among studies in this field [18], some cases of macroB12 have also been described in individuals with B12 results within the reference interval [17]. Our results show a positive correlation between the concentration of vitamin B12 and the presence of macrocomplexes. Thus, we do not recommend performing the screening of interferences in samples with normal vitamin B12 concentrations or in individuals previously diagnosed with the abovementioned diseases, unless there is a serious suspicion of B12 deficiency or other functional biomarkers of B12 status are altered, such as homocysteine and methylmalonic acid.

Regarding the economic impact of antibody-mediated interferences on vitamin B12 immunoassays, previous reports highlighted the current low awareness of physicians. Such a lack of awareness has made persistently increased vitamin B12 results lead to unnecessary medical referrals, costly and invasive complimentary tests, and delays in diagnosis [17]. According to our results, the implementation of our algorithm in a tertiary hospital would enable up to 45% of healthy individuals who do not require any further exploration to be identified, which points out its usefulness in terms of ruling out. This observation contributes to a growing body of evidence aiming to alert the IVD industry and medical professionals to the repercussion of such a phenomenon on diagnostic precision and patient safety.

As far as we know, our study is the first to evaluate the economic impact of an algorithm for the screening of interferences in Abbott's immunoassay for vitamin B12 and highlight its cost-effectiveness. In times of "right care" and efficient resource management as pillars for sustainable and quality healthcare, the active role of the laboratory is crucial in both economic and clinical terms.

Our study aims to help harmonize the clinical decisions associated with increased vitamin B12 results. The main strength of this evaluation is to propose a simple, cost-effective algorithm, based on GPC results as a reference method. However, the selection of individuals was based only on laboratory data and medical records. The clinical impact of the interferences was not assessed, neither was the specific structure of the immunocomplexes.

## CONCLUSION

Delgado JA, Pastor García MI, Jiménez NM, Petit GC, Pablo Cànaves JA, Robles J, et al. Challenges in the diagnosis of hypervitaminemia B12. Interference by immunocomplexes. Clin Chim Acta. 2023 Feb;541:117267. https://doi.org/10.1016/j.cca.2023.117267

The presence of antibody-mediated interferences, such as B12-immunocomplexes, is a relatively common phenomenon but little known by physicians. Owing to our study, a simple algorithm for the screening of macroB12 interferences was proven to be useful and reliable in ruling out healthy individuals and is highly cost-effective.

Delgado JA, Pastor García MI, Jiménez NM, Petit GC, Pablo Cànaves JA, Robles J, et al. Challenges in the diagnosis of hypervitaminemia B12. Interference by immunocomplexes. Clin Chim Acta. 2023 Feb;541:117267. https://doi.org/10.1016/j.cca.2023.117267

**Additional Information:**

**Consent for publication**

Consent to submit has been received explicitly from all co-authors, as well as from the responsible authorities. Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

**CRediT authorship contribution statement**

**JAD**: Conceptualization, Investigation, Methodology, Software, Writing, Supervision, Validation, Visualization. **MIPG:** Conceptualization, Investigation, Methodology, Writing, Supervision, Validation, Visualization. **NMJ**: Experimental Support, **GCP**: Experimental Support. **JAPC:** Software, Methodology, Experimental Support. **JR**: Methodology. **JMB:** Investigation, Methodology, Writing, Supervision, Validation, Visualization.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper**.**

**Funding**:

This work was supported by the scholarship by FENIN – Fundación José Luis Castaño – SEQC-ML.

**Acknowledgements**

Not applicable

Delgado JA, Pastor García MI, Jiménez NM, Petit GC, Pablo Cànaves JA, Robles J, et al. Challenges in the diagnosis of hypervitaminemia B12. Interference by immunocomplexes. Clin Chim Acta. 2023 Feb;541:117267. https://doi.org/10.1016/j.cca.2023.117267

**REFERENCE LIST**

1. Arendt JF, Nexo E. Unexpected high plasma cobalamin: proposal for a diagnostic strategy. Clin Chem Lab Med. 2013;51(3):489-96.

2. Albayrak D, Albayrak C. Clinical follow-up of children with high vitamin B12 values: should we worry?. Turk J Pediatr. 2021;63(6):1064-71.

3. Serraj K, Mecili M, Housni I, Andrès E. Hypervitaminémie B12: physiopathologie et intérêt en pratique clinique [Hypervitaminemia B12 (high level of cobalamin): physiopathology, role and interest in clinical practice]. Presse Med. 2011;40(12 Pt 1):1120-7.

4. Jammal M, Deneuville T, Mario N, Tiev K, Tolédano C, Josselin-mahr L, et al. Concentration plasmatique élevée de la vitamine B12 : un indicateur des maladies hépatiques ou tumorales High plasmatic concentration of vitamin B12 : An indicator of hepatic diseases or tumors. La Rev médecine interne. 2013;34(6):337–41.

5. Hoffmann-Lücke E, Arendt JF, Nissen PH, Mikkelsen G, Aasly JO, Nexo E. Three family members with elevated plasma cobalamin, transcobalamin and soluble transcobalamin receptor (sCD320). Clin Chem Lab Med. 2013;51(3):677-82.

6. Couderc AL, Puchades E, Villani P, et al. High Serum Vitamin B12 Levels Associated with C-Reactive Protein in Older Patients with Cancer. Oncologist. 2020;25(12):e1980-9.

7. Geissbühler P, Mermillod B, Rapin CH. Elevated serum vitamin B12 levels associated with CRP as a predictive factor of mortality in palliative care cancer patients: a prospective study over five years. J Pain Symptom Manage. 2000;20(2):93-103.

8. Wolffenbuttel BHR, Muller Kobold AC, Sobczyńska-Malefora A, Harrington DJ. Macro-B12 masking B12 deficiency. BMJ Case Rep. 2022;15(1):e247660.

9. Owen RA, Drake SK, Vanjani R, Huey ED, Grafman J, Horne MK 3rd. Markedly increased vitamin B12 concentrations attributable to IgG-IgM-vitamin B12 immune complexes. Clin Chem. 2006;52(11):2107-14.

10. Yang DT, Cook RJ. Spurious elevations of vitamin B12 with pernicious anemia. N Engl J Med. 2012;366(18):1742-3.

11. Tavares J, Baptista B, Gonçalves B, Horta AB. Pernicious Anaemia with Normal Vitamin B12. Eur J Case Rep Intern Med. 2019;6(2):001045.

12. Remacha AF, Zapico E, Sarda MP, et al. Immune complexes and persistent high levels of serum vitamin B12. Int J Lab Hematol. 2014;36(1):92-7.

Delgado JA, Pastor García MI, Jiménez NM, Petit GC, Pablo Cànaves JA, Robles J, et al. Challenges in the diagnosis of hypervitaminemia B12. Interference by immunocomplexes. Clin Chim Acta. 2023 Feb;541:117267. https://doi.org/10.1016/j.cca.2023.117267

13. Yang DT, Cook RJ. Spurious Elevations of Vitamin B 12 with Pernicious Anemia. N Engl J Med. 2012;366(18):1742–3.

14. Haddad RA, Giacherio D, Barkan AL. Interpretation of common endocrine laboratory tests: technical pitfalls, their mechanisms and practical considerations. Clin Diabetes Endocrinol. 2019;5(1):1–10.

15. Hattori N, Ishihara T, Matsuoka N, Saito T, Shimatsu A. Anti-Thyrotropin Autoantibodies in Patients with Macro-Thyrotropin and Long-Term Changes in Macro-Thyrotropin and Serum Thyrotropin Levels. Thyroid. 2017;27(2):138–46.

16. Šostarić M, Bokulić A, Marijančević D, Zec I. Optimizing laboratory defined macroprolactin algorithm. Biochem Medica. 2019;29(2):346–51.

17. Soleimani R, Favresse J, Roy T, Gruson D, Filleé C. Macro vitamin B12: An underestimated threat. Clin Chem Lab Med. 2020;58(3):408–15.

18. Jeffery J, Millar H, Mackenzie P, Fahie-Wilson M, Hamilton M, Ayling RM. An IgG complexed form of vitamin B12 is a common cause of elevated serum concentrations. Clin Biochem. 2010;43(1-2):82-8.

19. Biagetti B, Ferrer Costa R, Alfayate Guerra R, et al. Macroprolactin: From laboratory to clinical practice [published online ahead of print, 2021 Apr 23]. Macroprolactina: del laboratorio a la práctica clínica. Recomendaciones del grupo de trabajo de laboratorio de la SEEN y de la comisión de hormonas de la SEQCML sobre la medición e informe del resultado de la macroprolactina [published online ahead of print, 2021 Apr 23]. Endocrinol Diabetes Nutr (Engl Ed). 2021;S2530-0164(21)00082-3.

20. Sakai H, Fukuda G, Suzuki N, Watanabe C, Odawara M. Falsely elevated thyroid-stimulating hormone (TSH) level due to macro-TSH. Endocr J. 2009;56(3):435–40.

21. Veljkovic K, Servedio D, Don-wauchope AC. Reporting of post-polyethylene glycol prolactin: precipitation by polyethylene glycol 6000 or polyethylene glycol 8000 will change reference intervals for monomeric prolactin. Ann Clin Biochem. 2012;49:402–4.

22. Whitehead SJ, Cornes MP, Ford C, Gama R. Reference ranges for serum total and monomeric prolactin for the current generation Abbott Architect assay. Ann Clin Biochem. 2015;52(1):61–6.

23. Hom BL, Olesen H, Schwartz M. Turnover of 57Co-labelled vitamin B12-transcobalamin II and autologous 131-I-labelled IgG in a patient with antibody to transcobalamin II. Scand J Haematol. 1968;5(2):107-15.

Delgado JA, Pastor García MI, Jiménez NM, Petit GC, Pablo Cànaves JA, Robles J, et al. Challenges in the diagnosis of hypervitaminemia B12. Interference by immunocomplexes. Clin Chim Acta. 2023 Feb;541:117267. https://doi.org/10.1016/j.cca.2023.117267

24. Rodríguez JAD, García MIP, Bauça JM, Mullor RV, Barceló A. Persistently increased vitamin B12 concentration due to cobalamin macrocomplexes: a case report and review of the literature. Clin Chem Lab Med. 2020;58(10):e237-9.

25. De Haro L, Marquet J, Tonetti C, Zittoun J. Hypervitaminémie B12 sérique due à un anticorps antitranscobalamine II: À propos d'un cas. Rev Med Interne. 2001;22(11):1132–3.

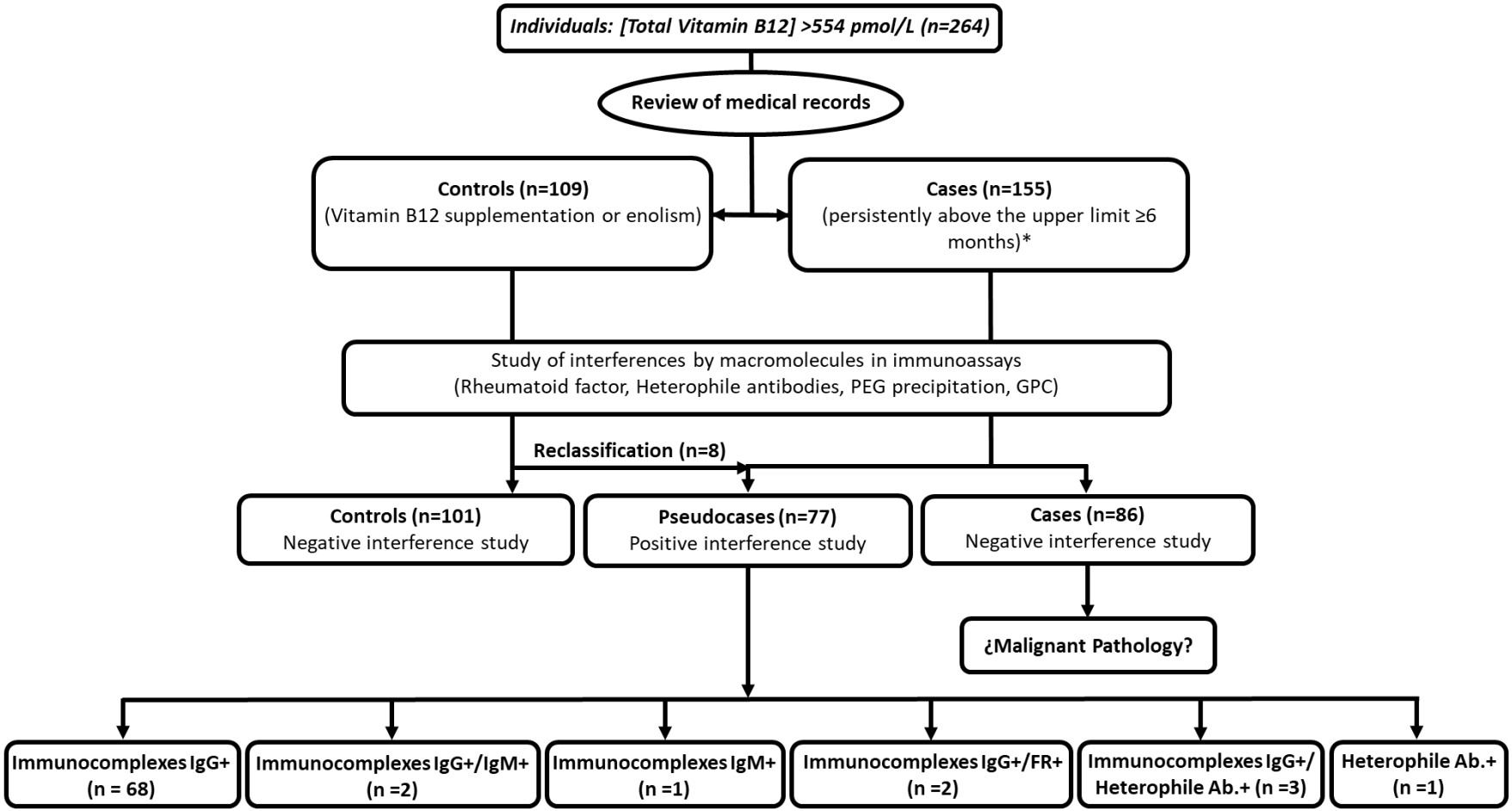
26. Carmel R, Tatsis B, Baril L. Circulating antibody to transcobalamin II causing retention of vitamin B12 in the blood. Blood. 1977;49(6):987-1000.

27. Remacha AF, Zapico E, Sarda MP, Rojas E, Simó M, Remacha J, et al. Immune complexes and persistent high levels of serum vitamin B12. Int J Lab Hematol. 2014;36(1):92–7.

Delgado JA, Pastor García MI, Jiménez NM, Petit GC, Pablo Cànaves JA, Robles J, et al. Challenges in the diagnosis of hypervitaminemia B12. Interference by immunocomplexes. Clin Chim Acta. 2023 Feb;541:117267. https://doi.org/10.1016/j.cca.2023.117267



**Figure 1.** Flow diagram for the individuals included in the study.




*Absence of other clinical or biochemical findings in their medical records that could explain such a concentration

Delgado JA, Pastor García MI, Jiménez NM, Petit GC, Pablo Cànaves JA, Robles J, et al. Challenges in the diagnosis of hypervitaminemia B12. Interference by immunocomplexes. Clin Chim Acta. 2023 Feb;541:117267. https://doi.org/10.1016/j.cca.2023.117267

Table 1. Descriptive statistics for the subjects included in the study and the results of the interference study.

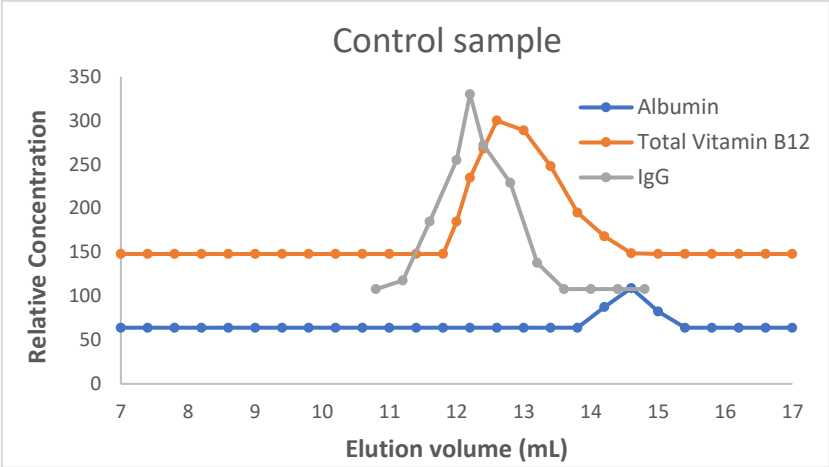
| | Vitamin B12, pmol/L (range, N) | Age, years (median, IQR) | Sex (M/F) | Vitamin B12, pmol/L (median, IQR) | % Precipitation (median, P95) | Heterophile Ab. (% of cases) | RF (% of cases) | Immunocomplexes (% of cases) |
|---|---|---|---|---|---|---|---|---|
| **Controls (n= 101)** | 554 – 886 (37) | 63 (48 – 70) | 17/20 | 756 (714 – 812) | 18 (31) | – | – | – |
| | 887 – 1181 (22) | 69 (59 – 77) | 12/10 | 968 (940 – 991) | 23 (40) | – | – | – |
| | 1182 – 1476 (16) | 70 (60 – 83) | 10/6 | 1270 (1240 – 1383) | 25 (38) | – | – | – |
| | >1476 (26) | 75 (59 – 85) | 11/15 | 1959 (1669 – 2888) | 16 (39) | – | – | – |
| **Pseudo-cases (n=77)** | 554 – 886 (8) | 71 (61 – 82) | 1/7 | 782 (692 – 866) | 57 (68) | – | 13% | 100% |
| | 887 – 1181 (15) | 73 (65 – 84) | 2/13 | 992 (951 – 1100) | 70 (80) | – | – | 100% |
| | 1182 – 1476 (12) | 77 (71 – 81) | 2/10 | 1279 (1227 – 1317) | 70 (79) | – | 8% | 100% |
| | >1476 (42) | 74 (63 – 81) | 10/32 | 2576 (2176 – 5180) | 81 (91) | 8% | – | 98% |
| **Cases (n=86)** | 554 – 886 (44) | 57 (48 – 78) | 11/33 | 723 (684 – 781) | 17 (30) | – | – | – |
| | 887 – 1181 (23) | 56 (45 – 73) | 7/16 | 974 (913 – 1049) | 19 (40) | – | – | – |
| | 1182 – 1476 (12) | 43 (28 – 66) | 3/9 | 1263 (1241 – 1315) | 26 (39) | – | – | – |
| | >1476 (7) | 60 (40 – 74) | 3/4 | 2059 (1625 – 2622) | 13 (33) | – | – | – |

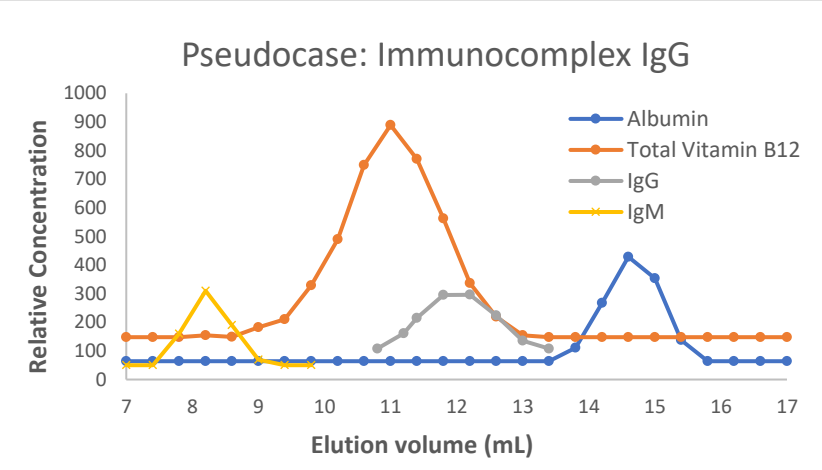
Abbreviations: M/F: Male/Female; IQR: interquartile range; P95: 95$^{th}$ percentile; Heterophile Ab.: Heterophile Antibodies; RF: Rheumatoid factor

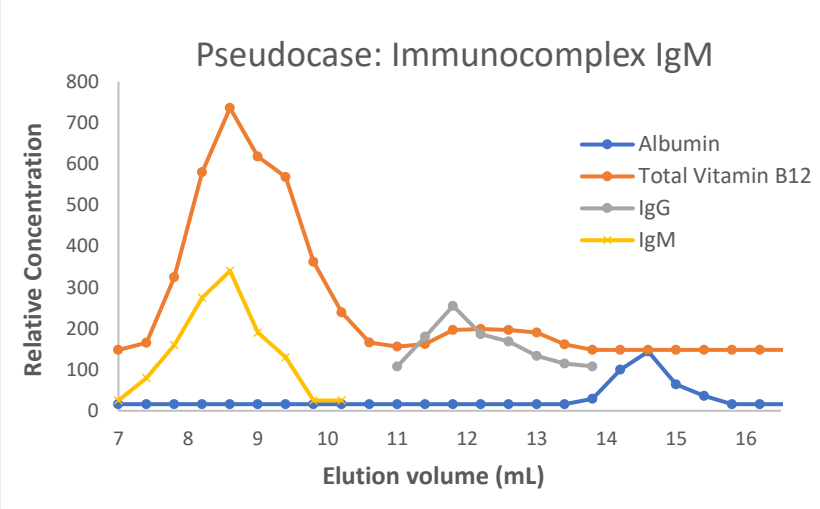
Delgado JA, Pastor García MI, Jiménez NM, Petit GC, Pablo Cànaves JA, Robles J, et al. Challenges in the diagnosis of hypervitaminemia B12. Interference by immunocomplexes. Clin Chim Acta. 2023 Feb;541:117267. https://doi.org/10.1016/j.cca.2023.117267

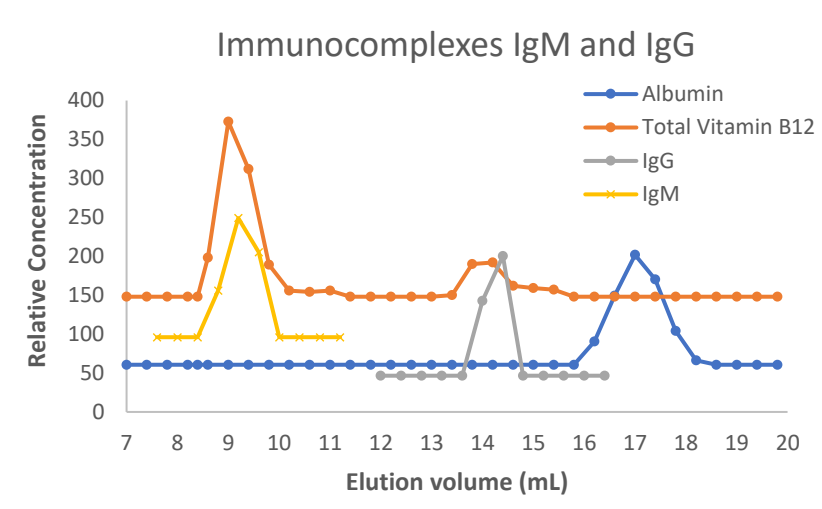


**Figure 2.** Chromatographic elution profile for Cases and Controls/Pseudo-cases. A) Control sample, B) Case with an immunocomplex B12-IgG, C) Case with immunocomplex B12-IgM, D) Case with immunocomplexes IgG and IgM.



Delgado JA, Pastor García MI, Jiménez NM, Petit GC, Pablo Cànaves JA, Robles J, et al. Challenges in the diagnosis of hypervitaminemia B12. Interference by immunocomplexes. Clin Chim Acta. 2023 Feb;541:117267. https://doi.org/10.1016/j.cca.2023.117267



**Figure 3.** Suggested algorithm for the screening of interferences due to vitamin B12 macrocomplexes in the clinical laboratory.