



# Investigation of Macro-Vitamin B12 interference: Proposed implementation of a polyethylene glycol precipitation procedure

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## Introduction

Measurement of Total B12 (cobalamin), is a mainstay of clinical diagnosis, primarily for the identification of deficient levels. However, B12 elevations (13%\*) are a frequent and underestimated anomaly and reportedly more common than deficient (2%\*) levels (MMUH B12 review 2020\* n = 11,510, RR 189 – 760pg/mL).<sup>1,2</sup> Increased B12 values may commonly reflect patients on supplementation but may also be indicative of haematological disorders, liver, kidney or intestinal disease.

Total B12 is measured by immunoassay, thus interference due to immune-B12 complex (macroB12), that may result in erroneously reported high B12 levels, should be considered, especially when the cause of a raised B12 is unknown.<sup>1-3</sup> Prevalence of macroB12 has been reported to be as high as 18%, yet this area seems to be understudied and in absence of routine reflex testing procedures, it may only be investigated following clinical uncertainty/discordance and liaison with the laboratory.<sup>1</sup> In our experience however, this approach does not seem effective, as inappropriate referral and further investigation has ensued for patients with subsequently confirmed macroB12.

## Aims

- To establish a method specific (Abbott) post-PEG reference range (RR) for B12
- To evaluate the prevalence of macroB12 interference
- To design a biochemical algorithm for investigation of raised B12, including macroB12 screening

## Methodology

### Subjects:

**Reference range study:** Lithium heparin (Sarstedt monovette) plasma samples (n = 121) from “healthy” staff volunteers (IRB approval and consented) used for macroB12 removal and establishment of a post-PEG B12 RR (+90% Confidence Intervals, CI) using the CLSI/IFCC robust approach.

**Exclusion:** Tukey statistical exclusion (n = 8) including patients with raised total B12 (n=6), B12 supplementation (n=1) and diabetes (n=1)

**MacroB12 prevalence study:** Lithium heparin (Sarstedt monovette) plasma samples from patients (n=116) attending MMUH (Aug-Sept '20) who had raised total B12 (>760 pg/ml) were used for evaluation of macroB12.

### Removal of macroB12 -Polyethylene Glycol Precipitation (PEG)

- Samples were treated with equi-volume PEG 25% W/V 6000 (Sigma product 4463) or phosphate buffered saline (PBS)
- Samples were vortexed for 30 seconds, incubated at room temperature (RT) for 10 minutes and centrifuged at 4000 r.p.m for 10 minutes (*ThermoFischer Scientific™ Heraeus Megafuge™ 16*)
- Samples were analysed for B12 on the Abbott Architect i2000®

### Macro-B12 Interpretation Criteria

- The presence of macroB12 was primarily evaluated by comparing the B12 result following PEG treatment against our newly established post-PEG B12 RR. We also estimated recovery (%) using the formula;  
 $(B12+PEG\ result / B12+PBS\ result) \times 100$

## Results

Using the above method, we established a post-PEG RR of 144-550 (CI: 122-170 + 522-574) pg/ml. The % recovery ranged from 58-105% with a RI of 64-102% (CI: 61-66% + 99-105%). Based on this RR, 8 patients with elevated B12 (n=116) had a post-PEG B12 <550 pg/ml supporting the presence of macroB12 as a major contributor to the total B12 concentration. The recovery (%) for such patients ranged from 11-64% (min to max). An extra 11 patients were identified with recovery <64% (range 11-63%) and with a post-PEG B12 ranging from 598 (11% recovery) to 15164 (50% recovery).

References  
1. Soleimani R, Favresse J, Roy T et al. Macro vitamin B12: an underestimated threat. *Clin Chem Lab Med*. 2020 Feb 25;58(3): 408-415  
2. Jeffery J, Millar H, MacKenzie P et al. An IgG complexed form of vitamin B12 is a common cause of elevated serum concentrations. *Clin Biochemistry*. 2010; 43: 82-88  
3. Schwartz M & Bastrup-Madsen P. A new vitamin B12 binding protein in serum causing excessively high serum vitamin B12 values. *Scand J Haematol*. 1968; 5:35-40

Table 1: MacroB12 prevalence: Patient samples (n = 116) with elevated total B12 (>760 pg/ml)

Total B12 Post- PEG B12 Analysis (Abbott Architect i2000®)		
MacroB12 Criteria/Definition	n	%
Total B12 < 550 pg/mL	8	7
Total B12 < 64% Recovery	19	16
< 550 pg/mL & < 64% Recovery	7	6

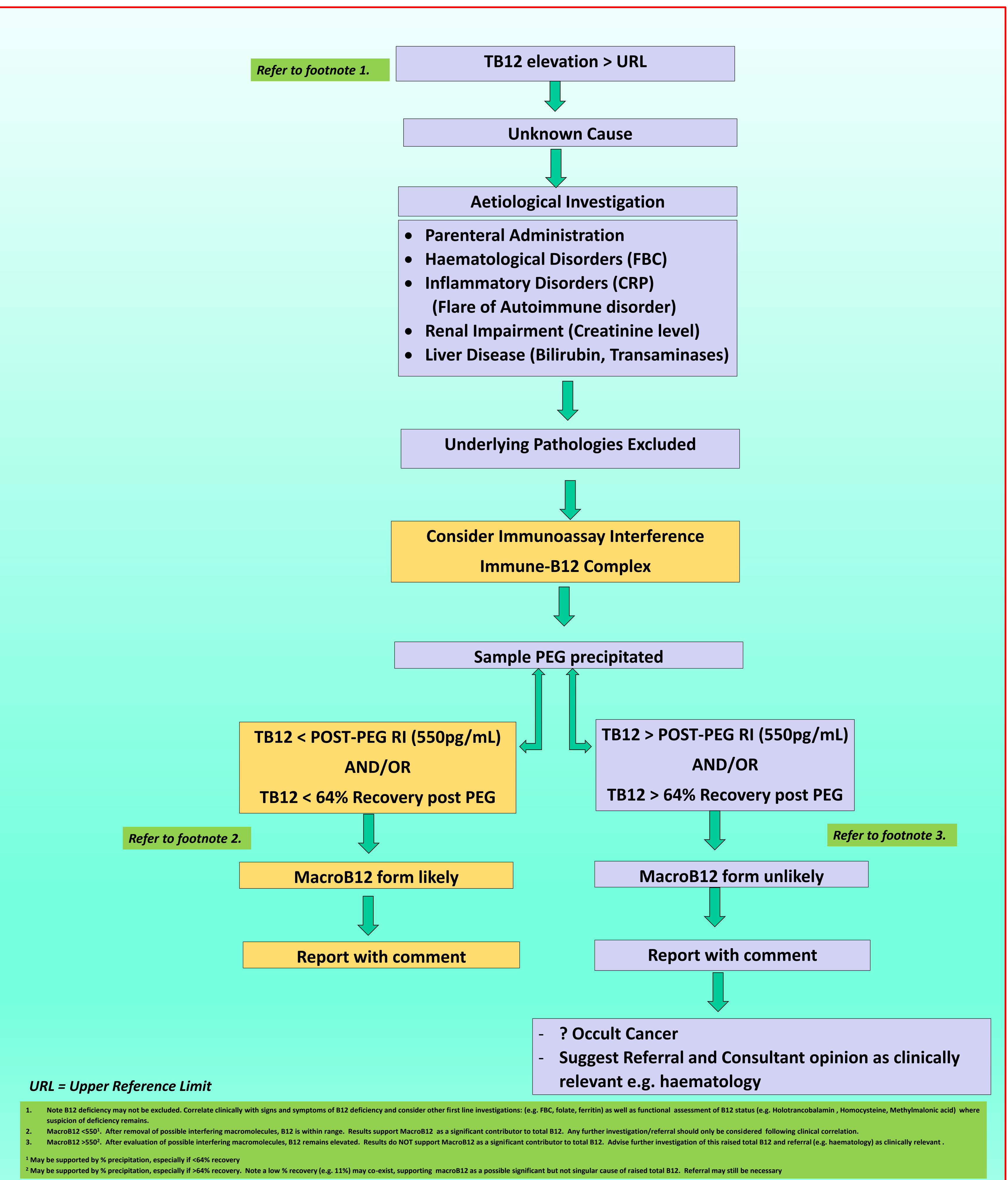


Figure 1. Proposed algorithm for raised B12 Investigations

## Discussion & Conclusion

Current diagnostic strategies for interpreting high B12 levels do not routinely consider MacroB12 interference. This is the first study we are aware of, that has established post-PEG B12 reference ranges for the Abbott Architect and has also estimated the prevalence of macroB12 in patients with raised total B12 using this methodology. Our estimated prevalence (7%) is however lower than reported previously using Roche Cobas study analysis which established a higher post PEG B12 RR (165-694 pg/ml) and recovery threshold (<68%).<sup>1</sup> We aim to corroborate our findings using Gel Filtration Chromatography and in the interim we propose an algorithm for the investigation of elevated B12 which incorporates macroB12 evaluation.

Although PEG precipitation may be considered a comparatively crude method for removing interfering macromolecules, it appears effective in this capacity for removing potentially interfering macroB12 molecules and shows potential for mitigating against misdiagnosis and mismanagement of patients with unexplained or clinically discordant raised total B12.