

Comparison of three PEG precipitation protocols for the detection of macrovitamin B12 interference

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Introduction

The presence of macrovitamin B12 (macroB12) in serum interferes in interpretation of total vitamin B12 (B12) result and can lead to misidentification of deficiency. Polyethylene glycol (PEG) precipitation is a non-specific method that removes macromolecules (protein polymers and antibody-bound analytes) allowing measurement of circulating vitamin B12 (i.e. B12 bound to transcobalamin and haptocorrin). Several PEG precipitation protocols have been described in the literature. The first aim of this study was the evaluation of the reproducibility of three different methods of PEG precipitation. The second aim was to compare the three protocols between them to determine whether they are interchangeable.

Material and Methods

We pooled sera from 124 random patient samples to obtain 5 pools of different concentrations (2165 ng/L, 1842 ng/L, 1480 ng/L, 790 ng/L and 522 ng/L).

Three protocols were compared:

- P1: PEG 6000 in distilled water, 1 hour at 37°C, centrifugation for 3 minutes at 2000 rpm
- P2: PEG 6000 in NaCl, 10 min at room temperature, centrifugation 30 min at 2000 rpm
- P3: PEG 8000 in PBS pH 7.4, 10 min at room temperature, centrifugation 30 min at 3715 rpm

Each pool was precipitated 20 times per protocol.

Total vitamin B12 was measured on the Abbott Alinity i system.

Statistical analysis:

The measurements are compared between protocols using the ANOVA test. The one-way Student's t-test is used to compare a measurement to its target value. The results are considered significant at a 5% uncertainty level ($p < 0.05$).

The calculations were performed using SAS statistical software version 9.4 and the graphs were created using R statistical software version 4.4.1.

Results

Three variables are described, namely the vitamin B12 measurement after precipitation (ng/L), the delta obtained by the difference between the pool value and the measurement, and the percentage loss given by the ratio $\text{delta}/\text{pool} * 100\%$. Repeatability was 6.75%, 6.70% and 6.61% for P1, P2 and P3 respectively.

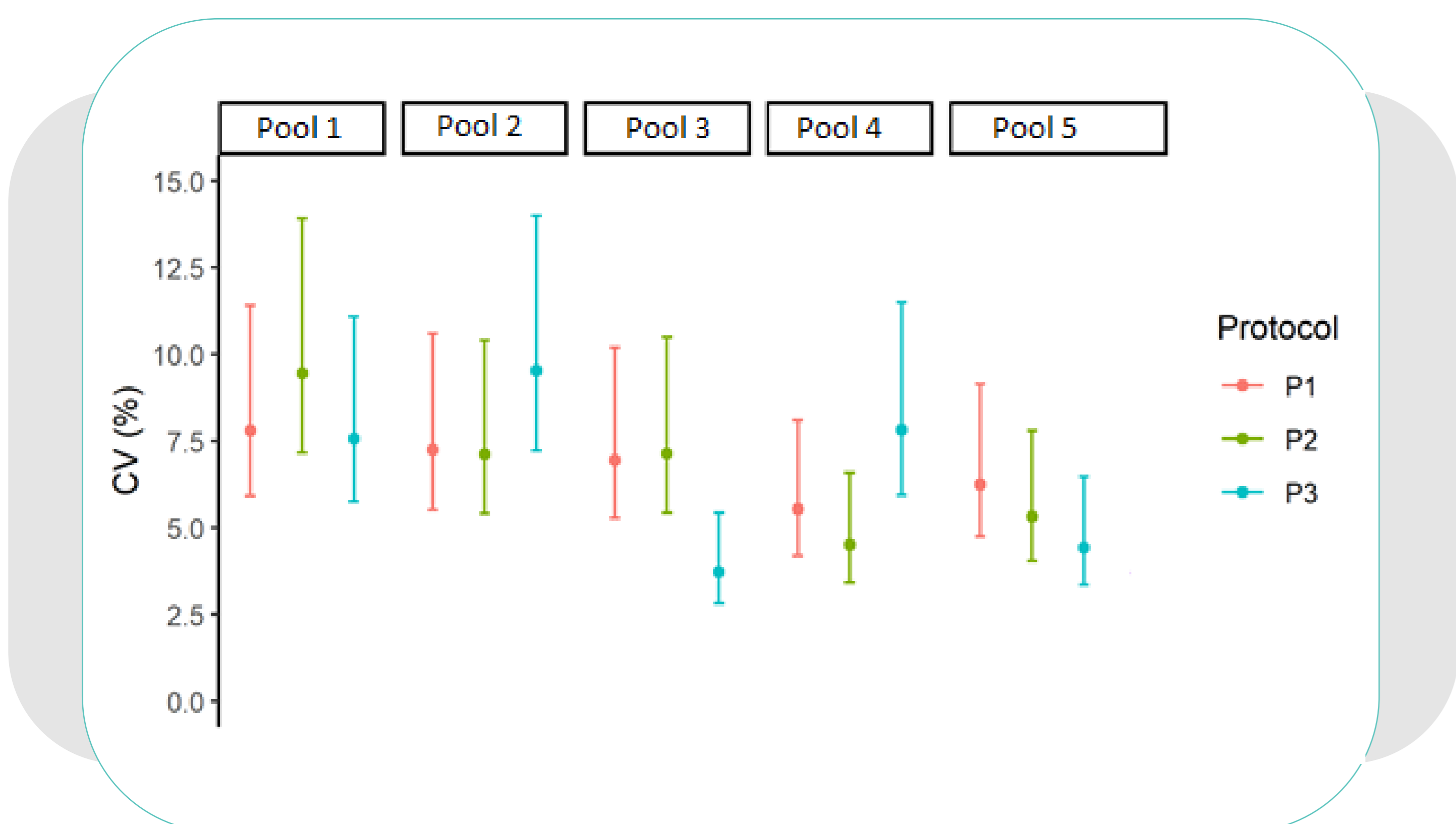


Figure 1 : Coefficient of variation (with 95% confidence interval) of the different protocols according to the pools

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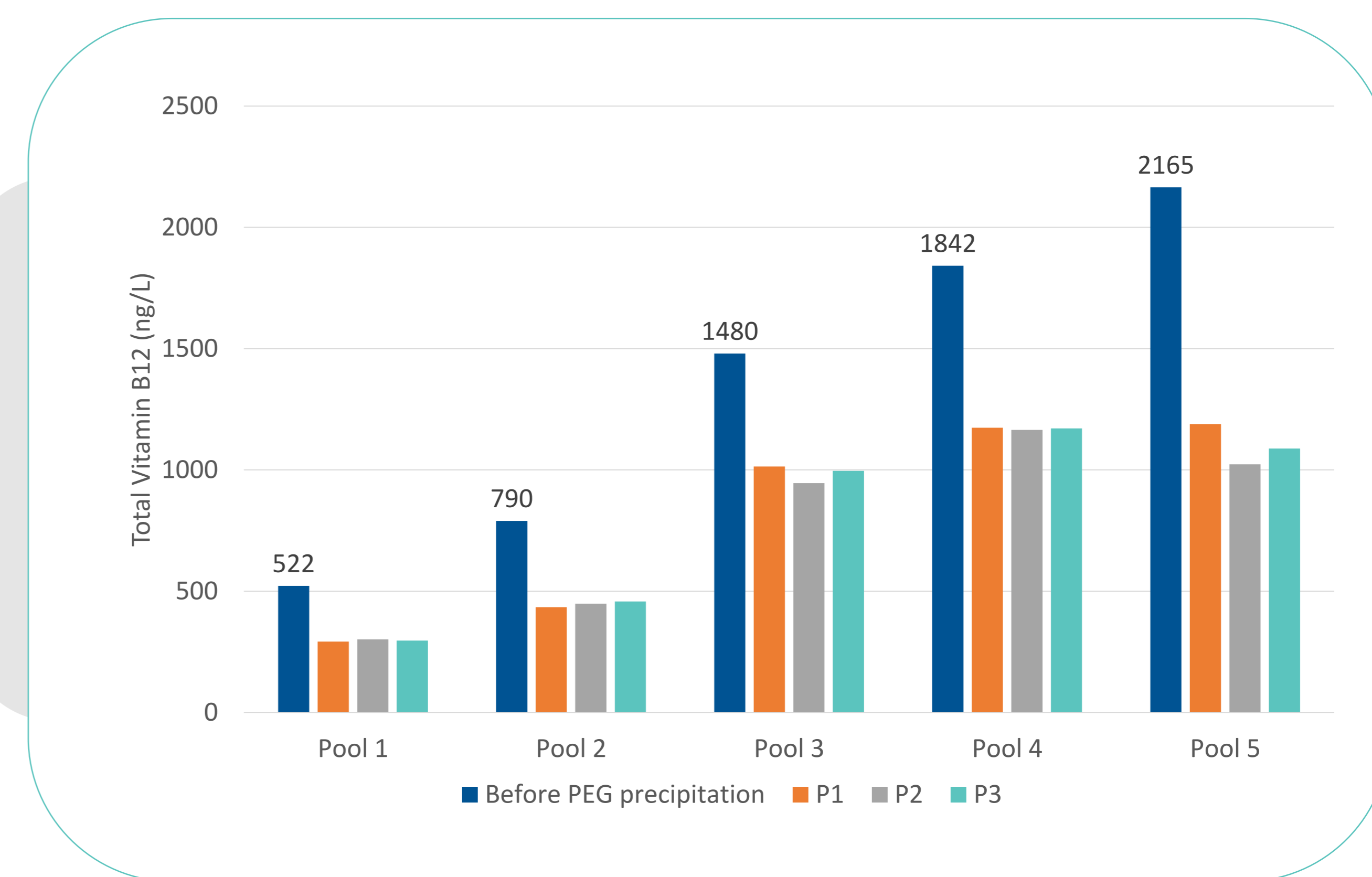


Figure 2: Comparison of vitamin B12 values before and after precipitation according to the three protocols

No significant differences were observed between the three protocols when comparing the delta using ANOVA. The mean percentage of B12 loss after PEG precipitation was respectively 59.6% (+/- 6.2%), 57.8% (+/- 6.7%) and 59.2% (+/- 6.5%) for P1, P2 and P3, respectively. Results after PEG precipitation are significantly different from results before PEG precipitation, in a systematic way, whatever the protocol used.

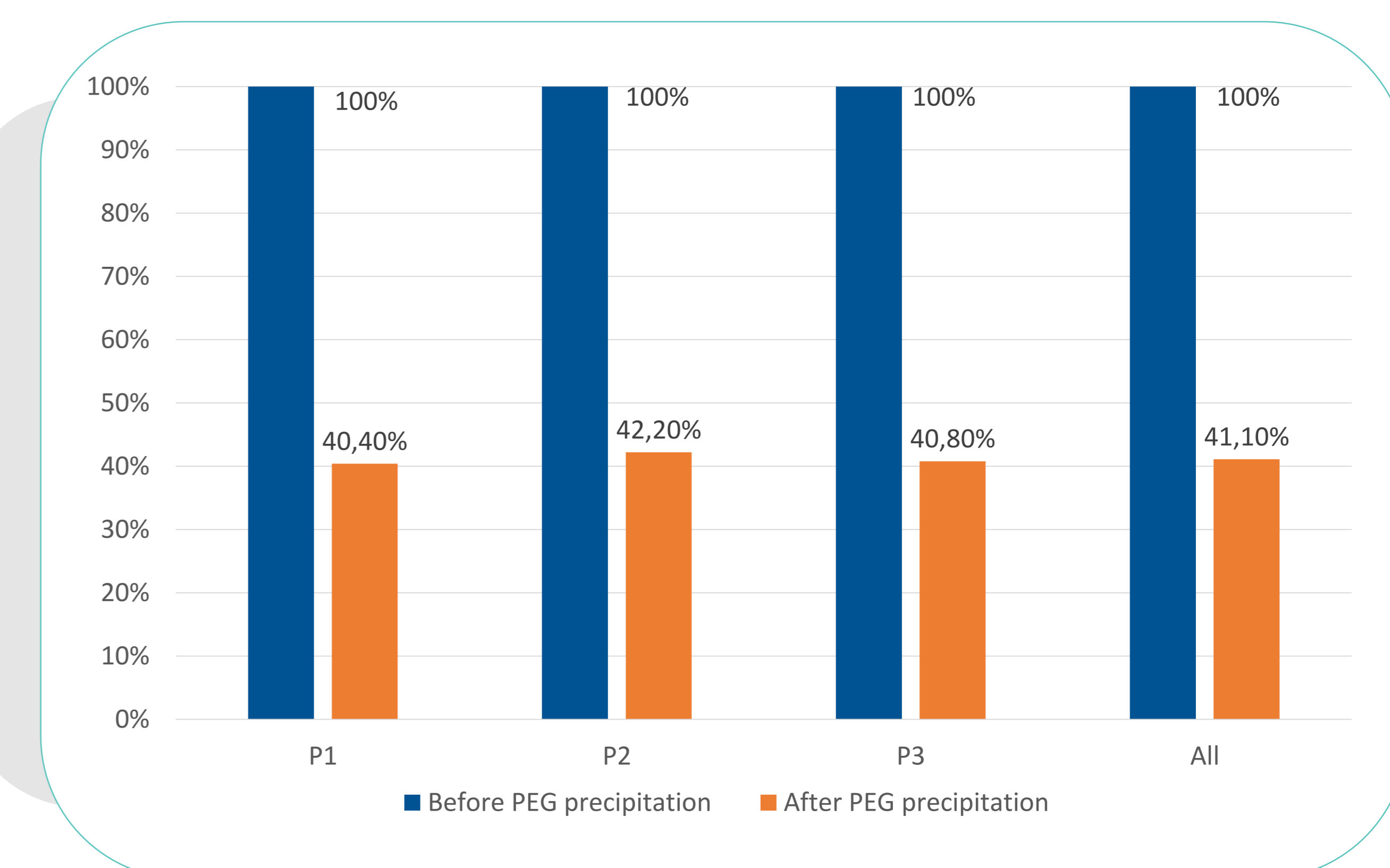


Figure 3: Recovery rate (%) of total vitamin B12 before and after PEG precipitation

Conclusions

The three PEG precipitation protocols demonstrated comparable and reproducible results and can therefore be used interchangeably, allowing comparison of results between laboratories for patient follow-up. On average, macromolecules represent 58.9% (+/- 6.5%) of the amount of B12, regardless of the initial concentration of B12. This high proportion underscores the risk of overlooking true deficiencies, as a normal total B12 result may falsely reassure clinicians. Since systematic PEG precipitation is not feasible in routine practice, alternative biomarkers such as holotranscobalamin, methylmalonic acid, or homocysteine, which are less affected by macromolecular interference, should be considered.