Intrinsic Factor Blocking Antibody Interference Is Not Detected In Five Automated Cobalamin Immunoassays

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Introduction

Several authors have recently reported false normal cobalamin (vitamin B12) results in patients diagnosed with pernicious anemia. Interference of native intrinsic factor blocking antibody (IFBA) with automated immunoassays, due to oxidized dithiothreitol, has been proposed as a cause of these falsely elevated results. Interference with cobalamin results is a concern, since almost 70% of pernicious anemia patients test positive for IFBA.

Objective

In light of a recent voluntary vendor recall of cobalamin reagent due to IFBA interference, our objective was to investigate five additional automated cobalamin immunoassays to determine if they were similarly affected.

Materials and Methods

• Six human serum pools were created (mean concentration):
  - High (>2000 pg/mL)
  - Normal (324 pg/mL)
  - Low (85 pg/mL)
  - High (>2000 pg/mL)
  - Normal (392 pg/mL)
  - Low (159 pg/mL)

Total cobalamin determined by Centaur XP
Cobalamin reference interval (210-911 pg/mL)
IFBA determined by ELISA, INOVA Diagnostics

• All serum pools were tested before and after serum immunoglobulin precipitation by 1:2 dilution with 25% polyethylene glycol (PEG) for total serum cobalamin, and for IFBA to confirm immunoglobulin precipitation.
• Sample pools were tested in triplicate on the same day by the following analyzers: Centaur XP and IMMULITE 2000 (Siemens Healthcare Diagnostics), ARCHITECT i2000SR (Abbott Diagnostics), UniCel Dxl 800 (Beckman Coulter Inc.), and Modular E170 (Roche Diagnostics). Kits donated by mfgs. listed.
• Total allowable error of mean ± 30% was used to evaluate changes in cobalamin concentrations.
• Serum from a patient with IFBA and untreated pernicious anemia which had previously generated false normal results with the Siemens Dimension Vista was tested untreated and PEG-treated with a representative assay (Centaur XP).

Results

• PEG treatment reduced IFBA results by 99%, which confirmed immunoglobulin precipitation.
• The average difference in measureable cobalamin concentration between the six untreated and PEG-treated pools using the five immunoassays was 16.9% (range 4.0 to 38.6%).
• All PEG-treated results were within the total allowable error (mean ± 30%) of the untreated pools, with one exception (low cobalamin - IFBA positive pool by the Centaur XP, 38.6%), however both treated and untreated pools would have qualitatively been classified as cobalamin deficient (<120 pg/mL).

• The low cobalamin - IFBA positive sample pool was below the AMR by the DxI (50 pg/mL) after PEG treatment. Correcting for dilution (x2), the sample result was presumed within the total allowable error of mean ± 30% (52 - 97 pg/mL).
• Similar results were observed for both the low and normal cobalamin - IFBA positive and negative, pools tested with the IMMULITE 2000. The lower limit of the AMR is 150 pg/mL, therefore correcting for dilution (x2) the normal cobalamin sample pools were presumed within the total allowable error of mean ± 30% (218 to 404 pg/mL and 272 to 506 pg/mL for IFBA positive and negative, respectively).

Conclusions

• Five automated cobalamin assays were evaluated; none showed a significant decrease in cobalamin concentration (mean ± 30%) after immunoglobulin precipitation by PEG.
• This was illustrated in serum pools, regardless of cobalamin concentration, along with a sample from a patient with untreated pernicious anemia which had previously generated false normal results with the Siemens Dimension Vista.
• Similar recoveries, regardless of IFBA presence or cobalamin concentration, suggest that these five automated cobalamin assays do not cross-react with IFBA.

While all results should be correlated with clinical signs and symptoms, laboratories may use this information to monitor clinical assay performance.