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Vitamin B12 immunoassay interference in a patient with multiple myeloma – troubleshooting in a two step reagent kit based on enhanced chemiluminescence testing

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ABSTRACT

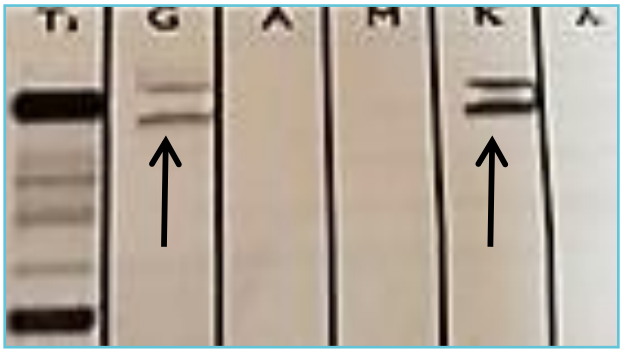
Immunoassays are widely used for quantification of serum analytes however they are subjected to interference by endogenous antibodies. The laboratory procedures used to identify these endogenous antibodies is the demonstration of response to dilution or use of nonimmunoglobulin protein to block the interfering antibodies or the use of an alternate immunoassay. We report a clinical-diagnostic situation where serum vitamin B12 determination was interfered in an immunoassay due to excess of endogenous antibodies from monoclonal gammopathy that resulted in excess of analyte concentration. Reporting of such cases may be beneficial when assaying sera of multiple myeloma to avoid false results and in addition to avoid costs due to unnecessary repeat testing and further delay reporting of results.

CLINICAL-DIAGNOSTIC CASE

Quantification of Serum B_{12} was ordered by the on-duty doctor from a forty-five years old female inpatient admitted at medical ward with provisional diagnosis of multiple myeloma.

The patient serum sample was processed for testing B_{12} using Vitros "ECiQ Immunodiagnostic system" Enhanced Chemiluminescence Immuno assay (ECI) after performing daily maintenance and running internal quality control samples from Bio-Rad, which were found to be within the acceptable range [Level 1 range = 268-608 pg/mL]. The analyser failed to give a result for the sample and the error message displayed "No Result." Repeated testing of the sample failed again. A new sample from the patient was requested and processed, yielding again no result. The test was repeated with a dilution series of this sample, after 25 folds dilution of the sample a result of 3512.0 pg/mL was obtained (Normal range: 239-931 pg/mL) [1].

Figure 1 Immunofixation showing monoclonal gammopathy in gamma globulin region and kappa light chains (*arrows*)



After reporting results to the clinician, the patient's medical records were reviewed, and it was observed that the patient was diagnosed with grade III Multiple Myeloma. Serum protein electrophoresis on cellulose acetate paper revealed a significant M-gradient. On immunofixation, the immunological nature of M-gradient was found to be a monoclonal gammopathy in gamma globulin region with corresponding light-chain correlates in kappa light chains [Figure 1]. Beta 2 microglobulin in serum was increased up to 11494 ng/mL (Reference: 609.0-2366.0 ng/mL) [1], based on which the grading of multiple myeloma was done. Beta 2 microglobulin was quantified using Siemens Nephelometer. There was absence of myeloproliferative disorder, eosinophilia, and iatrogenic intake of cyanocobalamin in this patient. Absence of heterophile antibody interference was confirmed using heterophile antibody blocking tube.

To eliminate the interference generated by endogenous antibodies from multiple myeloma, the serum of patient was then subjected to precipitation with Polyethylene Glycol (PEG) 6000 to remove interfering antibodies after performing control studies with another patient's serum

Table 1 Testing procedure and obtained values of serum vitamin B12

Testing of Serum B12	Value (pg/mL)
Undiluted serum sample (repeat testing)	Not determinable
Diluted serum sample (1:25)	3521
PEG precipitation (1:2)	887

(1:25) = 25 times dilution; (1:2) = 2 times dilution

sample. This resulted in the value of 887.0 pg/mL which was within normal range. (Normal range: 239-931 pg/mL) [1]. Values of serum vitamin B_{12} obtained after various methods applied in ECI is shown in Table 1.

DISCUSSION

Immunoassays are based on antigen-antibody reaction. Despite advances in immunoassay, they may be subjected to interference, depending on the assay used. According to International Federation of Clinical Chemistry (IFCC), interference in immunoassay is described as a systematic error of analytic measurement caused by a sample tested, which does not allow for a signal in the measuring system. [2] Interferences can rise from the presence of endogenous antibodies like heterophilic antibodies, anti-animal antibodies or autoantibodies. [3] In most cases, these interfering antibodies tend to produce false high values of the analyte tested. [4] Vitros Enhanced Chemiluminescence Immunoassay (ECI) is based on competitive binding immunoassay technique.

First, there is a competition between vitamin B_{12} present in the patient's serum sample with a Horseradish peroxidase (HRP)-labeled vitamin B_{12} conjugate for binding sites on a biotinylated *Intrinsic Factor*. When this competitive binding occurs, the vitamin B_{12} *Intrinsic Factor* complexes are formed which are captured by streptavidin on the wells. The complex bound with HRP thus formed is then measured by a luminescent reaction. This amount of complex bound with HRP is indirectly proportional to the concentration of vitamin B_{12} present. [5] We report a clinical-diagnostic situation where serum vitamin B_{12} testing was interfered in this immunoassay due to excess endogenous antibodies produced from monoclonal gammopathy which resulted in excess analyte concentration.

On average, 15-20 samples are analysed daily for Vitamin B_{12} in our Central Biochemistry Laboratory of the Institute of Medicine by Vitros Diagnostic immunoassay which is based on the principle of ECI. High serum vitamin B_{12} concentrations may be seen in myeloproliferative disorders, hyper eosinophilic syndromes, hepatic diseases, renal disease, and presence of heterophile antibodies or iatrogenic intake of cyanocobalamin for long duration. [6]

In this patient, myeloproliferative disorder was absent as bone marrow biopsy was normal. There was no raised eosinophil count in blood and patient had normal liver and renal function test. The patient was not taking cyanocobalamin during and six months before the sampling. Absence of heterophilic antibodies interference for the cause of the increased vitamin B_{12} in our patient was confirmed using heterophilic antibody blocking tubes. Possible cause for the excess vitamin B_{12} in this patient serum was due to high endogenous antibodies saturating all available sites on the IF in the first incubation step.

Subsequently, in the second incubation step there were no vacant sites on the IF for Vitamin B_{12} to bind. Thus, no signal was generated. In this case the patient has high endogenous antibodies from monoclonal gammopathy. Presence of excess IgG immunoglobulin also forms cobalamin complex and results in high value of vitamin B_{12} in serum and interferes with its assay. [7]

High vitamin B_{12} concentration in this patient was due to immune complexes composed of IgG as seen in immunofixation and to prove this we treated the serum with PEG and measured B_{12} in the supernatant. PEG precipitation test can be used as an alternative to size exclusion chromatography and is an easy method to confirm presence of immunocomplex. [8] An equal quantity of 25% PEG was added to 200 µl of the B_{12}-elevated serum sample.

After full mixing, the mixture was centrifuged at 1500×g for 30 minutes, and then the supernatant was isolated for B_{12} analysis. This gave a value of 887.0 pg/mL which was within the normal range. This decrease in serum B_{12} level after PEG treatment can be due to effect of PEG on assay system and not precipitation of interfering antibody.

So, control studies with other samples were done to investigate this observation and no effect of PEG was seen in the assay system. Thus, we present a case of positive interference in the Ortho B_{12} assay because of paraprotein interference in a patient with monoclonal gammopathy. To our knowledge, this particular paraprotein interference has not been previously reported.

Thus, for troubleshooting for assay interference when other samples can be quantitatively measured by two step reagent kit based on ECI, various steps can be taken.

Obtaining history of patient regarding the clinical status and dilution of sample should be done if there is a prior history of intravenous administration of Vitamin B_{12}. Likewise, the single step incubation method may be used. Others have also used heterophilic antibody blocking tubes, protein G–sepharose, size-exclusion High Performance Liquid Chromatography (HPLC), sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). [7]

But these methods are expensive and time consuming. The proper algorithmic knowledge is essential. Communication and collaborative approach between clinicians and laboratory staff is crucial in such cases yielding benefit for the patient. Furthermore, reporting these types of cases help clinicians and laboratory staffs to be aware of unnecessary repeated investigations or even false interpretation of results.

TAKE HOME MESSAGES/ LEARNING POINTS

- This case presents a positive interference in the Ortho B_{12} assay due to paraprotein interference in a patient with monoclonal gammopathy. Laboratory staff should be aware of such a phenomenon to avoid false results, unnecessary delay in reporting and unnecessary reagent wastage due to repeat tests.
- In such situations, the laboratory staff should consider dilution of sample and PEG precipitation before analysis.

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