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CASE REPORT

Undetectable Vancomycin Concentrations Utilizing a Particle Enhanced Turbidimetric Inhibition Immunoassay in a Patient with an Elevated IgM Level

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SUMMARY

Background: **A case of undetectable vancomycin concentrations with the use of a particle enhanced turbidimetric inhibition immunoassay is reported.**
Methods: **A 73-year-old woman with B-cell lymphoma, chronic neutropenia with myelodysplastic syndrome and elevated IgM levels displayed repeated undetectable vancomycin concentrations, despite appropriate empiric vancomycin dosing. The vancomycin concentrations were processed utilizing a particle enhanced turbidimetric inhibition immunoassay (PETINIA). Patients with high concentrations of paraproteins in their serum may have interference with the PETINIA. This may include patients with plasma cell dyscrasias and lymphoreticular malignancies associated with abnormal immunoglobulin synthesis.**
Results: **Repeated undetectable vancomycin drug concentrations prompted us to send a serum sample to an outside facility to utilize another standardized assay, enzyme multiplied immunoassay (EMIT), which resulted in a detectable vancomycin serum concentration. The patient's undetectable vancomycin drug concentrations with the PETINIA may have been due to abnormal immunoglobulin synthesis interference with the assay. A limited number of case reports have been published demonstrating undetectable or unexpectedly elevated vancomycin concentrations due to monoclonal immunoglobulin interference in patients with immunological disorders.**
Conclusions: **A 73-year-old woman with B-cell lymphoma, chronic neutropenia with myelodysplastic syndrome and elevated IgM levels may have had interference with a PETINIA resulting in undetectable vancomycin concentrations.**
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KEY WORDS

vancomycin, immunoassay, paraprotein, drug monitoring

INTRODUCTION

Vancomycin is a glycopeptide antibiotic that inhibits cell-wall synthesis. It is primarily indicated in patients with gram-positive infections involving methicillin-resistant *Staphylococcus aureus*. Pharmacokinetic studies in a variety of patient populations have demonstrated that a correlation exists between vancomycin trough concentrations and clinical efficacy and/or drug toxici-

ty. Although targeting vancomycin trough concentrations has been a debate for years, there is a clear relationship between drug concentration and clinical efficacy for vancomycin. A variety of commercial drug assays are available to test vancomycin drug concentrations, yet a specific assay has not emerged as a gold standard. Immunoassays are most often utilized to measure vancomycin concentrations as they are widely available and deliver results quickly. Clinicians should be aware of the limitations of these different assays and potential for erroneous results [1].

Only a few cases have been published describing undetectable or surprisingly elevated vancomycin concentrations due to monoclonal immunoglobulin interference in patients with immunological disorders. We describe a case where the vancomycin trough concentration was undetectable in a patient with B-cell lymphoma and chronic neutropenia and elevated IgM levels utilizing the particle enhanced turbidimetric inhibition immunoassay.

CASE REPORT

A 73-year-old African American woman with a history of small mature B-cell lymphoma/chronic lymphocytic leukemia with plasmacytoid differentiation and chronic neutropenia (baseline WBC 1 - 2 x 10^9/L) with myelodysplastic syndrome following chemotherapy completion in 2007, presented to our institution's emergency department with 10 days of increasing congestion, productive cough, right sided pleuritic chest pain, fever and chills for approximately 3 days. The patient's past medical history was significant for atrial fibrillation, coronary artery disease, hypertension, and hyperlipidemia. On admission, she was febrile (38°C), white blood cell count was 0.7 x 10^9/L, hemoglobin 7.2 g/dL, hematocrit 22%, platelet count 179 x 10^9/L, blood urea nitrogen 28 mg/dL, creatinine 1.4 mg/dL (baseline creatinine 1.0 mg/dL). The patient weighed 68.9 kg upon admission and height was 160 cm. The patient's immunoglobulins were measured during a previous hospitalization 3 years prior and IgG was normal at 1160 mg/dL (reference: 588 - 1573 mg/dL), IgM was elevated at 1370 mg/dL (reference: 57 - 237 mg/dL), and IgA was low at 32 mg/dL (reference: 46 - 287 mg/dL). Unfortunately, these were the most recent immunoglobulin studies at the time. In the emergency department she received a one-time 400 mg dose of intravenous moxifloxacin and a 2 liter bolus of 0.9% sodium chloride. A complete list of home medications prior to the patient's admission appears in Table 1. Medication allergies included penicillin (reaction: shortness of breath) and simvastatin (reaction: headaches).

On hospital day 1, the patient was empirically initiated on intravenous vancomycin with an initial dose of 1,250 mg (18 mg/kg), intravenous aztreonam 2,000 mg every 8 hours, oral doxycycline 100 mg every 12 hours, and oral oseltamivir 75 mg twice daily for treatment of right-lower-lobe pneumonia, sepsis in the setting of neutropenic fever, and urinary tract infection (Urinalysis: 3+ leukocytes, nitrite negative, 79 WBCs, and > 50 bacteria).

On hospital day 2, respiratory virus PCR, influenza A, B and respiratory syncytial virus were negative and oseltamivir was discontinued. Pneumocystis PCR, pneumococcal and legionella urine antigens were negative. Filgrastim 300 mcg daily was initiated due to acute on chronic neutropenia. The decision was made to start a vancomycin maintenance dose but due to acute kidney injury with rise in serum creatinine to 1.6 mg/dL, a random vancomycin concentration was ordered before initiating due to a dose administered the previous day. A sample was collected approximately 22 hours after the initial vancomycin dose in order to obtain a better understanding of her clearance and surprisingly, was found to be non-detectable (< 3.5 mcg/mL). As the result was unexpected, the pharmacist was concerned that there may have been an error with the dose and/or sample and so a 1000 mg (~15 mg/kg) vancomycin dose was ordered with a plan to obtain another random vancomycin concentration to check clearance.

On hospital day 3, serum creatinine had decreased to 1.2 mg/dL and a random vancomycin concentration obtained 11 hours after the 2nd dose of vancomycin was again reported as non-detectable (< 3.5 mcg/mL). At this point, the pharmacist was perplexed as to why the concentrations were undetectable. Did the patient have a significantly altered volume of distribution or clearance? Due to the patient's improving renal function and the concern for the severity of the situation, the pharmacist decided to implement an aggressive empiric vancomycin regimen with the goal to obtain troughs ~ 15 mcg/mL. Therefore, a 1500 mg (22 mg/kg) vancomycin dose was ordered to be followed by 1000 mg (~15 mg/kg) every 12 hours and another trough ordered prior to the 3rd dose of this regimen.

On hospital day 4, serum creatinine continued to improve to baseline of 0.9 mg/dL and vancomycin trough concentration was again reported as non-detectable (< 3.5 mcg/mL). With no explanation for the non-detectable concentration, the pharmacist contacted the laboratory department for input. It was determined that our institution utilizes a particle enhanced turbidimetric inhibition immunoassay to test vancomycin concentrations and the concern for assay interference was hypothesized. A decision was made to send a sample to another institution to utilize a different method of measurement, the enzyme multiplied immunoassay technique. Unfortunately, we did not retain a sample obtained at the same time to compare results between the two methods utilized. The vancomycin trough was collected just prior to the 3rd dose of the regimen as planned and was then sent to the outside laboratory.

On hospital day 5, serum creatinine remained stable at 1.0 mg/dL. We were unable to obtain the outside laboratory results for 24 hours. Therefore, with the concern of vancomycin accumulation due to entering the 5th day

Table 1. Prior to admission medications and medications at the time of initial vancomycin administration.

Medications prior to admission	Inpatient medications at time of initial vancomycin administration
metoprolol tartrate 12.5 mg every 12 hours	aztreonam 2,000 mg every 8 hours
amlodipine 5 mg once daily	doxycycline 100 mg every 12 hours
ferrous sulfate 325 mg three times daily	oseltamivir 75 mg twice daily
potassium chloride 10 mEq once daily	filgrastim 300 mcg daily
multivitamin once daily	aspirin 81 mg once daily
omega-3-fatty acids 1,000 mg once daily	pantoprazole 40 mg once daily
omeprazole delayed release 40 mg once daily	enoxaparin 40 mg once daily
aspirin 81 mg once daily	docusate 100 mg every 12 hours
	ferrous sulfate 325 mg twice daily
	potassium chloride 10 mEq once daily
	acetaminophen 650 mg every 6 hours PRN
	albuterol 0.083% nebulized solution four times daily PRN
	aluminum & magnesium hydroxide-simethicone 400 - 400 - 40 mg suspension every 6 hours PRN

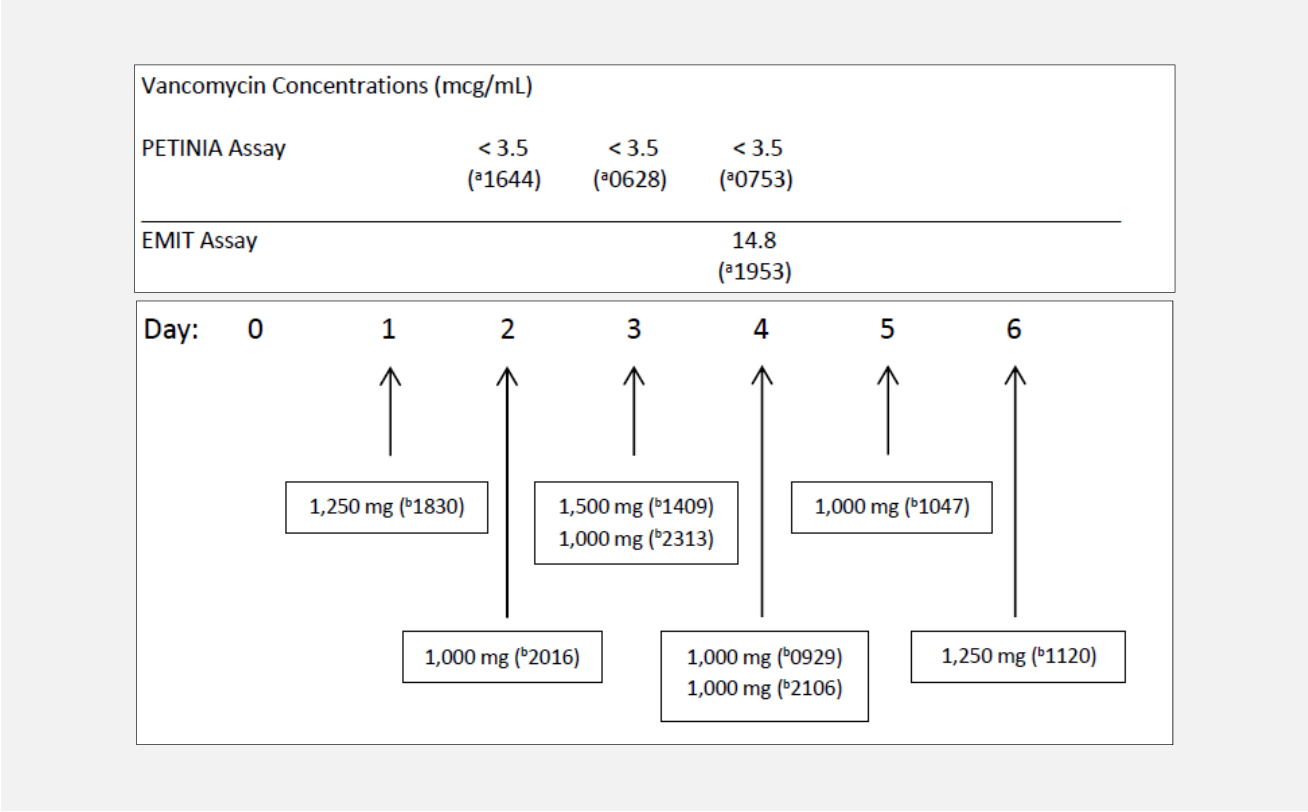

Figure 1. Timeline from admission to discharge of vancomycin administration and concentration.

[a] - Time sample collected, [b] - Time vancomycin dose administered.

of vancomycin treatment with no therapeutic drug monitoring guidance available, the decision was made to adjust the vancomycin dose based on population pharmacokinetics to 1250 mg (18 mg/kg) every 24 hours.

On hospital day 6, the trough concentration result from the outside laboratory became available and revealed a result of 14.8 mcg/mL, which was obtained 10 hours post-dose. This result, while not confirmatory without a

companion sample, strongly suggested that our institutions assay was confounded with interference. While the concentration was practically at goal level of 15 mcg/mL, it was noted to have been obtained 2 hours early. Because it was believed that accumulation would or had occurred, the decision was made to return the patient to the same regimen that produced the concentration of 14.8 mcg/mL, which was 1000 mg every 12 hours. The plan was to complete a 10 day course with no more concentrations to be measured unless renal function or clinical situation changed. Shown in Figure 1 are the doses and times of vancomycin administration as well as vancomycin concentrations collected from Day 0 to Day 6 of the patient's hospitalization.

DISCUSSION

There are several types of immunoassays commonly utilized to measure vancomycin concentrations. These include particle enhanced turbidimetric inhibition immunoassays (PETINIA), enzyme multiplied immunoassay technique (EMIT) competitive enzyme-linked immunoassays (C-ELISA), and fluorescence polarization immunoassays (FPIA). These immunoassays are generally robust and accurate and are the preferred method to measure vancomycin concentrations as they have rapid turnaround times and are widely available. Although rare, there can be interferences with these measurements and clinicians should be aware of their limitations. The two assays utilized in this case report are the PETINIA and the EMIT.

Particle enhanced turbidimetric inhibition immunoassays work by allowing particle-bound vancomycin to bind to specific antibodies in the sample, resulting in an insoluble aggregate which causes light scatter. Non-particle-bound vancomycin in the patient sample competes with the particle-bound drug for the antibody binding sites, inhibiting formation of the insoluble aggregates. The rate and amount of the particle aggregation is inversely proportional to the concentration of vancomycin in the sample [2].

Enzyme multiplied immunoassay technique works by way of competition between vancomycin in the patient's sample and vancomycin labeled with G6P-DH for antibody binding sites. G6P-DH decreases when bound to the antibody, so vancomycin concentrations can be measured in terms of G-P-DH activity [3].

Although rare, it is known that paraproteins, also called monoclonal immunoglobulins, have been found to interfere with clinical chemistry assays, leading to erroneous results. Included in these interference reports are spuriously elevated concentrations of total bilirubin and spuriously low direct bilirubin and high-density lipoprotein-cholesterol [4].

Even less well known, paraproteins can cause interference with vancomycin concentration measurements with a PETINIA through a few mechanisms: production of turbidity in the specimen during the course of reaction or analysis, binding of the paraprotein to a component of the assay system and/or binding of the paraprotein to the analyte itself. Paraproteins can lead to either falsely low or falsely high vancomycin concentrations. They can lead to falsely low vancomycin concentrations by promoting aggregation via non-specific mechanisms. They can lead to falsely high vancomycin concentrations by inciting error codes indicating aggregation rates that exceed those expected even in the absence of vancomycin. Patients with high concentrations of paraproteins in their serum may have interference with the particle enhanced turbidimetric inhibition immunoassay. This may include patients with plasma cell dyscrasias and lymphoreticular malignancies associated with abnormal immunoglobulin synthesis, such as multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease [5].

Our institution utilizes a PETINIA (Beckman Coutler, UniCel® DxC 600/800) platform to measure vancomycin concentrations. Per the manufacturer, interference may occur with serum samples from patients diagnosed with plasma cell dyscrasias and lymphoreticular malignancies associated with abnormal immunoglobulin synthesis, such as multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease. The manufacturer recommends that these samples be run by an alternate method [2].

In our case, the investigation of possible reasons for our patient's surprisingly low vancomycin concentrations led us to perform a literature search in which we discovered the possibility of interference with the assay that was originally used. We discovered that the PETINIA platform that our institution utilized could potentially be measuring a falsely low vancomycin concentration due to interference with the patient's high IgM levels. Although the immunoglobulin results we had were from 3 years previous, we thought this might be a possibility. Unfortunately, the immunoglobulins were not re-tested until a month later to confirm, but the results revealed an even higher IgM level of 1740 mg/dL (compared to 1370 mg/dL in 2012).

The pharmacist and lab analytical specialist collaboratively decided to send the patient's serum samples to another local laboratory for result confirmation by an alternative assay method. In this case, the enzyme multiplied immunoassay (EMIT) method (Ortho Clinical Diagnostics, Vitros 5600) was utilized. It uses antibodies that are specifically designed to bind the molecule(s) of interest (analyte) without binding to other substances in the sample. Its unique feature is the ability to detect this binding without resorting to a cumbersome separation of the bound component [3]. Unfortunately, the confirmatory results were delayed in returning to our institution and it was not until Day 6 of admission that we had accurate concentrations on which to base our therapeutic drug dosing. The confirmatory results revealed a vancomycin trough concentration that was not only detectable but also realistic. While we did not have a companion vancomycin concentration processed on our in-

stitution's assay from a sample obtained at the same time, we believe that the confirmatory results could be used to deduce that there was assay interference with our institution's PETINIA assay. We believe that the reason the vancomycin concentration was undetectable with the particle enhanced turbidimetric inhibition immunoassay utilized at our institution was due to the patient's abnormal immunoglobulin synthesis interference.

Competitive enzyme-linked immunoassays (C-ELISA) work by the involvement of at least one antibody with specificity for a particular antigen. Antigens from the sample are attached to a surface. Then, a further specific antibody is applied over the surface so it can bind to the antigen.

Fluorescence polarization immunoassay (FPIA) is a homogeneous competitive immunoassay method based on the increase in fluorescence polarization (FP) of fluorescent-labeled small antigens when bound by a specific antibody. This methodology does not use agglutination as the detection principle and therefore interference from paraproteins should not have an impact with results. However, there can be background interference in the serum sample and it requires a blank measurement.

Review of the Literature

Simons, et al. reported two cases of unusual vancomycin concentrations resulting from presumed paraprotein nonspecific aggregation leading to interference performed with a PETINIA. One case involved a patient with lymphoplasmacytic lymphoma and a surprisingly low vancomycin concentration, while the other case involved a patient with non-Hodgkin's lymphoma and a surprisingly high vancomycin concentration. Both patients had conditions associated with elevated IgM antibodies. Subsequently, both of these patients had specimens re-measured by using enzyme-multiplied immunoassay technique (EMIT). Resulting concentrations were consistent with the patient's renal function and dosing regimen [6].

LeGatt, et al. briefly described two cases in which inappropriately low vancomycin concentrations were obtained in patients when a PETINIA was utilized. One patient had a history of non-Hodgkin's lymphoma, hemolytic anemia, and systemic lupus erythematosus. When serum samples were analyzed with an alternative PETINIA and a FPIA, resulting vancomycin concentrations were supra-therapeutic. The other patient had a history of Felty syndrome, which is associated with seropositive rheumatoid arthritis. Subsequently, LeGatt, et al. conducted a study to evaluate the effect of different paraproteins (IgA, IgG, and IgM) and rheumatoid factor had on various vancomycin assays-FPIA, EMIT, and 2 PETINIA assays. They determined that IgA and IgG did not affect any of the vancomycin immunoassays. Rheumatoid factor did not interfere with vancomycin in plasma specimens but did result in elevated vancomycin concentrations in serum specimens in all assays. IgM did decrease vancomycin concentrations when the PETINIA was utilized. IgM did not affect the FPIA and the EMIT methods [7].

Gunther, et al. described two cases of undetectable vancomycin concentrations with a PETINIA. The first case involved a patient with multiple immune-related comorbidities resulting in elevated IgM levels. Subsequent appropriate vancomycin dosing led to undetectable or unsuspectingly low vancomycin concentrations. Eventually, the patient's serum was sent to an alternative laboratory and revealed supra-therapeutic levels with another brand of PETINIA and also with a FPIA. The second case involved a patient with Felty syndrome, characterized by presence of high levels of IgG and rheumatoid factor. After appropriate dosing of vancomycin was initiated, vancomycin concentrations were undetectable. Samples were then analyzed with a FPIA, with resulting therapeutic concentrations reported [8].

Florin, et al. reported a case of IgM interference with a competitive chemiluminescent microparticle immunoassay. The case involved a patient diagnosed with Waldenstrom's disease and a surprisingly elevated vancomycin concentration on the first day of therapy. Upon further dilution, lower initial concentrations were confirmed. Serum samples drawn prior to vancomycin initiation revealed elevated vancomycin concentrations, confirming interference with the assay. The patient's elevated monoclonal IgM concentrations were the suspected culprit. In order to eliminate the interference with IgM, the unbound vancomycin concentrations were measured after ultrafiltration of the patient's serum. The unbound concentrations were then utilized to calculate a total vancomycin concentration [9].

CONCLUSION

A 73-year-old woman with B-cell lymphoma, chronic neutropenia with myelodysplastic syndrome and elevated IgM levels displayed repeated undetectable vancomycin concentrations, despite appropriate empiric vancomycin dosing. The vancomycin concentrations were processed utilizing a PETINIA. Repeated undetectable vancomycin drug concentrations prompted us to send a serum sample to an outside facility to utilize another standardized assay, EMIT, which resulted in a detectable vancomycin serum concentration. Clinicians should be aware of the type of immunoassay that is utilized by their clinical laboratory to measure vancomycin concentrations and possible limitations or interferences that may occur. In our case, we were able to collaborate to determine a possible assay interference in which the patient's vancomycin concentration was undetectable with a PETINIA, possibly due to abnormal immunoglobulin synthesis interference. It is beneficial to develop a rapport between the clinician and laboratory personnel in order to investigate and communicate unexpected laboratory findings. If concentrations consistently do not represent what the clinician believes to be an accurate representation of the patient's clinical picture,

it may be helpful to communicate with the laboratory personnel to further investigate possible assay interferences leading to erroneous concentration findings. If possible, an alternative assay method should be utilized to confirm the results.

Key Points:

- There are several types of immunoassays utilized to measure vancomycin concentrations that are generally robust, accurate, have rapid turnaround times and are widely available.
- Although rare, there can be interferences with these assays and clinicians should be aware of their limitations.
- A few cases have described undetectable or surprisingly elevated vancomycin concentrations measured by immunoassays, which has been attributed to monoclonal immunoglobulin interference.

Declaration of Interest:

None of the authors have any conflict of interest that should be disclosed.

References:

1. Rybak MJ, Lomaestro BM, Rotschafer JC, et al. Therapeutic monitoring of vancomycin in adult patients. Am J Health-Syst Pharm 2009;66:82-98 (PMID: 19106348).

2. Synchron System(s) Chemistry Information Sheet. Vancomycin [package insert]. Brea, CA:Beckman Coulter, Inc;2014. Available at: https://www.beckmancoulter.com/wsrportal/techdocs?docname=/cis/A18566/%25%25/EN_VANC.pdf

3. Vitros Chemistry Products VANC Reagent [package insert]. Rochester, NY: Ortho-Clinical Diagnostics; 2015. Available at: https://www.cmmc.org/cmmclab/IFU/VANC_J21505_EN_I.pdf

4. Yang Y, Howanitz PJ, Howanitz JH, et al. Paraproteins are a common cause of interferences with automated chemistry methods. Arch Pathol Lab Med 2008;132:217-23 (PMID: 18251580).

5. Bakker AJ, Mucke M. Gammopathy interferences in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-3 (PMID: 17635066).

6. Simons SA, Molinelli AR, Sobhani K, et al. Two Cases with Unusual Vancomycin Measurements. Clinical Chemistry 2009; 55(3): 578-82. Available at: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.492.7446&rep=rep1&type=pdf

7. LeGatt DF, Blakney GB, Higgins TN, et al. The effect of paraproteins and rheumatoid factor on four commercial immunoassays for vancomycin: implications for laboratorians and other health care professionals. Ther Drug Monit 2012;34:306-11 (PMID: 22569352).

8. Gunther M, Saxinger L, Gray M, Legatt D. Two suspected cases of immunoglobulin-mediated interference causing falsely low vancomycin concentrations with the Beckman PETINIA method. Ann Pharmacother 2013;47:e19 (PMID: 23512662).

9. Florin L, Vantilborgh A, Pauwels S, Vanwynsberghe T, Vermeersch P, Stove V. IgM interference in the Abbott Vanco immunoassay: A case report. Clinica Chimica Acta 2015;447:32-33. Available at: https://www.researchgate.net/publication/276922494_IgM_interference_in_the_Abbott_iVanco_immunoassay_A_case_report