Endogenous Antibody Interference in Immunoassays

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Abstract

Immunoassays are important clinical tools for detecting drugs, tumor markers, hormones, and other proteins. Endogenous antibodies can interfere with these assays by blocking available antigen-binding sites thereby reducing detection of analyte or by linking components of the assay thereby feigning detection. Many methods exist to investigate, neutralize, or remove antibody interference in clinical testing. These include: (1) dilution of interfering antibodies and (2) application of Heterophilic Blocking Tubes. A 52-year-old patient with cardiac history presented to Fairview Southdale Hospital with chest pain and his plasma samples were subjected to troponin immunoassay. Although the troponin results were elevated, the patient's clinical presentation did not correlate with the immunoassay results. Aliquots of the patient's sample were sent to the Mayo Clinic for troponin testing using various techniques, all of which yielded normal results. These results suggest that antibody interference was responsible for the detection of troponin in this patient's sample. Using samples from the above patient as an example, this study discusses multiple techniques for the removal or neutralization of heterophile antibodies from blood samples prior to troponin immunoassay.

Background

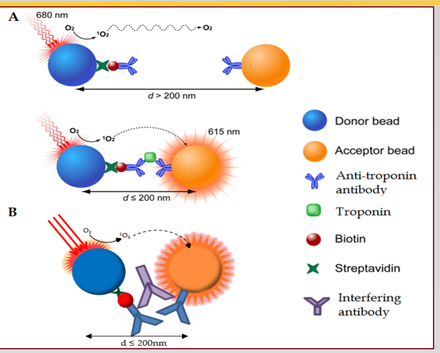

Figure 1. A. Sandwich immunoassay with LOCI® technology. Light stimulation of the donor bead excites oxygen which is transferred to the acceptor bead causing fluorescence if the analyte is present. If the analyte is not present, the excited oxygen does not cause fluorescence. **B.** Crosslinking of donor and acceptor bead due to interfering antibody which binds immunoassay antibodies allowing excited oxygen species to transfer to fluorescent acceptor bead. Adapted from Figure 1 of *Wu, F., Wang, L., Guo, Q., Zhao, M., Gu, H., Xu, H., & Lou, J. (2016). A Homogeneous Immunoassay Method for Detecting Interferon-Gamma in Patients with Latent Tuberculosis Infection. Journal of Microbiology and Biotechnology, 26(3), 588–595. https://doi.org/10.4014/jmb.1507.07102*

Immunoassays using anti-human antibodies against drugs, tumor markers, hormones, and other proteins are common practice in the modern clinical lab. Causes of immunoassay interference include endogenous heterophilic antibodies (HA) or human anti-animal antibodies (HAAA) that facilitate binding of components of the assay or interfere with binding between test antibodies and target antigens resulting in falsely elevated or decreased results, respectively.[1] One simple way to investigate whether or not interfering antibodies are present in an immunoassay is sample dilution. Typically, when interfering antibodies are present, serial dilution and assay of the sample will not produce a linear curve representative of analyte concentration.[2] Various methods have been developed to inhibit or remove interfering antibodies from immunoassay procedures. One method employs the Heterophilic Blocking Tube (HBT) to neutralize interfering antibodies. These tubes, manufactured by Scantibodies (Santee, CA), contain proprietary lyophilized reagent which is incubated with sample to neutralize interfering antibodies.[3-5] One issue with HBT is that human anti-murine antibodies (HAMA) have not been shown to be effectively neutralized.[2] Cardiac troponin I is a regulatory protein in cardiac muscle which can indicate heart damage if elevated.[3,6] Measurement of cardiac troponin I often relies on immunoassay and the presence of interfering antibodies in patient samples has been shown to produce false elevations in patient analyte in these types of assay.[1,3] A 52-year-old male patient with history of acute coronary syndrome presented to Fairview Southdale Hospital (Minneapolis, MN) with non-exertional chest pain. Cardiac troponin I was measured by immunoassay using a plasma sample obtained from on this patient. The result was elevated at 0.074µg/L (reference range: 0-0.045µg/L) using the Siemens Dimension Vista® System Flex® reagent cartridge (Newark, DE).[6] However, EKG and other clinical features did not indicate a cardiac event. The sample underwent further high sensitivity testing at the Mayo Clinic and troponin I levels were normal, so antibody interference was suspected in the patient's initial immunoassay at the Fairview laboratory. The Siemens Dimension Vista® System Flex® reagent cartridge, utilizes LOCI® technology in the form of chemiluminescent sandwich immunoassay with biotinylated monoclonal murine antibodies (Fig. 1).[6,7] This study investigates the possibility that interfering antibodies prevented accurate measurement of troponin by immunoassay and suggests methods of investigating and neutralizing antibody interference.

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Methods

Frozen lithium heparin plasma samples from the patient with suspected interfering antibodies were thawed. Three control samples from patients at Fairview Southdale Hospital were obtained: Control 1 with a slightly elevated troponin value (~1.0 ng/mL), Control 2 with moderately evelated troponin value (~5 ng/mL) Control 3 near the high end of linearity (~35 ng/mL). These three samples were used as control samples with no suspected antibody interference. Troponin I was measured using the Dimension Vista® 500 (Serial #DV331009) with the Cardiac Troponin I Flex® reagent cartridge (Siemens, Newark, DE)[6]. All tests used the same reagent lot number (19092BC). One milliliter of plasma from each of the four samples was incubated for an hour in Heterophilic Blocking Tubes (Scantibodies, Santee, CA)[5] and measured for troponin before and after. The patient's plasma sample was diluted by 1/4, 1/2 and 3/4 in Dimension® EXL™ CTNI Sample Diluent (Siemens, Newark, DE).[6] Undiluted sample, the three dilutions, and the diluent were tested for troponin levels, and a regression analysis was performed.

Results

	Pre-HBT troponin (ng/mL)	Post-HBT troponin (ng/mL)
Control 1	1.046	1.012
Control 2	4.406	4.272
Control 3	35.672	34.358
Patient	0.074	0.071

Table 1. Troponin concentrations of controls and patient sample before/after treating with Heterophilic Blocking Tubes.

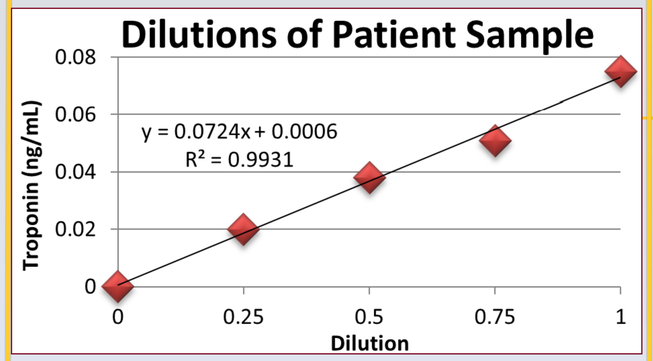

Figure 2. Troponin concentrations of undiluted patient sample, 3/4 dilution, 1/2 dilution, 1/4 dilution, and diluent. Regression yielded an R^2 value of 0.9931.

Discussion

The controls did not change much with HBT treatment, which is expected since it is unlikely that these samples have HA or HAMA. However, the patient sample also did not significantly change as it is still an elevated troponin result. Since it has been shown that HAMA are often not effectively neutralized with HBT,[2] and the troponin assay uses monoclonal murine antibodies,[6] it is possible that the patient's interfering antibodies are HAMA. Furthermore, the troponin values diluted in a linear fashion, which could suggest that there are no interference in the sample. However, the affinity of both the immunoassay antibodies and endogenous antibodies can affect linearity with dilution, so linearity does not prove absence of an interfering antibody.[2]

Other methods to consider when interfering antibodies remain an issue after HBT include: (1) protein A/G chromatography and (2) neutralization of HAMA by purified murine immunoglobulin. For experiments utilizing protein A/G columns: 1.5mL of sample and 1.5mL of Thermo Scientific Binding Buffer (0.1M phosphate, 0.15M NaCl, pH 7.2 PBS) will be mixed together and tested for troponin. This will be repeated for Controls 1-3. Then, 2mL of each mixture will be added to separate equilibrated NAb™ Protein A/G Spin Columns (Thermo Scientific, Rockford, IL).[8] The columns will be centrifuged for 1min at 1,000xg. The flow-through from each column will then be tested for troponin levels. In the case of neutralization of interfering HAMA: 1mg of purified lyophilized murine IgG (Sigma-Aldrich, St. Louis, MO)[9] will be added to 1mL of each of the patient sample, the three controls samples, and Dimension® EXL™ CTNI Sample Diluent (Siemens, Newark, DE),[6] and incubated for an hour at room temp. Then each sample will be tested for troponin level.

It is expected that dilution of sample with Binding Buffer will not affect the assay, so the troponin values of controls and sample before the spin column should be half of the undiluted values. After the samples are spun through the column, control troponin values should not change, but the patient sample should have a significantly decreased troponin value since the column should have removed the interfering antibodies. It is unknown whether troponin will be bound by the column, so it is important to run control samples through the column. If this column does bind troponin, another spin column with a different matrix should be considered, or perhaps protein A/G chromatography is not compatible with troponin immunoassays. Also, addition of the lyophilized IgG will not cause any dilution issues, so control values should remain the same. There should be no human troponin in the murine IgG, so the IgG in diluent should be negative for troponin. It is expected that after incubation, the patient's troponin result should be within normal range since the HAMA will have been neutralized. If these are not the results, the same procedure should be tried with other Immunoglobulin classes.

Conclusions

1. Investigating antibody interference is very complicated and unique to each patient
2. No single technique can be used to detect or neutralize antibody interference in every case
3. Lab results should always be considered in conjunction with clinical information

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