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Case Study

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HIGH DOSE HOOK EFFECT IN THYROGLOBULIN ASSAY: CASE STUDY

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ABSTRACT

The hook effect is common phenomenon in day-to-day work of a clinical laboratory and should not be neglected. Commonly followed laboratory methods in our country are immunometric and enzyme-linked immunosorbent assays where the prevalence of hook effect is more. In spite of huge prevalence of hook effect it is not well documented in most of the laboratory set ups. With the introduction of newer assay techniques like chemiluminescence, high-dose hook effect has only occasionally been observed. The thyroglobulin value for the patient who was known case of follicular carcinoma of the thyroid was obtained as 0.0 ng/ml during initial testing. Suspecting the false negativity the sample was processed in serial dilutions. Final value was obtained in 500 dilution as 64849.3 ng/ml.

KEYWORDS: Hook effect, Thyroglobulin, immunometric, antigen-antibody.

INTRODUCTION

The hook effect also called as prozone effect is a type of interference which can occur with certain immunoassays and nephelometric assays, resulting in false negatives or inaccurately low results. Other common forms of interference that include antibody interference, cross-reactivity and signal interference. The phenomenon is caused by very high concentrations of a particular analyte or antibody and is most prevalent in one-step (sandwich) immunoassays.[1] The hook effect is common phenomenon in day-to-day work of a clinical laboratory and should not be neglected. Commonly followed laboratory methods in our country are immunometric and enzyme-linked immunosorbent assays where the prevalence of hook effect is more. In spite of huge prevalence of hook effect it is not well documented in most of the laboratory set ups. With the introduction of newer assay techniques like chemiluminescence, high-dose hook effect has only occasionally been observed.[2,3]

Here we are presenting a case of high dose hook effect in thyroglobulin assay which we encountered in our practice.

CASE SUMMARY

60 years old female patient from was referred to laboratory for thyroid cancer panel II (Thyroglobulin, anti thyroglobulin, TSH) testing. The initial report showed values as follows: Tg 0.0 ng/ml, Anti Tg 0.3 IU/mL and TSH 32.8 microU/mL. The patient is a known case of follicular carcinoma of thyroid. Suspecting the false negativity the sample was processed in serial dilutions. Final value was obtained in 500 dilution as 64849.3 ng/ml (129.6986 X 500) and reported as same.

DISCUSSION

The hook effect is based on the saturation curve of antibody with antigen (Figure 1). It is caused by excessively high concentrations of analyte simultaneously saturating all the available sites from both capture as well as detector antibodies. Mostly the high-dose hook effect occurs in one-step immunometric (sandwich) assays but may be seen with some other assays, giving a decrease in signal at very high concentration of analyte.[4] We are using Access Thyroglobulin reagent for Beckmen Coultor DxI 600 Analyzer which is a one-step immunoenzymatic ("sandwich") assay. Analytes with very large concentration in immunoassay such as ferritin, growth hormone, hCG, PRL, Tg, tumor markers PSA, CA19.9, CA125; antigen-antibody reactions can go into antigen excess and result in falsely decreased results and potential misdiagnosis. In one step two-site immunoassays where capture and detection antibody are added simultaneously, free analyte and analyte bound to the labeled antibody compete for the limited number of antibody-binding sites of the detector and in the presence of very high analyte concentration will decrease in stead of increase label bound to the solid phase. According to kit literature the The Access Thyroglobulin assay does not demonstrate any "hook" effect up to 40,000 ng/mL.[5] The final value we got in our analysis is 64849.30 ng/mL which is above this range and was surely susceptible to

high dose hook effect. The sample was serially diluted in 10,20,50,100, 500 dilution. Final report was obtained in 500 dilutions 64849.30 ng/mL (129.6986 X 500). High-dose hook effect can be avoided by increasing the quantity of the reagent antibodies and by reducing the amount sample required for analysis or by sample dilution.[6] Careful assay design is necessary to ensure that the concentrations of both capture and detector antibodies are sufficiently high to cope with levels of analytes over the entire pathological range. It is common practice to re-assay samples at several dilutions as a check on the validity of the result.[7]

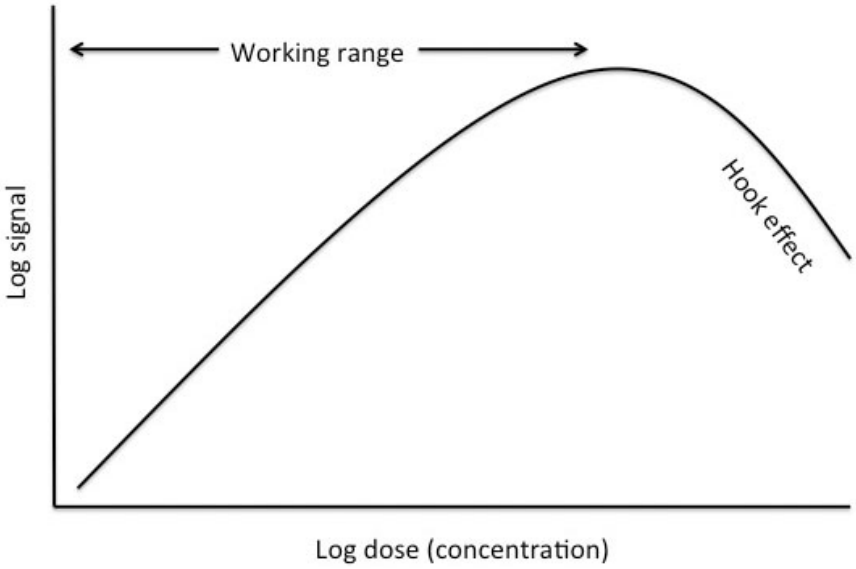

Figure No. 1. Mechanism of high dose hook effect.

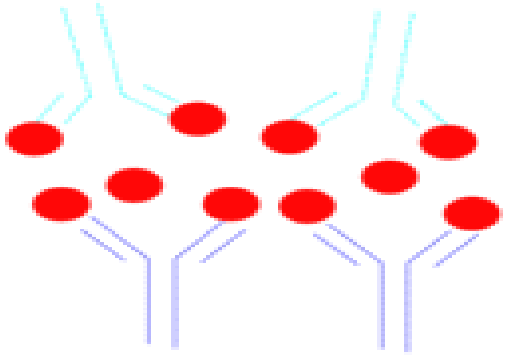

Figure No. 2. Analyte is binding to both capture as well as detection antibody leading to a reduction in formation of antibody-antigen-antibody complexes and a decrease in signal at higher concentrations of analyte

Ideally, as concentrations of analyte in plasma or serum increase, the response from sandwich immunoassays increases as well. The increase in signal should be linear with concentration of the analyte. However, as the concentration of analyte increases above a certain point, the system gets saturated and the signal begins to decline, the plot of which resembles a 'fish-hook' [Figure 1]. As a result, this phenomenon earned the name "high dose hook effect." Theoretically, this issue is only applicable to sandwich immunometric assays without a wash step between reagent additions. But, in all sandwich assays, the signal begins to plateau with high concentrations of analyte due to limiting amounts of reagent antibodies and rare samples with extremely high concentrations of analyte can even lead to the hook effect in assays with a wash step. Published reports document the prevalence of hook effect in immunoassays to be between 0.2 and 2%.[8]

CONCLUSION

As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made after pooling together all relevant clinical and laboratory fi ndings. False-negative results, even if they are extremely rare, may mislead or result in a delayed diagnosis and improper follow up or could have potential medical implications following mismanagement. Also, clinicians should understand the possibility of inaccurate results and women should be notified of the potential for false-negative or false-positive results wherever possible. In conclusion, high-dose hook effect has serious medical implications and

sample dilution is a simple method for detecting falsely low concentrations. Although modern assay methods have much improved reliability, physicians should still be aware of the potential for false-low due to the high-dose hook effect.

REFERENCES

1. Schiettecatte, Johan; Anckaert, Ellen; Smitz, Johan (2012-03-23). Interferences in Immunoassays.
2. Unnikrishnan AG, Rajaratnam S, Seshadri MS, Kanagasapabathy AS, Stephen DC. The 'hook effect' on serum prolactin estimation in a patient with macroprolactinoma. Neurol India 2001; 49: 78-80.
3. Agarwal M, Ananya D, Singh AS. High-dose hook eff ect in prolactin macroadenomas: A diagnostic concern. J Hum Reprod Sci 2010; 3: 160-1.
4. Fernando, S.A. & Wilson, G.S. (1992). Studies on the hook effect in the one-step immunoassay. J Immunol Methods, 151: 47-66.
5. Thyroglobulin [Kit Insert] Beckmen Coultor Access DxI600 fully automated Immunoassay Analyzer.
6. Cole, L.A.; Shahabi, S.; Butler, S.A.; Mitchell, H.; Newlands, E.S.; Behrman, H.R. & Verrill, H.L. (2001). Utility of commonly used commercial human chorionic gonadotropin immunoassays in the diagnosis and management of trophoblastic diseases. Clin Chem, 47: 308–315.
7. Davies, C. (2005). Concepts in The Immunoassay Handbook. 3nd edition, Elsevier Ltd., ISBN 008 044526 8, United Kingdom.
8. Musher DM, Hamill RJ, Baughn RE. Eff ect of human immunodeficiency virus (HIV) infection on the course of syphilis and on the response to treatment. Ann Intern Med 1990; 113: 872-81.