Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: The Hook Effect

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Slide 1:
Hello, my name is Yan Victoria Zhang. I am an Assistant Professor in the Department of Pathology and Laboratory Medicine at the University of Rochester Medical Center and Director of the Clinical Mass Spectrometry and Toxicology Laboratory and Associate Director of the Hematology and Chemistry Laboratory at Strong Memorial Hospital. Welcome to this Pearl of Laboratory Medicine on "The Hook Effect."

Slide 2:
In this Pearl, I will discuss what is the hook effect, how the hook effect affects testing results, prozone and postzone effect, and the hook effect in point-of-care testing.

Slide 3:
The hook effect is one of the most widely recognized limitations of immunoassays. It gives falsely low results with certain immunoassays in the presence of excess amount of analyte of interest.

As indicated in this slide, the x axis represents the analyte concentration, and the y axis represents the detection signal. When the analyte concentration is relatively low and is within the analytical range, the detection signal strength is linearly proportional to its concentration. For instance, the concentration of "a" generates the signal level of "c". As the concentration continues to increase, the relationship between analyte and signal evolves from this initial concentration-dependent relationship to a bell-shaped curve. The range of analyte concentrations where the signal starts to drop is called the hook point.

In the case of concentration "b", it generates the same signal as concentration "a" at level "c". Because immunoassays are calibrated in the linear range, the reported concentration for this sample is "a", which is lower than the real concentration "b".

This is called the Hook effect and only exists in one-step heterogeneous immunoassays.

Slide 4:
This slide is a quick review of heterogeneous immunoassays. It involves separation of bound and free analytes and can be one-step or two-step assay. Two-step assays involve a wash after the antigen is added and after label is added; one-step assays are just a wash after the label is added. The classic example of two-step assay is ELISA (enzyme-linked immunosorbent assay). Many immunoassays on current automated analyzers are one-step assays.

Slide 5:
In the typical two-step immunoassay or ELISA, capture antibodies are immobilized on a solid surface. Samples that contain the analyte of interest are incubated with the captured antibodies to form antibody-antigen complex. A wash step is used to remove the unbound antigen in the sample. Another antibody called detection antibody is added to the mixture. The detection antibodies bind to the antigen and form a "sandwich" with the previously formed antibody-antigen complex. Detection antibodies also have detecting agent attached, such as an enzyme which can react with its substrate to generate a signal for detection. The substrate is added after another wash step to remove any excess amount of detection antibodies. The concentration of the analyte in the sample is directly proportional to the signal.

Slide 6:
This slide illustrates the one-step immunoassay. In comparison to two-step immunoassays, the capture antibodies, analyte of interest (antigen), and detection antibodies are incubated together to form the "sandwich". Only one wash step is involved in the reaction which occurs before the addition of substrate to remove any unbound detection antibodies. Signals are detected and quantification is achieved based on the substrate and enzyme reaction.

Slide 7:
The hook effect happens when there is an excessive amount of analyte in one-step immunoassay.

This slide illustrates the mechanism. Where there is an excessive amount of analyte, they overwhelm the capture antibody; this allows the excess analyte to bind to the detection antibody directly, hence preventing the formation of the antigen-antibody sandwich. During the subsequent wash step, the detection antibodies that are not part of the sandwich structure are washed off. When substrate is added to the solution, only the small amount, if any, detection antibody that is bound to antigen will generate a signal for final concentration calculation, thus giving a falsely low result.

Slide 8:
On the other hand, in a two-step assay, the analyte is allowed to first bind to the capture antibody. After an appropriate incubation period (and the formation of the antibody-antigen complex), excess antigen is washed away. The antibody-antigen complex is then incubated with the detection antibody. Therefore, the maximum of the signal is dependent on the amount of capture antibody. When the antigen exceeds the amount of capture antibody, a saturated curved will be observed instead of a falsely low result.

Slide 9:
How are falsely low results corrected when the hook effect is present? In most laboratories, the correction of the hook effect involves serial dilution of the patient sample. If the hook effect is suspected, a patient sample is tested both prior to and after dilution. If the test results for the diluted samples are much higher than the undiluted sample, the hook effect is suspected.

Slide 10:
The hook effect is sometimes referred to as prozone or postzone effects which occur in the precipitin reaction. This slide shows the classic precipitin curve indicating the signal change when the antigen concentrations changes given the fixed amount of antibodies. The antibody and antigen forms the insoluble complexes in the equivalence zone when the optimal antibody and antigen ratio are met. When there is excess amount of antibody or excess amount of antigen, the insoluble structure cannot be formed and the soluble complex fails to generate the desirable results. When antibody is in excess, we call it the prozone effect. When the antigen is in excess, we call it the postzone effect. Those two terminologies are mainly used when the reaction happens in the solid phase, such as gel diffusion, immunoelectrophoresis. The hook effect is typically used for the automated immunoassays.

Slide 11:
The hook effect has been reported in various assays. Some examples include prolactin, growth hormone, thyroid stimulating hormone, gonadotropins, beta human chorionic gonadotropin and several tumor markers, such as prostate specific antigen, CA125, CA19-9, and calcitonin.

Slide 12:
Fortunately, the hook effect has become less common with assays on automated analyzers.

However, in recent years, there have been reports of what is called the "variant hook effect" occurring in point-of-care (POC) pregnancy devices. This occurs because there are several different variant forms of hCG including the free beta-subunit, and the core fragment of the beta-subunit. After about 5 weeks of pregnancy, the hCG beta core fragment is the predominant form of hCG in urine.

In contrast to the traditional hook effect, the variant hook effect happens when one antibody in the sandwich configuration is unable to recognize beta-core fragment, whereas the other antibody is able to readily bind to the same variant. When the beta core fragment is in excess of hCG, it will bind the antibody that recognizes it to the exclusion of hCG. In one study, a screening method was developed to qualitatively evaluate whether or not different POC hCG devices were susceptible to inhibition by beta hCG core fragment. Of the eleven devices tested, only two exhibited none to minimal susceptibility to this particular variant. Given the clinical significance of POC pregnancy testing, it is especially important that manufacturers of POC testing devices work to develop assays that are specific for the different variants of hCG to avoid falsely low results for those assays.

Slide 13:
In summary, the Hook effect is a phenomenon that gives falsely low results in the presence of excess amounts of analyte. The Hook effect only exists in one-step heterogeneous immunoassays. The Hook effect can be easily detected and corrected by serial dilution of a sample. Despite the efforts, the Hook effect still exists in modern assays. Care should be taken when interpreting low results, particularly for analytes such as tumor markers that can have very high circulating concentrations.

Slide 14: References

Slide 15: Disclosures

Slide 16: Thank You from www.TraineeCouncil.org
Thank you for joining me on this Pearl of Laboratory Medicine on "The Hook Effect." My name is Yan Victoria Zhang.