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**TITLE: Heterophile Antibodies**

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Hi, my name is David Lin. I'm a clinical chemistry fellow at the University of Utah. Welcome to this Pearl of Laboratory Medicine on "Heterophile Antibodies"

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First we'll go over the basic structure of antibodies and define what a heterophile antibody is. Then we'll quickly review immunoassay and look at some examples of *how* heterophile antibodies can interfere with immunoassays. And finally, we'll go beyond the theory and talk about how we can deal with heterophile antibodies in the real world, including how we can identify erroneous results and some strategies for the workup.

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Here's the basic structure of an antibody (Ab), which is defined as a blood protein produced in response to and counteracting a specific antigen. The portion of the figure shown in blue represents the Fc, or the constant region. And the part that's shown in pink represents the Fab or the variable region, which makes up the antigen binding sites. As you can probably tell from the figure, each antibody has 2 antigen binding sites, which allows the antibody to bind to specific antigens in a lock-and-key-like mechanism.

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So what is a heterophile Ab? If we look at the name semantically, the "hetero" part means "different" or "other than", and "philic" means "having an affinity for" or "loving".

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If we look into the literature, there are several different definitions of heterophile Abs. Here are some examples.

Some define heterophile antibodies as “antibodies produced against poorly defined antigens and that they are generally “weak” antibodies with multi-specific activities”. Others define heterophile antibodies as “Nonspecific antibodies that interact poorly with immunoassays antibodies”. And finally, others simply refer to them as “a group of defined endogenous Abs that react with many different molecules…”

You can see that these definitions do overlap a bit but also differ slightly.

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So WHERE do these heterophile antibodies come from? In many cases, heterophile antibodies can arise naturally in the body as the result of antigen diversity. Furthermore, the production of heterophile antibodies has been associated with patients with autoimmune or inflammatory conditions.

Heterophile antibody can also be found in healthy individuals, but typically to a lesser extent. Overall, it’s been estimated that these endogenous heterophile antibodies can be found in more than 10% of patients. The term “heterophile” antibodies is also often used to refer to human anti-animal antibodies, such as human-anti-mouse antibodies, or HAMA. And these could result from exposure to animal antigens in the environment, or if a patient has received therapy involving the use of animal-derived proteins. It’s been estimated that up to 40% of the general population have antibodies with affinity to animal antibodies, but not all of them will be problematic for laboratory testing.

One important thing to note is that there are papers in the literature that draw a distinction between heterophile antibodies and human anti-animal antibodies such as HAMA, largely because of differences in their origin and binding affinity. In other publications however, as well as in the clinical laboratory settings, these terms are often used interchangeably.

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For the purpose of this presentation, we’ll use “*heterophile Ab*” as an overarching term to describe Abs that can interfere with clinical immunoassays. And from a practical standpoint, laboratory’s main concern with heterophile abs is that they can interfere with clinical immunoassays and cause incorrect results

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So how do these heterophile antibodies interfere with immunoassays?

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Heterophile Abs, because of their multi-specificity, are capable of binding to different components of an immunoassay. This can include the antigen or the analyte of interest, such as the endogenous analytes from the patients, or even the labelled analyte from the assay reagent. They can also bind to antibodies in the immunoassay, such as the capture or the signal antibodies, as well as other components of an immunoassay, such as the conjugate and other parts of a detection system. But typically, when we talk

about heterophile antibodies interfering with immunoassays, most people tend to think about nonspecific binding to capture and or signaling antibodies in an assay.

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Now let's look at some scenarios. Heterophile antibodies can interfere with both competitive immunoassay and sandwich immunoassay. For the purpose of this presentation we'll focus on the sandwich immunoassay since that's what most people tend to think about when discussing heterophile Ab interferences. Let's first quickly review how a sandwich immunoassay functions in the absence of heterophile Ab.

The analytes from the patient are shown in green, and we have the capture and the signal Antibody from the assay reagent. The capture antibodies are located on the solid surface, and the signal antibodies are shown in blue. After incubation allowing the reaction to occur,

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the reagent and signal Abs will bind to the analytes from the patient's sample, essentially forming this "sandwich" structure. After a wash step

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the signal from the signal Ab is then detected, and the signal will be proportional to the amount of analyte present in the patient sample.

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Let's take a look at what happens when there's a heterophile Ab present that can bind to the capture Ab in the reagent.

This time, we have the heterophile antibody present in the patient's sample shown in red. In this case, after the incubation, these heterophile Ab can bind to the reagent capture antibody, which interferes with the interaction between analytes from the patient sample and the capture antibodies from the immunoassay.

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After a wash step, you can see how the presence of heterophile antibodies that cross-react with the capture antibody

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can lead to a decreased signal detected at the end. And since the signal is proportional to the analyte concentration, this translates to a falsely low result.

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Here's another similar example. In this case, the heterophile Ab present can cross react with the signal Ab in the reagent

The heterophile Abs can bind to the signal Ab

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And after a wash step, you can see how,

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similar to the previous case, decreased signal is detected, which again, translates to a falsely low result

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Let's take a look at another example, where the heterophile Ab is present, and the heterophile Abs can cross-react with both capture and signal Abs. Again, the heterophile Abs are shown red. During the incubation period, the heterophile Abs are able to cross-react with both the capture and signal antibodies in the assay, forming this sandwich like structure.

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After a wash step,

(Slide 21) this results in an increased amount of signal that's detected at the end, which translates to a falsely high result.

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So those were just some examples and they are not a comprehensive review of all the ways heterophile Abs can interfere with immunoassays. But the main point here is that heterophile Abs can cause false negative and false positive results. However, heterophile Ab typically cause false positive result in sandwich immunoassay. And this is important because it can lead to misinterpretation of the patient's result, and consequently, the wrong course of treatment may be given to the patient. The question now is how do we catch these "false" results?

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A lot of times though, when it comes to catching these misleading results, physicians are very often our biggest allies. A physician may contact the lab expressing concerns and asking questions such as "Is there something going on with your assay?", or "What's wrong with your lab test?" They might say, "This lab result doesn't make sense", or they may even ask, "are you sure this is my patient's result?" And what all of these boils down to is that there's clinical discordant result, and the result is either unexpectedly high or unexpectedly low. And this usually helps launch a workup in the laboratory.

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Communication is the key during the workup, and this can involve getting information from the clinical team. For instance, asking the provider questions such as
“Does the patient have any underlying autoimmune or chronic inflammatory condition?”
“has the patient been exposed to animal antigens or Abs” or
“Does the patient have other unexplained lab results?”

During the workup, the laboratory would ideally inform the physician regarding any follow-ups and findings. In general, there are several actions that can be taken during the workup.

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One of the first things that can be done when faced with a clinical discordant result, is to double-check the sample name and specimen type, since a sample mix-up could’ve caused the unexpectedly high or low results.

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Once a sample mixed up is ruled out, a dilution study could be carried out to check for possible high-dose hook effect or interfering substance.

In fact, it may also be possible to set up middleware rules to help flag extremely high or low results for review and follow-up.

An unexpectedly low result could be caused by a high-dose hook effect, in which a dilution experiment would reveal a higher result.

On the other hand, if there’s something in the sample that’s causing a positive interference and an unexpectedly high result, a dilution study could also help identify this, because a non-linear relationship is often observed during serial dilutions if interfering substance is present.

So let’s say the lab did a dilution study and nonlinear pattern was observed. For example, if the sample was diluted two fold but the signal detected did not decrease by 50% as expected. Now you have a higher suspicion of heterophile antibodies being present in the patient’s sample, so what else can you do?

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A commonly used approach that’s a bit more specific for identifying heterophile Abs is the blocking reagent study. Heterophile Ab blocking reagents consist of pooled proteins from different species. These blocking reagents are available commercially, and the components are very often proprietary, but in general, their purpose is to neutralize or inhibit heterophile Ab interference.

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And because blocking reagents can help neutralize or inhibit interference from heterophile Ab, which

tend to have weak non-specific binding, the idea is that you would run the same sample twice, once with blocking agent and once without, and then compare the results. And if you observe significantly different results (for instance, a 50% difference), then this would provide additional evidence that heterophile Abs are in fact present in the patient's sample.

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There are a couple of things to keep in mind however, the first is that the assay should be validated to ensure compatibility with the blocking reagent, and that the blocking reagent doesn't interfere with the assay itself. So if you were to test a sample from a healthy individual who does not have heterophile Ab, then you would expect to see the same result with or without blocking reagent treatment. Second, while blocking reagent study is commonly used to demonstrate the presence of heterophile Ab, it doesn't work all the time. It's been estimated that they may be ineffective in approximately 20-30% of cases because of the diversity in heterophile antibodies.

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So what else can we do? It's important to keep in mind that while one of the goals during the workup is to identify potential heterophile Ab interferences, the ultimate goal for the laboratory is to provide an accurate result for the clinical team to work with. Therefore, one strategy to consider is to re-test the same sample with a different assay, since an alternative assay that may have different susceptibility or resistance to patient's heterophile Ab. For example, this could be an immunoassay that uses Ab from different species, or a mass spectrometry-based assay, which is more resistant to heterophile Ab interferences. And the number of alternative assays available will depend on the analyte.

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In summary, heterophile Abs are non-specific Abs that can bind to multiple molecules. Falsely high or falsely low results may occur depending on the immunoassay type and the heterophile Abs. Suspicion of heterophile Abs is often initiated by clinicians due to unexpected results, and that's why communication between the laboratory and the clinical team is so important.

Lastly, Heterophile Ab blocking reagents can be used during the workup. These blocking reagents may help neutralize or inhibit heterophile Ab interference. That said, assay should be validated to ensure compatibility with the blocking reagent. And it's important to keep in mind that blocking reagents are not always effective, so re-testing sample with alternative platform may be useful.

(Slide 14) References

(Slide 15) Disclosures

(Slide 16) Thank You from www.TraineeCouncil.org
Thank you joining me on this Pearl of Laboratory Medicine on Heterophile Antibodies.