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Screening Method to Evaluate Point-of-Care Human Chorionic Gonadotropin (hCG) Devices for Susceptibility to the Hook Effect by hCG β Core Fragment: Evaluation of 11 Devices

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Guests:
Drs. Ann Gronowski and Robert Nerenz are from the Department of Pathology and Immunology at the Washington University School of Medicine in St. Louis.

Bob Barrett: This is the podcast from *Clinical Chemistry*. I am Bob Barrett.

Pregnancy tests such as the ones used to test urine in hospitals have been shown to give false-negative results in certain patients.

The April issue of *Clinical Chemistry* published a paper from a group led by Dr. Ann Gronowski at the Washington University School of Medicine in St. Louis, that reported a method to screen pregnancy devices for their susceptibility to these false negative results.

Today, we have Dr. Gronowski, who is Professor in the Department of Pathology and Immunology and Obstetrics and Gynecology, who led this research study, and her co-author Dr. Robert Nerenz, a fellow in the Department of Pathology and Immunology.

Now Dr. Gronowski let's start with you. We have talked to you before about these false-negative results in point of care and home pregnancy devices. Can you refresh our memory on what causes the false negatives?

Dr. Gronowski: Sure Bob! Pregnancy tests work by utilizing two antibodies that recognize the pregnancy hormone called the hCG. One antibody captures the hCG and the other antibody is linked to a signal molecule.

The problem is that there are actually a number of forms of the hCG molecule that can be found in urine. One of the forms called the hCG β-core fragment can inhibit the binding of hCG by the antibodies in the assay.

And in some women the concentrations of the β-core fragment become high enough that they cause negative results in women who are actually pregnant.

This is significant because the hCG β-core fragment is the most abundant form of hCG found in the urine after about five weeks of pregnancy.

Bob Barrett: This obviously doesn't sound like a very good thing. Why has it been difficult up till now to screen pregnancy tests for the false-negative?

Dr. Gronowski: Well, several things have made it difficult. First, not all women have enough hCG β-core fragment concentrations to cause a false-negative. So it's not like we can just go and take urine from any woman and test it with various devices.

Second, up until now we didn't know how much hCG β-core fragment was necessary to cause a false-negative and in what kind of ratio the hCG β-core fragment needs to be in relation to hCG. And finally the availability of purified hCG β-core fragment is limited.

Bob Barrett: Now let's turn to you Dr. Nerenz, give us an idea of how you address this problem in your study?

Dr. Nerenz: Well, first we started with two pregnancy devices that we had tested previously. And one of these we knew was very susceptible to false negatives and the other one seemed to be less susceptible.

Then we tested these two pregnancy devices with a wide range of hCG concentrations and a wide range of hCG β-core fragment concentrations.

And we were looking for combination of concentrations that would show the false-negative, but also because purchasing these purified hormones can be expensive, we wanted to find the lowest concentrations possible that were required to cause a false-negative result.

And we ultimately wanted to create a screening method that is easy and inexpensive, so we devised a method that can be performed using just two test cartridges.

On one, you test the solution of 500 picomoles per liter hCG and you should see a nice positive result. On the other you test a solution with 500 picomoles per liter of hCG, plus 500,000 picomoles per liter hCG β-core fragment.

The ideal device will show a nice positive signal with both solutions. If the second cartridge has a fainter signal than the first cartridge then that's evidence the device is susceptible false-negatives with hCG β-core fragment.

This screening is easy to perform and requires little in the way of reagents and devices.

Bob Barrett: So once you develop the screening method, did you look at a variety of hospital pregnancy devices?

Dr. Nerenz: That's exactly what we did. We tested 11 of the most commonly used point of care urine hCG devices according to proficiency surveys from the College of American Pathologists.

We tested them all at the same time and we had ten people who were not involved in any way with the study read the devices.

Bob Barrett: And how many did you find were susceptible to the false negative results?

Dr. Nerenz: Well, unfortunately we have found that 9 of the 11 hospital devices were subject to falsely low or even negative results due to the hCG β-core fragment.

We were able to classify the devices into three distinct groups: those with best performance, moderate performance and poor performance.

Now the two best-performing devices were the ICON 20 by Beckman Coulter and the hCG Combo by Alere.

The ICON 20 was the only device that actually showed stronger signal when the hCG β-core fragment was added to intact hCG which is what you should expect when more hCG of any kind is added to a solution.

The Alere device only modestly affected by the addition of hCG β-core fragment.

Now second, the group of devices with intermediate performance demonstrated noticeable susceptibility to the addition of hCG β-core fragment.

Devices in this group generated clear positive signal when tested with hCG alone, but that signal was noticeably diminished when tested with the solution containing both intact hCG and hCG β-core fragment.

Now lastly, the group of devices with the poorest performance consisted of the OSOM hCG Combo Test by Genzyme Diagnostics and the Elite Plus hCG by Cen-Med.

These devices were read as negative by all but two readers when used to test the solution containing both intact hCG and hCG β-core fragment.

Now these two devices present a clinically significant risk of false-negative results in patients after about five to seven weeks of pregnancy.

In fact, the OSOM has been shown previously by us to give false-negative results in pregnant patients, and a recent publication in the Journal of Emergency Medicine show that the Cen-Med generated negative results in a patient with molar pregnancy.

Bob Barrett: Did these results come as a surprise?

Dr. Nerenz: Yes, actually they surprised us quite a bit. I guess we thought that since this problem with hospital pregnancy devices was shown back in 2009, that very few devices at this point would still be affected.

We certainly didn't expect the majority of devices to be susceptible to false-negatives.

Bob Barrett: So Dr. Gronowski, what questions do these results raise?

Dr. Gronowski: This study raises a number of important questions. The first is a question that's been long debated and that is, "are monoclonal antibodies too specific for this type of tests?"

A lack of understanding of the exact analytical specificity of the antibodies can lead to problems. For analytes like hCG which are a heterogeneous mix of variant forms, one has to ask if polyclonal antibodies or a mix of monoclonal antibodies might actually be better.

There are also many unanswered questions about β-hCG core fragments: What causes some women to have higher hCG β-core fragment concentrations than others? How often do women achieve hCG β-core fragment concentrations that could cause a false negative result in the majority of devices that are subject to this effect?

Clearly more studies are needed. Until these questions are answered, we suggest that healthcare professionals choose qualitative hCG devices that are least subject to inhibition caused by increased β-core fragment concentrations to avoid the possibility of false-negative results.

We hope that the screening method that we described will help users choose devices and select device lot numbers. We hope that manufacturers will also use this information to develop devices that generate clearly positive signal even in the presence of high hCG β-core fragment concentrations.

Bob Barrett: Well finally let's look ahead, what needs to be done?

Dr. Gronowski: Well, as I have discussed with you before, we need to educate physicians, nurses, and other healthcare professionals that this is a problem.

Also, manufacturers need to make the possibility of false-negatives clearly visible in their package insert and state that when this is suspected, a simple dilution can yield a positive result if the patient is truly pregnant.

This is really important for sites that have no alternative way of testing for pregnancy.

And then finally in centers where Quantitative Serum hCG Testing is available, this should be the preferred pregnancy test.

Serum testing is not subject to the variant hook effect because hCG β-core fragment is not present in serum, and quantitative serum assays are much more analytically sensitive.

Bob Barrett: Drs. Ann Gronowski and Robert Nerenz are from the Department of Pathology and Immunology at the Washington University School of medicine in St. Louis. They have been our guests in this podcast on urine pregnancy testing from *Clinical Chemistry*.

I'm Bob Barrett, thanks for listening.