

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

The July issue of *Clinical Chemistry* published a paper from a group led by Drs. Ann Gronowski and Mitchell Scott at the Washington University School of Medicine, that reported false-negative results in point-of-care qualitative HCG devices, due to excess hCG beta-core fragment.

We are fortunate to have with us today Dr. Ann Gronowski, Associate Professor in the Departments of Pathology and Immunology and Obstetrics and Gynecology.

Dr. Gronowski, how can excess of a fragment of hCG cause a false negative result. This seems counterintuitive?

Dr. Ann Gronowski: That's a good question. I think that most laboratorians are familiar with the concept of the so-called hook effect. This occurs in sandwich assays where there are two antibodies that act like bread, and they form a sandwich with antigen in the middle. When there is a great excess of the antigen and there is no wash step to take away the excess, then sandwich formation is inhibited. What you end up with is a bunch of open-face sandwiches, if you will. This is a well-known phenomenon and can occur in both qualitative and quantitative hCG assays.

What we observed is slightly different, and to explain it, let me take a step back for a second and say something about hCG. In pregnancy, the hCG found in urine is comprised of intact hCG, which is a heterodimer, comprising alpha and beta subunits, as well as a number of other variants, including hyperglycosylated hCG, nicked hCG, free beta subunit, and the core fragment of beta hCG.

The fraction of total hCG that each of these variants represents in urine changes during pregnancy. For instance, hyperglycosylated hCG concentrations are high in early pregnancy and hCG beta-core fragment is high in mid-pregnancy urine. So this hCG heterogeneity makes measurement of this molecule difficult and confusing.

So in our paper we report that excess of one of these variants, the beta-core fragment, can cause a hook-like effect. It's different from a hook effect, because when you take purified beta-core fragment, and when that is tested on these devices, a positive result is not observed. In other words, the devices don't recognize this particular variant.

What we hypothesize is that one of the two antibodies does recognize the beta-core fragment, but the other does not. And when the beta-core fragment is present in high enough

concentrations, then it saturates the one antibody and prevents the formation of a sandwich.

We were able to demonstrate this by taking a random hCG positive sample and by adding excess hCG beta-core fragment, the positive band disappeared in a dose-dependent manner. Also, we did not see this effect in devices that are known to recognize the beta-core fragment.

Host: Has this finding ever been demonstrated before?

Dr. Ann Gronowski: Well, we've previously demonstrated a hook effect with hCG qualitative devices, but not the negative effect due to high concentrations of hCG variants. This type of inhibitory effect has been predicted by others in the literature, but to our knowledge it's not been demonstrated.

Experiments such as ours really were not possible before the development of highly purified hCG variants. In 2001, the WHO Expert Committee on Biological Standardization approved six hCG variant standards as the first WHO international reference reagents. These purified preparations will allow much better characterization of what point-of-care devices actually recognize and don't recognize.

Host: Well, do these findings have any clinical implications?

Dr. Ann Gronowski: First, it's important that clinicians are aware that these types of false-negative results can occur. I mentioned earlier that the hCG beta-core fragment is high in midterm pregnancy urine. That means urine from beyond about 5 to 8 weeks of gestation should not be used on certain devices because it has a much higher chance of producing a false-negative result.

Now, I know what your next question is likely to be, why would anyone test women at midterm, don't we just use these devices to try and detect pregnancy early in gestation?

Well, that's probably true for home pregnancy devices, where women purchase them to determine if they are pregnant, and that testing is probably done most frequently around the time of the missed menses. However, hCG testing in hospitals is often protocol driven and it's done regardless of patient history.

For instance, all women of childbearing age in the emergency room are tested before any diagnostic test or intervention that could adversely affect a fetus, or all women are tested before chemotherapy, etcetera.

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In the hospital laboratory, we see specimens sent for hCG testing there from 80-year-old women, from women with hysterectomies, and women who are many months pregnant. Our data indicates that we need to be certain that the devices we choose can recognize the hCG variants present in the patient population that we're going to be testing. As a result of our findings, our hospital switched their qualitative point-of-care hCG device to a different brand.

Host: So in your opinion, how can a hospital laboratory or physician's office using point-of-care hCG devices be certain that the device they use recognizes the necessary hCG variants?

Dr. Ann Gronowski: This is an important question. Publications by David Grenache's group have utilized the new purified International Reference Reagents to characterize several of the hospital point-of-care hCG devices, and our own group has used this material to characterize several over-the-counter home use hCG devices.

These types of reports can help professionals select their qualitative devices. If only early pregnancy urine is going to be tested, as in a fertility clinic, then any device that recognizes intact hCG is likely sufficient.

However, if the device is going to be used for more advanced pregnancies, as in a hospital setting, then a device that recognizes variants such as free-beta hCG and beta-core fragment should be selected. What's really needed is for manufacturers to better characterize what hCG variants their device recognize and to make that information available. To date that type of research has not been done.

When a negative result is suspected to be a false-negative, a simple dilution can be performed. The idea here is that as with the hook effect, you can get the variant antigen down to a concentration that does not block all the antibodies, and then a sandwich can form with the intact hCG.

Host: What about quantitative hCG tests? Are they also prone to this type of problem?

Dr. Ann Gronowski: In theory, yes. Quantitative hCG tests are also potentially subject to interference with high concentrations of certain hCG variants. Currently, our laboratory is actually examining this. hCG beta-core fragment is found almost exclusively in the urine and currently most quantitative hCG devices are FDA approved for use with serum only, therefore, there should be less of a problem with serum quantitative hCG assays. However, many laboratories have validated their

quantitative devices for use with urine to help when interpreting difficult cases.

In the August 2009 issue of *Clinical Chemistry*, Catherine Sturgeon and colleagues examined the ability of 16 different quantitative hCG assays to recognize four of the purified International Reference Reagent variants. Their report shows clearly that certain quantitative tests just don't recognize certain hCG variants.

This type of study will be key to interpreting discrepant hCG results and choosing hCG test for different clinical needs. We've written an editorial discussing this important work that appears in the same August issue of *Clinical Chemistry*.

Host: Well, it seems these data demonstrates that there is just a lot we still don't know about hCG testing?

Dr. Ann Gronowski: Absolutely. I think that our finding should be a call to arms for laboratorians to demand better standardization of hCG immunoassays, and at the very least, better characterization by manufacturers. We need to know which hCG variants each device recognizes, and if they're subject to interference with normal concentrations of hCG variants. Also, we need to have more discussion and research into what characteristics different hCG immunoassays should ideally have.

For example, what hCG variant should be recognized, and should all variants be recognized by all assays. We've got a long way to go in the improvement of hCG assays.

Host: Dr. Ann Gronowski is an Associate Professor in the Departments of Pathology and Immunology and Obstetrics and Gynecology at the Washington University School of Medicine, and has been our guest in this podcast from *Clinical Chemistry*. I am Bob Barrett. Thank you for listening.

Total Duration: 9 Minutes