Effect of Preanalytical Factors on the Stability of Maternal Serum Biomarkers and Calculated Risk for Trisomy 21, Trisomy 18, and Open Neural Tube Defect

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Guest: Dr. Robert Schmidt is an associate professor of pathology and director of the Center for Effective Medical Testing at the University of Utah.

Randye Kaye: Hello, and welcome to this edition of "JALM Talk" from *The Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host, Randye Kaye.

Prenatal screening is carried out during the first and/or second trimester of pregnancy, and provides a non-invasive way of assessing risk of fetal aneuploidy and/or neural tube defects. Now, typically, biochemical tests are performed that may include maternal serum, alpha-fetoprotein, human chorionic gonadotropin, unconjugated estriol, dimeric inhibin A, pregnancy-associated plasma protein A. As these tests are commonly only performed at reference laboratories, the type of sample that is stored, storage duration, and temperature of storage are pre-analytical factors that should be assessed.

An article called "Effect of Preanalytical Factors on the Stability of Maternal Serum Biomarkers and Calculated Risk for Trisomy 21, Trisomy 18, and Open Neural Tube Defect," which was published in the May 2017 issue of JALM, investigated these pre-analytical variables and if they could cause a misclassification of a pregnancy as high risk or low risk for Trisomy 21 or 18 and open neural tube defects.

One of the authors of this article is Dr. Robert Schmidt, an associate professor of pathology and director of the Center for Effective Medical Testing at the University of Utah, and he is our guest for today's podcast. Welcome, Dr. Schmidt.

Dr. Robert Schmidt: Thank you, happy to be here.

Randye Kaye: First question, I'd like to ask you if you would please describe the objective of your study, and the specific problem that your paper addresses.

Dr. Robert Schmidt: All samples have fairly stringent requirements for sample handling for maternal serum screening, so that samples should be refrigerated, it should serum rather than whole blood, it shouldn't be stored more than a week refrigerated. We often get calls from clients if they have violated one of

these, that they kept it at room temperature say for 48 hours, or they didn't spin it down rapidly and so forth. And so that's an inconvenience to patients that have to be redrawn, it adds to cost, and so forth.

So we conducted a stability study to see how the pre-analytical factors affected these analytes. So specifically, we let the samples stand at room temperature for various amounts of time and store them as whole blood rather than serum. For each sample, there were like 24 different conditions that we evaluated.

Randye Kaye: Okay. What else did you do when you approached the problem?

Dr. Robert Schmidt: On the surface, this kind of seems like a traditional sample stability problem, and people do these all the time. You have a single analyte you maybe keep it at room temperature for various amounts of time, see how the concentration changes. But this problem is a little more difficult because there are multiple analytes. And second, the analyte concentrations are used to calculate post-test risk. So just seeing how the concentration varies doesn't really tell you how the pre-analytical factors will affect the outcome.

So what we needed to do was look at how the variation in the pre-analytic conditions would affect post-test risk, which is really the thing of interest here.

Cases are classified as high risk or low risk relative to a decision point of risk of 1 in 270 pregnancies. What we really want to know is whether the pre-analytical factors affect the probability of misclassifying a pregnancy, not whether it just changes the concentration of an analyte.

So the challenge here is you would need a very large sample size to empirically estimate the misclassification rate. So we used a simulation to approach this, and we did this in two steps. The first step was we determined how the pre-analytical factors affected analyte concentrations. This is exactly what someone would do in a traditional stability study. And then we developed regression equations that predicted the analyte concentrations and the precision of this estimate based on the pre-analytical factors. So we could predict pretty well how the concentrations would change due to the pre-analytical factors.

And then the second step we used, we took data from the SURUSS (Serum, Urine and Ultrasound Screening Study) trial to simulate 10,000 pregnancies. The SURUSS trial was a big trial that was done years ago, looking at the distribution of all these analytes that go into the maternal

serum screening in a large population of women. This data from the study is commonly used for simulation studies to predict the accuracy of tests and so forth.

So we had 10,000 pregnancies and with biomarkers for each pregnancy that we got from the SURUSS trial. Then for each pregnancy, we used our regression models to predict the change in analyte concentrations that would occur if we subjected a sample from that pregnancy to different pre-analytical conditions.

So we have these 10,000 pregnancies. For each pregnancy, we could simulate what would happen if we, say, stored it to room temperature for four days or it was whole blood rather than serum and so forth. And so for each pregnancy, we simulated 50 different conditions. That way, we could look at whether the pre-analytical factors would affect the classification of the pregnancy as high risk or low risk.

Randye Kaye: Okay. So with all that, what did you find?

Dr. Robert Schmidt: What we found at the pre-analytical factors had a relatively small impact on misclassification. The baseline misclassification rate is pretty low and the pre-analytical factors increase that by about a third, I'd say. The incremental misclassification was about 1 in 200 pregnancies for Trisomy 21, and about the order of 1 in 1,000 for Trisomy 18.

Randye Kaye: Okay. In light of all these findings, now, are you going to change the sample handling requirements?

Dr. Robert Schmidt: We are looking into this. If the baseline misclassification rate is low and the increase in misclassification is modest then ultimately, it depends on whether the benefits, the fewer redraws and reduced costs are worth the cost associated with the misclassification.

The consequences of misclassification are pretty substantial. Failure to detect a high risk pregnancy or a false positive, calling a normal pregnancy high risk, and high risk pregnancies are generally confirmed by a diagnostic procedure, such as amniocentesis which is invasive, expensive and associated with some risk of pregnancy loss, so you may have to weigh all this up.

One possibility is to calculate the post-test risk on a mishandled sample and determine the distance that the result has from the decision point. We can, sort of, I think, identify a zone where it would be very likely that a result from a mishandled sample would be a misclassification. So you could sort of have a zone where you knew it was safe to give a result, and other ones would have to be redrawn. So

that's one thing. We're evaluating these alternatives at the moment.

Randye Kaye: Just from the study, is there anything more general that we can take from what you found?

Dr. Robert Schmidt: Well, yeah. I think nowadays, many, many prediction models are being published. They are coming out by the hundreds. And the approach we used for stability study could be used for any kind of predictive model like that. If you have any kind of model that's based on several different biomarkers, our approach could be used for that, so it's quite general I think, and that's I think one of the important things about the papers, sort of the methodological approach to doing sample stability studies where you have an outcome that's based on a mathematical model.

Randye Kaye: It sounds like there's so much to be learned from the approach that you took, not just from the results of the study.

Dr. Robert Schmidt: Yes. I think that's correct. Maternal serum screening is going to be supplanted by non-invasive prenatal testing, genetic methods, probably over the next five to ten years and there won't be much interest in this particular test at that time, but I think the approach we used is very general and can be used for a wide variety of tests.

Randye Kaye: Okay, very interesting. Well, thank you so much, Dr. Schmidt, for joining us today.

Dr. Robert Schmidt: Well, thank you.

Randye Kaye: That was Dr. Robert Schmidt from the University of Utah talking about the JALM article, "Effect of Preanalytical Factors on the Stability of Maternal Serum Biomarkers and Calculated Risk for Trisomy 21, Trisomy 18, and Open Neural Tube Defect," for this podcast.

Thanks for tuning in for "JALM Talk." See you next time and don't forget to submit something for us to talk about.