Development of Reference Materials for Noninvasive Prenatal Aneuploidy Testing by Massively Parallel Sequencing: A Proof-of-Concept Study

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Guest: Dr. Jonatan Blais is a practicing physician at Chaudière-Appalaches Integrated Health Center in Lévis, Quebec, Canada.

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.

Massively parallel sequencing technology has allowed DNA sequencing to become accessible and affordable in a wide variety of clinical settings from the diagnosis of rare diseases to the selection of effective treatments in cancer. Further, massively parallel sequencing presents opportunities to use noninvasive prenatal testing on pregnant women to identify fetal aneuploidies via the analysis of cell-free DNA.

However, as this technology becomes more widespread, there is a need for robust quality management and standardization among different laboratories performing the testing. While some guidelines have been published by professional laboratory organizations as a starting point, no laboratory quality guidelines have been established for massively parallel sequencing assays specific to noninvasive prenatal testing. In particular, standardized reference materials to allow for calibrations and value assignment are lacking.

An original research article published in the July 2019 issue of the *Journal of Applied Laboratory Medicine*, describes a proof-of-concept study which tested the possibility of using artificial plasma matrix spiked with fragmented genomic DNA from immortalized cell lines to produce reference material for noninvasive prenatal testing assays. The use of this synthetic reference material could allow for long-term supply of stable material and thus improve the quality and standardization of these new assays for the benefits of pregnant women and expecting families. The corresponding author of this study is Dr. Jonatan Blais, a practicing physician at Chaudière-Appalaches Integrated Health Center in Lévis, Quebec, Canada.

Dr. Blais is pursuing research in the field of molecular diagnostics, particularly topics related to applications of

circulating nucleic acids and issues related to analytical and clinical validity of molecular tests, and he is our guest today.

Welcome to the podcast, Dr. Blais. Let's start with this, what motivated you to conduct this study?

Dr. Jonatan Blais: The study is about reference material to be used in noninvasive prenatal testing, called NIPT. NIPT assays, let me just explain a bit briefly what it is. NIPT assays refer to genetic analysis usually via next generation sequencing of cell-free DNA circulating in maternal blood, a percentage of which originates from placental cells and thus reflect the fetal genome in order to detect aneuploidies, for example, Down Syndrome. And NIPT has been offered to pregnant woman in many countries now for several years. The tests that are used were developed and are offered as what's called "laboratory developed tests." And so none are regulated by the FDA, which leads some to raise concerns about quality control and one of the cornerstones of quality control and standardization is the availability of reference material that can be used to either calibrate or design external quality control programs. Unfortunately, there's no universally recognized reference material for NIPT as yet. Although recently, there is at least one commercial product that has become available. So we therefore wanted to explore the possibility of developing such material in a way that could in theory ensure an unlimited supply of material and that would be compatible with some approach of fetal fraction estimations, estimating the percentage of a circulating DNA that is of fetal origin.

Randye Kaye: All right. Thank you. Let's just dig a little deeper into this. So, we're looking at ideal reference material for non-invasive prenatal testing assays. So what properties would this reference material possess?

Dr. Jonatan Blais: Reference materials are usually defined as material with properties that are well-characterized metrologically speaking, that are stable, homogenous, and commutable, which means that they can be used on different platforms, or by different methods. In the case of NIPT, two issues make production of an ideal reference material challenging. Using biological samples is appealing since it avoids matrix effects, but even when pooling several samples together, that would require frequent resampling, which would modify its composition over time and thus, compromise its stability.

So, using an artificial plasma matrix, as we did, always spiked with the same genetic material ensures an unlimited supply of stable material, at the cost of possible matrix effect. But in the case of genetic analysis, this is often less of a problem, given that nucleic acids are typically analyzed after an efficient extraction process. So that first challenge

can be overcome. However, the second issue has to do with mimicking the parental allelic relationships between the mother and the fetus that characterizes NIPT samples, and that are the basis for some fetal fraction estimation based on variable genetic markers such as SNIPS, or single nucleotide polymorphisms that we call SNIPS. And so, that's why we decided to use DNA from cell lines of a mother and her child that had Down Syndrome. So that was a way for us to overcome the second challenge.

Randye Kaye: Now, let say we got widespread adoption of this type of reference material. What clinical benefits might we see?

Dr. Jonatan Blais: I think one of the most obvious benefits would be to provide an opportunity for standardization of risk scores across various assays currently being used. When a clinician receives a report, an NIPT report, this report would usually state that the risk of aneuploidy for a given pregnancy is for example 2% or 90%. And in the mind of the clinician, the assumption is that, if the test would have been carried out with a different NIPT assay, the value would have been essentially the same.

But without reference material, this kind of standardization cannot be formally established. The other very important benefit would be in terms of quality control of fetal fraction estimation. Both the limit of detection or limit of quantification, and the diagnostic performances of NIPT assays are critically determined by fetal fraction. So, ensuring an external quality control of this parameter is crucial. Of course, the external quality control of the aneuploidy risk estimate per se is also of paramount importance and would be facilitated by the availability of such reference material.

Randye Kaye: So, let's look a little bit into the future, and as more applications of massively parallel sequencing are realized, do you think that reference materials should be developed and available prior to the clinical introduction of a new test?

Dr. Jonatan Blais: Well, I don't think it would be desirable to delay the introduction of a test with demonstrable clinical utility and that's an important point, *with* demonstrable clinical utility, only because there is no available reference material meeting all the classical requirements of the definition. That being said though, I do think that the discussion about the issue of reference material should start way ahead of its clinical introduction. And if the development of an ideal material doesn't seem achievable in a realistic time frame, the best compromise or alternative solution should be actively pursued, explored, discussed, and planned. So, by the time the test starts to be used clinically, people have agreed on something that can fulfill at least some of the role

of a standard reference material. And unfortunately, that doesn't seem to be the way things usually go.

Randye Kaye: Great! Well, thank you so much for joining us today, Dr. Blais.

Dr. Jonatan Blais: It was my pleasure.

Randye Kaye: That was Dr. Jonatan Blais, from the Chaudière-Appalaches Integrated Health Center in Quebec, describing his original research article from the July 2019 issue of JALM, entitled, "Development of Reference Materials for Noninvasive Prenatal Aneuploidy Testing by Massively Parallel Sequencing: A Proof of Concept Study." Thanks for tuning in to this episode of "JALM Talk." See you next time, and don't forget to submit something for us to talk about.