Fetal Fraction Methodologies and Their Clinical Use: Results of a College of American Pathologists Exercise

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Guest: Dr. Glenn Palomaki is an Emeritus Professor of Pathology and Laboratory Medicine at the Alpert Medical School at Brown University in Providence, Rhode Island.

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

Cell-free DNA prenatal screening tests are used to assess whether a fetus is at increased risk for certain chromosomal abnormalities. A key factor in evaluating the quality and reliability of these tests is the measurement of the fetal fraction, the proportion of fetal cell-free DNA relative to maternal cell-free DNA in the blood sample. If the fetal fraction is too low, the test results may be inaccurate or even non-reportable. However, methods for estimating the fetal fraction and determining specimen adequacy vary across testing platforms and clinical laboratories.

The July 2025 issue of *JALM* features an article summarizing findings from sample challenges and supplemental questions on fetal fraction practices as part of a College of American Pathologists (CAP) Non-Invasive Prenatal Testing proficiency survey. The article's authors provide a global overview of current fetal fraction measurement practices and highlight opportunities for greater standardization in measuring and reporting the fetal fraction in prenatal screening for common trisomies.

Today we're joined by the article's corresponding author, Dr. Glenn Palomaki. Dr. Palomaki is an Emeritus Professor of Pathology and Laboratory Medicine at the Alpert Medical School at Brown University in Providence, Rhode Island. Welcome, Dr. Palomaki. Prenatal screening for common aneuploidies is now widespread. There is some controversy about test failures when the so-called fetal fraction is too low. What exactly is the fetal fraction?

Glenn Palomaki: Well, thank you for the introduction. And the maternal circulation has a large amount of maternal cell-free DNA and mixed in with that at about a 10 to 1 ratio, small amount is actually placental DNA, not fetal DNA, but placental DNA. And as you know from the experience of chorionic villus sampling,

sometimes the placenta has an abnormality that the fetus does not. So, this is definitely considered a screening test and not a diagnostic test.

So the ratio of this placental, or you can call it fetal cell-free DNA, to maternal cell-free DNA is called the fetal fraction. Again, we really should say the placental fraction, but we really want to know the answer to the fetus, so we've called it fetal fraction, and it is a screening test. I want to make sure everybody knows this is not a diagnostic test. It's a screening test. The diagnostic test would be a follow-up with preferably amniocentesis at 14, 15 weeks or later.

Randye Kaye: Why is fetal fraction measured and why is it important?

Glenn Palomaki: Well, there are multiple methodologies that different laboratories use. And we're not going to go into all the methods, but there are really four major methods. If the fetal fraction is too low or below the minimum that each laboratory sets, the idea is that there's insufficient material there to make a reliable call and the laboratories will often ask for another sample a week or two later when there's likely to be more, or a higher fetal fraction, and a reliable result can be obtained.

Randye Kaye: How is the fetal fraction used by these laboratories as part of clinical testing?

Glenn Palomaki: Well, the main reason for measuring fetal fraction is to determine from a laboratory's aspect whether the results are going to be reliable. So the laboratory would report a test failure if the fetal fraction result is lower than a cutoff that each laboratory sets on their own. But we did find that that cutoff varies widely between laboratories. Half the participants reported cutoffs of 1 to 3.9%, while the next most common cutoff was 4 to 6.9%. So to say that 4% is a common cutoff, yes, but it varies widely between laboratories. In fact, 16% of participants either don't even have a cutoff level or it's less than 1%. So there's wide variability by laboratory in the minimum required fetal fraction for clinical reporting.

Randye Kaye: I see. Well, how consistent were the within-laboratory fetal fractions over the three challenges?

Glenn Palomaki: Well, what we did was take each laboratory's three fetal responses and divided it by the median result of all the laboratories to create a MoM for the fetal fraction, a multiple of the median. When these were stratified by participants, the within-laboratory was really quite low for the majority. Almost 80% of participants were very consistent in their -- they weren't consistent between laboratories necessarily, but they were consistent within laboratories. There were a small

number of laboratories that were more than 20% below the participants for the 66, and 7 were more than 20% above the consensus of the 3 challenges. This just shows that there's wide variability in the definition of a test failure due to low fetal fraction across the laboratories that participated in the survey.

Randye Kaye: And so, what would you say is the whole takeaway message of this?

Glenn Palomaki: Well, first, it's important to recognize that fetal fraction measurements are an important component to testing, but they're laboratory specific. So, the idea that somebody can say, "Oh, well, if it's below 4%, it's no good. If it's above 2%, it's going to be okay." Those are laboratory specific decisions. And so, comparing fetal fractions between laboratories is also not recommended because of this variability. So, a single cutoff for an acceptable sample that's relevant for all laboratories simply is not possible.

Randye Kaye: All right. Thank you so much for joining us today, doctor.

Glenn Palomaki: You're welcome.

Randye Kaye: That was Dr. Glenn Palomaki, Emeritus Professor from the Alpert Medical School at Brown University, describing the *JALM* article "Fetal Fraction Methodologies and Their Clinical Use: Results of a College of American Pathologists (CAP) Exercise." 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.