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Letter to the Editor

Performance of non-invasive prenatal testing when fetal cell-free DNA is absent

Numerous studies have validated the accuracy of non-invasive prenatal testing (NIPT) using fetal cell-free DNA (cfDNA) to assess the risk of fetal aneuploidies early in pregnancy[1], and we have used this technology in our practice since 2012 in both low- and high-risk women[2].

We are aware that several factors influence the fraction of fetal cfDNA present in maternal blood. Such factors include gestational age and maternal weight[3], as well as methods of sample collection and shipping conditions that may lead to maternal cell hemolysis. Some commercial laboratories assert that the accuracy of cfDNA testing is influenced by the amount of fetal cfDNA relative to that of maternal cfDNA. In these laboratories that report fetal fraction, the performance claims for NIPT are based on testing that requires a minimal amount of fetal cfDNA to be present. We are also aware that some commercial laboratory providers assert that measurement of fetal cfDNA is unnecessary and that reliable results can be provided without prior knowledge of the amount of fetal cfDNA analyte in the sample.

In order to assess the reliability of NIPT, blood samples from two 44-year-old non-pregnant women were drawn and submitted to five American commercial laboratories offering NIPT. The first sample was sent in September 2014 and the second in October 2014. We reported the gestational age of both pregnancies as 12 weeks on each requisition form, and did not inform any of the five laboratories that the women were in fact not pregnant. Each laboratory was paid out-of-pocket and no third-party was billed. The NIPT results provided to us by each laboratory are presented in Table 1.

Two laboratories reported that there was insufficient fetal DNA present in the sample to provide a result. Three laboratories, two of which do not measure fetal fraction, provided test results suggestive of a genetically normal female fetus.

This example raises concerns about the need for quality standards in NIPT. We feel that the measurement of fetal cfDNA is a basic quality metric required to ensure reliable interpretation of test results. With karyotyping or fluorescence *in-situ* hybridization analysis, it is standard to require a minimum number of fetal cell colonies to be counted before reporting a result. It seems reasonable that for NIPT, an analogous control measure should be applied. While the promise of accurate performance with NIPT has been acknowledged widely in publications and realized in many clinical experiences, we urge professional medical and laboratory societies to set and enforce appropriate quality-control guidelines for NIPT that are consistent with standard laboratory practice as in other commercially available tests.

Disclosure

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Table 1 Non-invasive prenatal test (NIPT) results for two non-pregnant women from five commercial laboratories

	Patient 1		*Patient 2*	
Laboratory	*Test result available*	*Details*	*Test result available*	*Details*
Lab A	No	Insufficient fetal cfDNA for accurate NIPT evaluation	No	Insufficient fetal cfDNA for accurate NIPT evaluation
Lab B	No	Unable to report due to low fetal fraction (fetal fraction reported as 0.6%)	No	Unable to report due to low fetal fraction (fetal fraction reported as 0.6%)
Lab C	Yes	Negative, consistent with female fetus (fetal fraction 4.3% reported on request)	Yes	Negative, consistent with female fetus (fetal fraction 3.9% reported on request)
Lab D	Yes	No aneuploidy detected, two sex chromosomes (XX)	Yes	No aneuploidy detected, two sex chromosomes (XX)
Lab E	Yes	No aneuploidy detected, two sex chromosomes (XX)	Yes	No aneuploidy detected, two sex chromosomes (XX)

cfDNA, cell-free DNA.