777

Sample Specific Fetal Fraction Threshold for Non-Invasive Prenatal Testing

Amin R. Mazloom[1], Andrey Koch[1], Youting Sun[1], Jennifer Geis[1], Mark Whidden[1], Eyad Almasri[1], John Tynan[1], Timothy Burcham[1], Mathias Ehrich[1]
1. Sequenom, Laboratory Corporation of America® Holdings, San Diego, California 92121

Integrated GENETICS LabCorp Specialty Testing Group

sequenom

I. Abstract

Digital measurement of DNA copies in a maternal plasma sample has enabled Non Invasive Prenatal Testing (NIPT) to accurately assess Copy Number Variations (CNV) in the fetus even when fetal DNA fractional representation in the maternal blood is low (less than 0.1) and/or the size of a CNV is less than 10 Mb. Traditionally a lower threshold of 4% (fraction >= 0.04) has been applied to the fetal DNA fraction to qualify a sample for NIPT reporting. While a lower bound threshold of fetal fraction controls for a population based False Negative Rate (FNR), individual sample sensitivity is ascertained by the amount of fetal DNA fraction present in the plasma, as well as technical noise. While the fetal fraction is an uncontrolled biological factor, the latter is controlled by the process and the underlying technology. For instance in the case of Next Generation Sequencing (NGS) the noise in measurements of the dosage imbalance and/or the allelic imbalance is related to the number of short sequence reads that cover a genomic region, and is bounded at the lower end by theoretical limits of counting statistics. Here we present a method that compiles an NGS based estimate of fetal fraction and the observed noise level to create a Signal to Noise Ratio (SNR) measurement for each sample. This is then used as a quality metric to control for FNR. The SNR quality metric moves away from the population based cut-off of fetal fraction and allows for NIPT testing of samples with lower than 4% fetal fraction when the observed level of noise in the sample allows. The threshold of the aforementioned method is adjusted such that the sensitivity of the test remains unchanged. For instance, implementation of SNR method for MaterniT® 21 PLUS test enables the laboratory medical directors to report test results in certain cases when the fetal DNA fraction is as low as 2.5%. The proposed methods would result in a net positive experience for the patients by lowering the number of non-reportable cases, and also by reducing the expected turn-around time by eliminating unnecessary sample reruns or retesting (blood redraw) that would have resulted from a universal fetal fraction cut-off.

II. Introduction

DNA fragments found in maternal plasma comprise a disproportionate mixture of maternal DNA and placental DNA. The placental DNA fraction averages about 10% but can be less than 1%, depending on factors including maternal BMI, gestational age, size of the placenta, etc. Therefore, the NIPT (Non Invasive Prenatal Testing) signal is always embedded within a large maternal background. Other sources of controllable and uncontrollable noise during the sample processing and biological variability also attenuate the signal. In order to keep a high sensitivity in the NIPT, proper quality control (QC) metrics are used to account for signal attenuation.

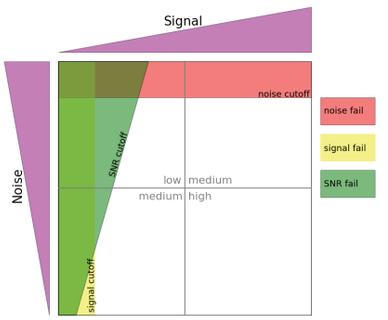
Our historical QC procedure is based on quality thresholds of several sample characteristics. If any characteristics fail quality thresholds, the sample is rejected or retested even though genomic events, if present, may be still detectable. **Fig.1** shows the effect of sample signal (as fetal fraction) and noise (as variance) on sample inclusion/failure rate. In order to avoid unnecessary retesting we propose a new QC metric based on the signal-to-noise ratio (SNR) concept used in signal processing applications [e.g. Meyer et al., 2013]:

$$SNR = \frac{Signal}{Noise}$$

Fig. 1, therefore, demonstrates how SNR, used in lieu of separate signal and noise cutoffs, helps to avoid rejecting samples with detectable signal and to increase sample inclusion rate.

The purpose of this study was to investigate the impact of SNR QC implementation on reducing the number of unnecessary retesting and rejecting of samples in a clinical laboratory setting. Therefore, we explore the effect of SNR QC on sample inclusion/failure rate and on the sensitivity of the test when it is used in place of a set of existing QC metrics.

Figure 1. Conceptual diagram of signal, noise, and SNR interplay and their effect on sample inclusion/ failure rate. SNR-dependent sensitivity level areas are marked by grey text



III. Methods

SNR formulation

SNR separates meaningful information (signal) from background information (noise). In statistical applications of SNR, signal is equivalent to a random variable expectation, and noise is equivalent to its variance. Here, cell-free DNA material (containing both maternal and fetal DNA) from pregnant plasma is tested for detection of genomic events in fetal DNA. The fractional fetal DNA in a sample determined by a linear model, as described in Kim et al., 2015, is treated as the signal in the QC procedure. The normalized number of sequencing reads falling in equally spaced bins across the genome (bin count), serves as a base variable in the QC and classification procedures. Median absolute deviation (MAD) of the bin counts across autosomal chromosomes is treated as the noise. Finally, SNR is termed as the ratio of fetal fraction less its measurement error and autosomal MAD:

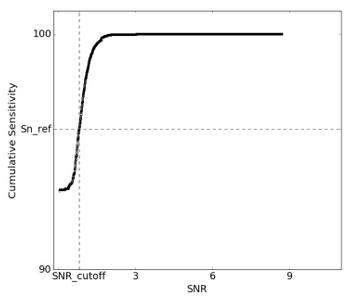
$$SNR = \frac{FF - FF\ error}{Autosomal\ MAD}$$

In classical SNR formulation, when signal and noise represent statistical properties of the same variable, meaningful signal would have SNR notably larger than 1. Because in our SNR formula above signal and noise are on different scales, SNR values do not adhere to a classical SNR range and meaningful signal could have SNR less than 1.

Finding SNR thresholds

In order to use SNR as a QC metric, the appropriate SNR threshold needs to be defined. While the method for establishing the SNR threshold is independent of the test type (MaterniT® 21 PLUS or MaterniT® GENOME), the threshold itself is not. In this approach a large number of fetal events, relevant to each test type, was simulated across large cohorts of negative or presumably negative consented clinical samples from each test. The cohort was selected to approximate the characteristics of a population of clinical samples. Simulations were performed by spiking artificial genome event signals into bin counts of euploid samples at a rate proportional to the sample's fetal fraction, thus ensuring the genome event strength stays within the population range. For MaterniT® 21 PLUS test, the signal was introduced to the whole chromosome 13, 18, and 21 bin counts to simulate trisomies in these chromosomes. For MaterniT® GENOME, the signal was introduced to the sub chromosomal regions across the entire genome. Event loci were selected based on the qualifying events reported in the International Standards of Cytogenetic Arrays (ISCA) database [ISCA database]. After this step, the augmented bin counts were normalized and passed on to the event identification software. The resulting sensitivities were compared to the reference historical sensitivity levels [Palomaki et al., 2011; Lefkowitz et al., 2016], corresponding to the QC failure rate currently used in the laboratory practice, and SNR thresholds (for each test) were found. The procedure of finding SNR thresholds for MaterniT® GENOME using historical sensitivity is illustrated on **Fig. 2**. Finally, SNR thresholds were applied to the clinical data and the new sample inclusion rates were compared to the old ones achieved by existing QC metric. A two-step quality control procedure, when only QC-failed samples were subjected to the SNR QC, was also performed.

Figure 2. SNR cutoff vs. Cumulative sensitivity of simulated samples for MaterniT® GENOME test



IV. Results

SNR vs. QC sample inclusion gain

Using the new SNR procedure allows to accept samples that would have been previously excluded, often because of a static fetal fraction cutoff. Comparing the two methods we find that we can include 0.24% more samples for MaterniT® 21 PLUS and 0.86% more samples for MaterniT® GENOME if all samples are tested against SNR cutoff. If SNR cutoff is applied to QC-failed samples only, we save 0.66% and 1.29% more samples, respectively. The sample inclusion gains by performing two different SNR QC procedures compared to the standard QC are summarized in **Table 1**.

Table 1. Sample inclusion gain summary

QC Metric	MaterniT® 21 PLUS %	MaterniT® GENOME %
SNR	0.24	0.86
SNR on QC-failed samples	0.66	1.29

V. Discussion

While the proposed method increases the sample inclusion rate, it has no adverse effect on the sensitivity of the test. Reduction in the overall turn-around time is another advantage of the SNR method which will have additional benefits, including: (a) net positive patient experience as it reduces the time to receive test results for the group of samples that would undergo unnecessary retesting (b) less hassle for the clinician as it reduces the frequency that they need to reach out to their patients and advise them about the delay, or worse when there is a need for a redraw. Accuracy of the observed fetal fraction is a function of noise. Therefore, a drawback of using the observed fetal fraction in the current quality control method is that it overlooks such dependency. Another interesting advantage of this method is the adaptability of its threshold to a particular event size or range, where a sample, disqualified for a smaller event detection, still may have sufficient quality to be tested for a larger event.

VI. Conclusions

- This new quality control metric based on SNR (signal-to-noise ratio) helps to reduce the number of clinical samples sent for retesting or rejected.
- From quality control application results summarized in **Table 1** we conclude that:
  - i. For MaterniT® 21 PLUS, the SNR metric, if used alone, saves 0.24% more samples than existing QC metric while an alternative procedure, SNR testing of only QC-failed samples, further increases the inclusion gain by 0.42%, totaling 0.66% more than the existing QC metric.
  - ii. For MaterniT® GENOME, the SNR metric, if used alone, saves 0.86% more samples than existing QC metric while SNR testing of only QC-failed samples increases the inclusion gain by additional 0.43%, totaling 1.29% more than the existing QC metric.
- Increase in sample inclusion rates achieved with the new QC metric based on sample SNR does not affect sensitivity of the MaterniT® 21 PLUS and MaterniT® GENOME assays.
- The use of SNR quality control should result in a net positive experience for the patient by lowering the number of non-reportable cases, and by reducing the expected turn-around time due to unnecessary sample reruns or retesting.

VII. References

Lefkowitz RB, Tynan JA, Liu T, et al. Clinical validation of a noninvasive prenatal test for genomewide detection of fetal copy number variants. *Am J Obstet Gynecol* 2016; 215:227.e1-16.

Meyer J, Dentel L, Meunier F (2013) Speech Recognition in Natural Background Noise. *PLoS ONE* 8(11):e79279. doi:10.1371/journal.pone.0079279

Palomaki GE, Kloza EM, Lambert-Messerlian GM, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med* 2011; 13:913-20.

Kim SK, Hannum G, Geis J, Tynan J, Hogg G, Zhao C, Jensen TJ, Mazloom AR, Oeth P, Ehrich M, van den Boom D, and Deciu C. (2015) Determination of fetal DNA fraction from the plasma of pregnant women using sequence read counts. *Prenatal Diagnostics*, doi:10.1002/pd.4615

ISCA genome database: http://dbsearch.clinicalgenome.org/search/

LabCorp