Information about Redraw Requests and Inconclusive Results with the Harmony prenatal test®

Introduction

Obstetric providers, patients, and laboratories share mutual interest in prenatal screening results that are accurate and easy to interpret. The probability score provided by the Harmony prenatal test® reflects the likelihood of trisomy in a sample with a correlating sensitivity and pecificity as demonstrated by prospective blinded studies of over 29,000 pregnancies.[1-12] A discussion of the Harmony test's probability score and relationship to positive predictive value is presented in the white paper Positive Predictive Value and Interpretation of Results of the Harmony Prenatal Test.[13]

While most samples submitted to the laboratory receive a result with probability scores, a small portion cannot be accurately evaluated due to either insufficient fetal fraction or not meeting quality control thresholds required for analysis. This usually does not represent a failure of the laboratory test, but instead reflects the complex nature of cell-free DNA and pregnancy biology. Samples receive a redraw request if the autosomal trisomy probability can not be assessed, or an inconclusive fetal sex and/ or sex chromosome aneuploidy (SCA) result if the sex chromosomes cannot be assessed. This paper addresses the integration of a redraw request or an inconclusive fetal sex and/or SCA report into clinical care by providing supplementary information regarding fetal fraction and quality thresholds.

Fetal Fraction

A pregnant woman's blood contains fragments of DNA from both the pregnancy and herself. The proportion of cell-free DNA fragments coming from the pregnancy is called the fetal fraction. A minimum of 4% fetal cell- free DNA in a specimen is necessary for accurate NIPT results.[1,14,15]

In a study looking at the effect of maternal weight and gestational age on fetal fraction, Wang et al. found that fetal fraction decreases with increased maternal body weight and increases with gestation, although the increase between 10-20 weeks gestation is small (averaging 0.1% per week).[16] The study also demonstrated that variance in fetal fraction seen in pregnancies is large even when maternal weight and gestational age are controlled for but that most pregnancies will have at least 4% fetal fraction after 10 weeks gestation. It is likely that other biological and environmental factors (such as infection, inflammation, and medications) also influence fetal fraction, but current data regarding the impact of these factors is very limited.

Some evidence shows a correlation between low fetal fraction and certain fetal aneuploidies (specifically trisomy 18 and trisomy 13), although the risk has been difficult to quantify.[11,17] A discussion of alternative screening and diagnostic testing is prudent for patients who either decline a second attempt at NIPT or who do not receive a result after two attempts.

Quality Control

Quality control measures ensure consistently accurate test performance so that the laboratory, patients and providers can have confidence in results. Each sample is assessed for quality and quantity of assay data, the consistency of the data within each chromosome, the number of informative loci for determination of fetal fraction, and the total amount of DNA in the sample. A result is not issued when quality control thresholds are not met. For each sample, a combination of unique biological and technical factors impact the quality of data obtained. Technical elements include unacceptable levels of variance in assay data related to specimen collection and/or processing. Biological factors encompass maternal, fetal, and other pregnancy-specific circumstances. Biological factors known to influence sample data are listed in Table 1. It is possible that there are biological circumstances with potential to influence sample quality that have yet to be identified.

Redraw Requests

About 2% of specimens submitted after 10 weeks gestation are issued a redraw request due to insufficient fetal fraction.[16] As described in the section discussing fetal fraction, maternal weight and gestational age are known to be correlated with fetal fraction. About two-thirds of pregnant women with a low fetal fraction from the first blood draw will have sufficient fetal fraction upon second attempt.[16] Table 2 details the percentage of patients with less than 4% fetal fraction upon redraw based on maternal weight. Providers may use this information in combination with clinical circumstances to determine the best follow-up for the patient.

About 1% of samples submitted receive a redraw request due to the sample not meeting thresholds for quality control.[18] It is not possible to rule out all biological factors influencing quality of data obtained from a sample, but it is important that a thorough review of clinical history is made prior to submitting a second sample for analysis. If review of history reveals a biological factor known to interfere with analysis, the laboratory should be notified. In some cases, the sample already submitted can be re-analyzed once clinical history information is updated by the lab. In other cases, submission of a

second sample will not be recommended. When review of history reveals no potential explanation for the sample not meeting QC thresholds, submission of a second sample for analysis will often produce a result.

Inconclusive Fetal Sex

An inconclusive fetal sex result indicates that the data obtained from the sample did not provide clear evidence of the presence or absence of the Y chromosome. Determining the presence or absence of the Ychromosome can be compromised by technical and biological factors (including benign variation in the structure of the Y chromosome and the quality of the DNA in the sample) without limiting reporting of the probability of trisomy. A sample that receives an inconclusive fetal sex result will also receive an inconclusive SCA result (if ordered). There is no evidence to suggest that an inconclusive fetal sex result corresponds to an increased risk (over the general population risk) for a sex chromosome aneuploidy in the fetus, but when analysis of Y chromosome is not clear, a SCA assessment cannot be made.

Inconclusive SCA

An inconclusive SCA result can be due to biological and technical factors influencing sex chromosome analysis that did not impact trisomy analysis. Technical factors are described in the Quality Control section above. Biological factors that can lead to an inconclusive SCA result, such as a demised co-twin, benign variations in the structure of the X or Y chromosome (copy number variants), and mosaicism for monosomy X or XXX in the mother or placenta are not uncommon in the general population, but can be difficult for the clinician to rule out.[19,20] It is for these reasons that submission of a second sample for analysis after an inconclusive SCA result may not yield a result for SCA. There is no evidence to suggest that an inconclusive SCA result corresponds to an increased risk (over the general population risk) for a sex chromosome aneuploidy in the fetus. An inconclusive SCA result indicates that the probability for fetal SCA has not been evaluated by the test. A decision regarding alternative methods of fetal sex chromosome aneuploidy assessment should be based on the patient's needs and any risk factors identified.

Summary

Most pregnant women receive complete results from cell- free DNA testing, indicating either a high or low probability for aneuploidy. As part of the laboratory's commitment to consistent and accurate performance, samples that do not meet quality control standards do not receive a probability assessment. Instead, these samples receive a redraw request (if autosomal trisomy probability cannot be evaluated) or an inconclusive fetal sex and/or SCA result if sex chromosomes cannot be assessed. The quality of data from a sample is affected by a combination of technical and biological factors, and while th e cause cannot always be identified, awareness of what factors can lead to these reports can help guide clinical follow-up.

Table 1
Biological factors influencing sample quality

Easily confirmed clinical history:

- Number of fetuses
- Biological relationship of egg donor to fetus
- Maternal history of organ transplant
- Consanguinity
- Demised co-twin

Clinical history that may/may not be known

- Maternal chromosome condition
- Vanishing twin
- Maternal malignancy or organ transplant

Clinically elusive factors

- Copy number variant (maternal, fetal or placental)
- Mosaicism (maternal, fetal and/or placental)

Table 2
Proportion of women with less than 4% fetal fraction on repeat blood draw[16]

Maternal Weight, kg	% with less than 4% fetal fraction on redraw
<90	29
90<100	39
100<110	41
110<120	41
120<130	61
130<140	61
140+	82

In Brief: Redraw Requests

Please refer to associated white paper for more comprehensive information

No Result Reported for trisomy 21, 18 and 13 (Redraw Request)

Possible reasons

Biological

- Too little fetal cell-free DNA present in the sample: "low fetal fraction (FF)"
  - Fetal fraction varies greatly from pregnancy to pregnancy
  - Fetal fraction is more likely to be low in pregnancies with higher maternal weight and early in gestation[16]
  - Studies have shown an association between low fetal fraction and fetal trisomies 18 and 13[11,17]

Technical

- Quality of data obtained from the sample does not meet laboratory standards
  - Strict standards are employed to ensure consistently accurate results
  - Unacceptable quality of data may be related to sample collection or processing

---

Discussion points

- Clinical context and patient's needs should be considered
- Second sample may be submitted for analysis
  - Most women will have sufficient fetal fraction upon redraw[16]
- The likelihood of a result with submission of a third sample has not been established
- Other options include conventional serum screening, fetal ultrasound and invasive testing

In Brief: Inconclusive Results

Please refer to associated white paper for more comprehensive information

Inconclusive fetal sex report

Additional information

Determining presence or absence of the Y chromosome can be compromised by factors which do not limit reporting of trisomy
- Quality of the sample data from the Y chromosome may be impacted by
  - quality and quantity of DNA in sample
  - features of the mother/baby/placenta/pregnancy

- When analysis of Y chromosome is not clear, a sex chromosome aneuploidy assessment cannot be made
- An inconclusive fetal sex result does not necessarily indicate an increased risk for sex chromosome aneuploidy in the baby (over the general population risk)
- The likelihood of a fetal sex result with submission of a second sample has not been established

---

Follow-up options

- Clinical context and patient's needs should be considered
- Fetal sex may be evaluated by ultrasound and/or invasive testing

In Brief: Inconclusive Results

Please refer to associated white paper for more comprehensive information

Inconclusive sex chromosome aneuploidy report

Additional information

Quality of the sample data from the X and Y chromosomes may be impacted by

- the amount of DNA in sample
- features of the mother/baby/placenta/pregnancy

- An inconclusive fetal sex result does not necessarily indicate an increased risk for sex chromosome aneuploidy in the baby (over the general population risk)
- A probability assessment for these conditions cannot be provided for the sample
- The likelihood of a sex chromosome aneuploidy result with submission of a second sample has not been established

---

Follow-up options

- Clinical context and patient's needs should be considered
- Invasive testing may be considered in some circumstances

Bibliography

1. Ashoor G, Syngelaki A, Wagner M, Birdir C, Nicolaides K. Chromosome-selective sequencing of maternal plasma cell-free DNA for first-trimester detection of trisomy 21 and trisomy 18. Am J Obstet Gynecol 2012;206:322.e1-5.
2. Nicolaides KH, Syngelaki A, Ashoor G, Birdir C, Touzet G. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. Am J Obstet Gynecol 2012;207:374.e1–6.
3. Norton ME, Brar H, Weiss J, et al. Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. Am J Obstet Gynecol 2012;207:137.e1–8.
4. Ashoor G, Syngelaki A, Wang E, et al. Trisomy 13 detection in the first trimester of pregnancy using a chromosome-selective cell-free DNA analysis method. Ultrasound Obstet Gynecol 2013;41:21–5
5. Brar H, Wang E, Struble C, Musci TJ, Norton ME. The fetal fraction of cell-free DNA in maternal plasma is not affected by a priori risk of fetal trisomy. J Matern Fetal Neonatal Med. 2013;26(2):143-145.
6. Gil MM, Quezada MS, Bregant B, Ferraro M, Nicolaides KH. Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies. Ultrasound Obstet Gynecol. 2013;42(1):34-40.
7. Verweij EJ, Jacobsson B, van Scheltema PA, et al. European non-invasive trisomy evaluation (EU-NITE) study: a multicenter prospective cohort study for non-invasive fetal trisomy 21 testing. Prenat Diagn 2013;33:996–1001.
8. Gil MM, Quezada MS, Bregant B, Syngekaju A, Nicolaides K. Cell-free DNA analysis for trisomy risk assessment in first-trimester twin pregnancies. Fetal Diagn Ther 2014;35:204– 1111
9. Hooks J, Wolfberg AJ, Wang ET, et al. Non-invasive risk assessment of fetal sex chromosome aneuploidy through directed analysis and incorporation of fetal fraction. Prenat Diagn. 2014;34(5): 496-499.
10. Nicolaides KH, Musci TJ, Struble C, Syngelaki A, Gil MM. Assessment of Fetal Sex Chromosome Aneuploidy Using Directed Cell-Free DNA Analysis. Fetal Diagn Ther. 2014;35(1):1-6.
11. Norton ME, Jacobsson B, Swamy GK, et al. Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med 2015;372:1589–97.
12. Stokowski R, Wang E, White K, et al. Clinical performance of non-invasive prenatal testing (NIPT) using targeted cell-free DNA analysis in maternal plasma with microarrays or next generation sequencing (NGS) is consistent across multiple controlled clinical studies. Prenat Diagn. 2015 Dec;35(12):1243-6.
13. Positive Predictive Value and Interpretation of Results of the Harmony Prenatal Test. https://www. ariosadx.com/files/7714/7558/5733/MM-00757_Webpage_PPV-Harmony_Test_Interpretation.pdf. Accessed July 20 2016
14. Jani J, Rego de Sousa MJ, Benachi A. Cell-free DNA testing: how to choose which laboratory to use? Ultrasound Obstet Gynecol. 2015;46:515-517.
15. Canick J, Kloza EM, Lambert-Messerlain GM, et al. DNA Sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations. Prenat Diagn. 2013 Jul;33(7):667-74.
16. Wang E, Batey A, Struble C, et al.. Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. Prenat Diagn. 2013;33(7):662-666.
17. Revello R, Sarno L, Ispas A, Akolekar R, Nicolaides KH. Screening for trisomies by cell-free DNA testing of maternal blood: consequences of failed result. Ultrasound Obstet Gynecol. 2016; 47: 698–704
18. Roche Diagnostics, data on file
19. Russell L, M, Strike P, Browne C, E, Jacobs P, A, X chromosome loss and ageing. Cytogenet Genome Res 2007;116:181-185.
20. Landy HJ, Weiner S, Corson SL, et al. The "vanishing twin": ultrasonographic assessment of fetal disappearance in the first trimester. Am J Obstet Gynecol. 1986 Jul. 155(1):14-9

The Harmony non-invasive prenatal test is based on cell-free DNA analysis and is considered a prenatal screening test, not a diagnostic test. Harmony does not screen for potential chromosomal or genetic conditions other than those expressly identified in this document. All women should discuss their results with their healthcare provider who can recommend confirmatory, diagnostic testing where appropriate.

MC--05394