Evaluation of Automated Cell-Free DNA Extraction Methods with the Harmony® Prenatal Test

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Abstract

Non-invasive prenatal testing (NIPT) utilizing cell-free DNA (cfDNA) from maternal blood is a highly sensitive and specific screening method for fetal aneuploidy. Extraction of highly fragmented, dilute cfDNA from large plasma volumes is difficult, but imperative in order to ensure accuracy for NIPT testing. This study compares the performance of three automated cfDNA extraction platforms when used with the Roche Harmony prenatal test. Remnant plasma samples from pregnant women were processed through the Harmony test using the MagNA Pure 24, MagNA Pure 96 and QiaSymphony SP/AS nucleic acid extraction platforms with the Ariosa cell-free DNA System (AcfS). The test results were compared to those generated from replicate sets of samples processed at the CAP-accredited Ariosa Clinical Laboratory using the CLIA-certified, lab-developed Harmony test. All cfDNA extraction methods resulted in 100% agreement in Trisomy 21, 18, and 13 results to those obtained by the Ariosa Clinical Laboratory, including concordant detection of 5 positive Trisomy 21 samples. All three platforms are therefore validated for performing cfDNA extraction prior to running the Harmony prenatal test on the AcfS.

Introduction

Screening for fetal aneuploidy using non-invasive prenatal testing (NIPT) is now recognized as a valuable option by the American College of Obstetrics and Gynecology (1) and the American College of Medical Genetics and Genomics (2) for all pregnant women. In utero, aneuploidy increases the risk for preterm labor and is associated with increased morbidity and mortality postnatally (3). Current first trimester screening utilizing biomarker and ultrasonography methods has false positive rates as high as 5% for which definitive diagnosis utilizing invasive methods (i.e. chorionic villus sampling or amniocentesis) is required (4). NIPT utilizing the presence of fetal cfDNA in maternal blood has a higher sensitivity and specificity, and therefore fewer false positives that require confirmatory invasive diagnosis (5). Due to the superior performance demonstrated in published studies and the rapid adoption of NIPT, NIPT testing is now available in over 60 countries worldwide, with the potential for increasing availability and uptake (5, 6).

cfDNA is released by many different tissues, including the placenta. It is highly fragmented and rapidly metabolized. Extraction of cfDNA is difficult given the small amount present in circulation, the fragmented nature, and rapid turnover. In pregnancy, both maternal and fetal (placental) cfDNA circulates in the maternal blood, with fetal cfDNA detectable reliably at 7 weeks gestation and increasing throughout pregnancy (5,7). In order to utilize fetal cfDNA for NIPT, it is crucial that the nucleic acid is effectively extracted. Automation of nucleic acid extraction allows for standardization and improves the reliability of downstream assays, including assays used for NIPT. Additionally, automation improves throughput by improving laboratory efficiencies and decreases risk for human error.

The Harmony® prenatal test (Ariosa Diagnostics Inc., San Jose, CA) is a validated screening test for use in pregnant women at ≥ 10 weeks gestation to assess risk for fetal trisomies 13, 18, and 21, sex chromosome aneuploidies (SCA) and the 22q.11.2 deletion (7,8). The Harmony test is provided as a laboratory-developed test (LDT) by the CAP-accredited Ariosa Clinical Laboratory. Since 2011 over 1.4 million pregnant women have been tested. The Harmony test is also available using the CE IVD (launched 2016) or Research Use Only (RUO) (launched 2015) Harmony kits on the Ariosa cell-free DNA System (AcfS). However, the AcfS does not include a cfDNA extraction platform and requires that laboratories develop their own cfDNA extraction method or use a commercially available platform.

The MagNA Pure 24 and MagNA Pure 96 Systems (Roche Molecular Solutions, Inc., Pleasanton, CA) are automated magnetic bead based platforms that efficiently extract nucleic acids from a variety of clinical sample types. These platforms are designed for clinical utility with standard processes described in the manufacturer's Package Insert. The QiaSymphony SP/AS (Qiagen, Germantown, MD) is also an automated magnetic-bead based extraction platform that provides customer flexibility, with protocols tailored to individual laboratories and applications. In this study, the performance of the MagNA Pure 24, MagNA Pure 96, and QiaSymphony SP/AS as platforms for cfDNA extraction for the Harmony prenatal test run on the AcfS was evaluated.

Methods

Subjects and Specimens

Remnant, anonymized plasma samples from 815 pregnant women with specimens submitted for Harmony prenatal testing by the Ariosa Clinical Laboratory were used for this study. Only samples from women with signed consent forms explicitly permitting the utilization of their remnant samples for research purposes were included. No identifying information was available for any patient. All subjects met the inclusion criteria for the Harmony test.

Maternal blood specimens were collected in either Roche Cell-free DNA or Streck Cell-free DNA BCT® collection blood tubes. Plasma was extracted after centrifugation for 10 min at 1600 rcf and was either immediately processed through cfDNA extraction or the plasma was stored frozen at -20ºC. Plasma volumes ranged from 2.5 – 4.7 mL.

cfDNA Extraction

Each subject had 2 replicate plasma samples; one replicate was extracted on the Ariosa Clinical Laboratory cfDNA extraction platform and one replicate was extracted with another automated extraction platform, i.e., MagNA Pure 96 (Roche), MagNA Pure 24 (Roche), or QiaSymphony SP/AS (Circulating DNA Kit, Qiagen). All extractions followed the manufacturer's instructions for use or the approved, validated protocols for the Ariosa Clinical Laboratory. Eight-hundred and fifteen (815) samples were extracted on the Ariosa Clinical Laboratory platform using the approved, validated protocols of the laboratory-developed test. The resulting cfDNA was eluted in a final volume of 150 µl for each sample and transferred to 96-well plates for immediate use in the Harmony LDT test.

Three-hundred and seventy-six (376) samples were extracted on the Roche MagNA Pure 24 using the manufacturer's protocol for the MagNA Pure 24 Total NA Isolation Kit and MagNA Pure cfNA Buffer Set. The resulting cfDNA was eluted in a final volume of 150 µl and transferred manually to 96-well plates. The cfDNA samples were stored frozen at -20ºC prior to use in the Harmony test on the AcfS.

One-hundred and eighty-nine (189) samples were extracted on the Roche MagNA Pure 96 using the manufacturer's protocol for the MagNA Pure 96 DNA and Viral NA Large Volume Kit and MagNA Pure cfNA Buffer Set. The resulting cfDNA was eluted in a final volume of 200 µL and 150 µl of each sample was transferred manually to 96-well plates. The cfDNA samples were stored frozen at -20ºC prior to use in the Harmony test on the AcfS.

Two-hundred and forty-seven (247) samples were extracted on the QiaSymphony SP/AS using customized protocols for the Circulating DNA Kit (circDNA4_CR21575_ID1498, circDNA4_CR21190_ID1498, circDNA4_CR21284_ID1588). The resulting cfDNA was eluted in a final volume of 150 µl and transferred to 96-well plates for immediate use in the Harmony test on the AcfS.

Harmony Prenatal Test and Data Analysis

All cfDNA samples were processed using the approved procedures of the Harmony prenatal test, with one replicate processed using the Harmony test performed at the CLIA-certified Ariosa Clinical laboratory and the other replicate using the Harmony RUO kit (Ariosa Diagnostics Inc., San Jose CA) on the AcfS following manufacturer's instructions for use. Probabilities for trisomy 21, trisomy 18, and trisomy 13 were generated only for samples that passed the quality metric thresholds for the Harmony Test. Fetal fraction measurements from the Harmony test were generated for each sample (9). Detailed descriptions of the Harmony test are previously published (10,11).

Harmony test results were compared for agreement between paired sample replicates where both samples passed Harmony QC criteria for agreement of trisomy probabilities for chromosome 21 (T21), chromosome 18 (T18) and chromosome 13 (T13). For each paired sample replicates, a linear regression of the Harmony measurements of fetal fraction (R2 coefficient) between the different extraction methods was calculated.

Results

The Harmony test conducted by the Ariosa Clinical Laboratory provided results for 809 of the subjects that were low probability for all three trisomies (T21, T18, and T13), 5 subjects that were high probability for trisomy 21, and 1 subject that was high probability for trisomy 13.

All three cfDNA extraction methods demonstrated 100% agreement of the Harmony test results for trisomy 13, 18, and 21 probabilities with the replicate sample processed at the Ariosa Clinical Laboratory, including 100% agreement for the 5 trisomy 21 positive subjects. The paired trisomy 13 subject was extracted with the QiaSymphony SP/AS and did not pass the Harmony test QC metrics, and therefore, could not be evaluated for agreement.

All three cfDNA extraction methods demonstrated highly correlated fetal fraction measurements with the replicate sample processed at the Ariosa Clinical Laboratory, with R2 values of 0.93 for the MagNA Pure 24, 0.92 for the MagNA Pure 96, and 0.90 for the QiaSymphony SP/AS (Table 1).

Table 1

Automated cfDNA extraction method	Agreement of Harmony Test Results (T13, T18, T21)	Harmony Fetal Fraction Linear Regression (R2)
MagNA Pure24	100%	0.93
MagNA Pure96	100%	0.92
QiaSymphony SP/AS	100%	0.90

Discussion

The goal of our study was to determine the ability of the MagNA Pure 24, the MagNA Pure 96, and the QiaSymphony SP/AS to extract cfDNA for the Harmony prenatal test. All three automated, bead-based cfDNA extraction methods, Roche MagNA Pure 24, Roche MagNA Pure 96 and Qiagen QiaSymphony SP/AS demonstrate high performance with the Harmony prenatal test with 200 to 400 pregnant women samples tested per extraction method. Each extraction method demonstrated 100% agreement with the Ariosa Clinical Laboratory for the Harmony prenatal test algorithm's calculation for probability of trisomy 13, 18 and 21. In addition, the fetal fraction measurements generated by the Harmony prenatal test were highly correlated across all tested cfDNA extraction platforms, demonstrating that the platforms provide comparable extraction of the highly fragmented, dilute cfDNA from large plasma volumes.

Conclusion

As NIPT continues to expand, the need for laboratories to perform high quality and high throughput testing will continue to increase. All three platforms evaluated here are validated for performing cfDNA extraction prior to running the Harmony prenatal test on the AcfS.

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