Development of Matched Maternal-fetal Non-invasive Prenatal Testing (NIPT) Reference Materials Compatible with a Broad Range of Assay Methods

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Introduction and Methods

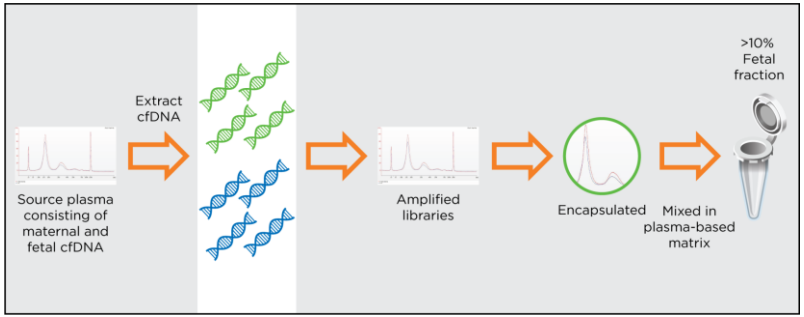
Problem: How to assure quality of NIPT tests and concordance of test results between various platforms?

Introduction

- There is limited availability of clinical samples which can be used for proficiency testing, assay validations, and run controls
- LGC SeraCare has developed a new technology and formulation to provide a sustainable source of patient-like NIPT reference materials
- The fetal-maternal match signifies that the material is compatible with a broad range of NIPT assays
- These materials are used by clinical labs to validate and monitor NIPT assays

Methods

- Antepartum and postpartum samples were obtained from patients with high-risk pregnancies under IRB approved protocols.
  - ccfDNA was isolated from ante- and post-partum samples
  - This isolated ccfDNA was amplified*
  - Encapsulated by a proprietary lipophilic procedure
  - Blended into a synthetic plasma to create patient-like samples.



**Patent pending WO2018/094183AI*

Results

Seraseq® NIPT reference materials (Figure 1 in red) demonstrated patient-like fragment distribution (for both maternal and fetal sizing) with a major peak of around 160 - 170 bp and minor peaks at 340 and 510 bp reflecting the pattern of endogenous ccfDNA (in blue).

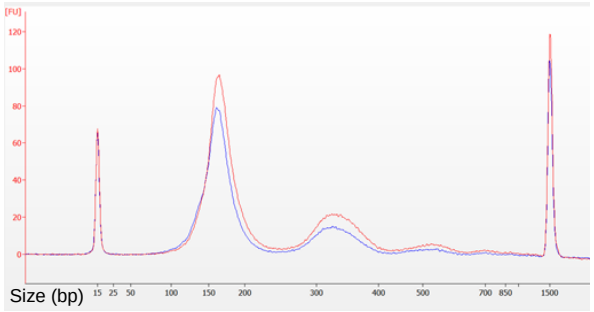


Figure 1. Representative DNA size profile of the SeraSeq 22q11 male-matched reference material (red) compared to normal human ccfDNA (blue) rendered on an Agilent BioAnalyzer.

Table 1. Comparison of test results by different assays on the same NIPT reference materials

	VeriSeq™	Verifi™	Harmony®	Panorama®
T21 male	11%	5.8%	7.5%	NT
T21 female	13%	NT	NT	NT
Euploid male	9.4%	NT	NT	7%
Euploid female	18%	12%	16.6%	NT
T18 male	NT	3.5%	5.3%	NT
T18 female	12%	NT	NT	5.3%
22q11 male	N/A*	NT	25.9%	26.2%

* Size of 22q11 microdeletion is approximately 2.8Mb, undetectable by a current version of VeriSeq test; NT – not tested

These materials are tested before release by at least one commercial NIPT assay (Table 1). As you can see there is some variation in the fetal fractions reported by the different tests.

Performance of Seraseq® NIPT Reference Materials

Table 2. Seraseq® NIPT materials have been extensively tested by multiple commercial NIPT assays and shown to generate expected data.

NIPT Test	Seraseq® NIPT aneuploidy materials
VeriSeq™	✓*
Harmony®	✓
Panorama®	✓
Verifi™	✓
Iona®	✓
Sage™	✓
Vanadis®	✓
Praena®	✓
NIFTY®	✓

* With exception of 22q11 reference materials

The data below shows actual results from VeriSeq™ testing:

- All genders were called correctly
- Fetal fractions were reported for each reference sample. They ranged from 9.4% for male euploid, to 18% for female euploid. Fetal fraction for trisomies was between 11.5 to 13%

Euploid female – 18%FF

Sample	Trisomy status	Fetal Gender	Fetal Fraction	Method	Pregnancy status	T13/T18/T21/XY	QC
104443-1 104443-2 104443-3	NO ANEUPLOIDY DETECTED	XX	0.18	ILMN Veriseq 48plax (pair end)	Singleton	NO ANEUPLOIDY DETECTED	PASS

Euploid male – 9.4%FF

sample ID	Trisomy status	Fetal Gender	Fetal Fraction	Pregnancy Status	T13	T18	T21	QC
seracare-euploid2	NO ANEUPLOIDY DETECTED	XY	9%	Singleton	NO ANEUPLOIDY DETECTED	NO ANEUPLOIDY DETECTED	NO ANEUPLOIDY DETECTED	PASS
seracare-euploid3	NO ANEUPLOIDY DETECTED	XY	10%	Singleton	NO ANEUPLOIDY DETECTED	NO ANEUPLOIDY DETECTED	NO ANEUPLOIDY DETECTED	PASS
seracare-euploid1	NO ANEUPLOIDY DETECTED	XY	9%	Singleton	NO ANEUPLOIDY DETECTED	NO ANEUPLOIDY DETECTED	NO ANEUPLOIDY DETECTED	PASS

T21 female – 13%FF

Sample	Trisomy status	Fetal Gender	Fetal Fraction	Pregnancy status	T13/T18/XY	T21	QC
1	ANEUPLOIDY DETECTED	XX	0.12	Singleton	NO ANEUPLOIDY DETECTED	ANEUPLOIDY DETECTED	PASS
2	ANEUPLOIDY DETECTED	XX	0.14	Singleton	NO ANEUPLOIDY DETECTED	ANEUPLOIDY DETECTED	PASS
3	ANEUPLOIDY DETECTED	XX	0.12	Singleton	NO ANEUPLOIDY DETECTED	ANEUPLOIDY DETECTED	PASS

T21 male – 11.5%FF

Sample	Trisomy status	Fetal Gender	Fetal Fraction	Method	Pregnancy status	T13/T8/XY	T21	QC
104442-4 104442-5	ANEUPLOIDY DETECTED	XY	0.11	ILMN Veriseq 48plax (pair end)	Singleton	NO ANEUPLOIDY DETECTED	ANEUPLOIDY DETECTED	PASS
104442-6	ANEUPLOIDY DETECTED	XY	0.12	ILMN Veriseq 48plax (pair end)	Singleton	NO ANEUPLOIDY DETECTED	ANEUPLOIDY DETECTED	PASS

T18 female – 12%FF

Sample	Trisomy status	Fetal Gender	Fetal Fraction	Method	Pregnancy status	T13/T21/XY	T18	QC
1	ANEUPLOIDY DETECTED	XX	0.12	ILMN Veriseq 48plax (pair end)	Singleton	NO ANEUPLOIDY DETECTED	ANEUPLOIDY DETECTED	PASS
2	ANEUPLOIDY DETECTED	XX	0.12	ILMN Veriseq 48plax (pair end)	Singleton	NO ANEUPLOIDY DETECTED	ANEUPLOIDY DETECTED	PASS
3	ANEUPLOIDY DETECTED	XX	0.12	ILMN Veriseq 48plax (pair end)	Singleton	NO ANEUPLOIDY DETECTED	ANEUPLOIDY DETECTED	PASS

Conclusions

- **Seraseq NIPT reference materials are:**
  - compatible with multiple testing platforms
  - patient-like thus allowing to perform an end-to-end QC
  - and preserve the fetal fraction observed in source pregnancy samples
- **Our ccfDNA amplification technology allows to construct materials for testing today and diagnostics in a future**
- **By using Seraseq NIPT materials it is possible to assure quality of the assay and objectively compare the results from different platforms**

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