Non-Invasive Prenatal Testing:
Laboratory Clinical Experience: 20000 Clinical Samples

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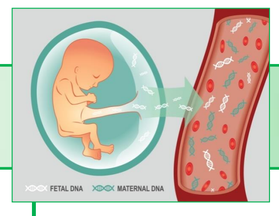

Introduction and Aims

Whole-genome sequencing (WGS) of maternal plasma cell-free DNA (cffDNA) can potentially evaluate all 24 chromosomes to identify abnormalities of the placenta, fetus, or pregnant woman. The objective of this study is to give a complete and robust clinical picture of the current performance of NIPT (non invasive prenatal testing) for trisomy 13, 18, and 21 and sex chromosomes aneuploidies as well as for the other chromosomes.

CE-IVD

Materials and methods

All data were generated in our AMES accredited laboratory from January 2017 to January 2019 in 20000 samples. The pipeline included automated library preparation (VeriSeq NIPT Microlab STAR, Illumina) and WGS sequencing on a Next550 (Illumina)..

VeriSeq NIPT Assay Software (www.illumina.com/NIPTsoftware) was used for data analysis of aneuploidy status of 13, 18, 21, X and Y chromosomes and fetal fraction of cffDNA. A in house algorithm was optimized to analyze other aneuploidies and subchromosomal aberrations. (From Bayindir B, et al. Eur J HumGenet 2015; 23:1286–93)

Results

Screen positive test results were reported in 343 cases, leading to a screen positive rate of approximately 1.7%, and confirmed by karyotyping or SNP-array following invasive prenatal diagnosis. Trisomies involving chromosomes 21 (N=152), 18 (N=50) and 13 (N=28) are most frequently reported (1.2%). 94 positive cases for aneuploidies involved sexual chromosomes (SCAs) were reported (**Table 1**). Finally we reported the performance of NIPT in twin pregnancies and in assisted reproductive technology (ART) (**Table 2**).

Table 1: Clinical performance based on clinical Experience

Overall performance n=20000	TP	FP	FN	Sensitivity % (95% CI)	Specificity % (95% CI)
T21	151	1	0	100	100
				(97.52,100.0)	(99.97, 100.0)
T18	44	6	0	100	99.97
				(91.97, 100.0)	(99.93, 100.0)
T13	24	4	0	100	99.98
				(86.2,100.0)	(99.95, 100.0)
SCAs	80	14	0	100	99.93
				(95.42,100.0)	(99.88, 99.96)
All	299	25	0	100	99.89
				(98.5,100.0)	(99.83,99.93)

Table 2: Clinical outcome data for twin and ART pregnancies

Multiple gestation n =441	TP	FP	FN	Sensitivity % (95% CI)	Specificity % (95% CI)
T21	5	0	0	100	100
				(56.55,100.0)	(99.13, 100.0)
T13	0	1	0		99.77
					(99.13, 100.0)
ART pregnancies n=984	**TP**	**FP**	**FN**	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**
T21	2	0	0	100	100
				(34.24,100.0)	(99.61, 100.0)
T18	2	0	0	100	100
				(34.24,100.0)	(99.61, 100.0)
T13	0	1	0		99.77
					(99.13, 100.0)
SCAs	7	3	0	100	99.69
				(64.57,100.0)	(99.10, 99.69)

We also identified 20 rare autosomal trisomies (RATs) and 6 structural abnormalities in 10500 samples, mainly trisomy 15 (n = 6), followed by trisomy 8 (n = 4) and trisomies 16 and 22 (each n=2) (**Figure 1, Table 3**).

Figure 1

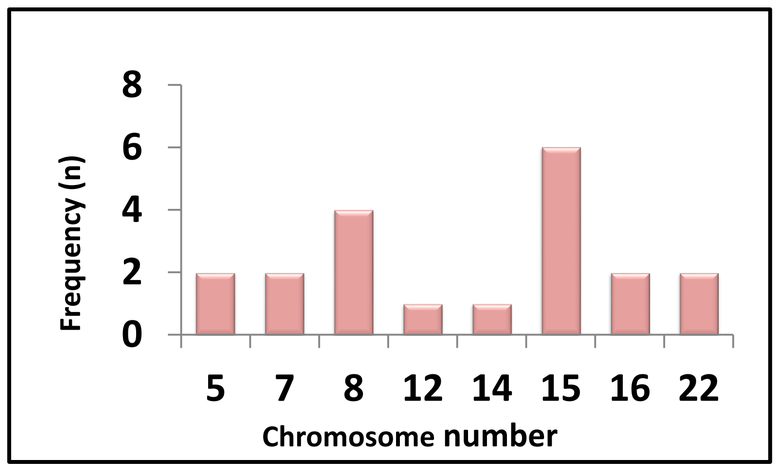

Table 3	N	Genetic Testing	Outcome
Trisomy 5	2	CPM (1), No testing (1)	LB (FGR)
Trisomy 7	2	CPM (2)	LB (FGR)
Trisomy 8	4	No testing (1), CPM (2)	MC (1), LB (2), FGR (1)
Trisomy 12	1	NAD (1)	MC (1)
Trisomy 14	1	CPM (1)	LB (1)
Trisomy 15	6	CPM (2), TFM (4), UDP (1)	MC (1), TOP (4), LB (1)
Trisomy 16	2	POC (1), TFM (1)	TOP (1), FSA, FGR (1)
Trisomy 22	2	POC (1), TFM (1)	MC (2)
Structural Abnormalities	6	CPM (4), TFM (2)	PN (2), LB (2)FSA, FGR, MT (2)

Abbreviations: CPM, confined placental mosaicism; FGR, fetal growth restriction; FSA, fetal structural abnormality on ultrasound; LB, live birth; MC, miscarriage; NAD, no abnormality detected; POC, trisomy confirmed on products of conception; PN, Postnatal anomalies found; TOP, termination of pregnancy, MT, maternal CNV

Conclusions

The performance characteristics were established in samples in which we analysed all 24 chromosomes with a minimum fetal fraction of 4%, and has been confirmed by our extensive clinical experience in the same clinical population.

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The authors have no conflicts of interest to declare