Eurofins Genoma NIPT performance data related to CE-IVD accreditation

Background

For many years now, Non-Invasive Prenatal Testing (NIPT) has become part of clinical practice and is used as a screening test by thousands of pregnant women in order to avoid invasive tests such as amniocentesis and chorionic villus sampling as much as possible and reduce the risks associated with them. NIPT analysis has expanded over time, allowing the study of not only trisomies 21, 18 and 13 (common fetal trisomies) and sex chromosome aneuploidies (SCA), but also microdeletion/duplication syndromes and genome-wide analysis that includes rare autosomal aneuploidies (RAAs) and partial deletions/duplications (1,2). Although, rarer fetal anomalies and partial deletions/duplications have a low prevalence, these anomalies can cause serious pregnancy complications and affect patient care. These complications include early miscarriage, fetal demise, intrauterine growth restriction, and birth defects (4).
NIPT based on massively parallel whole-genome sequencing, using paired-end sequencing (4), analyses small fragments of placenta- derived cell free DNA that are circulating in a pregnant woman's blood, allowing the detection of common aneuploidies, RAAs and deletions/duplications with high accuracy.
VeriSeq NIPT Solution V2 is a CE-IVD integrated platform and software that uses a CE-IVD protocol for genome-wide screening providing information on partial deletions/duplications >7Mb for all the autosomes and the aneuploidy status for all chromosomes.
Until now the potential to improve the performance of VeriSeq V2 protocol remained to be investigated.

Scope and design of the study

In this study, we aimed to validate the clinical utility of Eurofins Genoma Group's NIPT performances in the detection of genome-wide fetal anomalies including common trisomies, SCAs, RAAs, and partial deletion/duplication >7 Mb and <7 Mb.
We **retrospectively analysed 71,883** patients undergoing NIPT from the general pregnancy population collected over a 24-month period between December 2019 and December 2021. We applied **Illumina VeriSeq NIPT solution v2** in combination with our **proprietary algorithm, developed in-house (Fig.1)**

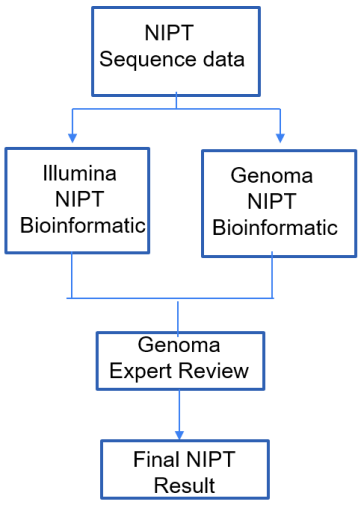

Fig.1 Analysis and interpretation of the results with Eurofins Genoma's algorithm

The results of NIPT were compare with those of the clinical outcome. Clinical outcomes, i.e., clinical truth, for study cases were determined by invasive prenatal diagnostic techniques (cytogenetic analysis after chorionic villus sampling (CVS) and/or amniocentesis), as well as by ultrasound and new-born physical exam. Positive NIPT results for fetal aneuploidy were considered confirmed when validated by either invasive prenatal diagnostics or an anomaly observed on ultrasound that matched the positive NIPT call.

Results

The results of this study show that the already excellent performances validated by Illumina are further improved when combined with the **test algorithm** designed by **our bioinformaticians** and **the experience of our professionals** allowing us to achieve an overall **very high sensitivity (99.49%) and specificity (99.88%**) (Table 1)**.**

Table 1. NIPT Performances for common aneuploidies, SCAs and other abnormalities in 71883 pregnancies

Positive cases *n*=1011 Total follow-ups *n*=868	**Triomy 21**	**Trisomy 18**	**Trisomy 13**	**SCA**	**Other abnormalities***	**Overall performances**
True positives	437	93	37	156	58	781
False positives	3	1	8	17	54	83
True negatives	71392	71775	71828	65598	46577	70872
False negatives	2	0	0	1	1	4
Sensitivity (95% CI)	**99.54%** (98.36% - 99.94%)	**99.9%** (96.11% - 100.00%)	**99.9%** (90.51% - 100.00%)	**99.36%** (96.50% - 99.98%)	**98.31%** (90.91% - 99.96%)	**99.49%** (98.70% - 99.86%)
Specificity (95% CI)	**99.9%** (99.99% - 100.00%)	**99.9%** (99.99% - 100.00%)	**99.99%** (99.98% - 100.00%)	**99.97%** (99.96% - 99.99%)	**99.88%** (99.98% - 99.99%)	**99.88%** (99.86% - 99.91%)
PPV (95% CI)	**99.32%** (97.92% - 99.78%)	**98.94%** (92.91% - 99.85%)	**82.22%** (69.82% - 90.24%)	**90.17%** (85.08% - 93.66%)	**51.79%** (45.08% - 58.42%)	**90.39%** (88.36% - 92.11%)
NPV (95% CI)	**99.9%** (99.99% - 100.00%)	**99.9%** (99.99% - 100.00%)	**99.9%** (99.99% - 100.00%)	**99.9%** (99.99% - 100.00%)	**99.9%** (99.99% - 100.00%)	**99.99%** (99.99% - 100.00%)

CI: Confidence Intervals; SCA: Sex Chromosome Aneuploidies.
Positive cases without follow-up that have been excluded from the positives reported in Table 1 (n°): T21 (49); T18 (14); T13 (10); SCA (36); Other abnormalities (34).
**Rare autosomal aneuploidies, segmental anomalies and microdeletions are included.*

In particular, for **common aneuploidies** such as **trisomy 21, 18 and 13 overall sensitivity and specificity** were **99.65%** and **99.99%**. **Sex chromosome aneuploidies** showed a high reliability for XXY (Klinefelter syndrome), XYY (Jacobs syndrome) and XXX (trisomy X) anomalies and a slightly lower reliability for monosomy X (Turner syndrome) (Table 2), confirming excellent overall **sensitivity (99.36%)** and **specificity (99.97%)** (Table 1).

Table 2. NIPT Performances for sex chromosome aneuploidies

Sex chromosome aneuploidies	X0	XXX	XXY	XYY
True positives	52	27	51	26
False positives	13	0	3	1
True negatives	65724	65775	65747	65776
False negatives	1	0	0	0
Sensitivity (95% CI)	**98.11%** (89.93% - 99.95%)	**99.99%** (87.23% - 100.00%	**99.99%** (93.02% - 100.00%)	**99.99%** (86.77% - 100.00%)
Specificity (95% CI)	**99.98%** (99.97% - 99.99%)	**99.99%** (99.99%- 100.00%)	**99.99%** (99.99% - 100.00%)	**99.99%** (99.99% - 100.00%)
PPV (95% CI)	**80%** (69.88% - 87.34%)	**99.99%** (99.99% - 100.00%)	**94.44%** (84.57% - 98.14%)	**96.3%** (78.55% - 99.46%)
NPV (95% CI)	**99.99%** (99.99% - 100.00%)	**99.99%** (99.99% - 100.00%)	**99.99%** (99.99% - 100.00%)	**99.99%** (99.99% - 100.00%)

CI: Confidence Intervals; SCA: Sex Chromosome Aneuploidies.
Positive cases without follow-up that have been excluded from the positives reported in Table 2 (n°): X0 (18); XXX (6); XXY (7); XYY (5).

The data obtained for rare trisomies reveal **high sensitivity and specificity (99.99%; 99.92%),** although with a low positive predictive value due to the high rate of feto-placental mosaicism and the risk of early spontaneous abortion found in these cases (Table 3). The clinical usefulness of detecting rare trisomies is confirmed in relation to the possible effects of feto-placental mosaicism on fetal growth and on the occurrence other pregnancy related complications, especially in the third trimester of gestation. It is also particularly useful in identifying uniparental disomy of chromosomes subject to imprinting, as in the case of trisomy 15 whose rescue has led to the identification of two cases of Prader-Willi syndrome.

Our NIPT analysis shows very good performances for the testing of segmental anomalies with dimensions greater than 7 Mb (sensitivity 99.99%; specificity 99.97%) and in this case too, the presence of false positives is attributable to feto-placental mosaicisms, as well as to the presence of maternal benign neoplasms such as uterine fibroids (Table 3).

The analysis of such a large statistical sample also allowed us to show for the first time the performances of the microdeletion syndrome tests, with promising results considering the rarity of the conditions investigated (sensitivity 83.33%; specificity 99.99%) (Table 3).

Table 3. NIPT Performances for rare autosomal aneuploidies, segmental chromosomal abnormalities, and microdeletions

Other anomalies	RAA	Segmental anomalies (>7 Mb)°°§§	Microdeletions* (segmental anomalies <7 Mb)§§
True positives	33	20	5
False positives	36	16	2
True negatives	46630	46681	28743
False negatives	0	0	1
Sensitivity (95%CI)	**99.99%** (89.42% - 100.00%)	**99.99%** (83.16% - 100.00%)	**83.33%** (35.88% - 99.58%)
Specificity (95%CI)	**99.92%** (99.89% - 99.95%)	**99.97%** (99.96%- 99.99%)	**99.99%** (99.99% - 100.00%)
PPV (95%CI)	**47.83%** (39.81% - 55.96%)	**55.56%** (43.37%- 67.11%)	**71.43%** (37.40% - 91.27%)
NPV (95%CI)	**99.99%** (99.99% - 100.00%)	**99.99%** (99.99% - 100.00%)	**99.99%** (99.99% - 100.00%)

CI: Confidence Intervals; RAA: Rare Chromosomal Aneuploidies.
Positive cases without follow-up that have been excluded from the positives reported in Table 3 (n°): RAA (25); Segmental abnormalities >7Mb (7); Microdeletions (2).
***Investigated microdeletions: Di George Syndrome, Cri-du-chat Syndrome, Prader-Willi Syndrome, Angelman Syndrome, 1p36 Deletion Syndrome, Wolf-Hirschhorn Syndrome, Jacobsen Syndrome, Langer-Giedion Syndrome, and Smith-Magenis Syndrome.*
§§Details are showed in Suppl. Table 1 and 2

With regard to the occurrence of false-negative results, these were in line with the international scientific literature on the limits of NIPT. An in-depth analysis of the few cases tested (<0.005%) confirmed the essential need to combine the NIPT study with an accurate ultrasound evaluation for correct and complete prenatal assessment.

This study demonstrates that our NIPT-algorithm is reliable and accurate when applied to maternal DNA samples collected from pregnant women. The performance data, which are superior to those of an already highly reliable protocol such as Illumina VeriSeq protocol, confirm the crucial importance of **data interpretation**, guaranteed by the experience of our biologists, geneticists and bioinformaticians, and the importance of having access to **a large and constantly updated sample pool** for the design of a valid test algorithm.

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