

## CLINICAL APPLICATION OF PAIRED-END MPSS FOR CFDNA SCREENING OF COMMON ANEUPLOIDIES IN AVERAGE RISK PREGNANCIES

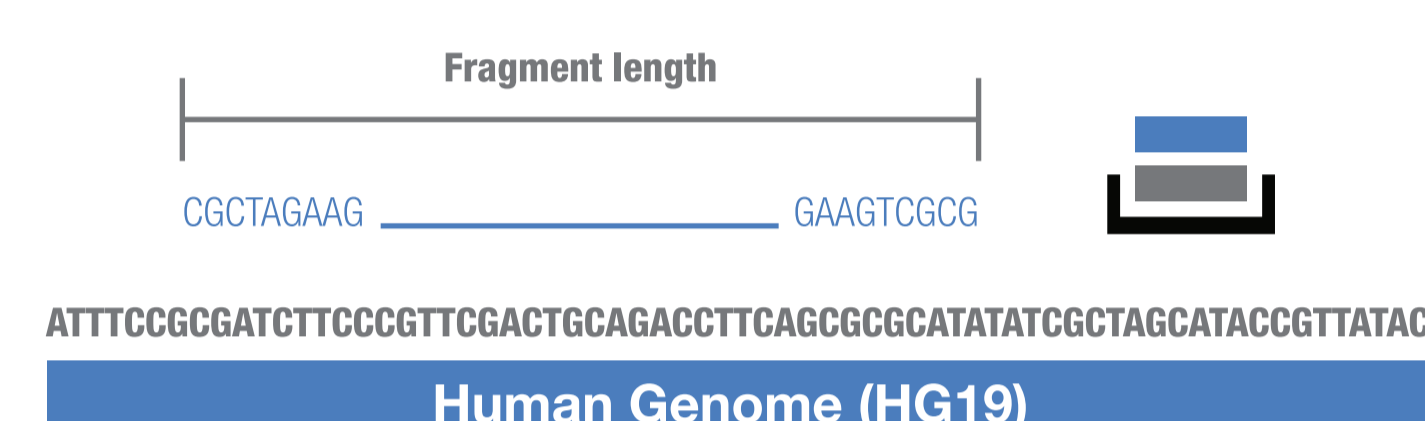
V. Cirigliano, E. Ordoñez, L. Rueda, S. Nicolas, M. Grau, I. Castilla, C. Puertollano, M. Lechuga, M. Cañadas  
**Department of Molecular Genetics. Synlab Diagnosticos Globales, Esplugues de Llobregat, Barcelona, Spain**

Paired-end MPSS allows digital counting of plasma cfDNA while also determining fragments length. cfDNA size differences can be used to estimate fetal fraction (FF) and to additionally applying counting statistics on short (fetal) fragments, thus potentially improving cfDNA screening performance. **NeoBona is the first test developed to exploit this approach. We evaluated its performance by screening a large cohort of consecutive average risk gestations.**

### METHODS

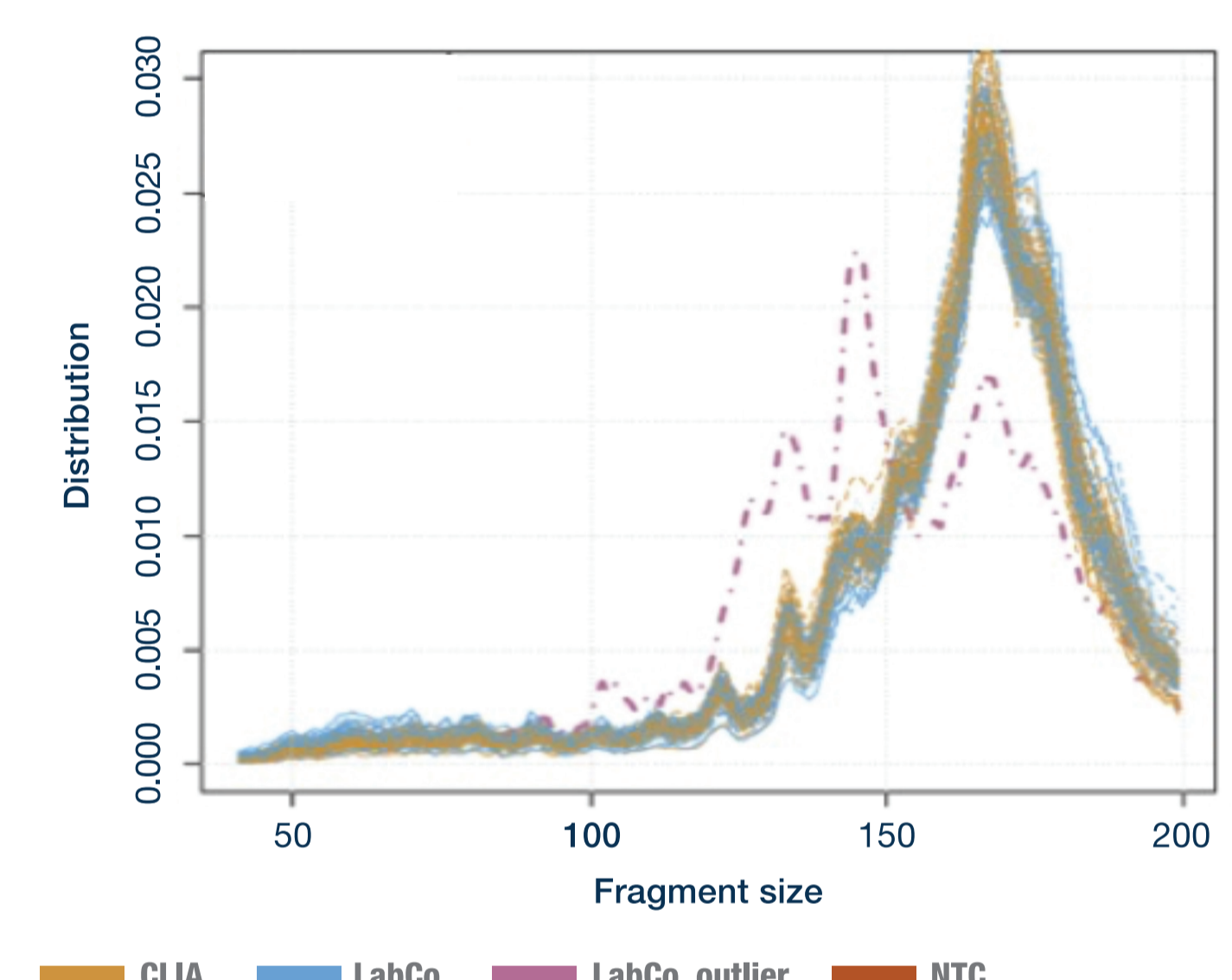
The new paired-end based MPSS of the NeoBona test provides input data for a novel analysis algorithm which generates a multicomponent Tscore value. Trisomy likelihood ratios (Tscore) for each chromosome of interest is calculated for each sample based on the resulting estimated fetal fraction, inter-chromosome statistics derived from both short and total fragments and the total unique sequencing counts (non excluded sites) on each chromosome.

#### Paired End Sequencing

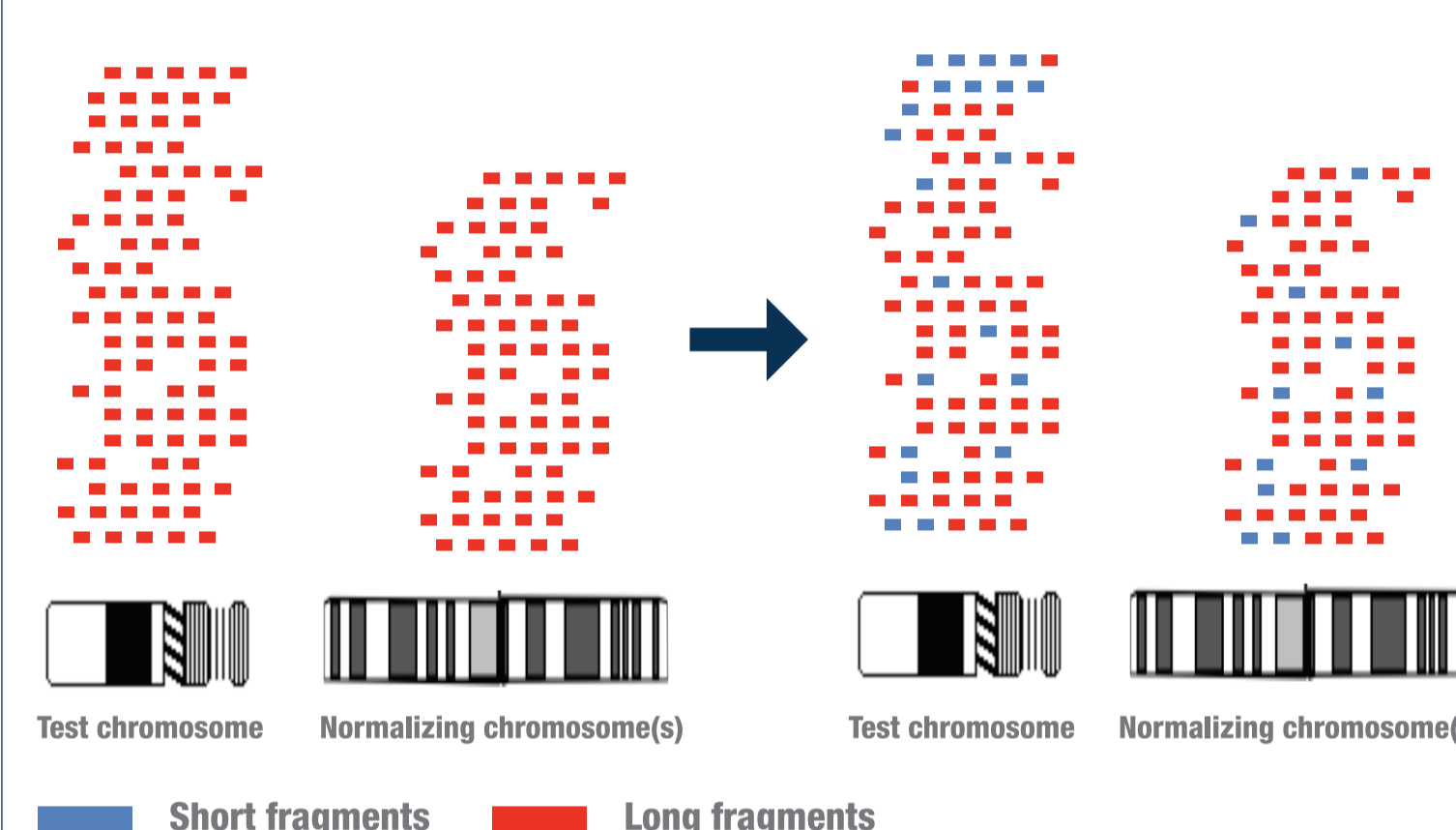


- Paired End Sequencing allows determining the length of cfDNA molecules thus fragment sizes distribution and fetal fraction.

#### Fragment Size Distribution



#### Analysis Method



#### Standard methodology

- Using **counting statistics**, determine if more than the expected number of reads mapped to the target chromosome

#### New methodology

- Using **counting statistics**, determine if more than the expected number of reads mapped to the target chromosome
- Using **fragment size statistics**, determine if the fraction of short fragments was higher on the target chromosome
- Combine** the counts and fragment size statistics for final scoring

- A total of 19151 pregnancies (575 twins) were screened for common trisomies. Sex Chromosome analysis and fetal sex determination was offered as an option in singleton cases. Samples were collected from several centres in European, South American and Middle East countries to be analysed in the molecular genetics department of Synlab in Barcelona, where cfDNA screening was performed.
- Blood samples were collected from pregnant women above 10 weeks of gestation regardless their risk category using Streck BCT tubes. Test was carried out using 1 mL of plasma processed in batches of 96 on a fully automated workstation (Hamilton Star, Hamilton Reno US) designed to handle plasma isolation, column based DNA extraction, set up of sequencing library, quantification, normalization and pooling (Fig.1). Sequencing libraries from each batch were collected in 2 separate pools of 48 double indexed samples which underwent paired-end MPSS for 2 sets of 36 cycles using the NextSeq 500 sequencers with TG NextSeq 500/550 High Output Kit v2. Sequencing outputs were analyzed using a novel bioinformatics approach generating chromosome specific Tcores where cut offs were applied (VeriSeq NIPT software v1.0.9, Illumina Inc., San Diego US).

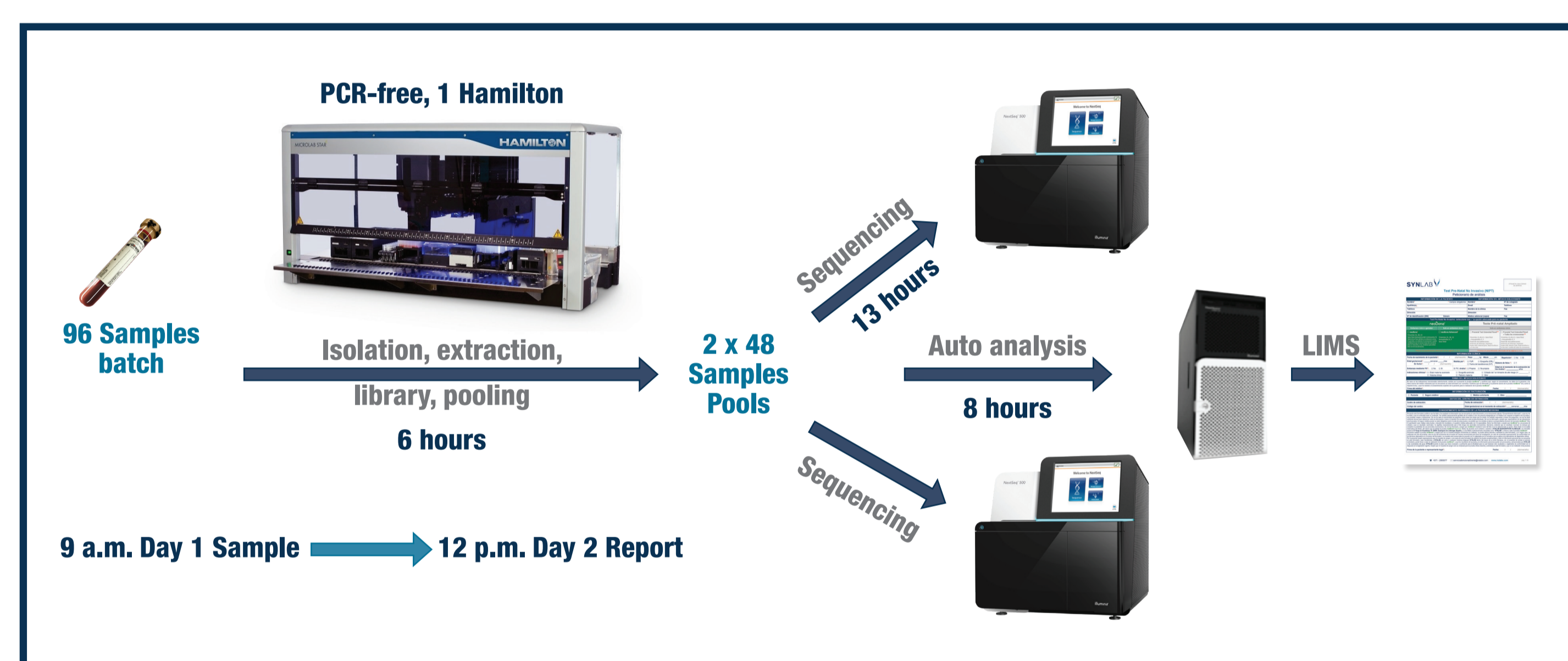


Fig.1. Workflow and Analysis Pipeline. Automated, PCR-Free Sample Prep, Paired-End Sequencing

### RESULTS

- Patients choose to only assess risk for autosomal trisomies in 8169 cases (42,7%), screening for XY aneuploidies was also requested for 10982 samples (57,3%). Test results were provided in 19017 (99,3%) gestations and 288 Trisomy 21, 63 Trisomy 18 and 27 Trisomy 13 were detected (Fig.2). Valid results could be obtained with FF below 4% in 1205 cases, including 23 trisomies which were correctly scored despite having fetal fractions between 0.8 and 3%.
- Invasive procedures were performed in 99% of pregnancies at high risk for trisomy, 5 false positives were observed for Trisomy 21, 2 for Trisomy 18 and 3 for Trisomy 13 (FPR 0.03%, 0.01% and 0.02% respectively). One Trisomy 21 was missed in a male pregnancy with 7% FF (DR 99.7%). XY aneuploidies were reported in 36 cases, follow-up with prenatal diagnostic procedures was only available for 14 with 4 false positive results (FPR 0.13%). Vanishing twins of discrepant sex were suspected in 5 cases and 4 samples with maternal X aneuploidies were also identified. Samples were redrawn in 240 out of 334 test failures and valid results could be obtained in 83,3% of cases (Failure Rate: 0,7%).

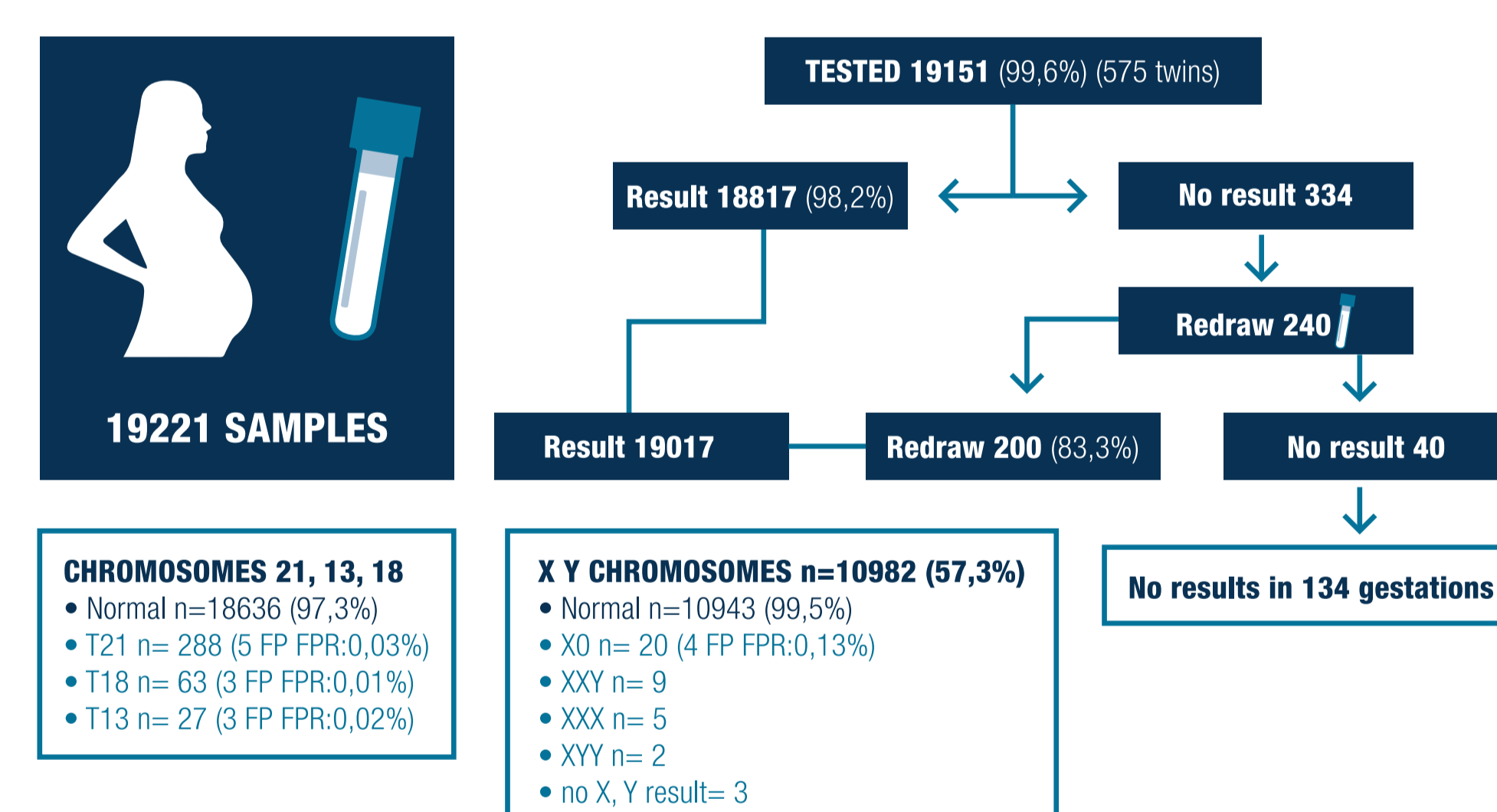


Fig.2. Clinical application of NeoBona test in 19.151 consecutive samples. FP: False positive; FPR: False positive rate.

### CONCLUSIONS

- Paired-end MPSS proved highly efficient for aneuploidy screening of average risk pregnancies, allowing detecting chromosome aneuploidies with extremely low false positive rates (cumulative 0,06% for autosomal trisomies).
- Despite the low overall FPR for XY aneuploidies observed in the course of this study, extending cfDNA based screening to sex chromosomes in general population should be considered with caution as it still resulted in a 2-fold increase of the cumulative FPR.
- The novel bioinformatic approach including cfDNA size distribution and size based counting statistics, allowed detecting all autosomal trisomies with fetal fractions below 4%, while also reducing FPR.
- Removing the need of a lower limit of FF allowed cfDNA analysis to be successful on a high proportion of clinical cases thus extending the benefits of cfDNA screening to a larger population of pregnancies.

Table 1. Results of NeoBona test.

	FN	FP	DR	FPR
<b>T21 n=288</b>	1	5	99.6%	0.03%
<b>T18 n=63</b>	-	2	100%	0.01%
<b>T13 n=27</b>	-	3	100%	0.02%

**FPR 0,06%**

Results of screening for XY aneuploidies

	n	Amnio	FP	FPR
<b>45,X</b>	20	9	4	0.13%
<b>47,XXY</b>	9	5	0	0.00%
<b>47,XXX</b>	5	3	0	0.00%
<b>47,XYY</b>	2	2	0	0.00%
<b>Mat</b>	3			
<b>Total</b>	36	19	4	

**FPR 0,13%**

FN: False negative; FP: False positive; DR: Detection rate; FPR: False positive rate.

**Contact Information**  
vincenzo.cirigliano@synlab.es  
www.neobona.com

**www.synlab.es**

European Human Genetics Conference.  
Milan, Italy | June 16-19, 2018

