First clinical application of paired-end MPSS for cfDNA screening of aneuploidies
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Objective
To evaluate the performance of the NeoBona test, a new paired-end MPSS based assay, for cfDNA aneuploidy screening in average risk pregnancies.

Methods
A total of 6000 consecutive samples were collected from pregnant women above 10w of gestation (188 twins), regardless their risk category. Samples were analysed using the NeoBona test, which allows simultaneous assessment of fetal fraction, cfDNA fragments size distribution and chromosome counting statistics, through a novel Tscore value reflecting the likelihood for chromosome aneuploidy. Chromosome specific pre-established cut-offs were applied at Tscores to classify normal and aneuploid cases. Samples were tested and reports issued within 5 days from collection.

Results
Nineteen cases were not suitable for analysis, 5981 were tested and results obtained for 5856 (98%). A total of 96 trisomies 21, 17 trisomies 18 and 13 trisomies 13 were identified, in 5 cases with FF between 1 and 3%. Invasive procedures were performed to confirm the results in 98% of cases. Two false positive results were observed for trisomy 21 and one for trisomy 13 (FPR 0.03% and 0.02% respectively). Screening for sex chromosomes aneuploidy was carried out in 57% of samples tested and 15 such cases were identified. Invasive procedures were performed to confirm the results in 9/15 cases (45, X n=4; 47, XXY n=3; 47, XXX n=1 and 47, XYY n=1) and one false positive result was observed (45, X; FPR 0.05%). No follow up for normal pregnancies was available yet, being the great majority still ongoing. A total of 125 cases failed to provide result (2 twins), sample redraw following test failure provided valid results in 81% of cases. Thus, the NeoBona test showed overall success rate of 99.6% with a redraw rate of 2%.

Conclusion
Paired-end MPSS coupled with the new analysis algorithm of the NeoBona test, proved highly efficient, allowing cfDNA analysis to be successful on a high proportion of clinical cases. The new multifactorial Tscore allowed detecting aneuploidies with confidence even at fetal fractions as low as 1% with reduced false positive rates. Eliminating the need of a fixed lower limit at fetal fraction for reportable cases has the potential to extend the benefits of cfDNA screening to a larger population of pregnancies.