Performance of targeted cell-free DNA (cfDNA) analysis with microarray quantitation for assessment of fetal sex and sex chromosome aneuploidy risk

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The accuracy of targeted cell-free DNA (cfDNA) testing with DANSR™ and FORTE™ for trisomies 21, 18 and 13 has been well demonstrated and is consistent across next

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generation sequencing and microarray quantitation methods.[1] Targeted cfDNA analysis for fetal sex chromosome aneuploidy (SCA) has also been validated and shown to have high specificity in prospective studies.[2,3] This study expands upon the available published data by describing the performance of targeted cfDNA analysis of the X and Y chromosomes using microarray quantitation for assessment of SCA probability in singleton pregnancies and fetal sex in twin and singleton pregnancies.

Samples of banked maternal plasma from 791 singleton and 51 twin pregnancies were obtained as part of ongoing multi-center clinical studies (NCT02201862 and NCT01451671) and from a sample bank at King's College London. Single cell-free Roche, Streck BCT-DNA, or EDTA collection tubes were available for each sample. Collection and processing differed from commercial protocols only in that all samples were frozen prior to analysis and available specimen volumes were lower than standardly used. Patient consent and fetal karyotype information was obtained for all samples. The cohort included 15 singleton pregnancies with sex chromosome aneuploidy.

Targeted cfDNA analysis with microarray quantitation was performed as previously described under a blinded protocol.[4] Y-chromosome specific DANSR assays were used to evaluate fetal sex in twin and singleton pregnancies. Results were reported as male or female, depending on concluded presence or absence of Y-chromosome fragments. In twin pregnancies, a result of male indicates the presence of at least one male fetus. Fetal SCA analysis was performed on samples from singleton pregnancies using X- and Y-specific DANSR assays followed by FORTE analysis adapted for this purpose.[2,4,5] A probability cut-off of 1 in 100 for non-disomic genotypes was used for calculation of

sensitivity and specificity.

Fetal sex assessment was performed for singleton and twin pregnancies; SCA analysis was performed in singletons. Average gestational age was 16.7 weeks. 51 samples had insufficient fetal fraction or failed to pass quality control thresholds. 748 of 752 singleton and 39 of 39 twin samples yielded fetal sex results. Fetal fraction averaged 13.4%. Predicted fetal sex was consistent with karyotypic sex in 786/787 cases (99.9% concordance) (Table 1). All twin fetal sex cfDNA results accurately reflected either the presence of two female fetuses (18 cases) or at least one male fetus (21 cases).

742 samples were eligible for SCA assessment. All sex chromosome aneuploidies were correctly identified (100% sensitivity; 95% CI 79.6-100%) (Table 1). 740 out of 742 disomic (XX or XY) pregnancies were correctly classified as low-risk for SCAs (99.7% specificity; 95% CI: 99-99.9) (Table 1).

In summary, targeted cell-free DNA analysis performed with high accuracy for fetal sex in twins and singletons, and correctly identified all SCAs with high specificity. A limitation of using these banked samples is that the positive predictive value observed in this enriched cohort would not be translatable to a routine prenatal screening population. In addition, the number of samples passing quality thresholds may be lower than standard due to irregular sample volumes. Ultimately however, this study provides a valuable supplement to the currently available data supporting the use of targeted cfDNA analysis for fetal sex and SCA assessment and substantiates previous conclusions that the performance of this methodology is robust across quantitation platforms.

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Table 1 Performance of cfDNA screening across publications

PUBLICATION	FETAL SEX n/N % (95% CI)		SEX CHROMOSOME ANEUPLOIDY‡ n/N % (95% CI)						
	Accuracy*		45,X		47, XXX§		47, XXY§		Euploid
	Singleton	Twin	DR	FPR	DR	FPR	DR	FPR	Accuracy*
CURRENT	747/748 99.9 (99.3-100)	39/39 100 (91-100)	13/13 100 (77.2-100)	1/742 0.1 (0-0.8)	1/1	1/742 0.1 (0-0.8)	1/1	0/742 0 (0-0.5)	740/742 99.7 (99-99.9)
Hooks et al[2]	414/414 100 (99.1-100)		26/27 96.3 (81.7-99.3)	2/380 0.5 (0.2-1.9)	1/1	2/380 0.5 (0.2-1.9)	6/6	0/380 0 (0-1)	378/380 99.5 (98.1-99.9)
Nicolaides et al[3]	109/110 99.1 (95-99.8)		43/47 91.5 (80-96.6)	0/172 0 (0-2.2)	5/5	1/172 0.6 (0.1-3.2)	1/1	0/172 0 (0-2.2)	115/116 99.1 (95.3-99.9)

* Accuracy is defined as concordant cfDNA and karyotype resut

‡ DR = detection rate, FPR = false positive rate

§Sensitivities for the individual SCAs cannot be concluded from this data based on small number of affected pregnancies