An innovative non-invasive prenatal testing (NIPT) assay offers the potential for a low-cost, highly-accurate aneuploidy screen in the global population

Alka Chaubey[1], Kate Hanauer[1], Justin Leighton[1], Yong Wee Wong[2], Chan Lee Yin[2], Madhuri Hegde[1]

1. PerkinElmer Genomics, Pittsburgh, PA 2. DNA Lab, Kuala Lumpur, Malaysia

Background

Noninvasive prenatal testing (NIPT) for screening of common aneuploidies has been an effective and established test in clinical practices across the United States since 2011. With demonstrated advantages over conventional serum screening, most notably a high detection rate with low false positive rate, the American College of Medical Genetics and Genomics recommends informing all pregnant patients of the option of NIPT irrespective of a priori risks. Several methodologies are available for NIPT in the clinical space; however, the cost and necessity of complex bioinformatics of these methods limits the widespread acceptance of NIPT in routine prenatal care, particularly in regions outside the United States. Additionally, a high no-call rate of PCR-based NIPT methodologies prevents many patients from obtaining reliable NIPT results. In an effort to develop a cost-effective assay that allows for more effective screening globally, we validated the Vanadis® NIPT, which utilizes rolling-circle replication rather than PCR, as a laboratory developed test (LDT) for the detection of trisomies 13, 18 and 21 in a laboratory in Malaysia.

Methods

A total of 324 samples were analyzed by Vanadis® NIPT technology to complete LDT validation for aneuploidy screening for a laboratory in Malaysia. 216 samples were utilized for analytical sensitivity and specificity performance metric calculations in 3 separate runs. 108 samples were run for accuracy and precision studies which included SeraSeq T21, T18 and T13 reference materials and a set of normal plasma samples. Assay technology is described in Figure 1. Sample types included in this validation study are maternal plasma from confirmed trisomies, maternal plasma from pregnancies negative for an aneuploidy and SeraSeq reference material. The following performance metrics were performed in this study: analytical sensitivity and specificity, no-call rates, accuracy and precision. Quality assessment and automated data analysis was performed and samples were classified as either low or high risk based on Z score cutoffs of 3.5 for chromosome 21 and 3.15 for chromosomes 18 and 13. Samples failing quality assessment were classified as no-call. In addition to aneuploidy screening, 214 samples had fetal sex classification performed.

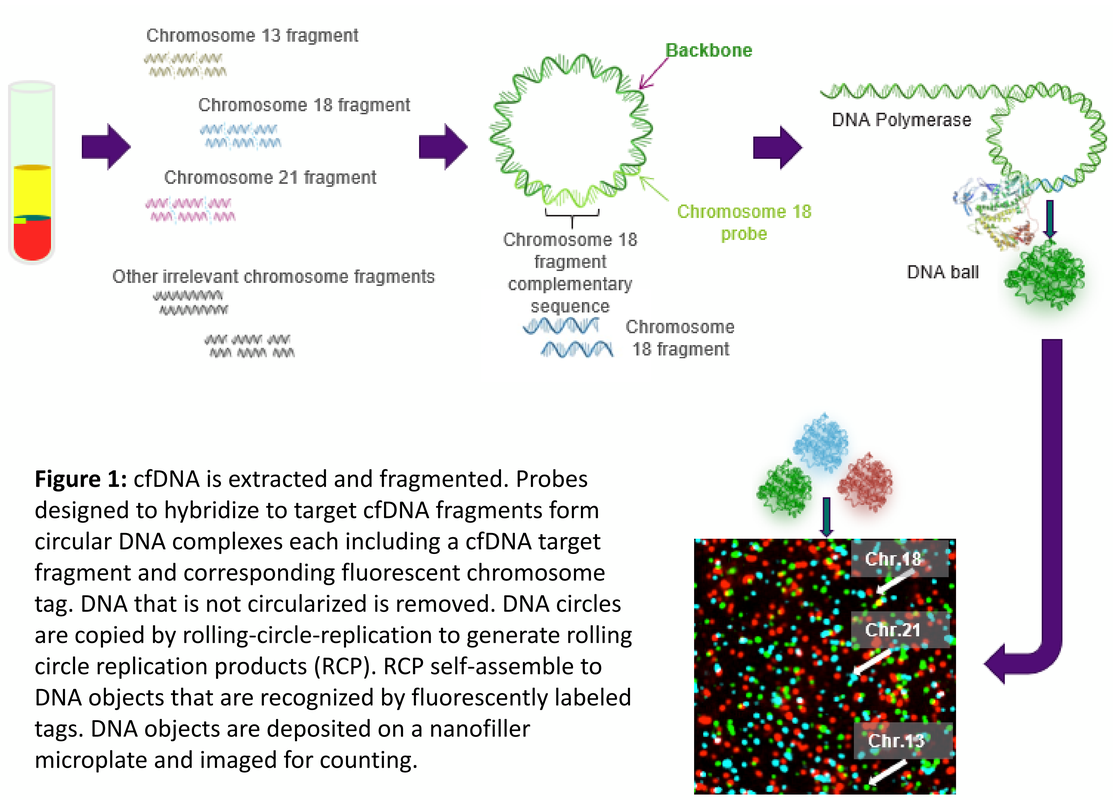

Figure 1: cfDNA is extracted and fragmented. Probes designed to hybridize to target cfDNA fragments form circular DNA complexes each including a cfDNA target fragment and corresponding fluorescent chromosome tag. DNA that is not circularized is removed. DNA circles are copied by rolling-circle-replication to generate rolling circle replication products (RCP). RCP self-assemble to DNA objects that are recognized by fluorescently labeled tags. DNA objects are deposited on a nanofiller microplate and imaged for counting.

Results

- All trisomy 21, 18 and 13 samples were accurately classified as high risk with no false negatives.
- All negative controls that received results were accurately classified as low risk with no false positives.
- Two samples had a “no-call” result. Both no-call samples were normal controls.
- Fetal sex was accurately classified in 213/214 samples.
- High accuracy and precision was observed as demonstrated in table 2

Analytical Sensitivity and Specificity

Table 1: Analytical performance of data from LDT validation in laboratory in Malaysia

Overall Analytical Sensitivity	Overall Analytical Specificity
100%*	100%**
23/23 trisomic samples were accurately characterized as high risk	207/207 disomic samples were accurately characterized as low risk

* Data represents the samples used for LDT validation for the Malaysia lab only and hence fewer positive samples were included in this validation dataset

** Due to non-availability of additional specimen to repeat this assay (as per protocol for no-calls); the 2 no call samples are excluded from this calculation.

Accuracy and Precision

Table 2: Evaluation of chromosome ratios and Z-scores of trisomic reference material samples. Each run (S1 and S2) contained 2 SeraSeq T21, 2 SeraSeq T18 and 2 SeraSeq T13 samples as well as 2 T21 positive maternal plasma samples. Highlighted values illustrate minimal variability between chromosome ratios and z-scores of each trisomy sample type, thereby providing evidence of a highly precise and accurate assay.

		Ratio Chr21	Ratio Chr18	Ratio Chr13	Z-score Chr21	Z-score Chr18	Z-score Chr13
T13	Run S1	0.9783387	0.9718246	1.0524666	-5.346807	-6.946361	9.416352
	Run S1	0.9766053	0.9689793	1.057569	-5.638743	-7.462917	10.219249
	Run S2	0.987389	0.972556	1.0418071	-3.175446	-5.965193	6.588632
	Run S2	0.9868658	0.9722992	1.0426514	-3.599153	-6.4164	6.919315
T18	Run S1	0.9872672	1.0268105	0.9866226	-3.099374	6.574897	-2.367882
	Run S1	0.9849351	1.0297707	0.9861553	-3.72701	7.428212	-2.471082
	Run S2	0.9864187	1.0319333	0.982641	-3.986559	7.861121	-2.862751
	Run S2	0.9865758	1.0313774	0.9830057	-3.936862	7.718378	-2.801883
T21	Run S1	1.0509314	0.9726279	0.9789224	13.14143	-6.970543	-3.816419
	Run S1	1.0451627	0.9679742	0.9889637	11.668511	-8.168245	-2.002305
	Run S2	1.0495404	0.9698716	0.9829848	15.956639	-7.728575	-2.868633
	Run S2	1.0448024	0.973876	0.9832728	14.388086	-6.698017	-2.818696
T21 Pool	Run S1	1.056112	0.9694646	0.9774262	14.629959	-7.849753	-4.106047
	Run S1	1.050963	0.9782631	0.9732537	13.663308	-5.767551	-4.930934
	Run S2	1.051843	0.9745506	0.9761626	16.300267	-6.424337	-3.989128
	Run S2	1.0509772	0.9753557	0.97614	15.807414	-6.163549	-3.977235

Discussion

- The Vanadis® NIPT assay meets, and in some cases exceeds, the performance of PCR-based NIPT assays.
- By eliminating PCR, the Vanadis® NIPT achieves high-precision allowing for the assay performance to be maintained in samples without a fetal fraction cutoff (e.g 4%) thereby providing a minimal no-call rate.
- By eliminating the expenses of PCR and advanced bioinformatics, Vanadis® NIPT is well-suited to meet the needs for general population NIPT on a global scale.

Acknowledgement

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