Non-Invasive Prenatal Screening by Vanadis LifeCycle® Platform

Suresh Shenoy , Robert Guajardo, Catherine Hammes, Shawn Bennett, Donti Taraka, Madhuri Hegde.
Revvity Omics, Pittsburgh, PA, USA.

BACKGROUND

Non-invasive prenatal screening (NIPS) for screening of common aneuploidies has become the standard of care in the past decade. The American College of Obstetricians and Gynecologists (ACOG) has revised its position on noninvasive prenatal testing (NIPT) and is now recommending prenatal aneuploidy screening for all pregnant patients regardless of age or other risk factors. ACOG previously recommended use of screening only in individuals 35 and older or with other known risk factors. Therefore, it is critical to implement a highly automated low cost walk away technology to make NIPS accessible to all women. NIPS has demonstrated a high detection rate with a low false positive rate in screening for aneuploidies; a significant advantage over conventional serum screening methods. In 2019, we validated the Vanadis® NIPS -a non-PCR based - Rolling Circle Replication (RCR), cost-effective, highly precise assay with a short turnaround time (7 days) test using plasma for the effective screening of the common trisomies 13, 18 and 21 as a laboratory developed test (LDT). We have now implemented globally this test in our laboratories in the US, Malaysia and Sweden, with RVTY's Plus91® laboratory information system, and Revvity's Vanadis LifeCycle®. This has permitted a seamless workflow starting with intake of blood samples from pregnant women and finishing with a clinical report. The report contains risk values for chromosomes 13, 18, 21 trisomies computed by Vanadis LifeCycle® that uses Vanadis® NIPS-determined z-scores for normalized chromosomal ratios and the mother's demographics. Additionally, the NIPS report, when requested, contains fetal sex classification. Using our integrated workflow, since late October 2020, our laboratory has screened for aneuploidies in chromosomes 13, 18 and 21 and has issued 804 NIPS reports. Of the cases reported, we saw one case for T21 (0.12%), and 2 cases (0.37%) cases with an increased risk for T18 and none for T13 There were eighteen borderline T18 positive calls (2.24%); all turned out to be negative for T18 upon testing the second sample from the mother. Finally, there were twenty-one "no calls" (2.61% "no call" rate) of which only 2 cases remained unresolved when the second specimen was tested. This methodology has significant advantages over the NGS based methodologies and requires low capital investment therefore making it globally accessible to populations of broad economic strata.

METHODS

A total of 804 plasma samples from mothers between their 1st and 2nd trimester of pregnancy were subjected to the Vanadis® Aneuploidy screen for T13, T18 and T21 and for fetal sex determination. The normalized chromosome ratio scores from the assay were handed off from Vanadis® system software to LifeCycle® for z score calculation of chromosome ratio scores for chromosomes 13, 18 and 21, interpretation of the z-scores, and for assignment of risk percentages for each trisomy. 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 (Tables 1 and 2). Samples failing quality assessment were classified as no-call. Assay technology is described in Figure 1. In addition to aneuploidy screening, samples had fetal sex determined.

RESULTS & DISCUSSION

- The Vanadis® assay is a novel rolling circle replication-based method for NIPT testing and meets, and in some cases exceeds, the performance of PCR-based NIPT assays. The high precision of the system is derived from efficient purification of cell free DNA (Figures 1 and 2), its quantitative conversion to rolling circle products (RCP; Figures 1 and 3) and the high number of RCP (Figure 1) counted for each chromosome. The precision is demonstrated by the low coefficient of variation in chromosomal ratio scores (Figure 4) which allows the facile detection of ratio score outliers (i.e., aneuploidy cases, Figure 5).
- Table 1 contains the summary of our first 804 cases. The data matches the expected range of aneuploidy cases and distribution of male and female fetuses in the USA. This test is associated with low "no call" and "borderline T18" rates. All the "borderline T18" cases proved to be "screen negatives" upon the testing the second plasma tube of the patient. Only two of the 21 "no calls" repeated as no calls upon testing the second plasma tube from the patient, and for three "no calls' a second plasma tube was not available.
- By complete automation and seamless bioinformatic reporting tools, this Vanadis® NIPT assay is well-suited to meet the needs for a low cost and a low complexity assay for general population NIPT on a global scale.

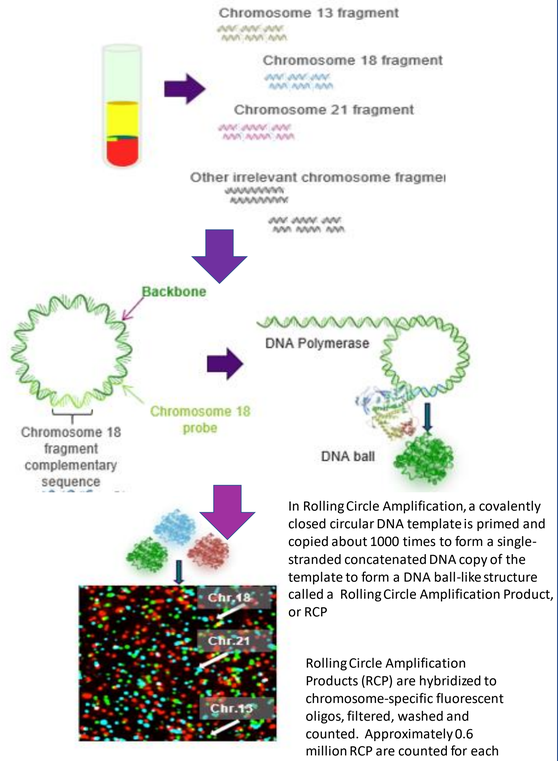
	T13	T18	BORDERLINE T18	T21	NOCALLS	FALSE Positive	MALES	FEMALES	M/(M+F)	F(M+F)
Number of cases	0	3	18	1	21	1	414	366	0.53	0.47
Percent of cases	0.00%	0.37%	2.24%	0.12%	2.61%	0.12%				

Table 1. Summary of findings in first 804 cases subjected to Vanadis Aneuploidy Test at RVTY Pittsburgh. All borderline T18 cases tested as "screen negatives" with the second tube result. Only 5/21 "no call" persisted as "no calls" (two repeated as "no calls" using the 2nd tube result, and for three cases a second tube was not available) for an overall "no call" rate of 0.62% (5/804)

REFERENCES

Imaging Single DNA Molecules for high precision NIPT. Fredrik Dahl et al., Nature Scientific Reports, (2018), 8:4549

Figure 1. Schematic of Vanadis® and LifeCycle® Workflows



In Rolling Circle Amplification, a covalently closed circular DNA template is primed and copied about 1000 times to form a single-stranded concatenated DNA copy of the template to form a DNA ball-like structure called a Rolling Circle Amplification Product, or RCP

Rolling Circle Amplification Products (RCP) are hybridized to chromosome-specific fluorescent oligos, filtered, washed and counted. Approximately 0.6 million RCP are counted for each chromosome.

Vanadis® System Software computes normalized chromosomal ratio scores

Using ratio scores, and in-built logic calibrated by user, LifeCycle® software computes and interprets chromosomal z scores as risk percentages and issues clinical reports

VANADIS

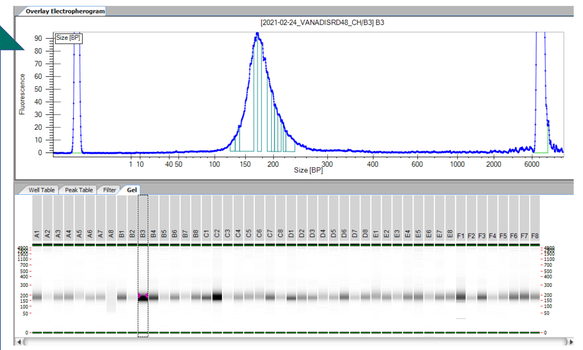


Figure 2. cfDNA purified on Vanadis® shows characteristic ~170 bp size range. cfDNA size analysis was performed on Revvity LabChip™ Gx Touch HT and analyzed using the associated LabChip™ Gx Reviewer software.

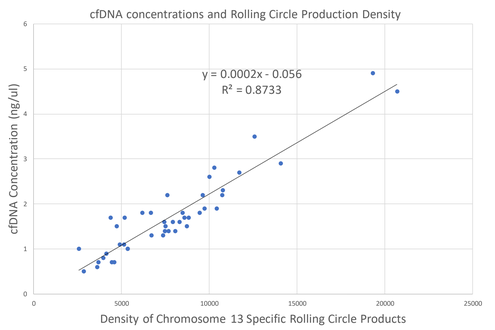


Figure 3. Strong correlation between cfDNA input and generation of Rolling Circle Product by Vanadis® . cfDNA concentrations were measured fluorometrically on Revvity Victor²™D; RCP density was obtained from the Vanadis® output

LIFECYCLE

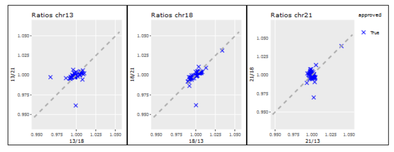


Figure 4. Ratio Plots show tight cluster "of screen negatives", (i.e., all chromosomal ratios ~1) and presence of screen positives, if any.

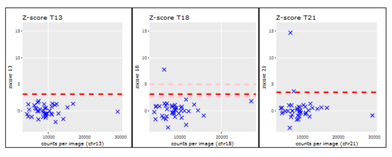


Figure 5. Z-Score Plots with preset threshold values discriminate screen positives (above red dashed line), screen negatives (below red dashed lines) and borderline T18 calls (in between pink dashed lines)

Condition	Z-score – 1st run	Z-score – 2nd run	Post-Test Risk	Interpretation
Trisomy 21	<1.00	Not Applicable	Less than 0.01%	Screen Negative
	1.00 to 1.99	Not Applicable	Less than 0.01%	Screen Negative
	2.00 to 2.99	Not Applicable	0.01%	Screen Negative
	3.00 to 3.49	Not Applicable	0.02%	Screen Negative
	3.50 to 4.99	Not Applicable	50%	SCREEN POSITIVE
	≥5.00	Not Applicable	Greater than 90%	SCREEN POSITIVE
Trisomy 18	<1.00	Not Applicable	Less than 0.01%	Screen Negative
	1.00 to 1.99	Not Applicable	0.02%	Screen Negative
	2.00 to 2.69	Not Applicable	0.1%	Screen Negative
	2.70 to 5.00	< 2.00	0.15%	Screen Negative
	2.70 to 5.00	>= 2.00	29%	SCREEN POSITIVE
	2.70 to 3.14	Second tube not available	0.30%	Screen Negative
	3.15 to 5.00	Second tube not available	2.0%	SCREEN POSITIVE
	>5.00	Not Applicable	Greater than 90%	SCREEN POSITIVE
Trisomy 13	< 1.00	Not Applicable	Less than 0.01%	Screen Negative
	1.00 to 1.99	Not Applicable	Less than 0.01%	Screen Negative
	2.00 to 3.14	Not Applicable	0.05%	Screen Negative
	3.15 to 3.99	Not Applicable	1.0%	SCREEN POSITIVE
	4.00 to 4.99	Not Applicable	50%	SCREEN POSITIVE
	≥5.00+	Not Applicable	Greater than 90%	SCREEN POSITIVE

Table 2. Interpretation of Z scores of normalized chromosomal ratios used by LifeCycle® to generate clinical reports

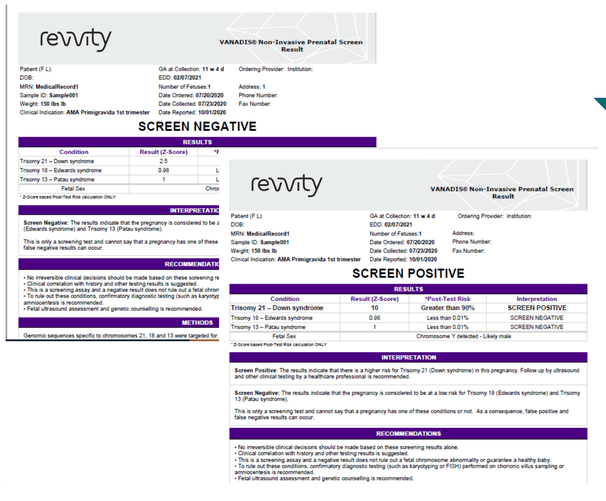


Figure 6. Examples of clinical reports generated by LifeCycle®