Improved Vanadis® cfDNA Platform for Detection of T13 T18 and T21 and Sex Chromosome Abnormalities

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BACKGROUND

The American College of Obstetricians and Gynecologists (ACOG) is recommending prenatal aneuploidy screening for all pregnant patients regardless of age or other risk factors. To address this need, in 2021, we expanded our validation of the Vanadis® cell-free DNA (cfDNA) - a non-PCR based, and automated assay for the screening of trisomies 13, 18 and 21 - as a laboratory developed test (LDT) within our global laboratory sites in USA, Sweden, India, and Malaysia. We integrated the Vanadis® cfDNA system and Vanadis LifeCycle® reporting software with our Plus91® laboratory information management system for a simpler workflow, starting with intake of blood samples from pregnant women and finishing with clinical reports for >2000 cases. In 2021, we also validated new Vanadis instrumentation and assay components that enabled us to add screening of sex chromosome abnormalities (SCA) to the integrated Vanadis® cfDNA system.

METHODS

Cell-free DNA from maternal plasma samples was extracted through an automated system, followed by conversion of selected regions of chromosomes 13, 18, 21, X, and Y to fluorescently labelled rolling circle products (RCP). These RCPs were filtered, washed, and counted for each chromosome RCP in separate channels on a specialized imager. The RCP counts were converted to normalized chromosomal ratio scores which were then used to evaluate risk for SCAs, trisomies 13, 18 and 21, and for fetal sex determination

RESULTS & DISCUSSION

- In the last 12 months, our USA laboratory has tested over 2000 cases with a first tube “no call” rate of just 1.98% (45/2265; compared to 0.7 – 8.1% “no call” rate found with other non-invasive prenatal screening (NIPS) systems) with none of the cases lost due to mechanical failures. This "no call" rate drops to <0.2% upon testing of the second tube. In the absence of birth outcomes and through use of positive and negative reference materials we have established an analytical sensitivity for T13, T18 and T21 of 97.4% (38/39), 95.2% (40/42) and 96.7% (29/30) respectively, an overall specificity of 100% (24/24), and a fetal sex determination accuracy of 100% (24/24). During this period, through improvements in pre- and post -analytical workflow practices, we have reduced our turnaround time from 7 to 5 days. This year, we also validated new Vanadis instrumentation and assay components that enabled us to add screening for sex chromosome abnormalities (SCA) to the integrated Vanadis® cfDNA system. The validation results indicate that the assay detected the SCA group of conditions (XO, XXX, XXY) with 99% (284/287) specificity and 80% (16/20) sensitivity and was associated with an improved performance in the T13, T18, and T21 detection component of the test. The sensitivity for the SCA group using Vanadis® cfDNA (80%) compares favorably with that seen on NIPS conducted on other available platforms (47 -100%) with the additional advantage of being a PCR-free, automated technology needing minimal operator involvement.
- A non-NGS based NIPS assay facilitates the wider adoption of NIPS and availability to all women irrespective of the ability to pay for the services. Although this is a smaller study, this methodology also demonstrates a lower "no call" rate and increased sensitivity for detection of SCA. Confirmatory testing for all positive samples should be performed irrespective of the technology used keeping in mind that these are screening methods albeit offering higher sensitivity than biochemical markers.

PERFORMANCE CRITERIA	DEFINITION	OBSERVED RESULTS
ACCURACY for Chromosomes 13, 18, 21, combined	(TP + TN)/All Results	1.0 (325/325)
PRECISION for chromosomes 13, 18 ,21, combined	TP/TP + FP	1.0 (9/9)
ANALYTICAL SPECIFICITY for Chromosomes 13, 18, 21, combined	TN/TN + FP	1.0 (296/296)
ANALYTICAL SENSITIVITY for Chromosomes 13, 18, 21, combined	TP/TP + FN	1.0 (9/9)
Positive Predictive Value (Precision) for chromosomes 13, 18, 21, combined	TP/TP + FP	1.0 (9/9)
Negative Predictive Value (NPV) for Chromosomes 13, 18, 21, combined	TN/TN + FN	1.0 (296/296)
False Negative Rate (FNR) for 13, 18, 21, combined	FN/FN + TP	0 (0/9)
False Positive Rate (FPR) for 13, 18, 21, combined	FP/FP + TN	0 (0/296)
Autosomal No Call Rate (1 tube value)	No Call/All	0.006 (2/327)

Table 1. Clinical Performance of autosomal aneuploidies using the SCA inclusive assay

CONDITION	DEFINITION	OBSERVED RESULTS (Frequency)
SCA (any type) SPECIFICITY	TN/TN + FP	0.99 (284/287)
SCA (any type) SENSITIVITY	TP/TP + FN	0.8 (16/20)
SCA No Call Rate (1 tube Value)	No Calls / All	0.030 (9/296)

Table 2. Clinical Performance of sex chromosome aneuploidies using the SCA inclusive assay

PERFORMANCE CRITERIA	DEFINITION	RESULTS
ACCURACY	Fetal Sex Correctly Determined/ All chr 13, 18, 21 negative samples with live birth outcomes	0.983 (291/296)

Table 3. Accuracy of the fetal sex determination using the SCA inclusive assay

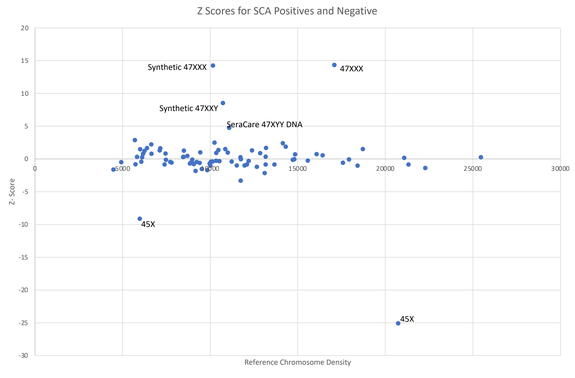

Figure 1. z-scores of SCA positive and negative samples

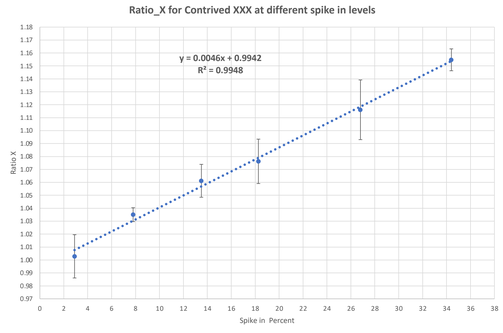

Figure 2. Linear response of contrived 47XXX plasma sample in the SCA assay

Spike Percent (Expected Value)	Average RatioX	SD_ RatioX	Observed Spike in Value (Ratio-1)*2	Concordance of Observed/Expected Spike in Values	Frequency of ratio X > or = 1.01
2.9	1.00	0.02	0.568	0.20	3 of 5
7.8	1.04	0.01	7.008	0.90	5 of 5
13.5	1.06	0.01	12.244	0.91	10 of 10
18.27	1.08	0.02	15.25	0.83	10 of 10
26.8	1.12	0.02	23.22	0.87	5 of 5
34.4	1.15	0.01	30.944	0.90	5 of 5

Table 4. Linear response of contrived XXX plasma sample in the SCA assay

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REFERENCES

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