WHITE PAPER

Vanadis® NIPT system: Proof of principle data for trisomy screening

Introduction

Analysis of cell-free DNA for prenatal screening requires very precise quantification of DNA molecules, and so far has only been achieved through expensive analysis platforms. This has limited the access to high-performance prenatal testing for pregnant women since the tests are costly and mainly available at advanced service laboratories. Herein we describe a simple microplate-based technology, which is presently in development, to precisely quantify cell-free DNA molecules. Our technology will enable cost-effective and high-throughput prenatal screening by eliminating the need for microfluidics, microarrays and DNA sequencing. We present early clinical research data showing the successful identification of trisomy 21 in maternal plasma.

Fully Automated and Cost Efficient cfDNA Platform

The possibility to precisely analyze fetal aneuploidies in maternal DNA was first enabled by next-generation sequencing. The precision in sequencing comes from the ability to read single molecules, while the specificity stems from the associated bioinformatics that map these reads to discrete positions in the human genome. That is, the advanced bioinformatics analysis enables separation of data derived from the target chromosomes of clinical interest from data generated from other irrelevant chromosomes or sample preparation artefacts. Since the specificity is achieved through sorting algorithms, sample preparations themselves can have low or minimal target specificity. For example, in assays based on whole genome sequencing only ~1.5% of the generated data is derived from chromosome 21. Thus 98.5% of the data generated by sequencing needs to eventually be separated and discarded through bioinformatics analysis.

The initial results in this case are big data sets containing vast amounts of information that must be sieved through by advanced bioinformatics tools to find the small fraction of relevant sequence data. Current workflows are difficult to automate and require large

> Our technology is designed to enable cost-effective and high-throughput prenatal screening by eliminating the need for microfluidics, microarrays and DNA sequencing.

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capital investments and highly specialized staff for operation. There must be a more data- and cost-efficient way to measure the presence of a chromosome.

We have developed Vanadis® technology to solve the cost, workflow and data analysis bottlenecks of NIPT. The technology is designed to eliminate the need for advanced DNA analysis platforms like microarrays or sequencing and replaces them with a microplate reader.

This reduces the cost of the analysis since the reader does not require any consumables or reagents to operate. The data complexity is reduced and the reported data is the number of counts per chromosome for each sample, thereby simplifying analysis. The Vanadis technology only targets the relevant chromosomes for direct analysis by counting and also eliminates PCR amplification. PCR amplification introduces assay variability, and laboratories have to introduce stringent time- and space-consuming routines to avoid contamination by amplification products. The NIPT technology can readily be automated using a liquid handling system for cost-effective high-throughput analysis, allowing results in 3 days from the receipt of the blood samples.

Vanadis Technology

The Vanadis technology converts target chromosomes into digital objects during the sample preparation to facilitate simple readout and data analysis (see Figure 1). The Vanadis technology is based on a proprietary, patent-pending molecular technology that very precisely selects thousands of DNA fragments from the target chromosomes using thousands of complementary DNA probes. The probes are used together with DNA modification enzymes including DNA ligase and DNA polymerase to very precisely create DNA circles that then are copied into long repeated DNA chains. The DNA chains collapse into spherical DNA objects that can be labeled with fluorescent probes. The probes are designed so that all DNA objects created from the same chromosome fragments are labeled with the same color. One sample is processed in one reaction well in a 96-well microplate. In the last step the labeled DNA objects are imaged through the bottom of the well using a microplate scanner.

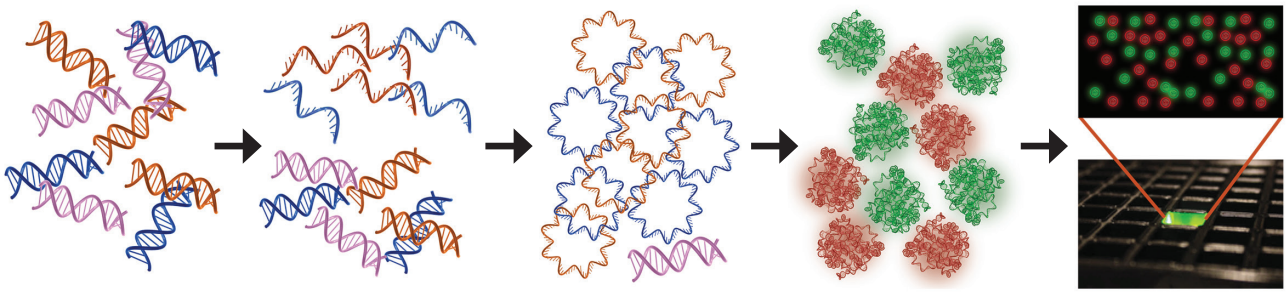
It can be fully automated using liquid handling systems for cost effective high-throughput analysis, making it possible to report results in 48 hours from the receipt of the samples.

Proof of Principle and Clinical Research Data

To demonstrate the Vanadis technical approach 441 de-identified blinded samples were analyzed, of which 17 were positive T21 samples, 6 positive T18 and 6 positive T13 samples (see Figures 2 and 3). Samples were collected in STRECK® tubes from pregnant women during gestational weeks 10–18. Plasma from one blood tube was used for DNA extraction and analysis. DNA was extracted using an optimized, single-step bead-based DNA extraction protocol. DNA was then subjected to the Vanadis technology to convert target DNA fragments from chromosome 21, 18, 13 and a reference chromosome into objects labeled with different fluorophores for downstream counting. Each microplate well corresponded to one patient sample, and for all samples an average of 450,000 counts were generated for each chromosome. Samples were classified by comparing each sample to the average counted ratio. Samples three standard deviations from the mean were classified as positive. After proof of principle data analysis, 104 samples from singleton high risk pregnancies at weeks 11–22 were collected prospectively. Samples were taken from women classified as high risk for trisomy 21 before invasive testing. Samples were analysed blindly. 13 positive trisomy 21 pregnancies were classified correctly with no false positives (Figure 4).

Discussion

We describe a new platform for screening of aneuploidies by measuring cfDNA from maternal blood. Initial data suggests that that Vanadis technology will enable high-performance, high-throughput screening of T21, T18 and T13. By eliminating the need for a complex readout platform and measuring chromosomes directly without amplifications, the cost and complexity is reduced dramatically. The Vanadis solution will enable decentralized cost-efficient testing of cfDNA, which is needed to provide all pregnant women access to high-performance prenatal screening.



cfDNA is extracted from maternal plasma.

Thousands of targeted cfDNA fragments are captured and converted to DNA circles.

Rolling circle replication products are labelled with chromosome specific fluorophores.

The labeled objects are individually counted using a microplate scanner.

Figure 1. Vanadis® NIPT Technology.

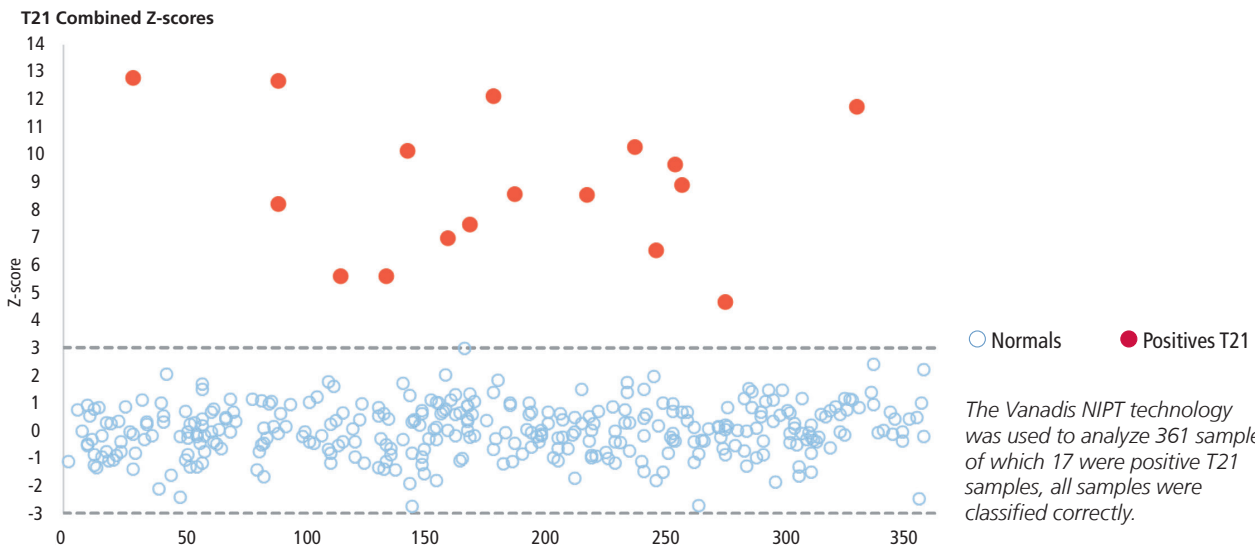


Figure 2. Proof of concept T21 Analysis.

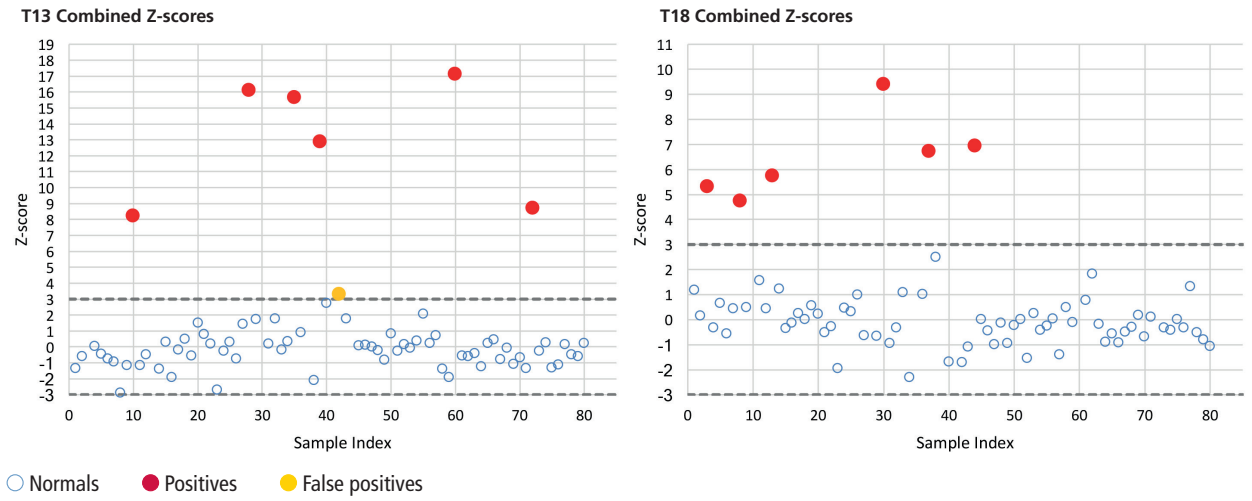


Figure 3. Proof of concept T13 and T18 Analysis.

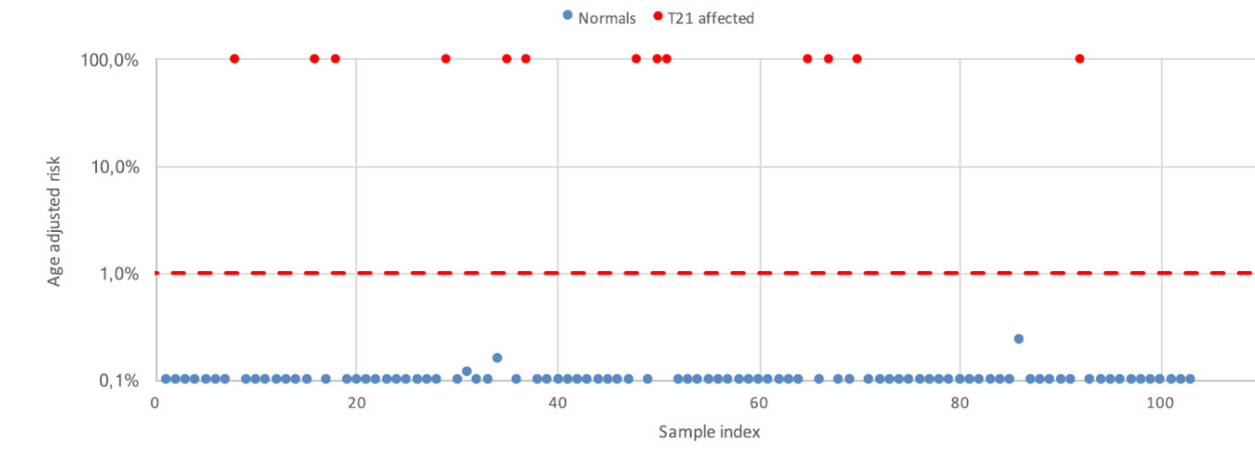


Figure 4. Prospectively collected high risk sample cohort. Samples provided by Kypros Nicolaides from King's College Hospital (London, UK).[1]

Reference

1. Dahl et al. (2018) Imaging single DNA molecules for high precision NIPT. Nature Scientific Reports.

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