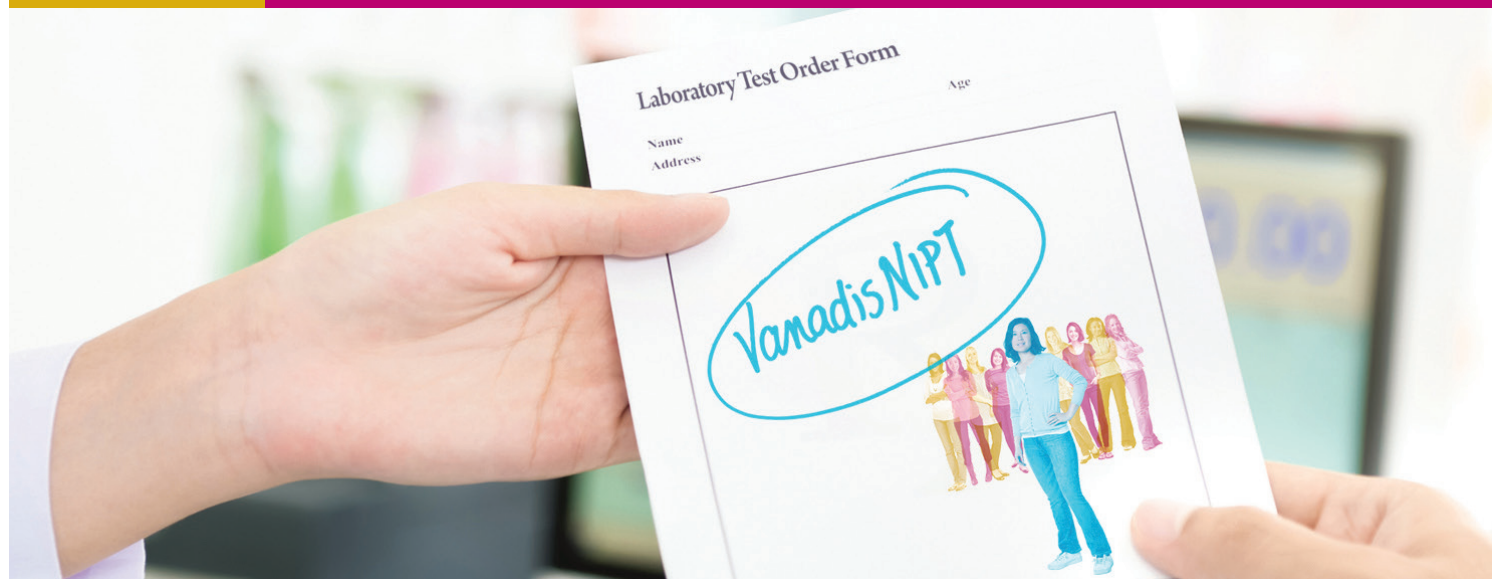# Vanadis® NIPT: An Automated System for High-Precision Prenatal screening

Introduction

Traditional prenatal aneuploidy screening methods, such as maternal serum marker screening and ultrasound imaging, are correlated with a high number of false positive results, leading to unnecessary invasive procedures.[1] Non-invasive prenatal testing (NIPT), which is based on the analysis of placental cell-free DNA (cfDNA) circulating in maternal blood, provides a highly accurate screening option and reduces the need for invasive testing. NIPT has been rapidly introduced to clinical use allowing for high-performance screening of chromosomal abnormalities, including trisomy 21 (T21, Down syndrome), trisomy 18 (T18, Edwards syndrome), and trisomy 13 (T13, Patau syndrome).

In recent years, several sequencing-based technologies, including targeted sequencing, whole genome sequencing, and microarrays, have been applied to address the increasing demand for NIPT. These technologies have demonstrated high performance, achieving a detection rate of approximately 99% for T21. However, the proportion of non-reportable results, i.e. "no-call rate", varies between the currently available NIPT systems, with reported values as high as 6%.[3] The high no-call rate, coupled with the complexity, cost, and reliance on bioinformatics skills, has hampered the introduction of NIPT into routine prenatal care.

This paper describes the importance of assay precision in achieving high detection rate of fetal aneuploidies. Furthermore, the paper shows how the Vanadis® NIPT platform, aims to address the existing limitations of NIPT technologies.[2]

The Importance of High Assay Precision

The proportion of placental to maternal cfDNA, referred as fetal fraction (FF), is approximately 10% on average and increases over the course of pregnancy. FF is also affected by other factors, such as maternal weight and the presence of aneuploidies.[4,6] In some NIPT platforms, samples with FF below 4% are excluded to ensure a reportable result. Using this cut-off, however, approximately 3–5% of sampled women are left without a screening result.[3,6] According to a recent study, samples with low FF have the same rate of T21 but an even higher rate of T18 and T13.[4] Other studies have reported manifold increase in the incidence of these fetal aneuploidies in samples with low FF.[6-8] Thus, with current sequencing-based NIPT, a significant proportion of potential aneuploidies may remain unidentified.

High-precision NIPT platforms provide a higher overall detection rate of trisomies. Figure 1 illustrates the interdependence of NIPT assay precision and detection rate. Low-precision platforms are likely to miss fetal T21 in samples with low FF. In order to achieve ≥99% detection rate, these samples need to be converted into no-calls. If the assay precision is high (low coefficient of variation, %CV), ≥99% of T21 cases can be identified without eliminating the low FF samples.

Vanadis® NIPT eliminates the need for:

- Manual liquid handling
- Microfluidics
- Microarrays
- PCR amplification
- Sequencing
- Big data handling

The Automated NIPT Platform for High-Precision Screening

Vanadis NIPT is designed to enable cost efficient high-performance screening of fetal aneuploidies. As opposed to complex and expensive sequencing-based platforms, Vanadis NIPT uses standard microplate formats and offers highly automated sample processing from the primary blood tube to the result report (Figure 2). To achieve high precision and simple data analysis, the underlying Vanadis technology converts target chromosomes into digitally quantifiable objects. The system significantly reduces complexity and labor intensity compared to sequencing-based NIPT technologies, and one laboratory employee can operate the system at a throughput of 2,000-20,000 samples per year. The hands-on time needed to run one set of samples including blood centrifugation steps is less than three hours and results can be obtained in approximately 48 hours from the receipt of blood samples.

Sequencing- and microarray-based NIPT systems require amplification of the target DNA by PCR-based methods to produce a sufficient amount of material for the analysis. PCR, however, introduces a significant increase in the assay variability, and thus compromises the precision of the test. Vanadis NIPT is the only screening assay targeting specific chromsomes without using PCR amplification. Instead, target fragments are directly captured using probes before being converted to circular DNA objects that are replicated to form DNA bundles that are labeled for counting.

Eliminating PCR improves the assay precision and also removes the requirement to separate the workflow into pre- and post-PCR areas.

In addition, by targeting thousands of chromosomal positions, the Vanadis platform quantifies, on average, 650,000 molecules per chromosome. This is over three-fold greater than sequencing, and corresponds to approximately 70 million sequencing reads per sample. The high precision achieved through incorporating high-yield counting and elimination of PCR improves the detection rate and reduces the no-call rate.
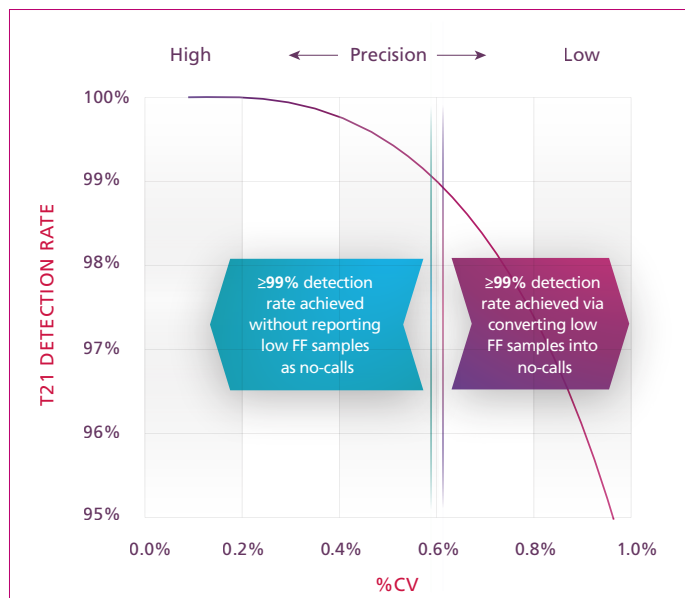

Figure 1. T21 detection rate as a function of assay precision. The figure is based on a theoretical model of FF distribution in a population of >10,000 pregnant women. The detection rate is the proportion of positive samples above a cut-off, which is defined in order to achieve a false positive rate of 0.33%. The coefficient of variation, %CV, is given as a measure of precision.
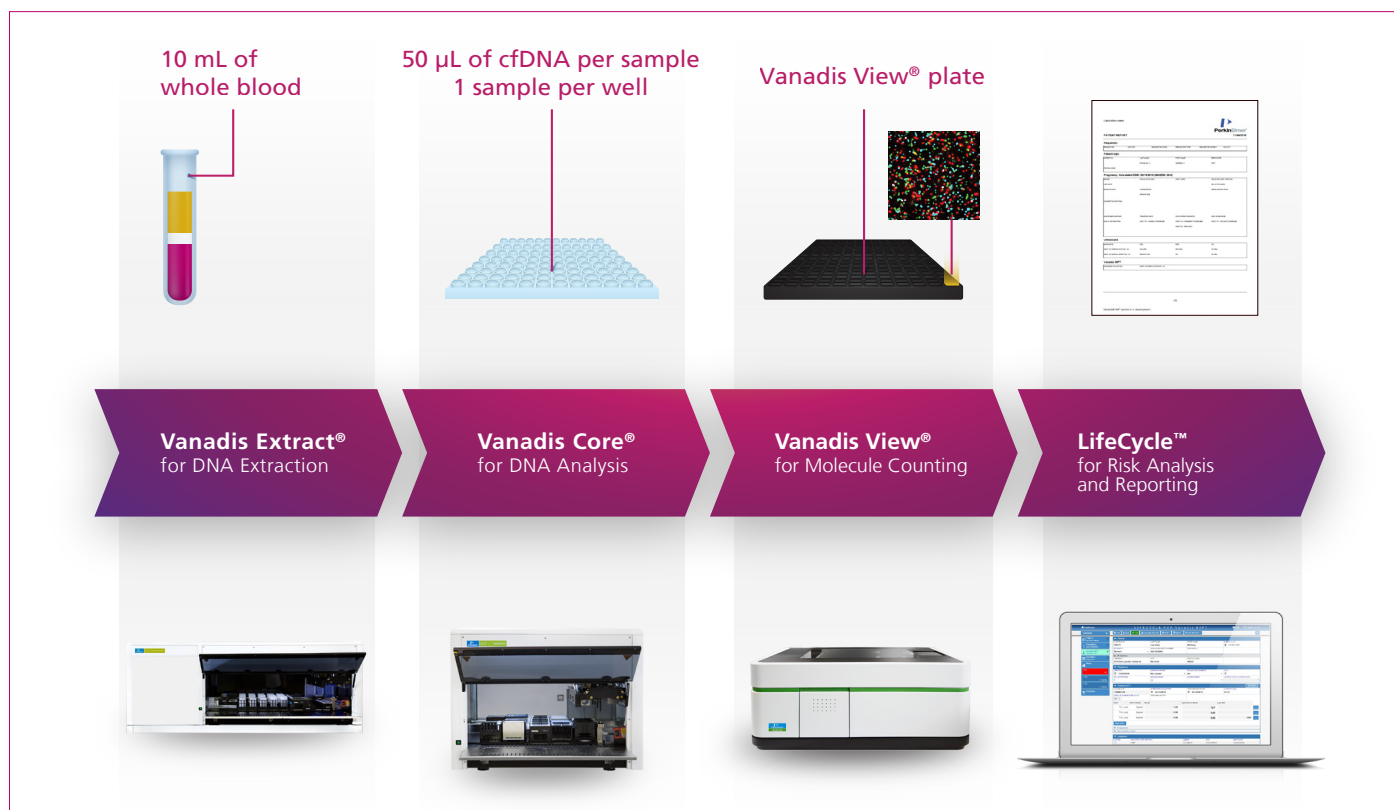
Built-in Quality Control

Figure 2. The workflow of the Vanadis NIPT system. The automated Vanadis technology provides an optimized system for cfDNA extraction and processing to ensure high sample quality and high detection rate of T21, T18, and T13.

To ensure consistent quality, the Vanadis NIPT system includes comprehensive built-in quality control procedures. The quality assurance begins from drawing and handling of the blood sample. Samples for Vanadis NIPT are collected from week 10 onwards and plasma should be separated within five days of the sample draw. This ensures the integrity of the sample and helps to avoid maternal DNA contamination caused by hemolysis during sample storage.

In addition to the automated sample handling, the Vanadis NIPT DNA extraction is designed to eliminate the risk of buffy coat disturbance, another potential source of contaminating maternal DNA. When extracting fetal cfDNA, it is critical to pipette only the upper plasma layer, and to leave the buffy coat intact. To eliminate buffy coat disturbance and other manual errors, Vanadis NIPT entails an automated plasma monitoring function for optimized cfDNA extraction. All individual tubes are imaged with a camera to measure the corresponding plasma volume. This ensures that only plasma will be transferred from the blood tube (Figure 3).

To enable control of overall performance and continuous quality assurance, Vanadis NIPT system monitors standard deviation (SD) of a targeted chromosome ratio over time (Figure 4).

Summary

The Vanadis NIPT system aims to address the current limitations of NIPT by providing an automated, cost-efficient, and high-precision NIPT platform with built-in quality control. Vanadis NIPT is designed to eliminate the need for complex and expensive sequencing-based technologies as well as PCR amplification, enabling precise measurement of chromosomal abnormalities with a minimal number of no-calls.
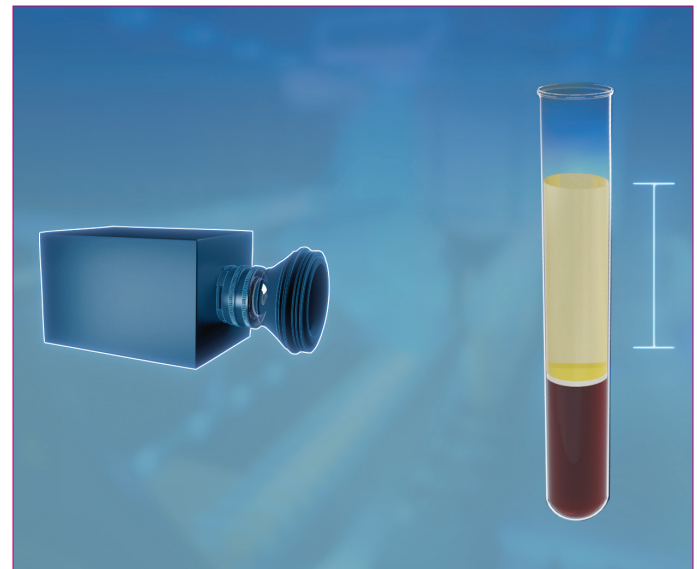

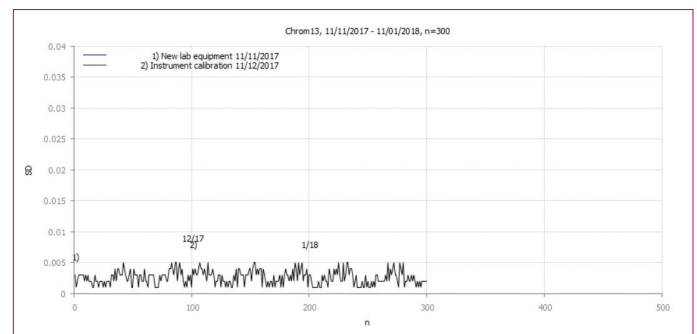*Figure 3.* Plasma volume is monitored by a camera to avoid contamination by buffy coat.

Figure 4. The Z-Score SD plot displays the standard deviations for measurements of a certain chromosome in order to monitor the quality of the assay.

The quality of the Vanadis NIPT system is ensured at each step of the workflow:

1. **Automated** sample handling and plasma pipetting eliminates manual errors such as buffy coat disturbance and sample mix-up
2. **Verified** detection with internal controls of predefined amounts of DNA and trisomy DNA ensures reliable and accurate measurements
3. **Controlled** overall performance by monitoring the distribution of affected versus non-affected pregnancies over time ensures high detection rate and correct sample handling

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