# Noninvasive Prenatal Testing Expands Globally

By developing its NGS-based Prenatalis test, MVZ Martinsried now offers a complete set of prenatal screening services to its patients.

## Introduction

Advances in genetic analysis and cell screening techniques have transformed prenatal testing over the last 2 decades, enabling noninvasive fetal screening for genetic anomalies. The latest next-generation sequencing (NGS)-based screening assays support noninvasive prenatal testing (NIPT) using a maternal blood sample. The development of new NIPT techniques coincides with an increasing number of women delaying childbirth until later in life.[1-2] Pregnant women over the age of 35 run a higher risk of having babies with genetic anomalies including Klinefelter syndrome, a chromosomal condition affecting physical and cognitive development in males, and trisomy 21, better known as Down syndrome.[3]

Amniocentesis was the first diagnostic test used to identify the presence of these and other genetic conditions in pregnancies. The procedure is performed between 15–20 weeks gestation and involves inserting a long thin surgical needle into the womb to obtain a sample of amniotic fluid. The test is highly accurate in identifying chromosomal abnormalities.[4] However, the invasive procedure comes with a risk of infection, preterm labor, and miscarriage,[5] and is not suitable for screening a larger population or testing all pregnancies.

Towards the end of the 20th century, Researchers began identifying several biomarkers (including PAPP-A, ß-hCG, and AFP) linked to fetal chromosomal anomalies in the serum of pregnant women. The Fetal Medicine Foundation (FMF) in the UK and Germany later found that pregnancy-associated plasma protein-A (PAPP-A) and free beta-human chorionic gonadotrophin (ß-hCG) quantification assay results together with ultrasound measurements of nuchal translucency, yielded data providing a valid risk score for chromosomal disorders such as Down syndrome. Based on the risk score, the mother may be referred for additional, invasive procedures. Still in use today, this screening test is inaccurate and provides a high level of false positive results, leading to unnecessary invasive procedures.

High-throughput next-generation sequencing (NGS) and new blood plasma isolation technologies now enable sensitive NIPT that is benefiting thousands of women worldwide. The latest tests use cell-free fetal DNA (cfDNA), accessible through a simple maternal blood sample, to screen for potential genetic conditions in the fetus. They offer improved accuracy over current serum screening methods for common aneuploidies, reducing false positives, and can be performed any time after 10 weeks gestation. NGS-based NIPT provides women with accurate information earlier in the pregnancy without risking unnecessary risky invasive procedures.

Hanns-Georg Klein, MD, a medical geneticist and Chief Executive Officer of the MVZ Martinsried Center for Human Genetics and Laboratory Medicine, develops innovative molecular genetic screening tests. In 2014, Dr. Klein and his colleagues created their own NGS-based NIPT assay, the Prenatalis test. iCommunity spoke with Dr. Klein about the promise of molecular genetics in prenatal screening and why MVZ Martinsried developed the NGS-based Prenatalis test.

**Q: Why did you decide to study molecular genetics?**

**Hanns-Georg Klein (HGK):** I'm a medical doctor and did a postdoc in the United States in the early 1990s after receiving a fellowship from the German Research Council and a Fogarty Fellowship at the National Heart, Lung, and Blood Institute (NHLBI). While there, we characterized the molecular defects of some rare lipid disorders like fish-eye disease, which causes the eyes to become cloudy.

The early 1990s marked the beginning of the molecular genetic diagnostics field and there was a lot of enthusiasm at the National Institutes of Health about molecular genetics. That was when the Human Genome Project started and everyone wanted to be a part of it. I certainly did, and was on a small research team that performed sequencing. I started with radioactive sequencing methods, like single-strand sequencing with subcloning. It was a complicated procedure and not many people could perform it. This was way before any kind of automated sequencing. When I returned to Germany in 1993, I continued sequencing and did a subspecialization in laboratory medicine and medical genetics. I've been working in genetics ever since.

**Q: What types of testing and services does MVZ Martinsried offer?**

**HGK:** The MVZ Medical Care Center in Martinsried started as a medical genetics laboratory, focusing on rare diseases. Some of our first procedures involved sequencing large genes linked with rare diseases, such as fibrillin-1, a gene associated with Marfan syndrome.

**Dr. Hanns-Georg Klein serves as a clinical pathologist and medical geneticist, and as Chief Executive Officer and Medical Director at the MVZ Martinsried Center for Human Genetics and Laboratory Medicine in Munich, Germany.**

Over the years, we have expanded our portfolio. We became involved with cytogenetics and clinical genetics testing when my colleague, Dr. Imma Rost, joined the team. Later, we added subspecializations like immunogenetics, mainly human leukocyte antigen (HLA) typing, but also clinical pathology, molecular pathology, and, most recently, microbiology and virology.

> We started offering NGS-based NIPT because the first trimester screen just wasn't good enough. It generated a substantial number of false positive and false negative results, and didn't offer high sensitivity and specificity.

Q: What types of IVF and prenatal testing do you perform?
HGK: We perform several genetic tests that support *in vitro* fertilization (IVF) procedures for women who are over 35 or are having difficulty becoming pregnant. These tests include polar body diagnostics (PBD) and preimplantation genetic screening (PGS). They're used to identify viable eggs and embryos for IVF procedures, increasing the chances of a successful pregnancy. Polar bodies are produced after an oocyte (egg cell) undergoes its first cell division. PBD testing detects aneuploidies—the presence of an abnormal number of chromosomes—and monogenic diseases. In contrast, PGS is used to detect genetic anomalies in trophoblast cells that make up the outer layer of an early stage embryo.

We also perform NIPT, recently developing the Prenatalis test to replace several older technologies.

Q: Who are your customers and where are they located?
HGK: Most of our customers are located in Germany and in other German-speaking nations such as Austria and Switzerland. While we don't receive many samples from abroad, we are happy to expand our customer regions.

Our customers are physicians, human geneticists, and gynecologists who refer samples to us. Those doctors come from private practice, universities, and large hospitals. We are also involved in clinical research studies.

Q: What technologies were you using 10 years ago for prenatal screening?
HGK: For the last 25 years, prenatal screening has been performed using invasive diagnostic procedures, such as amniocentesis. There were some noninvasive screening procedures, like the triple test and the quadruple test, but these were not very accurate and led to high false positives. Ten years ago we started performing first trimester screening, measuring PAPP-A and hCG in a woman's plasma. The results of these tests and nuchal translucency ultrasound provided a risk score for common trisomies. These tests had their limitations, but it was the best that we could do at the time for noninvasive prenatal screening.

Q: Why did you decide to offer NGS-based NIPT?
HGK: We started offering NGS-based NIPT because the first trimester screen just wasn't good enough. It generated a substantial number of false positive and false negative results, and didn't offer high sensitivity and specificity. We wanted to move to a technology that offered higher accuracy and the new NGS-based tests offer that.

Q: What drove the decision to develop the Prenatalis test?
HGK: We developed the Prenatalis test so that we could deliver a complete set of prenatal screening services. Prenatal diagnostics is important for us and NIPT is a piece of that puzzle. It's not everything, certainly. We'll need ultrasound and, to confirm findings, we might even need invasive diagnostics. Yet, NGS-based NIPT is one piece of the prenatal diagnostic and screening spectrum that every professional genetics lab should offer patients.

Q: What process did you follow in developing your Prenatalis test?
HGK: To develop the Prenatalis test, we purchased a sequencing system, a server, and a service and reagent supply agreement so that we could perform the testing in our laboratory. The server is the computer hardware needed for calculating disease risk scores. We created our own algorithm to filter the relevant data from unnecessary information, and to keep the data in our own laboratory as well.

In August 2014, we began developing the test. We designed our first test standard and prepared our accreditation procedure, and in January 2015 our Prenatalis test became a routine part of our NIPT offering. In June 2015, we submitted the last required document (quantification of fetal fraction) for the accreditation of the Prenatalis test from the Deutsche Akkreditierungsstelle (DAkkS), Germany's national accreditation body.

> With our Prenatalis test, we have enough samples to perform 1 sequencing run per week—so our turnaround time is about 5 working days.

Q: How did you perform clinical validation of the Prenatalis test?
HGK: For clinical validation, we tested 60 samples. We started inhouse validation with 2 positive and 2 negative examples of each parameter, identifying trisomy 13, trisomy 18, trisomy 21, and so on, that had been confirmed by invasive testing. We obtained 100% concordance.

Q: What is the time to results with the Prenatalis test?
HGK: With our Prenatalis test, we have enough samples to perform 1 sequencing run per week—so our turnaround time is about 5 working days. All the samples received by Monday at noon are reported back on Friday.

Q: What are the benefits of the Prenatalis test?
HGK: The Prenatalis test has high accuracy, sensitivity, and specificity for the most common trisomies. We're also planning to use NIPT to identify microdeletions that can cause fetal abnormalities. So for high-risk populations, women with positive serum screens, abnormal ultrasounds, or who are over 35 years old, we now have a true alternative to traditional invasive first trimester screening.

> The Prenatalis test has high accuracy, sensitivity, and specificity for the most common trisomies.

Q: What new services will MVZ Martinsried offer in the future?
HGK: My vision is to create services for all subspecialties involved in laboratory diagnostics, and to identify and develop applications based on findings from current genome research.

## References


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