Expanded noninvasive prenatal testing looking beyond trisomies T21, T18, and T13

Sequencing with the VeriSeq™ NIPT Solution v2 enables comprehensive insights reducing the need for invasive tests

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Dr. Pascale Kleinfinger is Deputy Director of the Genetics Department at Laboratoire Cerba in France where she has been involved in prenatal testing for over 23 years. She is an expert in prenatal medicine, including the use of next-generation sequencing (NGS) for noninvasive prenatal testing (NIPT). NIPT involves sequence analysis of cell-free DNA (cfDNA), released into the maternal bloodstream by placental-cell apoptosis, to screen for fetal chromosomal abnormalities. Dr. Kleinfinger also cohosts a working group with the Association of French-Speaking Cytogeneticists to recommend policies and standards for the use of NIPT in clinics in France and elsewhere.

We spoke with Dr. Kleinfinger about her experience in prenatal testing, the progress she sees with NIPT and expanded NIPT, and how the Illumina VeriSeq NIPT Solution v2 is helping her lab meet the needs of pregnant women in France.

Q: Can you tell me about Laboratoire Cerba and your role in the company?

Pascale Kleinfinger (PK): The Laboratoire Cerba has existed as a specialized clinical pathology laboratory since 1967. We have about 650 employees and are part of Cerba HealthCare, a worldwide leader in the field of diagnostic testing. The Cerba laboratory mainly operates as a reference laboratory for analyses that are not carried out by the practice where the patient samples come from.

I am a Deputy Director of the Department of Genetics, which includes eight geneticists and about 60 technicians. The department works directly with the obstetricians that collect the patient samples.

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Q: Why did Cerba decide to offer NIPT?

PK: NIPT started at the Cerba laboratory in 2013. We were the first French medical laboratory, and one of the first European medical biology laboratories, to offer this test. According to the report of the Agence de la Biomedecine, during the 2010s, approximately 45,000 invasive prenatal tests were performed every year in France.[1] That amounted to almost 6% of pregnant women, with as many as 12% in regions such as Île-de-France. It was clear to all health professionals that we needed to reduce the number of invasive procedures, especially given that the positive predictive value (PPV) of traditional screening was less than 5% at that time. When NIPT came along, it became apparent that this test was more sensitive and more specific than anything we had to offer at the time.

Q: How have referring clinicians responded to NIPT?

PK: At first, responses to NIPT were extremely varied. Some people were very much opposed to implementing NIPT and others were very supportive. We had to travel around France to educate obstetricians and gynecologists about NGS, NIPT, and how the screening works.

Now, clinicians understand the test and its limitations better. They also understand that NIPT should not be offered to certain populations, for example in the case of an abnormal ultrasound finding. As a result, the clinicians have really made the test their own. For the people we work with today, NIPT works well in their practices as the standard of care.

Q: How do you collaborate with your centers and referring hospitals?

PK: We work with two types of prescribers. First, there are the hospital prescribers in multidisciplinary centers where there is a higher level of collaboration. We present our clinical reports to them and we work closely with them to develop health care policies. Then, there are the more common individual prescribers, with whom we talk through each individual case and give advice. We are available on demand. When they need our support they call us.

Our collaborations make it possible to improve the individual care and attention provided to patients. They also allow us to think about health policy development, such as the needs within the population and the needs of prescribers.

Q: Can you provide an overview of the NIPT landscape in France?

PK: NIPT is a genetic test that is subject to strict legislation in France. For the test to proceed, the blood sample must be accompanied by a consent form signed by the patient and a certificate from the prescriber stating that he or she has properly informed the patient of the benefits and limitations of genetic testing. A woman also cannot visit a practice and decide for herself to have this test.

The test should only be carried out following an ultrasound scan, ideally in the first trimester, between 11 and 13 weeks of gestation, and only if there are no ultrasound anomalies. In particular, we are looking for a nuchal translucency (NT) measurement of less than 3.5 millimeters. Legislation in France has granted funding for NIPT for pregnancies at a certain risk of trisomy 21 only. This test will be 100% funded if the patient is at risk of trisomy 21 based on a risk assessment from the maternal serum screening of greater than 1 in 1000, with a special rule for patients with a risk between 1 in 2 and 1 in 50, who will be offered the option to have an invasive sampling method instead of NIPT. Obviously, all positive NIPT results would still need to be confirmed by an invasive diagnostic procedure.

Q: Tell us about your recent publication on implementation strategies for expanded, or genome-wide, NIPT.

PK: When NIPT became available around 2013, I wondered what would be possible in the future with regard to chromosomal abnormalities other than trisomy 21. We started a multicenter collaboration in France for the collection of samples with rare aneuploidies and structural unbalanced rearrangements for the day when someone would come up with a technical solution. The scope of the project wasn't always clear because we used to consider trisomy 21 to be the most common anomaly, and the only one for which screening may be of interest. However, we did already extend the screening to anomalies that were less common, including trisomy 13 and trisomy 18.

Initially, these other chromosomal abnormalities were not well known and we were under the impression that they were rare anomalies without a real place in public health. However, every year in France, a national report is published and this report showed that some of these anomalies were not so rare at all. People started producing publications, including the Lindquist publication in 2018[2] that showed that the prevalence was potentially much higher.

When Illumina launched the VeriSeq NIPT Solution v2, which also has the option to screen for rare autosomal anomalies and partial deletions and duplications larger than seven megabases, we examined our first cohort of biobanked samples from 192 pregnancies for a retrospective study. The aim was to assess the sensitivity

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and specificity of the VeriSeq NIPT Solution v2 assay. Of these samples, 42 had abnormalities and 150 had no abnormalities. Two of the samples had placental mosaicism, which affects 1-2% of pregnancies, and their prevalence varies depending on the type of chromosomal abnormality.[3] For example, they are unusual for trisomy 21, more frequent for trisomy 18, and common in trisomy 7.

For copy number variations, we were able to see that there was a good correlation between the size of the variation detected by expanded NIPT and the size of the variation when assessed by an array. We found an overall specificity of 99.3% and a sensitivity of 88.1%.[3]

We then decided to look at a second cohort of 3000 pregnant women who were referred for NIPT and for whom we did not have fetal karyotypes. For this cohort, we focused on chromosomal abnormalities that were consistent with a fetal anomaly, such as anomalies that are unlikely to be found in relation to placental mosaicism—trisomies 8, 9, 12, 14, 15, 16, and 22 and significant unbalanced chromosomal anomalies. When testing was limited to this list, the rate of positive results was only 0.57%,[3] which indicates only a very small number of women that would require confirmatory invasive testing.

Q: What are the benefits of expanded NIPT for patients?

PK: The main advantage is to enable an earlier diagnosis. For example, we were able to detect an isochromosome 12p via NIPT in a patient in the first trimester. There is no doubt that signs would have appeared on the second trimester ultrasound, which, in France, is carried out at around 22 weeks. Thanks to NIPT followed by confirmatory testing, we can now find this much earlier.

Eventually, we will be able to detect anomalies other than 13, 18, and 21 that we haven't been able to test for up until now. And, potentially, we will be able to prevent certain risks for pregnancy complications in the third trimester related to placental mosaicism, such as trisomy 16 that is associated with risks of preeclampsia, intrauterine growth restriction, and fetal demise. I think there will be a framework that will allow for better management of these. In the near future, the Biomedicine Agency, which is our supervisory authority, will bring together professionals to define how to prescribe this test.

Q: How does Illumina support your laboratory and the adoption of VeriSeq NIPT Solution v2?

PK: The collaboration with Illumina is excellent. They provide technical help and availability that suits our laboratory perfectly. We are provided with updates on literature to support our work. Illumina also helps us to be in contact with other laboratories that are carrying out the same tests so data that would be rare individually can be compiled.

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Intended use statement

The VeriSeq NIPT Solution v2 is an *in vitro* diagnostic test intended for use as a screening test for the detection of genome-wide fetal genetic anomalies from maternal peripheral whole blood specimens in pregnant women of at least 10 weeks gestation. VeriSeq NIPT Solution v2 uses whole-genome sequencing to detect partial duplications and deletions for all autosomes and aneuploidy status for all chromosomes. The test offers an option to request the reporting of sex chromosome aneuploidy (SCA). This product must not be used as the sole basis for diagnosis or other pregnancy management decisions. Noninvasive prenatal testing (NIPT) based on cell-free DNA analysis from maternal blood is a screening test; it is not diagnostic. Further confirmatory testing is necessary prior to making any irreversible pregnancy decision.

Learn more

VeriSeq NIPT Solution v2, www.illumina.com/products/by-type/ivd-products/veriseq-nipt.html

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