# Next-gen arrives for next (prenatal) generation

## Anne Paxton

July 2013—In his 25-year practice career, Texas obstetrician James Maher, MD, has performed several thousand amniocenteses, using a long needle to draw amniotic fluid from the uterus of higher-risk pregnant women to rule out certain fetal chromosomal abnormalities—trisomy 21, or Down syndrome, in particular.

Even in good hands, the procedure can lead to complications, says Dr. Maher, who supervises residents at Texas Tech University.

But with the new generation of noninvasive prenatal screening (NIPS), made possible by next-generation DNA sequencing, there will likely be a lot fewer of the invasive procedures in Dr. Maher's future, and that of OB/GYNs across the country.

By ordering one of the four screening tests now available in all 50 states, OB/GYNs are finding they can confidently rule out the most common autosomal aneuploidies, trisomy 21, 18, and 13, with a cell-free fetal DNA test of maternal serum as early as 10 weeks of pregnancy—as opposed to the 16 weeks required for an amniocentesis.

"For prenatal aneuploidy screening and detection, noninvasive genetic testing is the most dramatic technological advance in the past couple of decades," Dr. Maher says, noting that NIPS has a sensitivity of 99.98 percent and is extraordinarily specific as well.

"Instead of saying to a mom, you can either do a non-sensitive, non-specific blood test, the maternal serum test, or do an amniocentesis, now we have something in the middle that is going to give you very precise information without putting the baby at risk. It's an exponential improvement."

The sharp rise in the volume of NIPS testing suggests the OB/GYN world shares that view. The culmination of 30 years of research, NIPS has been available only since late 2011, yet tens of thousands of the tests have been performed, and some hospitals report that their amniocentesis rate has already dropped by 40 to 50 percent.

However, genetics experts warn that like all laboratory tests, NIPS must be used with appropriate caution. "While NIPS is a fantastic technology compared to our old methods, the limitations should not be overlooked," says Martha Dudek, MS, genetic counselor with the Division of Maternal Fetal Medicine at Children's Hospital Vanderbilt in Tennessee.

As a genetic test, NIPS requires pretest and posttest counseling, and presents clinicians, genetic counselors, and patients with new challenges in understanding test results and weighing them to decide how to manage pregnancies. Cost, turnaround time, negative and positive predictive value, reporting format, informed consent, and confirmation testing throw additional factors into the equation.

In interviews with CAP TODAY, laboratory experts and clinicians explain how they are taking advantage of this prenatal screening breakthrough—and steering through the web of complex medical and ethical issues associated with it.

Four companies currently share the market for noninvasive prenatal tests, which are all laboratory-developed tests for screening and do not have approval of the Food and Drug Administration as diagnostic tests. Sequenom makes MaterniT21 PLUS, which detects X and Y chromosomal material in addition to trisomy 21, 18 (Edwards syndrome), and 13 (Patau syndrome), and was performed more than 61,000 times in its first year, 2012.

Panorama, made by Natera and the most recently launched test (March 2013), uses a proprietary bioinformatics technology called NATUS to test for the same abnormalities. Verinata Health, owned by gene-sequencing company Illumina, makes Verifi, while Ariosa Diagnostics offers the Harmony test.

These platforms vary according to the targets for sequencing: amplified regions throughout the genome, chromosome-specific regions, or single-nucleotide regions, according to the American College of Medical Genetics and Genomics. List prices range from $2,700 for Sequenom's test to $795 for Ariosa's Harmony.

Since the 1980s, the most common prenatal screening regimen has combined maternal age, sonographic measurement of the fetal nuchal translucency, and measurement of maternal serum screening markers in the first and second trimesters. And for the vast majority of maternal patients, who are considered average risk, the maternal serum test—testing alpha fetoprotein, human chorionic gonadotropin, unconjugated estriol, and, if it is a quadruple test, inhibin A—continues to be offered routinely.

**Dr. Wick**

"The NIPS test is validated for high-risk patients—those who are already over 35, who have a positive first-trimester aneuploidy screen or ultrasound abnormalities, or who have a previous pregnancy affected with aneuploidy for chromosomes 13, 18, or 21. At Mayo, we are offering the test to that population," says Myra Wick, MD, PhD, geneticist and OB/GYN at Mayo Clinic, Rochester, Minn.

The first-trimester maternal serum screening has a relatively high number of false-positive results, especially for women over 40. "So if the woman is 36, I'll offer her the noninvasive testing, rather than the first-trimester screening."

The extent of first-trimester screening differs substantially from state to state. In California, for example, under state law, all pregnant women must be offered a first-trimester screening, with the assurance that if an invasive test is indicated later, costs beyond $200 will be covered.

For the population served in Rochester, Minn., on the other hand, "only 25 to 30 percent of our pregnant patients elect for first-trimester screening, while in other parts of the country, that percentage is much higher," Dr. Wick says.

The response of pregnant women to the noninvasive testing has been enthusiastic. "Patients are very happy with the noninvasive option, and several patients have asked for noninvasive testing at their first OB visit," Dr. Wick says. Although the test hasn't been clearly validated in lower-risk populations, she believes the data will come out shortly and probably show that the noninvasive testing has much better sensitivity and specificity than the first-trimester screening test.

Mayo has the capability to do next-generation sequencing, but not in the context of prenatal testing. "There are intellectual property and patent issues associated with NIPS testing, and we are not performing the test at Mayo. We collect the samples on site and send them out for testing. However, the laboratories currently offering clinical NIPS testing may partner with other labs in the future, allowing for 'in-house' testing."

While insurers such as Aetna and UnitedHealth Group are willing to cover the test for higher-risk mothers, others do not, and coverage depends on which state you're in, Dr. Maher says. "Some states will allow companies to say we will guarantee your out-of-pocket expense is no more than $200; we will work with your insurance."

"But in other states, and Texas is one, that's not legal. The insurance company would say you're giving the patient a better discount than it is getting. So in different states, mothers have to make different calculations."

**Dr. Maher**

Despite the tests' expense, some OB/GYNs who have started employing the tests are confident that noninvasive prenatal testing could soon become a standard part of the test options for all of the nation's several million annual pregnancies, not just those classified as high risk. The reason is that in the trials completed so far, the NIPS tests are so much more accurate.

"I'm hoping we can offer the test to the general obstetric population in the next year or so," says Dr. Wick. Dr. Maher also supports widening the target market for the test.

Historically, he notes, "The way of guidelines has been to pick on moms when they turned 35. It's like the warranty expired and you're old; you need an amniocentesis." In 2007, however, the American College of Obstetricians and Gynecologists came out with revised guidelines saying every woman is entitled to be offered prenatal screening.

Saying that every woman should be offered aneuploidy screening doesn't mean they have to have it, Dr. Maher adds. "Your job is to basically be the waiter and present the menu; it's up to the woman to decide if any of those options meet her needs."

Next-generation sequencing is what allows NIPS tests to detect chromosome abnormalities. At Illumina, for example, "They have this million dollar machine called an autosequencer, which does DNA sequencing through synthesis," Dr. Maher says.

"NGS is a variation on the old Sanger sequencing where you have a strand of DNA that you want to sequence. You get this soup of cell-free DNA from the maternal serum and you take and anneal it or staple it down to the substrate, so it has fragments of DNA that are 150 to 170 base pairs long, and the machine automatically mixes in these reagents and can sequence these little strands of DNA one bit at a time."

"After 25 or 35 cycles of sequencing, they have this humongous terabyte of information, and using very fast computers and very clever algorithms and having the DNA code, which was cracked with the human genome project, they can say here's what the sequence is from the start of chromosome 1 all the way to the very end of the XY chromosome."

"So this magical machine takes a 30-million-piece jigsaw puzzle and puts it back together into a complete replication of the DNA pool you're looking at, the sample from the mom, through NGS."

Down syndrome, chromosome 21, makes up slightly more than half of all the chromosome abnormalities. "That's why most of the tests focus on the sensitivity and specificity of Down syndrome—because that's where the bang is," Dr. Maher says. "Then if you add 13, 18, and the sex chromosome aneuploidies, you get up to about 85 percent, and the other 19 chromosomes combined make up 15 percent of chromosome abnormalities, which are rare and almost always lethal."

Since cell-free DNA sequencing produces 30 million copies of the sequence, "if you find that the number of copies that map to chromosome 1, 2, 3, 4, 5, and on up are all coming up with a certain number and when you get down to chromosome 21 you're seeing 10 percent more of that than all the other ones, you can say this is statistically significantly unlikely."

"There's less than a one in 10,000 chance that this 10 percent excess in DNA at chromosome 21 is just a statistical fluke. The much more likely answer is there's too much DNA there because the baby, who is the contributing factor for the extra DNA, has an extra copy of chromosome 21, and this baby has Down syndrome."

As the clinical trials have revealed, there are interfering factors that can throw off results. “It’s an overwhelmingly reasonable assumption that most moms are normal, but it’s not impossible for the mother to carry an abnormal quantity of certain chromosomes from tumors, or from what’s called mosaicism.” Mosaicism is where either the mother, baby, or placenta carries some cells with a different chromosome number than the majority of the cells. “For instance,” Dr. Maher says, “if 99 percent of the cells sampled carried 46 chromosomes, but one percent of the cells had an extra copy of a chromosome, the tissue would be mosaic. The cell-free DNA test could pick up a mosaic and report as ‘aneuploid’ as a result of the low quantity of abnormal cells.”

The research arm of ARUP Laboratories has been involved for several years with researching cell-free fetal DNA, Elaine Lyon, PhD, medical director of molecular genetics, says. “We’ve been watching the field unfold, and we were involved with contributing samples to a large prospective study of high-risk pregnancies, although we haven’t brought the test in clinically yet.”

**Dr. Lyon**

NGS has been necessary to develop noninvasive aneuploidy screening, Dr. Lyon believes. “Scientists have tried several methods that could work, but they didn’t fit well in the laboratory workflow. Even though NGS is still relatively new within the clinical laboratory, it is fitting within a workflow.” Eventually, she thinks ARUP Labs will want to bring NIPS in-house, but for the moment it seems to make better sense to let the technology mature a little in the field.

“I don’t think NIPS is replacing the serum testing for the moment. Right now it’s being used in conjunction with serum testing, so the results showing a high risk typically would have amniocentesis. NIPS is helping to reduce the number of amniocenteses, which I think is one of the reasons for using it.”

“But the results so far from this noninvasive testing have a very high sensitivity and specificity, even though it’s still considered a screening test.” She expects that in a few years NIPS will become diagnostic and receive approval from the Food and Drug Administration.

“I think NIPS is a phenomenal development in terms of patient care in maternal fetal medicine,” says John C. Spinosa, MD, PhD, medical director of Scripps Medical Laboratories in San Diego and former medical director of Verinata Health. “It gives an answer that patients can actually use in pregnancy determinations, but also, by letting a whole population of high-risk mothers stop being exposed to a marginally risky procedure, amniocentesis, it heralds the need we talk about in medicine, of using our resources to take care of patients in the best and safest way possible while still paying attention to resources.”

He believes Verinata, for example, has done a good job by emphasizing that clinical data about its test Verifi so far only relates to high-risk patients. “I’m pretty confident Verifi will end up working well as a general tool, but you have to pay attention to how it’s brought out and performance parameters.”

Sensitivity and specificity of a test are independent of the prevalence of a disease or condition, so they can be high while positive or negative predictive value may not be. “You can think that a test is performing well when it’s just a matter of the prevalence of disease in a population, so it’s important to roll out the test conservatively where you can actually see the studies to back it up.”

On this score, the Verifi test has so far been performing with extremely high accuracy. “The performance is not at the level of karyotyping, but it’s very close to that level and will reach it over time as the test evolves,” Dr. Spinosa says. That there are already four companies offering NIPS, he adds, “probably indicates there’s a real need for

safer prenatal testing that's more specific. It's not an artifactual market."

**Dr. Spinosa**

Pathologists need to be much more aware of NIPS, in his view, even if they are not performing the tests now. "One reason is that it makes sense that the initial marketing has been geared to maternal fetal medicine, but the differences among the tests and the reasons why you might argue one is better or not are entirely of non-interest to maternal fetal medicine."

"Those specialists are not used to conducting their practices on the technical details of controlled trials and validation studies. But that's all in the wheelhouse of pathologists who are actually involved in the laboratory."

He argues that pathologists need to represent the interests of the health care system in evaluating the usefulness of NIPS assays. For example, although spending on the assays might increase the pathology budget marginally, the total cost to the health care system might drop significantly because of other costs that are avoided as a result of using NIPS.

For Genevieve L. Fairbrother, MD, chief of staff at Northside Hospital in Atlanta, adopting NIPS as a screening test for the general population was a no-brainer. "Nuchal translucency ultrasound evaluation along with a blood draw is not performed until 11 weeks and it provides a 92 percent to 95 percent trisomy 21 pickup rate, whereas the Ariosa Harmony test can be performed at 10 weeks with a 99.9 percent pickup rate and less than .1 percent false-positive rate."

"Before, if I had a thousand pregnant patients 30 years old, I could anticipate that three would be carrying a Down syndrome-affected pregnancy, but current aneuploidy screening would produce an additional 50 false-positives. From the start, I was on board with the noninvasive screening test and its one in a thousand false-positive rate."

In her view, it doesn't make sense that the test would be somehow better for a high-risk person and less valuable to a patient at low risk. "Why would a 22-year-old not be offered as accurate a test as a 35-year-old, especially if there was no risk to the pregnancy?"

Northside, a community hospital system that does 18,000 deliveries per year, was a beta site for the screening test, and the high sensitivity/high specificity results found in clinical trials were borne out in her patient population, Dr. Fairbrother says. "I think noninvasive prenatal screening is a game changer. It will become the standard of care, because it doesn't require any special expertise."

"It's a simple blood draw with a low false-positive rate, and you get results in the first trimester—you're not waiting on an ultrasound and more blood work before you come up with something."

NIPS is expensive, Dr. Fairbrother admits. "But it's not as expensive on a population level when you consider all those under 35-year-olds with false-positive results who would be offered unnecessary genetic counseling, referral to high-risk doctors, extra ultrasound, and invasive testing."

"It always takes a while to adopt new technology," she adds. "But I think once patients understand the test, they're going to demand it, and it will become universally accepted." Easing the path will be the fact that the test involves a simple blood draw.

**Dr. Fairbrother**

“The standard of care tends to be connected to access. If you’re in a rural or underserved part of the country where you don’t have access to specialized ultrasound, that is a barrier to health care. And the standard tends to the lowest common denominator, because you can’t hold physicians to a standard that is unachievable. But there’s no place in the country that cannot draw blood.”

The test also works for almost all pregnancies including twins and donor egg singleton pregnancies, she notes; the only exception is donor egg twin pregnancies.

Has the availability of NIPS affected genetic counseling in her practice? “We explain to all our patients what screening and invasive tests are available and what it can and can’t tell them. We continue to offer first-trimester ultrasound and maternal serum alpha fetoprotein at 15 to 20 weeks, and a mid-trimester ultrasound as well.”

“We encourage our patients to take advantage of aneuploidy testing, but it is a personal choice.” She is seeing fewer patients who need an amniocentesis or chorionic villus sampling. A high-specificity test is a great boon to patients, she says. “I can tell you fear of a false-positive sends people into a tailspin.”

She particularly likes the Harmony results reporting. “They incorporate in their algorithm the fetal fraction of cell-free DNA along with maternal and gestational age, so by knowing how much of the cell-free DNA belongs to the fetus in maternal bloodstream, it allows them to do what I like to call a binary approach.”

“It’s a very clear ‘It is or it isn’t.’ For example, if you know the cell-free fetal fraction is four percent by analyzing the reference chromosomes, then when you find that the fetal fraction of chromosome 21 is 50 percent increased to six percent, that’s clear. You are more confident it’s an affected pregnancy. It’s not a random cutoff that determines an affected pregnancy.”

NIPS is likely to edge out many tests commonly used now, says Philip D. Cotter, PhD, co-founder of ResearchDx and laboratory director of Pacific Diagnostics Clinical Laboratory in Irvine, Calif. “I see that noninvasive prenatal screening will pretty much kill off the prenatal cytogenetics business, to the same extent that microarray testing has significantly impacted constitutional blood karyotyping,” he says, noting that over the last five years he’s seen a dramatic shift in referral patterns to microarray as the first test of choice.

**Dr. Cotter**

“I know there is a lot of verbiage and community concern around just testing high-risk pregnancies with NIPS, but I think realistically this will ultimately become the first prenatal screen.” As far as the potential market for test orders, he thinks, “from a screening perspective, we’re barely getting started.”

The most likely tradeoff for the test makers, Dr. Cotter believes, is to minimize the NIPS false-negatives at the cost of having some false-positives. “No test is perfect, no matter how much we like to think so, and in doing the least amount of harm you’d want to minimize false-negatives, and the only way to do that is probably to adjust the ratio.

So you push a few people into invasive procedures, but quite frankly those people were going to have one anyway."

As to the differing technologies for the NIPS tests on the market, "More than anything, the choice between technological approaches among the test maker is, I think, a price point issue," Dr. Cotter says. "The sheer amount of sequencing you have to generate is what drives your costs, so by sequencing less, the price point is much lower."

Dr. Cotter predicts the market will start diverging as more service providers start doing their own testing, just as academic institutions started running their own chromosome microarrays. "At some point, I wouldn't be surprised if the companies started producing kits."

"More and more laboratories have next-generation sequencing capability, and I don't know if it's viable to put NIPS on a MySeq, but those are about $150,000 each, or $600,000 to $700,000 for a high-throughput HiSeq model, and the average laboratory is not going to be able to go out and buy one." But inevitably, he says, the larger laboratories and institutions are inclined to internalize those kinds of tests.

Choices about genetic testing can create complex dilemmas for pregnant women. "NIPS is bringing a lot of ladies in who would not have had an amniocentesis but are still very anxious about the possibility of having a baby with Down syndrome," says Dr. Maher.

"A lot of times they say, 'I'm not going to terminate so I'm not doing an amnio, but I will have a lot of sleepless nights staring at the ceiling tiles, hoping the baby is okay.' This blood test may be a way to give them a window into what's going on with the baby, because it's the not knowing for sure that drives people crazy."

The termination issue is linked to all prenatal diagnostic testing, Dr. Maher emphasizes. "The information doesn't trigger termination, it triggers a discussion between the doctor and the patient and the family. And by getting more precise information in the hands of patients at a much earlier gestational age if they do decide to terminate, it's potentially a lot safer for the pregnant mom."

However, even more than previous prenatal testing, NIPS forces medical providers to "step up our game" on pretest counseling, Dr. Maher adds.

In offering NIPS, Children's Hospital Vanderbilt in Tennessee has followed most institutions in choosing to focus on high-risk patients, says Martha Dudek. She notes that the Maternal Fetal Medicine Society and the National Society of Genetic Counselors have statements saying the screen is not appropriate for the average-risk population, which as a rule does not receive genetic counseling either.

But there's been a large uptake by patients who are offered NIPS. It has allowed a lot more Vanderbilt patients the opportunity to have more information in a pregnancy that's been identified at risk than previously.

"Abortion is not really readily available in Tennessee for post-16-week pregnancies, so for amniocentesis that would pretty much rule out termination, but with NIPS they can get a diagnosis earlier. A lot of patients in this population in the South do not consider interruption of pregnancy an option, but they would still like information to be better prepared for the pregnancy," she says.

With any test there are always challenges for a genetic counselor, Dudek adds. "I think we've been able to implement a good protocol to address the benefits and limitations of all patient options. But one challenge has been patients who have an ultrasound abnormality or other pregnancy anomalies."

"In those cases, an invasive test like amniocentesis microarray is really a much better option in terms of informativeness, but if the patients are aware of the noninvasive testing, it's hard to get across the limitations of it and why it is not a good option for them."

On the other hand, "Patients are seeking reassurance that most likely the pregnancy is healthy, and with this test I

have a 99 percent chance of telling them the baby doesn't have a problem, which can really lessen a lot of stress for them and may even have positive effects on the pregnancy." Just learning of an abnormality early on can help them be better prepared if they continue the pregnancy, she adds.

The average two-week turnaround for NIPS can be something of a concern, Dudek says. "Anytime you are talking to a patient about a pregnancy, it's always ideal to give results sooner, ideally the next day, but I don't think it's realistic."

"The sequencing alone takes three days, then there's logistics of getting the sample to them and interpretation. A week is pretty darn good. With an amniocentesis, on the other hand, if you have FISH, you can have results within 24 to 48 hours."

It took about a year after NIPS became available for the insurers to start agreeing to reimburse for the test, Dudek notes. "Reimbursement is extremely important to patients, and since then it's been a lot easier to reassure patients they will have coverage," since the out-of-pocket cost can be as high as $3,000 for patients whose policies exclude NIPS.

Because of NIPS' high level of accuracy, she feels even more strongly about the importance of written consent. "While it's a blood test, patients really need to understand exactly what the test is testing for and be sure they really want that information, because there's no turning back once the information is there. But it's completely optional for them to have the test."

It's equally important for providers to have a good understanding of the merits and possible drawbacks of the test too. "It's new and challenging, and it's changing. That's why it would be very difficult for a provider to be on top of it at the same level as a specialist in prenatal genetics. Fortunately, a lot of the companies do have medical liaisons that are genetic counselors whom they can call as resources."

NIPS is likely the first major step toward eventual application of whole fetal genome/whole fetal exome sequencing, according to the American College of Medical Genetics and Genomics. But in the near term, the obvious first improvements in NIPS will be ones of content, Dr. Cotter says.

"As the price point lowers, you can afford to do more sequencing and more targeted approaches, and you could start to add in some of the other less common but still not insignificant aneuploidies. At some point, you could step it up and start thinking of the test as a low-density array. If you had appropriate targets every five megabases across the genome, you could start to replicate what a karyotype gets you."

"Noninvasive prenatal screening is a surprisingly robust and very viable alternative to some of the current prenatal procedures, depending on the patient's indications," Dr. Cotter says. "For more advanced maternal age, it's probably a better idea to go down the karyotype microarray route because you want to rule out a lot of smaller rearrangements. But for the general population, I think this is an amazing technological advancement, and really an impressive screen."

"We're now at the level of information we can get with 25 or 30 million copies of the DNA," Dr. Maher points out. "But if you move that up to a billion copies of the DNA, now you can tell not only is there a whole extra chromosome or one missing, but you can get what are called sub-chromosomal deletions or duplications, and those may be linked to certain abnormalities."

"So that's the next step: basically to do this array CGH [comparative genomic hybridization] on the NIPS specimen. And it's been done. It just requires a 10-fold increase in the number of sequences you look at to have the mathematical precision to say whether or not there's something there."

"But if you have a family history of a previous child with Angelman syndrome or a mom carrying a gene for DiGeorge anomaly, it will probably be technically feasible, in the not-too-distant future, to do deep sequencing to actually detect these submicroscopic copy number variations. And you wouldn't be able to get that information from a straightforward karyotype from just amniocentesis." This advance is already technically feasible, and he

predicts in five years or sooner, the cost will have dropped enough to make it economically feasible too.

“NIPS is just another tool in our armamentarium. But it’s an extraordinarily sensitive and specific tool,” Dr. Maher emphasizes. And NIPS is evolving rapidly. In just the past 18 months, he notes, citing the Verifi test as an example, new reagents and improvements have substantially reduced the cost of reagents and improved the fidelity of the test. “So I think we will eventually get to the point where if you see an abnormality and your standard test is normal, this test to give you a ‘deeper peek’ will be part of the package.”

*Anne Paxton is a writer in Seattle. As part of the molecular pathology checklist, CAP’s Laboratory Accreditation Program has adopted new requirements for maternal blood screening to detect fetal aneuploidy using NGS technologies. The checklist additions address information required on requisitions, quality control, monitoring, and reporting. More details on the new requirements will appear in an upcoming issue of CAP TODAY.*