For prenatal NGS labs, new accreditation requirements

Anne Paxton

September 2013—With the 2013 edition of the Laboratory Accreditation Program checklist, the College moves to a new level in its effort to ensure the highest-quality practices in clinical laboratories' use of next-generation DNA sequencing.

Expanding on last year's debut of next-generation sequencing checklist requirements, the new molecular pathology checklist, released July 29, includes a focus on NGS for maternal plasma to identify fetal aneuploidy. Phase I requirements address information needed on requisitions, monitoring of assays and targeted disorders, and reporting, while phase II requirements address quality control.

Noninvasive prenatal screening is the right place to start in setting detailed checklist requirements for NGS, says Glenn Palomaki, PhD, a member of the Biochemical and Molecular Genetics Resource Committee of the CAP and American College of Medical Genetics and Genomics. "This is by far the largest application for NGS right now. It is likely that more pregnant women are going to choose to have this test in the coming year than all the other tests using NGS technology."

Dr. Palomaki

In the U.S., the new NGS prenatal section will be applicable to just four companies: Sequenom, Verinata Health, Natera, and Ariosa Diagnostics, which are the only laboratories that currently offer noninvasive prenatal screening based on circulating cell-free DNA. A few other CAP-accredited laboratories offer the testing outside the United States.

"In 2012, we published the first checklist requirements specifically for next-generation sequencing," says Karl Voelkerding, MD, chair of the CAP NGS Work Group and professor of pathology, University of Utah, and medical director for genomics and bioinformatics, ARUP Laboratories. "The requirements were written so that they could generally apply to genetics/inherited disorders, oncology, and other emerging NGS-based testing areas. But we didn't divide them into specific areas."

"Now the process is that each year, when the overall CAP checklist is published, we will incorporate specific, topic-focused accreditation requirements. This year our NGS Work Group was asked to review the area of using NGS to screen for fetal aneuploidy in pregnant women."

The added checklist requirements are new for laboratories doing NGS testing, says Dr. Palomaki, an epidemiologist who has been working in prenatal screening for more than three decades.

"The new requirements are modeled after those already in the chemistry checklist," he says. For the 200 to 300 maternal serum screening laboratories in the U.S. that have been operating for the past 20 years, the data collection, monitoring, and QC parameters would not be considered new. "But much of this will seem new to the four prenatal NGS companies."

The NGS Work Group that helped develop the checklist additions didn't want to be highly prescriptive. "We didn't want to mandate that everyone ought to follow the checklist exactly," says Dr. Palomaki, who was a contributor to the group. But the hope is that the laboratories performing prenatal NGS, given guidance from the checklist, will

head in the right direction and gain useful clinical information from the data collection as well.

For example, the new checklist requirement (MOL.34915) that requisitions include a gestational age estimate, based on either ultrasound measurements, first day of last menstrual period, or estimated date of delivery, is important because clinical validation studies have been conducted mainly on samples drawn over 10 to 20 completed weeks' gestation, Dr. Palomaki points out.

If requisitions show a lot of samples being collected at 24 weeks, or before 10 weeks, it could indicate a new use for what is considered a screening test. "You might want to investigate if more than a certain percentage of samples are from late in gestation. If a large number are being done later, labs may want to document these new patterns of practice so we can detect inappropriate testing. Alternatively, there may be a whole new way to use this test that we're not anticipating."

Maternal weight, another piece of the clinical history now required on requisitions in checklist item MOL.34917, may be even more significant, because it has a strong impact on fetal fraction. The analytic performance is reduced when fetal DNA levels are inadequate. "It's not as simple as a dilution effect," Dr. Palomaki explains. "But in fact, for women over 130 kg [285 lbs], the test has about a 50 percent failure rate. So you can reach the point where it may not be reasonable to offer the test."

"I don't think any professional standards have emerged as to how this issue should be handled. Should physicians routinely inform women over a certain weight that the test is more likely to fail? Should the test not be offered to women over a certain weight?" Although the ACMG and the American Congress of Obstetricians and Gynecologists have said maternal weight is an important factor, neither has made recommendations on how to take it into account, Dr. Palomaki says.

"So clearly we'd like to have more information about this relationship. The commercial laboratories should be looking over their data to see if there is a maternal weight above which they're unhappy with the proportion of successful tests. They may decide they want to say something in their educational materials."

Another situation in which more information would be helpful is that of multiple gestations. Data about multiple gestations are required (MOL. 34919), if the laboratory accepts such samples.

"We have a limited experience with twins, and it is probably up to the laboratory whether it feels confident in offering the screening to this population. There's insufficient information to justify screening triplets at this time, but we'd like to learn more about twins," Dr. Palomaki says.

Even more complicated are in vitro fertilization cases. The checklist (MOL.34918) recommends collecting parentage data, because closer scrutiny is needed. "IVF pregnancies are sometimes associated with an early fetal demise or a 'vanished twin.' What if the vanished twin had Down syndrome?"

While the test may work perfectly well in this group, he says, "this is a population of women who are very concerned about their pregnancy and very much want to avoid an invasive procedure with its associated potential for fetal loss. This group is also more likely to have the resources to afford a test like this. There is an extra onus on the community to make sure that for this vulnerable group, the test works well."

The new checklist also requires collecting data about family history and prior pregnancy risk (MOL.34920 and MOL.34922). While some companies offer the test only to high-risk patients, others will perform the test for women in the general pregnancy population. Given this wide range in prior risks, laboratories should at least have this information available when making an interpretation, Dr. Palomaki says.

"For example, should the DNA test in a 30-year-old with a negative serum screening test be interpreted the same way as a 38-year-old with a positive integrated test? Are they simply 'positive' or 'negative?' The interpretation should account for the prior risk, when possible.

"The checklist doesn't mandate that labs incorporate prior risk into their report, but if they collect the information,

they should realize that not all positives and negatives are created equal." Dr. Palomaki believes that a number of informative conclusions could potentially be drawn from these collected data that could improve the final risk estimate.

Often, he notes, physicians are provided with test results and have to assemble the interpretation themselves. If a woman has a nuchal translucency measurement and maternal serum screening showing she is at high risk, for instance, and then has the NGS test, the physician may wonder what the negative result on the molecular test means in the context of the prior test results.

"Interpretation of both positive and negative tests is not so straightforward. That's one of the reasons why the professional organizations have suggested that these issues be more fully explored before we move to testing in the general pregnancy population."

Two phase II checklist items on quality control (MOL.34923 and MOL.34924) are among the additions and require that labs monitor test performance limits and QC parameters, and include positive and negative controls in each analytical run. Longitudinal monitoring of the assays, another checklist requirement (MOL.34925), will help with a process called epidemiologic monitoring, Dr. Palomaki says.

"What that means is that most of the pregnancies, and tests, will be normal even in the high risk setting. This can be used to actually help monitor your assay." One company uses a laboratory performance index known as a z-score; two others use something similar. "This score can be monitored as an indicator of assay quality, and when it does not meet expectations, the reference data may not be appropriate. In any event, the laboratory should develop a monitoring scheme, if possible, and have an associated action plan when epidemiological monitoring indicates a potential problem."

He is hopeful that collecting those data will help improve the tests' performance. "There are numerous reports from serum screening labs showing what happened to their assays month by month because they monitor results carefully. When one month's results appeared to be out of control, they went back and found, for example, the problem occurred because they had switched to new reagents.

"Because of this, we now suggest that laboratories preview their serum screening reagents when they are received and assess them using a method comparison before using them clinically. Testing has improved because of this practice. We've learned these things because people were willing to share their findings, including their remedies. Whether that will be true for NGS laboratories, I don't know."

Dr. Voelkerding

Monitoring of targeted disorders is another important quality assurance measure that the checklist now requires (MOL.34926). At least quarterly, laboratories should calculate and review the percentages of women with positive results for each targeted disorder such as Down syndrome or Turner syndrome, test failure rates due to such factors as low fetal fraction, and rates of "inconclusive" test results.

Longitudinal monitoring is a challenging area for clinical laboratories, Dr. Voelkerding points out. "The importance of NGS testing for fetal aneuploidy is that if there is a positive result, such as presence of trisomy 21, this should trigger a discussion between the pregnant mother/couple and the care provider with respect to confirmatory testing."

The mother may elect the use of a confirmatory followup method, he says. “In this case, amniocentesis coupled with cytogenetic analysis would be considered standard of care.”

But some believe that the confirmatory method may eventually not be needed. “Will the NGS test become the standard of care so that one can rely on the NGS result and not need an amniocentesis? The only way to determine this is to do longitudinal studies wherein NGS results are compared to those obtained by amniocentesis and cytogenetic analysis.” Important to note, Dr. Voelkerding says, is that cytogenetic analysis may identify other chromosomal abnormalities not currently detected by NGS.

Longitudinal studies are essential going forward, he says. “While laboratories offering NGS-based testing for fetal aneuploidy have performed validation studies, longitudinal studies would provide a larger database to determine the sensitivity and specificity of this testing in clinical practice.”

Finally, the new checklist contains a requirement (MOL.34927) that laboratories’ reports include qualitative and/or quantitative test results for each target chromosome (for example, z-score, fetal fraction, likelihood ratio), reference ranges or cutoff values as appropriate, and a summary set of risks/categorical interpretations.

Variation in these specific test results can make a big difference in a test’s information content, Dr. Palomaki says. “I and others have been suggesting that labs report not only the test interpretation but also the test results. Two of the labs now routinely include fetal fraction on patient reports, one result of testing. But I don’t know of any labs that include results such as the z-score or normalized chromosome value.” However, current practice has been considered satisfactory for CLIA and CAP inspections, he says. “But the checklist suggests that if actual test results are available, they should be reported along with the final interpretation.” This would be similar to serum screening where the raw assay values and the results in multiples of the median are mandated, in addition to the Down syndrome risk.

He gives an example of why including these results is important. “If the fetal fraction were four percent, most of these tests will be reliable, but at 10 to 20 percent, the performance will be better. At 20 percent or higher, even fewer errors would be expected to occur.” This has implications for reporting results, as knowing the fetal fraction for each test may help practitioners better understand the individual interpretations. “For example, as they gain experience, they may clearly see more ‘no calls’ made among women with lower fetal fractions that tend to have higher maternal weights.”

The checklist requirement doesn’t stipulate that the companies have to change their reporting, he says, because it uses the language “as appropriate.” But he hopes the companies will begin issuing more informative and detailed results of the testing, not just assessment of risk.

It’s significant that the College is looking at this type of prenatal testing that is being introduced rapidly into clinical practice, Dr. Palomaki believes. “For the first time, there are specific checklist requirements for the labs offering this type of NGS prenatal screening. This type of testing is a very sensitive area for patients, yet is one of the most common applications for NGS right now. CAP is looking out for physicians and patient care by quickly introducing checklist requirements that are relevant to prenatal screening.”

The NGS-related checklist requirements will continue to evolve, Dr. Voelkerding says. “We initially responded to this new area for labs with a series of general checklist requirements and we are also progressively adding yearly revisions, including diagnostic topic-specific requirements, in response to feedback from the field, to continually improve the checklist as new applications are being developed.”

NGS proficiency testing is next, he adds. “We are now working in parallel toward launching proficiency testing challenges for the diagnostic community for assays based on NGS. That will include PT challenges for genetics/inherited disorders and oncology,” and for fetal aneuploidy, NGS PT is under discussion.

Anne Paxton is a writer in Seattle.