






Focus



Considerations for Adding Molecular Testing to Your Lab

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The Papanicolaou (Pap) screening test has long been proven effective in early detection and diagnosis of cervical cancer and precancerous lesions. In the 1990s, liquid-based cytology changed the Pap test in terms of collection and evaluation. The past decade has seen molecular testing move from a reference test to an in-house test in many laboratories. Many laboratories are now wondering whether they can effectively and efficiently bring in molecular testing. Depending on state regulations, the types of molecular tests, staff abilities, and administration support, the answer is a definitive YES.

High-risk HPV testing has grown in the past few years to stand with the Pap test as an integral part of cervical cancer screening. It appears to be here to stay, and is positioned for continued growth into the foreseeable future. The question isn't whether your cytology lab can afford to bring HPV testing in-house; it is whether you can afford not to. If you don't, someone else will. Wait too long and you may find yourself trying to win back your clients from another lab, or you may face political battles with other departments, such as microbiology, within your (affiliated) hospital.

TF Tim recalls two HPV testing implementations:

"In the first lab, which was relatively low-volume, I advocated bringing HPV testing in-house after our volume grew to where I knew it would be profitable. A few months earlier, the lab director of our affiliate hospital invited me to discuss the addition of HPV testing to their clinical lab. They were more interested in obtaining tests that could be run utilizing PCR. This was a lucky break. Had they realized the benefits of bringing HPV testing into the clinical laboratory, we probably would not have had the opportunity to acquire it for ourselves. Our implementation went very smoothly. Within a month, three cytologists were running weekly HPV tests. Although we were not efficient in kit use because of our low volume, we now had our feet firmly planted in a new, growing market."

"The high-volume laboratory where I now work was a bit more complicated. HPV testing was already being done by a lab that we share close ties with. Many hurdles had to be overcome and diplomacy skills tested before implementation. There seemed some hesitancy due to uncertainty over whether cytotechnologists could run the test. We succeeded in bringing high-risk HPV testing and HPV 16/18 genotyping in-house, largely due to good leadership and support by our operations manager and medical director. We now have a thriving new section of our laboratory, which affords us much needed diversification, given the uncertain future of the Pap test. Our current monthly volume approximates the yearly volume at my former lab, and I expect the current volume will only increase, as we plan to automate very soon."

There are several important points to consider when implementing HPV testing.

Evaluate Throughput: Make sure you can run enough tests to be profitable. A simple break-even analysis can help project when you will move into the black. Start-up costs should also be considered, but remember the old saying, "You need to spend money to make money."

Assess Researcher Personality: You will need to train people who are organized and detail oriented, as running this test requires a great deal of concentration. They don't need to be molecular biologists, but a basic knowledge of molecular biology helps when troubleshooting. Most cytotechnologists should already be familiar with these concepts.

Consider Training Individually: When training, validating, and insuring competency, remember that not all five-day training runs are created equal. In a best case scenario, train each staff member individually until competent. If staff are new to molecular testing, it may be best to run short until they are competent and comfortable with the protocols.

MS recalls her experience with staff training:

"Our lab had been running HPV tests in-house for several years and looked to change methodology. We wanted to train two individuals (a cytotechnologist and a microbiologist) for HPV testing and looked to set up a once weekly run schedule. We trained and validated under the ASR product using shortened runs. Once validated, we split testing so that each person ran HPV testing once every other week. We experienced test and run failures intermittently for several months, which caused a sense of frustration for all. Root cause analysis showed that our main problem was due to technique memory. The lesson learned from our initial testing trials was that it would be better to perform five runs over five days, rather than over five weeks in order to reinforce technique with knowledge. We were able to fully realize this notion as we trained our third testing team member (another cytotechnologist)."

Verify Pipetting Skills: Pipetting competency is key to efficient molecular testing. Whereas cytology involves non-graduated pipettes for specimen transfer, molecular testing—because of its microliter volumes—has more stringent demands. Having a steady hand is important, as even slight volume deviations in molecular testing can lead to costly errors for one test or a full run.

Learn From Failed Runs: Failed runs will inevitably occur, whether due to contaminated reagents, faulty kit lots, defective instruments, human error, or an unidentifiable problem.

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Molecular Testing Principles:

1. Unravel the double helix.
2. Add target specific nucleotide sequences (oligonucleotides) known to hybridize to HPV and human genomic DNA.
3. Add a structure specific enzyme that cleaves when the specific target sequence of interest is present to generate fluorescent signal.
4. Measure the fluorescent signal produced by the cleavage to determine presence of the specific target sequence.
5. Measure whether this is a specific fit of host DNA to the specific probe.

DNA Isolation: Breaking apart the nuclei to free the both human genomic and viral DNA that is present in the cell population.

Hybridization: Once the DNA has been isolated, the next step is to add target specific oligonucleotides that hybridize to the HPV and human genomic DNA sequences.

Signal: Once hybridization occurs, a specific structure is formed between the oligonucleotides and target sequence of interest. This structure is recognized by the enzyme and cleavage of the probe oligonucleotide occurs. The cleaved portion of the probe then hybridizes to a FRET oligonucleotide once again forming a specific structure recognized by the enzyme. The FRET oligonucleotide is cleaved causing the detection of fluorescent signal. Signal is generated only if the specific target sequence is available.

Thermocycler: Equipment

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When they occur, it is important to document the incident and learn from it, rather than assign blame. Taking a punitive approach to failed runs will only create more failed runs from nervous cytotechnologists and/or other testing staff. **Use Vendor Tech Support:** It is also important to have a good working relationship with your vendor's technical support staff. Regular communication with them often leads to excellent service. You may even receive a free kit if you can show a failed run occurred from defective equipment or reagents. We talk to our technical support staff regularly and the service we receive is always excellent. Remember, it is in their best interest to support you in any way they can, so use technical support often – they are there to help you. Vendor tech support can also help you adjust to the initial learning curve for HPV testing, which has different troubleshooting guidelines from cytology. While your technical support reps are experts in HPV testing, they may not be as familiar with the differences between molecular and cytology. There's no question too basic to ask your vendor's technical support staff.

Address the Area: Another important consideration is your physical space. You should designate a location with low traffic and few distractions. Avoid interrupting staff with questions, phone calls, or other duties. As mentioned earlier, the person running the test needs to stay focused. Therefore it is essential to make sure you have enough staff to keep up with the regular cytology workload while one is tied up performing HPV testing.

Don't Buy Cheap Supplies: Although it may be tempting to conserve funds, it is generally unwise to buy inexpensive supplies such as pipettes and disposables such as tips and 96-well plates. Use the manufacturer recommended consumables when you can. One or two failed runs of expensive reagents can quickly erase alleged savings on subpar disposables. You can't afford to perform too many full runs twice. Technical staff are often not fully aware of reagent costs versus reimbursement for the test performed.

Create Troubleshooting Guides: With the new jargon, it can be difficult to troubleshoot quickly, which may lead to several meetings to review things from scratch. A guide can streamline problem review and help distinguish areas of competency from those that could use additional training.

Management Can't "Coast": Supervisors and managers should know the basic principles behind molecular testing, as well as how the calculations are made and interpreted. Whereas many cytologists may believe in the absolute nature of numbers and calculations, there will always be variation in DNA amounts (both human and viral). Review your confidence and correlation statistics. If it's unclear what results are OK, good, and great in terms of the calculations, ask your vendor reps for help.

Conclusion

Don't be afraid of change, embrace it. This is a time of transition and opportunity. What you do during this time will impact your laboratory for many years to come.

that maintains specific heating and cooling temperatures required for the reaction to occur.

Fluorescent Plate reader: Measures the target-specific fluorescent signal generated during the enzymatic reactions

FAM/FOZ: This is the specific signal to noise ratio that is calculated to determine if the target of interest is present.

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