New accreditation program checklist section: Imaging mass spec scores its own quality standards

Anne Paxton

October 2018—It happened for next-generation sequencing. It was an important step for in vivo microscopy. And now it's taking place with imaging mass spectrometry. The milestone: development and adoption of a set of specialized checklist requirements for laboratories that want CAP accreditation.

Dr. Lehman

Imaging mass spectrometry, an adjunct methodology to help pathologists analyze areas of interest in tissue specimens, is, at this point, used in a small number of research laboratories in the U.S., says CAP Checklists Committee member Christopher M. Lehman, MD, clinical professor of pathology, University of Utah College of Medicine, and medical director of the University of Utah Hospital Laboratory. But the technology is swiftly moving toward clinical use, and the CAP decided it was time to set standards that laboratories could adopt for the use of imaging mass spec (IMS). The new checklist requirements, released in August, are part of the 2018 chemistry and toxicology checklist.

"IMS gives you the ability to do mass spectrometry on a slide or a piece of tissue, and then overlay the MS results onto, say, an H&E-stained slide, so you can see not only the morphology of the tissue but also where the molecules of interest are showing up. With IMS, you can actually look at the lipids, proteins, and peptides of interest that are associated with specific pathological or histological traits on the slide," Dr. Lehman explains.

Since the subject-matter experts consulted by the Checklists Committee were not familiar with the creation of checklists, "to help them we supplied them with existing checklists including the molecular, microbiology, and chemistry checklists," Dr. Lehman says. "Many of the IMS checklist items were derived from these checklists."

Obviously, the experts doing research on IMS know how they do it. But to develop the checklist, he says, they had to create a mental process map and think through the steps and the quality control points. "We said to them, 'If you were going into another researcher's lab to evaluate how they performed mass spectrometry imaging, what would you want to see to be assured that they were doing it correctly.'"

Most of the checklist requirements will make sense to people who operate mass spectrometers or who already do microscopy. They include how to operate the instrument, what would be a proper control, and how to identify an area you are interested in analyzing, Dr. Lehman says. "Those already exist in the checklist but had to be adapted for IMS." He expects the checklist to be revised as more is learned about how to use IMS.

IMS has become a mainstream research tool in certain circles all over the world, says Jeremy L. Norris, PhD, managing director of the National Research Resource for Imaging Mass Spectrometry at Vanderbilt University School of Medicine. He and Richard Caprioli, PhD, director of Vanderbilt's Mass Spectrometry Research Center, were the two IMS experts consulted by the Checklists Committee.

There are many R&D applications of IMS in the pharmaceutical industry, including use of IMS to characterize drug distribution and drug metabolism in animal studies. However, "The use of IMS to look at biological tissue is something that originated with Dr. Caprioli," Dr. Norris says. "It's becoming apparent that there are going to be real applications for diagnosis of human disease and use in the clinical lab."

The Mass Spectrometry Research Center is already at that stage. “IMS is a pretty robust R&D tool. Now that experience is being applied to develop a new clinical diagnostics lab with IMS as the core technology.” Dr. Norris is also CEO of Frontier Diagnostics, which he co-founded with Dr. Caprioli in 2014 expressly to develop some of the diagnostic capabilities of the technologies the MSRC has developed and to eventually launch commercially available clinical assays.

IMS has advantages over traditional MS assays, which often homogenize the sample, then extract the proteins, then do the analysis. “In that process, you’ve lost all of the sample’s spatial information,” Dr. Norris points out. By contrast, IMS is part of a unique combination of tools that preserves that spatial information. With IMS, “what we do is collect molecular information from vivisections, like those the pathologists are looking at under a microscope, but we preserve the spatial information from the samples. So we are using MALDI MS, which already has a toehold in the clinical laboratory through several applications, to add a molecular dimension to microscopy.”

Dr. Norris

“We can work collaboratively with pathologists to identify groups of cells that potentially might be diseased, then we can collect unique signatures from each of those. Within one section, you can do multiple areas of analysis and you can accomplish that very quickly. Then you use that data to ask: Are these specific cells cancerous, or not? Tissues are heterogeneous, and this allows us to collect that additional molecular data while preserving that heterogeneity.”

Dr. Norris views IMS as building on the existing knowledge base in pathology. “With this application, we’re not necessarily claiming we’re going to replace what pathologists do, but we are adding the capability to get an extra dimension of information that can help in the areas of the tissue that might be too ambiguous to make a definitive call.” Much of what is unique about the assays Frontier is developing has to do with how the sample is prepared, Dr. Norris says. “The software systems tie the sample preparation in together with the instrumentation and the data analysis to get the job done efficiently. The innovation is less the instrument itself and more about how you integrate it into the workflow.”

In many ways the IMS work at Vanderbilt is bringing the molecular age to the field of anatomic pathology, in his view. That’s how he thinks it will be revolutionary. “There certainly are molecular assays that are done in AP. What we have the opportunity to do is, on a single platform, develop methodologies that can be applicable in many areas of AP without making a lot of change in the reagents we use. So we can potentially have one instrument platform that can serve many different subspecialties using a common analytical method.”

In helping to create the checklist, he and Dr. Caprioli saw their role as identifying the unique areas for which IMS requires special consideration and making sure the checks and balances are in place. “We try to think through what are all the possible ways that one might prepare or set up the instrument and to make sure that the checklist items are general enough to cover those possibilities—without being so loose that they aren’t effective.”

In addition to the more standard checklist elements such as the need to tune and calibrate the instrument, use appropriate consumables, and retain data, the new IMS checklist addresses those unique areas. “We’re detecting a rather broad number of peptides in each analysis we do,” Dr. Norris points out. “And so we had additional instrument QA or QC in the checklist to make sure the instrument is sensitive, plus steps for identifying the region of interest within the tissue, steps for data analysis, and protocols to ensure we are able to pick up a false-negative or false-positive.”

Frontier plans to launch its IMS platform in its own CLIA-certified laboratory first, Dr. Norris says. “Over time, there is an opportunity to provide labs with an FDA-approved platform, with kits suited to certain assays, and reagents and protocols to do the assay correctly.” He expects the platform will be able to perform multiple assays because “you have to have critical mass with the number of applications to justify purchasing the instrument and having it in the lab.” For example, since there are many types of skin cancer, the company plans to have multiple assays in the area of skin cancer, each with its own assay label.

“One of the powers of the IMS technology is that there is no chemical labeling requirement, so we don’t have any antibody we have to link to, we don’t have a specific probe we use, and we detect molecules in their native state. This makes it universal, in that any tissue type can go into the instrument and be analyzed,” Dr. Norris points out.

He believes Frontier will enter the market with its first IMS assay within a year, and he hopes it will be the first company to do so. “I think we’ll have a laboratory-developed test for which the clinical utility studies are completed and we can provide evidence that it’s effective for solving a problem, and we will be selling the test to clinical labs.”

When the IMS checklist project was initiated, John D. Pfeifer, MD, PhD, professor of pathology and immunology and vice chair for clinical affairs at Washington University School of Medicine, was chair of the CAP Personalized Health Care Committee. In that role, he helped bring Drs. Caprioli and Norris in to speak to the committee two years ago about the potential uses for the technology.

The same committee had followed a similar process when developing the in vivo microscopy checklist, Dr. Pfeifer says. “That technology matured to the point that there is an IVM committee now. And we realized that IMS was at about the same stage in its development, and that labs that do it would welcome guidance to make sure they have a quality operation. Given my experience with the process for IVM, I was comfortable helping to lead it again for IMS.”

Dr. Caprioli demonstrated to the committee that IMS technology had a technical piece and an informatics analysis piece that could be used to discover information from tissue sections that could not be obtained from the tissue slides by conventional microscopy or histochemistry, Dr. Pfeifer says. “Through IMS, there were reproducible patterns of molecules—they could be lipids, small metabolites, proteins, a number of things—that were characteristic of specific normal tissues or disease states. He also showed that with appropriate validation you could build a test that would have clinical utility around that.”

Dr. Pfeifer

IMS is still in a category of boutique testing, Dr. Pfeifer says. “It still requires a specialized instrument to perform, and platform manufacturers have only recently become interested in the space. It’s one of those things that only specialized centers have been able to do because the necessary equipment has been expensive and you needed a certain infrastructure.” Now, a number of manufacturers are developing, or already have, instruments that are cheaper and have the required functionality, he says. “I think now we’re in a situation where the technology is poised for growth.”

As with any other test, he says, “there are a few labs that do the hard work to show it is reproducible and has utility. Then, based on the work of those labs, commercial entities that manufacture the platforms, the mass spectrometers, develop something you can buy and put in your lab. They’re starting to do that, which will, of course, engender more widespread use of the technology.” Dr. Pfeifer is not aware of any applications pending at

the FDA at this time.

IMS' impact could be significant, he says. "We all know that routine immunohistochemical stains are powerful tools for diagnosis and prediction. But we're also aware there's other information in tissues that you can't discover by light microscopy. IMS is another tool in the kit."

Migration from research to clinical use is taking place already, Dr. Pfeifer says. "It's become clear that IMS is almost certain to have clinical utility in some settings, and the labs that are doing it are looking for guidance to know what sort of testing would meet high-quality criteria."

Although it's early in IMS' career as a clinical test, Dr. Pfeifer says "that's the point at which the CAP should be getting involved in these things"—just as the CAP did some five years ago with in vivo microscopy. "If it's coming out of a research environment, a lot of the people there are creative, brilliant people. But they're not pathologists, so they're uncertain about what it looks like to develop a quality test from a regulatory perspective. So the first goal is to help them. The second goal is to make sure it's done right."

Potentially, Dr. Pfeifer says, some other group could develop a checklist and offer accreditation that isn't at the same standard as what the CAP offers. In fact, "The CAP explored the boundary of being too late with NGS sequencing. Labs were already using these checklists from molecular testing that were generally correct but did not reflect the intricacies or unique aspects of NGS."

"The good news," he adds, "is the field didn't suffer." The CAP "moved in and embraced NGS" as a new technology. "And for in vivo microscopy, the CAP was right there where it needed to be, with a checklist."

The committee was able to draw on earlier checklists to assemble the IMS checklist, Dr. Pfeifer notes. Those included, for example, the mass spectrometry checklist for toxicology or clinical chemistry, and the telepathology checklist. Also helpful were experts who use mass spectrometry in toxicology, clinical chemistry, and microbiology and who use telepathology, as well as those who understand the computational components (bioinformatics) of NGS.

The new checklist addresses the analytical data analysis procedure in requirement CHM.21445, which calls for "a written procedure that describes and identifies the algorithms and steps that make up the data analysis process used to analyze, interpret, and report test results." The committee might pay even more attention to data analysis in future editions of the checklist, Dr. Pfeifer says. "Everybody is talking about computer-assisted diagnostics, image analysis, artificial intelligence, machine learning, and so on. People are developing algorithms that will soon be in routine use. So we're getting close to the point that the CAP might want a checklist for validating those types of algorithms." Although it's still too early for that checklist, he says, it will have some of the same themes as NGS and IMS. "Operationally, the IMS checklist is just the latest example of the evolution of computationally intensive processes to help get better diagnoses."

Optimizing patient care is always the No. 1 purpose, Dr. Pfeifer says. "Pathologists are, after all, laboratory physicians. One of the CAP's primary missions is to support pathologists in that role by providing guidance to perform high-quality testing. In my view, if the CAP ever stops doing that for emerging technologies, its reputation and relevance as an organization will come into question."

The usefulness of IMS technology in coming years, Dr. Lehman says, depends on how quickly any laboratory develops its own test for that purpose. "This is a new and interesting area and we don't yet know what contribution it will make to the diagnosis or prognosis of disease. It's at a very early stage. And I think the College is being very proactive in getting some initial checklist items prepared for the first couple of labs that have an LDT or for a manufacturer that wants to offer an FDA-approved test."

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