A preanalytics push in accreditation checklists

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September 2021—Taking steps to protect the integrity of specimens is at the heart of new and revised requirements in this year's edition of the accreditation program checklists, set for release Sept. 22.

A CAP team made up of members of the Checklists, Personalized Health Care, and Cytopathology committees collaborated to incorporate into the checklists the evidence-based recommendations set forth in a 2019 article on preanalytics and precision pathology (Compton CC, et al. *Arch Pathol Lab Med.* 2019;143[11]:1346–1363).

Many of the new and revised requirements, which are in seven checklists, are aimed at improving the quality of tissue and blood specimens that may undergo molecular testing for patients with cancer. The aim of others is to improve the preanalytic quality of specimens used for all types of testing.

The problem of faulty preanalytics that compromise the molecular integrity of specimens is long-standing, says Carolyn C. Compton, MD, PhD, who was a member of the CAP Preanalytics and Precision Pathology Project Team that determined what revisions or new requirements were needed. "It's a problem that's kept translational research and product and drug development from moving forward. It's been pervasive across all of biomedicine, making it extremely hard to fix," says Dr. Compton, professor of life sciences at Arizona State University, medical director of the ASU Biodesign clinical testing laboratory, and professor of laboratory medicine and pathology, Mayo Clinic College of Medicine.

Addressing the problem, she says, requires getting to the point of care.

"From the very moment a specimen comes out of or off of a patient we need to control preanalytic variables. Only professionals who handle specimens can specifically identify and remedy the problems."

As the revisions took shape, the team was mindful of the impact of changes on daily practice. Says team member and Checklists Committee member Amer Mahmoud, MD: "We tried to make sure we didn't include something that would create an impossible burden on labs. Language was carefully considered and crafted to make it the best guardian of quality yet practical and not burdensome."

And the team invited feedback from others. "We didn't just dictate things. We sent our thoughts to other committees for comments. When we received feedback, we'd meet to discuss various concerns and points of view and edit the requirements accordingly. The work was fully vetted by others," says Dr. Mahmoud, a hematopathologist and molecular genetic pathologist, TriCore Reference Laboratories and Presbyterian Hospital, Albuquerque, NM, and clinical assistant professor of pathology, University of New Mexico.

In the anatomic pathology checklist is a new requirement that says labs must have a process to ensure optimal total fixation time in formalin for specimens clinically suspected or otherwise known to contain malignancy (ANP.10039 Total Fixation Time), because specimens of this type are likely to be submitted for ancillary testing. "This is one of the most important requirements, and I believe it will grab the most attention," Dr. Mahmoud says. "It went through multiple iterations because we wanted to include the best possible evidence pertaining to fixation time, while making sure not to penalize any lab unnecessarily."

Dr. Compton

“We know from our literature review,” Dr. Compton says, “that overfixation”—periods exceeding 24 to 36 hours—“start to create artifactual mutations in DNA. The cross-linking between molecules causes DNA damage that can be read on an assay as contributing to tumor mutational burden, a predictive biomarker for immunotherapy for cancer. So TMB must be reflective of the disease state and not an artifact of a process to which you subjected a specimen.” With the rise in the use of immunotherapy, she adds, “this has become critical.”

Ideally, the goal would be to treat all specimens the same, because whether a specimen contains malignancy isn’t known until after it’s analyzed, “so total fixation time is important,” says Harris S. Goodman, MD, Checklists Committee chair and member of the preanalytics team and Commission on Laboratory Accreditation. “Right now, however, this requirement is limited to specimens suspected of containing malignancies, but at some point it will apply to all specimens. This is just the start.”

The team’s members know this will be hard to do given the workflows in typical AP labs. “Specimens get tossed into buckets into which they may sit all day long, and we don’t track that,” Dr. Compton says. “Now we have to come up with a process, a system, to track this. The workflows, the settings on the tissue processors, the tracking of time zero in the formalin—these are all things that have to be followed and will likely require a workflow revision. When a lab is inspected, they will have to show how they have done this. We’re not telling labs how to do this, just that they have to do it.”

Another new requirement calls for labs to monitor the quality of the formalin used for fixation (ANP.10041 Quality of Formalin). “This will probably catch some people off guard,” says Dr. Goodman, who is chief of the Department of Pathology, Alameda Health System Highland Hospital, Oakland, Calif. “Labs need to make sure formalin is good for fixing specimens. Until now, it’s been a huge preanalytic variable.”

Dr. Compton says the fixative shown to have the best performance overall is 10 percent neutral (pH 7.0) phosphate-buffered formalin. “Here’s the problem: It has to be neutral, so its pH has to be measured on a regular basis, and it has to be 10 percent formalin by volume from a saturated formaldehyde solution—a four percent formaldehyde solution,” she says. If the labs that mix their own formalin rather than purchase it from manufacturers don’t have the pH correct, Dr. Goodman says, “it will not fix the specimens properly.”

Dr. Compton says laboratories could be compromising the quality of their formalin without realizing it. “If you are trying to cut down on costs by using old formalin over again or dumping formalin that contains a decalcification chemical into a formalin bucket, that creates problems. You may not be treating fixative with the absolute respect it needs because you are unaware of the consequences.”

Among the revised requirements is ANP.22969 Report Elements, which says that for IHC and ISH tests that provide independent predictive information, the patient report must include information on specimen processing, the antibody clone/probe, the scoring method used, and the limitations relating to suboptimal preanalytical factors that may have an impact on results. The laboratory performing the gross examination of the specimen must record the cold ischemia time and length of time in fixative.

“This is huge,” Dr. Compton says of the revision. “Before this, control and reporting of these variables were only required for testing in breast cancer specimens, but if it was important for one kind of molecular test for one kind of cancer, it’s likely to be important for all molecular tests in all cancers.

“And it’s a must,” she continues. “Not only are we requiring control over cold ischemia time and fixation time, but we are requiring people to report those parameters as part of the history of that specimen. Until now, we had no way of knowing what had happened to a specimen because we didn’t record it.”

Dr. Mahmoud calls the requirement to report the limitations relating to suboptimal preanalytical factors that may impact results “an extremely important change.”

Dr. Mahmoud

“It is very important for the person signing out reports on predictive markers to be aware of preanalytic issues that could impact the accuracy of the result,” he says, “and these revisions bring everyone’s attention to the two important factors of cold ischemia and total fixation time.” The same reporting requirement has been added to the molecular pathology and cytogenetics checklists (MOL.39295 and CYG.47880). “The stakes are high when you’re talking about predictive markers,” Dr. Mahmoud says.

ANP.22983 Fixation—HER2 and ER Breast Cancer Predictive Marker Testing specifies that cold ischemia time should be one hour or less. In this revised requirement, the CAP “strongly recommends” specimens be fixed in 10 percent neutral phosphate-buffered formalin for at least six hours and up to 72 hours at room temperature and that specimens be fully submerged. The group acknowledges that a fixation time greater than the 24 to 36 hours, as put forward in the revised checklists, may be required for fatty specimens like breast tissue. Information about fixative, the actual fixation time, and the cold ischemia time for each specimen must be recorded as part of the permanent specimen record in the pathology report.

MOL.39358 and CYG.48932 Fixation—HER2 (ERBB2) Breast Predictive Marker Testing similarly require monitoring of cold ischemia time (one hour or less) and fixation time.

Histology processing requirements in the anatomic pathology and biorepository checklists also were updated. ANP.23100 and BAP.07200 Tissue Processor Solutions were revised to say that “when solutions are changed, they must be entirely replaced with new solution and not just ‘topped off.’”

“The main reason tissue specimens degrade while in paraffin blocks is because of hydrolysis, which occurs when water remains in the specimen due to inadequate dehydration during tissue processing,” Dr. Compton says. Alcohol baths in tissue processors can become contaminated with spillover from formalin buckets. “If you don’t change the alcohol baths faithfully, dehydration of the tissue is compromised. Therefore, this requirement says they must be entirely replaced on a regular basis.”

ANP.23350 and BAP.07400 Paraffin Baths, Flotation Baths, and Embedding Stations now recommend the use of high-quality, low-melt paraffin because low-melt paraffin is removed more efficiently during de-paraffinization and/or antigen retrieval, which is essential for molecular analysis.

Also revised is ANP.11670 Specimen—Gross Examination, which now says “the ideal thickness for specimen sections submitted in cassettes is 5 mm or less.”

“If you don’t cut the specimen thinly enough,” Dr. Compton says, “it won’t get fixed on the inside. It will be raw. We want the formalin to be able to penetrate the entire thickness of the specimen.”

ANP.12500 Record and Material Retention—Surgical Pathology says paraffin blocks used for patient diagnostic, prognostic, and/or predictive purposes must be stored (for 10 years) in a manner that “preserves their identity and integrity,” and tissue blocks must be stored in a temperature-controlled, pest-free environment.

“The quality of the storage is important,” Dr. Compton says, “and not all institutions have space for storage so they farm it out. If you are paying someone to store your blocks, they will have to provide you with evidence that they’ve maintained temperature control and that rats aren’t gnawing through the paraffin blocks in their facility in order for you to pass a CAP inspection.”

In the laboratory general checklist is a revision to GEN.40100 Specimen Collection Manual Elements—Clinical

Pathology Specimens. “In this revision we’re calling out phlebotomy draw order, as well as fill volume and proper mixing,” Dr. Compton says. “Some tubes in which you’re drawing blood for a molecular study have additives in them. The ratio between the additives and the blood in the tube is important and has been precisely calculated to get optimal outcomes. If you don’t fill a tube to the proper level or you don’t mix the additive with the blood thoroughly enough, you won’t get optimal results.”

GEN.40115 Specimen Collection Manual Elements—Surgical Pathology and Cytopathology Specimens is a new requirement that “puts CAP’s stamp on the importance of preanalytical factors for surgical pathology and cytopathology specimens,” she says.

The requirement lists the seven elements for which instructions must be included in the manual, including special timing for collection, type of collection container and amount of specimen to be collected, and types and amounts of fixatives or special media, among others. “Before, the checklist required some of the seven points but only suggested others. Now they are all mandated,” Dr. Compton says.

In this requirement is a note that addresses fixation and cold ischemia time, she says, noting the use of the word “must.” “These requirements will come as no surprise to anyone. They’re well known, but now they are being enforced. This is a big stick and a big step forward.”

Says Dr. Mahmoud: “Cold ischemia time and fixation are the two preanalytical factors that most affect molecular testing. These are the biggies that show up throughout revisions and demand a lot of attention. For instance, the note spells out types and amounts of fixatives, such as 10 percent neutral buffered formalin. Some hospitals collect specimens in nonconformance to that 10 percent, send them for molecular testing, and it never works. It is a disservice to patients who could never have the chance to get that testing done.”

Requiring records on cold ischemia time and fixation generated a lot of discussion, Dr. Goodman says, “because many labs are not able to control or obtain the data pertaining to cold ischemia time and when the specimen was placed in fixative. But the idea is to make sure people acquiring specimens are aware of this. I think for labs to be compliant they’ll have policies and procedures in place on how to handle specimens, educate other physicians, and make sure their OR staff, gastroenterology staff, pulmonology staff, and others are aware of them.”

In the all common checklist, COM.06300 Specimen Rejection Criteria requires labs to define and follow criteria for the rejection or special handling of specimens that do not meet established laboratory criteria for the requested test, and to retain records of these specimens in the patient/client report or quality management records or in both. Eight examples of specimens that do not meet established preanalytic parameters are provided, such as broken slides or specimens submitted beyond their stability time limits.

“Each lab must define its own criteria for rejection based on the kind of tests it does and with knowledge of the types of preanalytical factors that can compromise or preclude getting the right molecular analysis result,” Dr. Compton says. “If a specimen must be rejected, that’s serious. It means the patient will not get an answer.” The people who procure and handle the specimen, including those outside the purview of the pathology department, may be implicated. With feedback from pathology, she says, they will get the message it’s their fault. “This assigns responsibility to everyone in the chain of custody. Colleagues handling specimens in the operating room or clinic suite may need to change their own practices to ensure that the molecular quality of their patients’ specimens is preserved.” To pathologists who want to run tests on good specimens and get the right answers for patients, she says, “the entire upstream process is important.”

This requirement indicates that laboratories have choices to make, Dr. Goodman says. “For example, if I receive a specimen that’s hemolyzed, I cannot measure the potassium in it, but I can measure other analytes like sodium. So I have a choice: reject the entire specimen, analyze for sodium and not potassium, or analyze everything and add a disclaimer noting hemolysis may affect the result.”

Dr. Goodman

In surgical pathology, the choice to analyze or reject is a more difficult one, he says, “because we can’t just take out another gallbladder or breast mass, in the same way lab medicine could request another urine or blood sample.” Even though a specimen may not have been handled perfectly, it still may be good for some analysis, he says. “This requirement establishes that if you decide to analyze a less than optimal specimen, information must be recorded to indicate there may be a problem. We must make sure doctors understand there’s an anomaly.”

The preanalytics team weighed in on the COM.30750 Temperature Checks requirement, noting patient specimens, reagents, and controls may be stored in a frost-free freezer only if protected from thawing and that thermal containers within the freezer can be used. It also says: “Repeated freeze-thaw cycles contribute to biomolecular degradation and are detrimental to biospecimen quality” and that avoiding freeze-thaw altogether by aliquoting specimens before freezing is prudent.

“This is a data-driven recommendation to keep freeze-thaw cycles to a minimum,” Dr. Compton says, adding that data show that freezing and thawing disrupt the molecular integrity of the sample. “If you’ve done this three times, it’s like putting a little grenade into the middle of the molecule and blasting it apart. You’ve destroyed your sample. We want people to aliquot it up front, freeze all of the aliquots, and only thaw each aliquot once.”

In the molecular pathology checklist, MOL. 32365 Specimen Preservation/Storage now says the same about repeated freeze-thaw cycles contributing to degradation and avoiding it altogether. And it says peripheral blood specimens shouldn’t be frozen, unless otherwise validated.

In the cytopathology checklist is a new section on predictive markers, the requirements of which are similar to those in the anatomic pathology checklist. “This section was added,” Dr. Goodman says, “to take into account labs that perform predictive markers on cytopathology specimens. While it’s rare for there to be a cytology lab doing this without an anatomic pathology lab associated with it, this covers such a lab if that is the case.”

In the new section consisting of six requirements, ranging from report elements to cytology slide and block storage, is “nothing revolutionary or unique,” he says. “We just want users of the cytology checklist to be aware of information and language adapted from the ANP checklist. In short, as more testing for predictive markers is done on cytopathology specimens, we want to make sure it is done correctly.”

And with more ancillary testing performed now on cytology specimens, Dr. Mahmoud says, other cytopathology requirements were revised to include language that is more inclusive of specimens other than tissue.

The key takeaway from the checklist additions and revisions, Dr. Goodman says, is that “preanalytic variables affect our results in all areas of pathology. If we don’t have the right raw materials on which to perform our tests, the rest doesn’t make any difference. We need quality specimens to get the right results.”

Dr. Compton says it’s an initiative that changes the standard of care by improving the quality of specimens for patient care and translational research “in one fell swoop.”

“It has never before been required to record what happens to a specimen on its way to analysis in a lab. Now we must document these important preanalytics. Now every specimen will have a history.”

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