NCCCP Site Participation in the Formalin Fixation Project

Adequate tissue fixation is essential not only for preserving cellular morphology and diagnosing cancer, it is also critical for the accurate identification of protein profiles and molecular nucleic acid signatures used to personalize prognosis, prediction, and therapy for patients with cancer. Very little standardization of tissue fixation exists among pathology laboratories in the United States and elsewhere. Although non-formalin fixatives have been used in diagnostic pathology, 10% phosphate-buffered formalin without "proprietary additives" remains the "gold standard" for tissue fixation and diagnostic immunohistochemical (IHC) testing.[1] Studies have also shown that development of RNA-based assays from formalin-fixed, paraffin-embedded tissue is feasible; however, greater attention to tissue handling and processing is essential to improve the quality of biospecimens.[2]

In 2007 the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) published recommendations for HER2/neu testing in breast cancer.[3] This pivotal paper recommended that breast tissue be fixed in formalin for at least 6 hours and no longer than 48 hours. More recently, ASCO and CAP published the complementary Estrogen Receptor/Progesterone Receptor Guideline that updated the total time in formalin to between 6 and 72 hours.[4]

In January 2009 the NCCCP Biospecimens Subcommittee initiated discussions on best practices for the collection, fixation, and processing of biospecimens for IHC and molecular testing. Participation in the formalin fixation project was voluntary. Although not an NCCCP subcontract deliverable, sites agreed to collect this information to establish a baseline for community hospitals' capabilities to follow *NCI Best Practices.* The intent was to establish protocols that would allow pathology laboratories to provide "high-quality" biospecimens for histologic diagnosis, molecular research, and construction of targeted therapies for patients with cancer.

The 2007 ASCO/CAP HER2 guideline and a working draft of the ASCO/CAP ER-PgR guideline served as the foundation for developing and implementing NCCCP's formalin-fixation best practices for the collection and preservation of tissue biospecimens.[4]

Development and Implementation of Formalin Fixation Best Practices

The Biospecimens Subcommittee provided a forum for sharing ideas and strategies for the implementation of the best practices based on the experiences of the participating NCCCP sites, and then allowed for benchmarking progress among the sites. Stakeholders included pathologists, laboratory staff, and pathologist assistants. It was essential to develop cooperation with a wide range of hospital departments and clinicians, including surgeons, medical oncologists, radiation oncologists, interventional radiologists, anesthesiologists, and others. The rationale for NCCCP site participation was that the development of infrastructure at local sites could support the collection of high-quality biospecimens for enhanced patient care.

Formalin fixation time is calculated from the time the biopsied or dissected (from resection) specimen is placed in formalin until the time it is removed from formalin, including the time in formalin during processing. A 6 to 72 hour formalin fixation time was mandated. This required the following:

1. The cooperation of the pathology department and the nursing/OR staff
2. Education on terminology
3. Revision of the pathology specimen requisitions.

A change in tissue preparation workflow (e.g., specimen cut-off times and weekend coverage) was necessary to ensure appropriate fixation times.

To develop the process at NCCCP sites, standard data elements were included in pathology reports, such as "Formalin fixation time is 6 to 72 hours" or "Total time in formalin is _____." The data to calculate total time in formalin include date and time specimen is placed in formalin and date and time specimen is removed from formalin. The first datum point (date and time placed in formalin) is provided by the clinician and/or OR staff or, in some instances, the pathology department if the specimens are received fresh. The second datum point (date and time removed from formalin) is determined by the pathology department. The actual times could be maintained on the specimen requisition or on the report, but were not required on the final reports.

Implementation at some NCCCP sites required a new mindset regarding turnaround times of surgical specimens to accommodate for appropriate fixation times. A few sites had to make weekend staffing changes. The ASCO/CAP guidelines for reporting predictive markers in breast carcinoma were used to educate staff about requirements that made these changes necessary. Several of the sites added templates for reporting the fixation times on the pathology reports to laboratory information systems; other sites developed programs for fixation monitoring. Sites trained pathologists and histology staff on placement of tissue in the appropriate processors with specific programmed times in formalin.

Success was monitored by the reporting of "formalin fixation time" on the pathology report—another requirement of the more recent ASCO/CAP guidelines. This requirement was instituted predominantly for breast carci-

noma cases, with some NCCCP sites planning to include fixation times on all pathology reports.

Pathology assistants monitored requisitions for the appropriate data elements. Histology managers worked closely with the OR staff leadership to ensure success. When documentation was not present, communication by phone or email between the pathology and histology staff and the OR staff ensured timely feedback and correction of any deficiencies.

While implementation of changes to ensure 6 to 72 hour total time in formalin became part of the normal work flow at NCCCP sites, barriers to the process included:

- Lack of understanding of the critical nature of the process by OR staff and OR technicians
- Competing priorities, such as specimens delivered fresh for intraoperative consultation or frozen section
- Tissue processors that may require different start times for standardization of time in formalin
- Commercial anatomic laboratory information systems (LIS) in the community currently do not have searchable fields for formalin fixation times and are not easily customizable for this feature; therefore, additional work was needed for the pathology assistants to dictate times and for the transcriptionists to type the data.

NCCCP sites provided educational tools to support the implementation of standard fixation times. For example, the Biospecimens Subcommittee offered presentations on the scientific significance of fixation time, focusing attention on the molecular process of fixation. The subcommittee also audited NCCCP sites for adherence to best practice fixation times by requesting percentages of specimens fixed within the 6 to 72 hour time interval as a deliverable. Data from early in the process and during implementation allowed sites to benchmark with other community hospitals.

Implementation of the 6 to 72 hour "formalin fixation time" requirement varied greatly among NCCCP sites. Several pilot sites started with policy and procedure development while other sites already had policies in place.

Costs to incorporate these process changes were not measured at any of the NCCCP sites. Associated costs may include education time of staff, reprinting requisitions, staff time to document data elements, and time for pathology assistants to dictate information. A potential cost is modifications to the LIS that would help with time calculations and provide automated recording of data on reports and audits. Implementation of LIS changes may help decrease the staffing costs to provide these data, especially if defined fixation times are required on all specimen types.

Staff place vials in a cryotray.

Lessons Learned and Recommendations

While many pathology laboratories have adopted the ASCO/CAP recommendations for formalin fixation of breast specimens, it is important to note that these are not "mandates." Although the ASCO/CAP recommendation for a minimum of 6 hours of formalin fixation was based on a study by Goldstein and colleagues[5] that looked at estrogen receptor staining in invasive breast carcinoma, an earlier study examining the effect of prolonged formalin fixation on breast biomarkers found that HER2/neu was stable for up to 20 days and ER/PR staining for up to 57 days.[6] Therefore, individual laboratories are free to use alternative fixation guidelines as long as they validate their protocols against the recom-

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mended guidelines. In addition, while the NCCCP focused largely on formalin fixation times, delay to fixation (cold ischemia time) may have a negative impact on the identification of biomarkers. The recent ASCO/CAP ER-PgR guideline recommends that the delay to formalin fixation not exceed one hour and that biospecimens not be stored overnight at 4° C prior to fixation.[7]

Participating NCCCP sites conducted formalin fixation studies in 2009 and 2010. Study results indicated that laboratories in 2009 were able to calculate formalin fixation times in the majority of breast specimens, and 2010 data suggested that formalin fixation documentation was improved on all case types.

Many factors led to success among the different NCCCP sites challenged with maintaining and recording formalin fixation times. Communication, cooperation, and collaboration among multiple service areas, based on the knowledge that there is good scientific rationale for changing practice, proved important. Sites had to determine how to efficiently accomplish the goal of implementation within the context of limited resources (see "Steps to Implement Formalin Fixation Times at an NCCCP Site" at right).

The Biospecimens Subcommittee recommended ongoing educational events for the NCCCP network sites. With increased emphasis on formalin fixation studies, the goal for continued education is to break barriers to practice changes, improve tissue handling procedures, and implement changes in the community hospital setting that will advance molecular research to support genomically informed medicine.

References

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Fixation Time Documentation

Diagnostic pathology laboratories are tasked to keep track of the exact time that a breast biospecimen has been fixed in formalin and the 2010 ASCO/CAP ER-PgR guideline requires that this information be included in the surgical pathology report.

An alternative approach, based on the process set up at an NCCCP-hospital-affiliated laboratory, is recommended for those laboratories that find it difficult to document the exact time of fixation: Once minimum and maximum fixation time guidelines were established by the individual laboratory, a policy was established that ensured all breast biospecimens satisfied the fixation requirements. For example, if a laboratory follows the 2007 ASCO/CAP "6 hour" minimum fixation recommendation, then the combined time of "pre-tissue processor" and "tissue processor" formalin fixation must add up to 6 hours. Therefore, it is necessary for the surgeon, interventional radiologist, and pathology staff to document the FCT (time biospecimen is placed in formalin) on the pathology requisition for all breast biospecimens. Knowing the FCT and when the tissue processor is started, a decision would be made as to whether the biospecimen is set-up that day or held until the next day for processing. Continual surveillance of FCT compliance should occur and feedback be given to those individuals not documenting the FCT for their patient's biospecimen.

While achieving the fixation time goal was a challenge for NCCCP sites with limited resources, all sites felt their accomplishments far outweighed the challenges. Most sites gained compliance with the ASCO/CAP guidelines and expanded the process from breast tissue to all or most tissue types with the knowledge that patients benefit from optimally processed tissue. It has been suggested that as implementation of the *NCI Best Practices* continues to grow, documented FCT may be necessary for other types of cancer that require immunohistochemical and/or molecular studies for diagnosis, prognosis, or research.

Other obstacles were encountered primarily when NCCCP sites had to change long-established processes. Workflow in the histology labs needed adjustment to accommodate for the minimum 6 hour and maximum 72 hour specimen fixation times. Simple changes included training staff to calculate fixation times. A web-based calculator was identified for use. The tool is available online at: *http://www.timeanddate.com/date/timeduration.html*).

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Steps to Implement Formalin Fixation Times at an NCCCP Site

- ✔ Meet with hospital committee (Cancer Care Committee) to initiate working group.
- ✔ Educate working group on significance of initiative.
- ✔ Revise requisition and ordering process to include date/time of removal of specimen(s) on all cases and date/time formalin added.
- ✔ Educate OR staff and other hospital areas that submit biopsies to include time of removal and time formalin added.
- ✔ Educate pathology assistants and pathologist to document time formalin added on cases sent fresh or for frozen sections.
- ✔ Use training tools/signs in OR and outpatient surgery areas and radiology.
- ✔ Determine time out of formalin on all processors in histology department. Develop a chart based on what time tissue is placed in formalin will allow for appropriate time in fixation (6 to 72 hours). Load processors appropriately.
- ✔ Develop canned text to be placed on all reports to indicate fixation time. Example: Pre-analytic factors: Time in 10% phosphate-buffered formalin is between 6 and 72 hours. Pre-analytic factors: Time in 10% phosphate-buffered formalin is greater than 72 hours (74 hours, 10 minutes).
- ✔ Train histology staff to calculate the time with aid of online time and date duration calculators and to indicate which canned text to use.
- ✔ Train transcriptions to enter canned text codes from times/codes as documented by histology staff.
- ✔ Monitor process. Identify locations not providing times appropriately for further education. Surgeons/OR may need to be educated to not leave specimens in OR without formalin until case finished.
- ✔ Develop methods to calculate overall formalin fixation rates.
- ✔ Work with anatomic pathology laboratory information systems to allow for time entries, calculations, and automated documentation.

In the future, hopefully, nationally recognized LIS companies will automatically include solutions to include time to decrease the manual calculations, coding, and transcription.

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