The quantity and quality of tumor specimens are central to successful comprehensive genomic profiling (CGP) using the OmniSeq INSIGHT® test. Control of preanalytic variables and optimal tissue stewardship are critical to achieving accurate and reproducible laboratory testing. As prominent examples, the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) have published guidelines for hormone receptor and HER2 testing in breast cancer, both of which included recommendations for tissue handling. ¹ ⁴

Published guidelines for molecular testing of tumor tissue, in particular next generation sequencing procedures, have lagged because of the newness and dynamic nature of the technology, limited published empirical data, and assay-specific dependence due to heterogeneity of methods and performance characteristics of individual tests. Many variables can impact the ability to obtain reliable results. Based on our experience with CGP, we are providing our best practices that will maximize the likelihood of successful OmniSeq INSIGHT testing and minimize issues with DNA/RNA quality and quantity that can lead to partial or complete test failures.

A. Documentation

**Recommendation:**
Referring facilities should track and record key elements of the preanalytical history (e.g., time to tissue fixation, time in formalin, temperature, fixative, decalcification status and agent, etc.) of each specimen intended for CGP. ³

B. Specimen Acquisition

**Recommendation 1:**
Since tissue acquisition is critical to successful CGP, well-trained staff should be involved in biopsy acquisition, triage and rapid on-site evaluation of tissue adequacy during minimally invasive procedures. ²

**Recommendation 2:**
If possible, based on the clinical situation, facilities should consider obtaining one or two separate core biopsies specifically designated for molecular testing. In addition, small specimens can be divided among multiple blocks rather than a single block, with specific designation of one block for CGP and/or molecular testing. ²

C. Specimen Transport

**Recommendation:**
To avoid errors and delays in tissue processing, implementation of a standard operating procedure for transport of specimens to pathology is recommended. ²
D. Formalin Fixation

Recommendation 1:
Immediate stabilization of specimens at low temperature in quality-controlled, 10% neutral buffered formalin is recommended whenever possible. In situations in which immediate immersion in formalin cannot be achieved, specimens should be submerged in formalin as quickly as possible. In general, the “delay to formalin fixation,” also known as “cold ischemia time,” should be less than one hour, in keeping with the ASCO/CAP guidelines for ER/PR/HER2 testing in breast cancer.1-4

Note: Cold ischemia time (or at minimum, times in excess of 60 minutes) should be recorded in the pathology report.

Recommendation 2:
Many laboratories choose to purchase a commercially available 10% neutral buffered formalin solution that is known to be reliable and of high quality. Because formalin pH is a critical variable in optimization of nucleic acid quality, formalin pH should be measured before use and periodically thereafter. Formalin should be maintained in accordance with CAP requirements for reagent handling. For internally prepared formalin, histology laboratories should pay strict attention to concentration.2,3

Note: Acid decalcification should not be used with specimens intended for OmniSeq INSIGHT testing. However, submitting the soft portion of bony tissue without decalcification is acceptable. EDTA specimens may also be acceptable.

Recommendation 3:
When specimens are placed in formalin prior to sectioning, bivalving specimens at the center should be considered to allow direct contact between the formalin and the tumor.

Recommendation 4:
To improve uniformity and speed of fixation, specimens should be cut as thin as possible (<5 mm).3

Recommendation 5:
The time the tissue is in formalin should be at least six hours and a maximum of 24 hours at room temperature (25 °C) for non-fatty specimens.2,3

Note: Some specimens with high fat content such as skin or breast tumors may require fixation times up to 48 hours. These preceding recommended times are shorter than the 72 hours the ASCO/CAP guidelines allow for ER/PR/HER2 testing.1,4

Recommendation 6:
Specimens should be completely submerged in formalin, with a preferred volume to tissue mass of 10:1 and a minimum ratio of 4:1.3

Recommendation 7:
The fixation time should be recorded in the pathology report, especially for cases in which time in formalin is longer than 24 hours.3

E. Tissue Processing/Paraffin Embedding

Recommendation 1:
Tissue processing laboratories should strictly adhere to standardized protocols for reagent processing, processor function and the use and maintenance of high-quality reagents—particularly in alcohols—during processing of formalin-fixed tissue.3

Note: Laboratories must not “top off” processor chambers with nonstandard solutions. Timer settings should be monitored to ensure total time in formalin does not exceed targets.

Recommendation 2:
Low-melting-point (<60 °C) paraffin wax should be used to ensure adequate deparaffinization.3
F. Specimen Use and Sectioning

Recommendation 1:
Although the OmniSeq INSIGHT test can often achieve results with much smaller specimens, a tumor surface area of at least 5 mm² (with a tumor fraction of 20% or more) is preferred.²

Recommendation 2:
Efficient cutting of 5-µm sections, performed by experienced technicians using specialized microtomes that make use of the whole tissue specimen, is essential. If possible, specimens from the surface portion of an FFPE block should be avoided when selecting samples for OmniSeq INSIGHT testing.² This may decrease the possibility of false positive results, analogous to the edge effect observed in surgical pathology.

Note: Extra sections may be cut at the time of morphologic sectioning to avoid tissue waste.

Recommendation 3:
To avoid carryover contamination, microtome blades should be changed and water baths should be cleared of residual material between cases.

Recommendation 4:
Tissue management protocols, including immunohistochemistry and other ancillary testing, should consider the need for downstream testing to ensure the availability of adequate amounts of material for successful results.²

Recommendation 5:
For large specimens in which there is concern about inadequate formalin fixation in the central portions, sections from the superiorly fixed and less necrotic peripheral zone should be submitted. As a general rule, necrotic tissue should be avoided or minimized to the extent possible.

G. Storage and Transport

Recommendation 1:
Paraffin blocks should be stored in dry and pest-free conditions at room temperature (25 °C).³

Recommendation 2:
Specimen labeling, storage and shipping should be standardized and continuously monitored for efficiency and adherence to protocols.²,³

References

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