Practical Guide To Ordering Molecular Tests (BRAF, EGFR, and KRAS)

Sample quality is a critical factor for obtaining high quality results in molecular testing. To achieve good quality samples, attention must be given at all points of sample processing and selection to features such as adequate fixation, tumor sufficiency, block selection and clinical characteristics of the patient. Thus, it is important that a pathologist be involved at all steps of sample processing and selection with a view to molecular testing. Below is a list of specific specimen and process characteristics necessary for successful molecular testing.

1. Molecular tests for BRAF, EGFR and KRAS are validated from formalin fixed paraffin embedded tissue and cytology cell blocks.

2. The optimal specimen should contain at least 0.5 cm x 0.5 cm of tumor to improve the chances for successful amplification.

3. There should be sufficient tissue in the block to cut 10 x 5 micron sections to improve the chances for successful amplification.

4. Optimal tumor cellularity is 20% of tissue content to prevent false negative from high benign DNA content. Samples with tumor content as low as 5% may be tested. However, the ability to detect low level mutations is reduced.

5. Specimen decalcification or heavy metal fixation (ie. B5 or B+) results in a specimen with unamplifiable DNA or PCR inhibition. Such specimens are generally rejected.

6. Fixation requirements include minimum time from biopsy to formalin fixation and a fixation time of less than 48 hours. Prolonged ischemic time, poor fixation or overfixation results in poor quality DNA.

7. Dual testing of two primary tumors when present is recommended.

8. It is sufficient to test the most representative specimen in cases of primary versus metastasis (ie. the specimen with sufficient tumor content, cellularity and adequate fixation). If both meet these criteria, it is recommended to test metastasis.

9. Testing of the invasive component of colonic adenocarcinoma produces a higher yield than the adenoma component.

10. In lung adenocarcinoma, repeat EGFR testing may be necessary in previously mutation positive cases based on the clinical judgement of the physician to rule out secondary resistant mutations.

11. It is **highly recommended** that a pathologist review the specimen for adequacy and best block selection before sending for testing to prevent cancelation due to specimen inadequacy and for good turnaround time.