The College of American Pathologists offers these templates to assist pathologists in providing clinically useful and relevant information when reporting results of biomarker testing. The College regards the reporting elements in the templates as important elements of the biomarker test report, but the manner in which these elements are reported is at the discretion of each specific pathologist, taking into account clinician preferences, institutional policies, and individual practice.

The College developed these templates as educational tools to assist pathologists in the useful reporting of relevant information. It did not issue them for use in litigation, reimbursement, or other contexts. Nevertheless, the College recognizes that the templates might be used by hospitals, attorneys, payers, and others. The College cautions that use of the templates other than for their intended educational purpose may involve additional considerations that are beyond the scope of this document.

**TEMPLATE FOR REPORTING RESULTS OF BIOMARKER TESTING OF SPECIMENS FROM PATIENTS WITH CARCINOMA OF THE COLON AND RECTUM**

Completion of the template is the responsibility of the laboratory performing the biomarker testing and/or providing the interpretation. When both testing and interpretation are performed elsewhere (eg, a reference laboratory), synoptic reporting of the results by the laboratory submitting the tissue for testing is also encouraged to ensure that all information is included in the patient’s medical record and thus readily available to the treating clinical team.

**BIOMARKER REPORTING TEMPLATE**

**Colon and Rectum**

Select a Single Response Unless Otherwise Indicated.

Note: Use of this template is optional.

+ Data elements preceded by this symbol are not required.

+ RESULTS

+ Immunohistochemistry (IHC) Testing for Mismatch Repair Proteins (select all that apply) (note A)
**IHC Interpretation**

- **No loss of nuclear expression of mismatch repair (MMR) proteins:** low probability of microsatellite instability—high (MSI-H)*

- **Loss of nuclear expression of MLH1 and PMS2:** testing for methylation of the MLH1 promoter and/or mutation of BRAF is indicated (the presence of a BRAF V600E mutation and/or MLH1 methylation suggests that the tumor is sporadic and germline evaluation is probably not indicated; absence of both MLH1 methylation and of BRAF V600E mutation suggests the possibility of Lynch syndrome, and sequencing and/or large deletion/duplication testing of germline MLH1 may be indicated)*

- **Loss of nuclear expression of MSH2 and MSH6:** high probability of Lynch syndrome (sequencing and/or large deletion/duplication testing of germline MSH2 may be indicated)*

- **Loss of nuclear expression of PMS2 only:** high probability of Lynch syndrome (sequencing and/or large deletion/duplication testing of germline PMS2 may be indicated)*

* There are exceptions to the above IHC interpretations. These results should not be considered in isolation, and clinical correlation with genetic counseling is recommended to assess the need for germline testing.

**Loci Testing**

- **Mononucleotide panel (select all that apply)**
  - BAT-25
  - BAT-26
  - NR-21
  - NR-24

- **NCI panel (select all that apply)**
  - BAT-25
  - BAT-26
  - D2S123
  - D5S346
  - D17S250

- **Other (specify):**

**MLH1 Promoter Methylation Analysis** (note B)

- **MLH1 promoter hypermethylation absent**
- **MLH1 promoter hypermethylation present**
- **Cannot be determined (explain):**

**KRAS Mutational Analysis** (note C)

- **No mutation detected (wild-type KRAS allele)**
- **Mutation identified (select all that apply)**
  - Codon 12
  - Codon 13

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<table>
<thead>
<tr>
<th>Codon 13 mutation</th>
<th>Specific codon 13 mutation not stated</th>
<th>Other codon 13 mutation (specify):</th>
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<tr>
<td>Gly13Asp (GGC&gt;GAC)</td>
<td>Gly13Arg (GGC&gt;GCG)</td>
<td>Gly13Cys (GGC&gt;TGC)</td>
</tr>
<tr>
<td>Gly13Ala (GGC&gt;GCA)</td>
<td>Gly13Cys (GGC&gt;GCA)</td>
<td>Gly13Val (GGC&gt;GTC)</td>
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<tr>
<td>Specific codon 13</td>
<td>Other codon 13 mutation (specify):</td>
<td>Cannot be determined (explain):</td>
</tr>
<tr>
<td>Codon 61</td>
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<td></td>
</tr>
<tr>
<td>Gly61Leu (CAA&gt;CTA)</td>
<td>Gly61Val (GCA&gt;GTA)</td>
<td>Gly61Ile (GCA&gt;GTC)</td>
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<tr>
<td>Specific codon 61</td>
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<tr>
<td>Codon 146</td>
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<td></td>
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<tr>
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<td>Ala146Thr (G436A)</td>
<td>Ala146Thr (G436A)</td>
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<tr>
<td>Specific codon 146</td>
<td>Other codon 146 mutation (specify):</td>
<td>Cannot be determined (explain):</td>
</tr>
</tbody>
</table>

**BRAF Mutational Analysis (note B)**

- No mutations detected (wild-type BRAF allele)
- Mutations identified:
  - **BRAF** V600E (c.1799T>A)
  - Other **BRAF** V6000 mutation identified (specify):
  - Cannot be determined (explain):
- Other **BRAF** mutation:
  - Other **BRAF** mutations identified (specify):
  - Cannot be determined (explain):

**PIK3CA Mutational Analysis (note D)**

- No mutations detected (wild-type PIK3CA allele)
- Exon 9 mutation present (specify):
- Exon 20 mutation present (specify):
- Cannot be determined (explain):

**PTEN Expression Analysis (by immunohistochemistry)**

- Positive cytoplasmic and/or nuclear expression
- Negative for cytoplasmic and/or nuclear expression
- Cannot be determined (explain):

**PTEN Mutational Analysis**

- No mutations detected (wild-type PTEN allele)
- Exon 1–9 mutation present (specify):
- Cannot be determined (explain):

**Multiparameter Gene Expression/Protein Expression Assay**

- Specify type:
  - Low risk
  - Moderate risk
  - High risk
  - Recurrence score:

**METHODS**

**Dissection Method(s) (select all that apply) (note F)**

- Laser capture microdissection
  - Specify test name:
- Manual under microscopic observation
  - Specify test name:
- Manual without microscopic observation
  - Specify test name:
- Cored from block

**BRAF Mutational Analysis**

- Mutations Assessed (select all that apply):
  - V600E
  - Other **BRAF** V600 mutation identified (specify):
  - Other (specify):
- Testing Method (select all that apply):
  - Direct (Sanger) sequencing
  - PCR, allele-specific hybridization
  - Real-time PCR
  - Immunohistochemistry for V600E gene product
  - Other (specify):
+ Immunohistochemistry
+ In situ hybridization
+ Direct (Sanger) sequencing
+ Duplication/deletion testing (MLPA)
+ Other (specify): 

**COMMENTS**

Note: Fixative type, time to fixation (cold ischemia time), and time of fixation should be reported if applicable in this template or in the original pathology report.

**EXPLANATORY NOTES**

A: Mismatch Repair Testing: Microsatellite Instability and Immunohistochemistry.—Detection of defective mismatch repair (MMR) in colorectal carcinomas is important for detection of Lynch syndrome (hereditary nonpolyposis colorectal cancer syndrome), which accounts for approximately 2% to 3% of all colorectal carcinomas and has clinical implications for treatment of the affected patient and family members. Microsatellite instability (MSI) testing can be used to cost-effectively screen patients with colorectal cancer for possible Lynch syndrome. Patients with a microsatellite instability–high (MSI-H) phenotype that indicates MMR deficiency in their cancer may have a germline mutation in 1 of several DNA MMR genes (eg, MLH1, MSH2, MSH6, or PMS2) or an altered EPCAM (TACSTD1) gene. After appropriate genetic counseling, patients may want to consider testing to identify the causative heritable abnormality. An MSI-H phenotype is more frequently observed in sporadic colorectal cancer (approximately 15% of cases) due to somatic abnormalities, usually hypermethylation of the MLH1 gene promoter. The specificity of MSI testing can be increased by using it primarily on at-risk populations, such as patients with colorectal cancer who are younger than 50 years, or patients with a strong family history of Lynch-associated tumors (eg, colorectal, endometrial, gastric, or upper urinary tract urothelial carcinoma), but with sacrifice of sensitivity, since a sizeable minority of cases lack these clinical characteristics.

Microsatellite instability testing of tumor DNA is generally performed with at least 5 microsatellite markers, generally mononucleotide or dinucleotide repeat markers. In 1998, a National Institutes of Health consensus panel proposed that laboratories use a 5-marker panel consisting of 3 dinucleotides and 2 mononucleotide repeats for MSI testing. Recent data suggest that dinucleotide repeats may have lower sensitivity and specificity for identifying tumors with an MSI-H phenotype. As a consequence, there has been a move toward including more mononucleotides and fewer dinucleotides in MSI testing panels. Many laboratories now use a commercially available kit for MSI testing with 5 mononucleotide markers.

Microsatellite instability testing is frequently done in conjunction with immunohistochemical (IHC) testing for DNA MMR protein expression (ie, MLH1, MSH2, MSH6, and PMS expression). If DNA MMR IHC has not been performed, this testing should be recommended for any case that shows an MSI-H phenotype, because this information will help identify the gene that is most likely to have a germline mutation (eg, a patient whose tumor shows loss of MSH2 and MSH6 expression, but retention of MLH1 and PMS2 expression, is likely to have an MSH2 germline mutation). If the results of DNA MMR IHC and MSI testing are discordant (eg, MSI-H phenotype with normal IHC findings or abnormal IHC findings with MSS phenotype), then the laboratory should make sure that the same sample was used for MSI and IHC testing and that there was no sample mix-up. However, MSI-H may not occur in colorectal cancers of patients with germline MSH6 mutation. Intact expression of all 4 proteins indicates that MMR enzymes tested are intact but does not entirely exclude Lynch syndrome, as approximately 5% of families may have a missense mutation (especially in MLH1) that can lead to a nonfunctional protein with retained antigenicity. Defects in lesser-known MMR enzymes may also lead to a similar result, but this situation is rare.

Any positive reaction in the nuclei of tumor cells is considered as intact expression (normal), and it is common for intact staining to be somewhat patchy. An interpretation of expression loss in tumor cells should be made only if a positive reaction is seen in internal control cells, such as the nuclei of stromal, inflammatory, or nonneoplastic epithelial cells. Loss of expression of MLH1 may be due to Lynch syndrome or methylation of the MLH1 promoter region (as occurs in sporadic MSI colorectal carcinoma). Genetic testing is ultimately required for this distinction, although a specific BRAF gene mutation (V600E) is present in many sporadic cases, but not familial cancers. Loss of MSH2 expression strongly suggests Lynch syndrome. PMS2 loss is often associated with loss of MLH1 and is only independently meaningful if MLH1 is intact. MSH6 is similarly related to MSH2. One should also keep in mind that nucleolar staining or complete loss of MSH6 staining has been described in colorectal cancer cases with prior radiation or chemotherapy, and a significant reduction of MSH6 staining has been described in a small percentage of colorectal carcinomas with somatic mutations of the coding region microsatellites of the MSH6 gene in MLH1/PMS2-deficient carcinomas.

B: MLH1 Promoter Hypermethylation Analysis and BRAF Mutational Analysis.—Defective MMR in sporadic colorectal cancer is most often due to inactivation of the MLH1 gene promoter by hypermethylation (epigenetic silencing). The V600E mutation of the BRAF gene may be present in up to 70% of tumors with hypermethylation of the MLH1 promoter. In colorectal cancer, this mutation has been associated with a limited clinical response to epidermal growth factor receptor (EGFR)–targeted therapies (cetuximab or panitumumab). Analysis for somatic mutations in the V600E hot spot in BRAF may also be indicated for tumors that show MSI-H, as this mutation has been found in sporadic MSI-H tumors, but not in Lynch-associated cancers with MLH1 or MSH2 mutations. BRAF V600E mutations have been described in probands with monoallelic PMS2 mutations. Direct testing of MLH1 promoter hypermethylation and/or the use of BRAF V600E mutational analysis before germline genetic testing in patients with MSI-H tumors and loss of MLH1 by IHC may be a cost-effective means of identifying patients with sporadic tumors for whom further testing is not indicated.

C: KRAS Mutational Analysis.—The presence of the K-ras gene (KRAS) mutation has been shown to be associated with lack of clinical response to therapies targeted at EGFR, such as cetuximab and panitumumab. While clinical guidelines for KRAS mutational analysis are evolving, current provisional recommendations from the American...
Society of Clinical Oncology are that all patients with stage IV colorectal carcinoma who are candidates for anti-EGFR antibody therapy should have their tumor tested for KRAS mutations. Anti-EGFR antibody therapy is not recommended for patients whose tumors show mutations in KRAS codon 12, 13, or 61, but data on codon 146 are currently insufficient.

**D: PIK3CA Mutational Analysis.**—PIK3CA mutations activate the PI3K-PTEN-AKT pathway that is downstream from both the EGFR and the RAS-RAF-MAPK pathways. PIK3CA mutation and subsequent activation of the AKT pathway have been shown to play an important role in colorectal carcinogenesis and have been associated with KRAS mutation and microsatellite instability. PIK3CA mutation has further been associated with poor survival in resectable stage I to III colon cancer, with the adverse effect of PIK3CA mutation potentially limited to patients with KRAS wild-type tumors. PIK3CA mutations have been associated with resistance to anti-EGFR therapy in several studies, but not in others. The reasons for the discrepancy are not clear. Mutations of exons 1, 9, and 20 of the PIK3CA gene represent >95% of known mutations.

A European consortium recently suggested that only PIK3CA exon 20 mutations are associated with a lack of cetuximab activity in KRAS wild-type tumors and with a shorter median progression-free survival and overall survival. By contrast, exon 9 PIK3CA mutations are associated with KRAS mutations and do not have an independent effect on cetuximab efficacy. More studies are needed to establish the prognostic and predictive roles of PIK3CA exon-9 and exon-20 mutations.

**E: PTEN Mutational Analysis.**—The role of PTEN loss in colorectal cancer progression and therapy is unclear. It has been suggested that loss of PTEN expression, as determined by immunohistochemistry, is associated with lack of benefit from cetuximab in metastatic colorectal cancer. Loss of PTEN has been found to co-occur with KRAS, BRAF, and PIK3CA mutations. The recorded frequency of loss of PTEN expression varies from 19% to 36%, with some studies reporting an effect on response rate and survival, whereas others found an effect only on progression-free or overall survival. Moreover, data on the loss of PTEN expression are not concordant in primary and metastatic tumors. There is currently no standardized method for PTEN expression analysis by immunohistochemistry.

**F: Dissection Method.**—Please denote the manner in which the tissue was dissected and specify the biomarker test only if different dissection methods are used for different tests.

1. Laser capture microdissection: Use of a laser-equipped microscope to isolate and retrieve specific cells of interest from a histopathologic region of interest.
2. Manual under microscopic observation: Hematoxylin-eosin slide is examined under a light microscope and marked by a pathologist for subsequent tumor dissection and retrieval.
3. Manual without microscopic observation: Hematoxylin-eosin slide is examined without a microscope and marked by a pathologist for subsequent tumor dissection and retrieval.
4. Cored from block: Area of interest is cored from a paraffin-embedded tissue block.
5. Whole tissue section: No tumor enrichment procedure used for tissue retrieval.

**References**