COLLEGE of AMERICAN PATHOLOGISTS

Template for Reporting Results of Biomarker Testing of Specimens From Patients With Carcinoma of the Colon and Rectum

Template web posting date: December 2014

Authors
Angela N. Bartley, MD, FCAP
Department of Pathology, St. Joseph Mercy Hospital, Ann Arbor, MI
Stanley R. Hamilton, MD, FCAP
Division of Pathology and Laboratory Medicine, University of Texas MD Anderson Cancer Center, Houston, TX
Randa Alsabeh, MD, FCAP
Beverly Hills, CA
Edward P. Ambinder, MD
Department of Medicine (Medical Oncology and Hematology), Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY
Michael Berman, MD, FCAP
Department of Pathology, Jefferson Regional Medical Center, Jefferson Hills, PA
Elaine Collins, MA, RHIA, CTR
St. Paul, MN
Patrick L. Fitzgibbons, MD, FCAP
Department of Pathology, St. Jude Medical Center, Fullerton, CA
Donna M. Gress, RHIT, CTR
American Joint Committee on Cancer (AJCC), Chicago, IL
Jan A. Nowak, PhD, MD, FCAP
Department of Pathology and Laboratory Medicine, NorthShore University HealthSystem, Evanston, IL
Wade S. Samowitz, MD
Department of Pathology, University of Utah, Salt Lake City, UT
Yousuf Zafar, MD, MHS
Department of Medicine, Division of Medical Oncology, Duke University Medical Center, Durham, NC

For the Members of the Cancer Biomarker Reporting Workgroup, College of American Pathologists

© 2014 College of American Pathologists (CAP). All rights reserved.

The College does not permit reproduction of any substantial portion of these templates without its written authorization. The College hereby authorizes use of these templates by physicians and other health care providers in reporting results of biomarker testing on patient specimens, in teaching, and in carrying out medical research for nonprofit purposes. This authorization does not extend to reproduction or other use of any substantial portion of these templates for commercial purposes without the written consent of the College.

The CAP also authorizes physicians and other health care practitioners to make modified versions of the templates solely for their individual use in reporting results of biomarker testing for individual patients, teaching, and carrying out medical research for non-profit purposes.

The CAP further authorizes the following uses by physicians and other health care practitioners, in reporting on surgical specimens for individual patients, in teaching, and in carrying out medical research for non-profit purposes: (1) **Dictation** from the original or modified templates for the purposes of creating a text-based patient record on paper, or in a word processing document; (2) **Copying** from the original or modified templates into a text-based patient record on paper, or in a word processing document; (3) The use of a **computerized system** for items (1) and (2), provided that the template data is stored intact as a single text-based document, and is not stored as multiple discrete data fields.
Other than uses (1), (2), and (3) above, the CAP does not authorize any use of the templates in electronic medical records systems, pathology informatics systems, cancer registry computer systems, computerized databases, mappings between coding works, or any computerized system without a written license from the CAP.

Any public dissemination of the original or modified templates is prohibited without a written license from the CAP.

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.
The inclusion of a product name or service in a CAP publication should not be construed as an endorsement of such product or service, nor is failure to include the name of a product or service to be construed as disapproval.

CAP Colon and Rectum Biomarker Template Revision History

Version: ColonBiomarkers 1.2.0.1

Summary of Changes
Microsatellite Instability
Changed MSI response from Indeterminate to Cannot be determined

Biomarker Reporting Template

Template web posting date: December 2014

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.

COLON AND RECTUM

Select a single response unless otherwise indicated.

Note: *Use of this template is optional.*

+ RESULTS

+ Immunohistochemistry (IHC) Testing for Mismatch Repair (MMR) Proteins (select all that apply) (Note A)
+ ___ MLH1
  + ___ Intact nuclear expression
  + ___ Loss of nuclear expression
  + ___ Cannot be determined (explain): ______________________
+ ___ MSH2
  + ___ Intact nuclear expression
  + ___ Loss of nuclear expression
  + ___ Cannot be determined (explain): ______________________
+ ___ MSH6
  + ___ Intact nuclear expression
  + ___ Loss of nuclear expression
  + ___ Cannot be determined (explain): ______________________
+ ___ PMS2
  + ___ Intact nuclear expression
  + ___ Loss of nuclear expression
  + ___ Cannot be determined (explain): ______________________
+ ___ Background nonneoplastic tissue/internal control with intact nuclear expression

+ IHC Interpretation
+ ___ No loss of nuclear expression of 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, and, if negative, sequencing and/or large deletion/duplication testing of germline *MSH6* may be indicated)[#]
+ ___ Loss of nuclear expression of MSH6 only: high probability of Lynch syndrome (sequencing and/or large deletion/duplication testing of germline *MSH6* 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)[#]

+ Data elements preceded by this symbol are not required.

 # 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.

+ Microsatellite Instability (MSI) (Note A)
+ ___ MSI – stable (MSS)
+ ___ MSI – low (MSI-L)
  + ___ 1% - 29% of the National Cancer Institute (NCI) or mononucleotide markers exhibit instability
  + ___ 1 of the NCI or mononucleotide markers exhibit instability
  + ___ Other (specify): ________________________
+ ___ MSI – high (MSI-H)
  + ___ ≥30% of the NCI or mononucleotide markers exhibit instability
  + ___ 2 or more of the NCI or mononucleotide markers exhibit instability
  + ___ Other (specify): ________________________
+ ___ MSI – cannot be determined

+ Loci Testing
+ ___ Mononucleotide panel
  + BAT-25
  + ___ Stable
  + ___ Unstable
  + ___ Cannot be determined (explain): ___________________________
  + ___ Not performed
  + BAT-26
  + ___ Stable
  + ___ Unstable
  + ___ Cannot be determined (explain): ___________________________
  + ___ Not performed
  + NR-21
  + ___ Stable
  + ___ Unstable
  + ___ Cannot be determined (explain): ___________________________
  + ___ Not performed
  + NR-24
  + ___ Stable
  + ___ Unstable
  + ___ Cannot be determined (explain): ___________________________
  + ___ Not performed
  + Mono-27
  + ___ Stable
  + ___ Unstable
  + ___ Cannot be determined (explain): ___________________________
  + ___ Not performed
+ ___ NCI panel
  + BAT-25
  + ___ Stable
  + ___ Unstable
  + ___ Cannot be determined (explain): ___________________________
  + ___ Not performed
  + BAT -26
  + ___Stable
  + ___ Unstable
  + ___ Cannot be determined (explain): ___________________________
  + ___ Not performed
  + D2S123
  + ___ Stable
  + ___ Unstable

+ ___ Cannot be determined (explain): ___________________________
+ ___ Not performed
+ D5S346
+ ___ Stable
+ ___ Unstable
+ ___ Cannot be determined (explain): ___________________________
+ ___ Not performed
+ D17S250
+ ___ Stable
+ ___ Unstable
+ ___ Cannot be determined (explain): ___________________________
+ ___ Not performed

+ ___ Other (specify): _____________________________
+ ___ Stable
+ ___ Unstable
+ ___ Cannot be determined (explain): ____________________________

+ *MLH1* Promoter Methylation Analysis (Note B)
+ ___ *MLH1* promoter hypermethylation present
+ ___ *MLH1* promoter hypermethylation absent
+ ___ Cannot be determined (explain): ___________________________

+ *KRAS* Mutational Analysis (Note C)
+ ___ No mutation detected
+ ___ Mutation identified (select all that apply)
+ Codon 12
+ ___ Gly12Asp (GGT>GAT)
+ ___ Gly12Val (GGT>GTT)
+ ___ Gly12Cys (GGT>TGT)
+ ___ Gly12Ser (GGT>AGT)
+ ___ Gly12Ala (GGT>GCT)
+ ___ Gly12 Arg (GGT>CGT)
+ ___ Codon 12 mutation, not otherwise specified
+ ___ Other codon 12 mutation (specify): ___________________
+ Codon 13
+ ___ Gly13Asp (GGC>GAC)
+ ___ Gly13Arg (GGC>CGC)
+ ___ Gly13Cys (GGC>TGC)
+ ___ Gly13Ala (GGC>GCC)
+ ___ Gly13Val (GGC>GTC)
+ ___ Codon 13 mutation, not otherwise specified
+ ___ Other codon 13 mutation (specify): ___________________
+ Codon 61
+ ___ Gln61Leu (CAA>CTA)
+ ___ Gln61His (CAA>CAC)
+ ___ Codon 61 mutation, not otherwise specified
+ ___ Other codon 61 mutation (specify): ___________________
+ Codon 146
+ ___ Ala146Thr (G436A) (GCA>ACA)
+ ___ Codon 146 mutation, not otherwise specified
+ ___ Other codon 146 mutation (specify): ___________________

+ ___ Other codon (specify): _______________________
+ ___ Cannot be determined (explain): ___________________________

+ *NRAS* Mutational Analysis (Note C)

+ ___ No mutation detected
+ ___ Mutation identified (select all that apply)
    + Codon 12
    + ___ Gly12Asp (GGT>GAT)
    + ___ Gly12Val (GGT>GTT)
    + ___ Gly12Cys (GGT>TGT)
    + ___ Gly12Ser (GGT>AGT)
    + ___ Gly12Ala (GGT>GCT)
    + ___ Gly12Arg (GGT>CGT)
    + ___ Codon 12 mutation, not otherwise specified
    + ___ Other codon 12 mutation (specify): ___________________
    + Codon 13
    + ___ Specific codon 13 mutation (specify): _________________
    + ___ Codon 13 mutation, not otherwise specified
    + Codon 61
    + ___ Gln61Lys (CAA>AAA)
    + ___ Gln61Arg (CAA>CGA)
    + ___ Codon 61 mutation, not otherwise specified
    + ___ Other codon 61 mutation (specify): ___________________

    + ___ Other codon (specify): _____________________
+ ___ Cannot be determined (explain): ____________________________

+ *BRAF* Expression (by immunohistochemistry) (Note B)
+ ___ Positive cytoplasmic expression
+ ___ Negative for cytoplasmic expression
+ ___ Cannot be determined (explain): ____________________

+ *BRAF* Mutational Analysis (Note B)
+ ___ No mutations detected
+ ___ *BRAF* V600E (c.1799T>A) mutation
+ ___ Other *BRAF* mutation (specify): _______________
+ ___ Cannot be determined (explain): ____________________________

+ *PIK3CA* Mutational Analysis (Note D)
+ ___ No mutations detected
+ ___ Exon 9 mutation present (specify): __________________
+ ___ Exon 20 mutation present (specify): ____________________
+ ___ Cannot be determined (explain): ____________________________

+ *PTEN* Expression (by immunohistochemistry) (Note E)
+ ___ Positive cytoplasmic and/or nuclear expression
+ ___ Negative for cytoplasmic and nuclear expression
+ ___ Cannot be determined (explain): ____________________

+ *PTEN* Mutational Analysis
+ ___ No mutation detected
+ ___ Exon 1-9 mutation present (specify): __________________
+ ___ Cannot be determined (explain): ____________________________

+ **Multiparameter Gene Expression/Protein Expression Assay**
+ Specify type: _______________________________________
+ Results:
+ ___ Low risk
+ ___ Moderate risk
+ ___ High risk

+ Data elements preceded by this symbol are not required.

+ 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
    + Specify test name[#]: ______________________________
+ ___ Whole tissue section (no tumor enrichment procedure employed)
    + Specify test name[#]: ______________________________

[#] *If more than 1 dissection method used, please specify which test was associated with each selected dissection method.*

+ Microsatellite Instability (MSI)
+ Number of MSI markers tested (specify): ________

+ Cellularity
+ Percent tumor cells present in specimen: ______%

+ Whole Genome or Exome Sequencing
+ ___ Whole genome sequencing (specify): ____________________________
+ ___ Whole exome sequencing (specify): ____________________________

+ *MLH1* Promoter Methylation

+ Testing Method
+ ___ Methylation-specific real-time polymerase chain reaction (PCR)
+ ___ Other (specify): ___________________________

+ *KRAS* Mutational Analysis

+ Codons Assessed (select all that apply)
+ ___ 12
+ ___ 13
+ ___ 61
+ ___ 146

+ Testing Method(s) (select all that apply)
+ ___ Direct Sanger sequencing
+ ___ Pyrosequencing
+ ___ High-resolution melting analysis
+ ___ PCR, allele-specific hybridization
+ ___ Real-time PCR
+ ___ Other (specify): ___________________________

[#] *Please specify in Comments section if different testing methods are used for different codons.*

+ *NRAS* Mutational Analysis

+ Codons Assessed (select all that apply)
+ ___ 12
+ ___ 13

+ ___ 61

+ Testing Method(s) (select all that apply)
+ ___ Direct Sanger sequencing
+ ___ Pyrosequencing
+ ___ High-resolution melting analysis
+ ___ PCR, allele-specific hybridization
+ ___ Real-time PCR
+ ___ Other (specify): ___________________________

Please specify in Comments section if different testing methods are used for different codons.

+ *BRAF* Mutational Analysis

+ Mutations Assessed (select all that apply)
+ ___ V600E
+ ___ Other *BRAF* V600 mutation (specify): ______________________________
+ ___ Other (specify): _____________________________

+ Testing Method(s) (select all that apply)
+ ___ Direct Sanger sequencing
+ ___ PCR, allele-specific hybridization
+ ___ Pyrosequencing
+ ___ Real-time PCR
+ ___ Immunohistochemistry for V600E gene product
+ ___ Other (specify): ____________________________

+ *PIK3CA* Mutational Analysis

+ Testing Method(s)
+ ___ Direct Sanger sequencing
+ ___ Other (specify): ________________________

+ *PTEN* Expression and Mutational Analysis

+ Testing Method(s) (select all that apply)
+ ___ Immunohistochemistry (specify antibody): ________________________
+ ___ In situ hybridization (specify probe): ________________________
+ ___ Direct Sanger sequencing
+ ___ Duplication/deletion testing (MLPA)
+ ___ Other (specify): ________________________

+ COMMENT(S)

____
____

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.

Gene names should follow recommendations of The Human Genome Organisation (HUGO) Nomenclature Committee (www.genenames.org; accessed February 10, 2015).

All reported gene sequence variations should be identified following the recommendations of the Human Genome Variation Society (www.hgvs.org/mutnomen/; accessed February 10, 2015).

Explanatory Notes

A. Mismatch Repair Testing: Microsatellite instability and Immunohistochemistry
Detection of defective mismatch repair in colorectal carcinomas is important for detection of Lynch syndrome (hereditary nonpolyposis colorectal cancer syndrome [HNPCC]), 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 colorectal cancer patients for possible Lynch syndrome. Patients with a microsatellite instability-high (MSI-H) phenotype that indicates mismatch repair deficiency in their cancer may have a germline mutation in one of several DNA mismatch repair (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 (about 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 colorectal cancer patients 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),[1] but with sacrifice of sensitivity, since a sizeable minority of cases lacks these clinical characteristics.

MSI 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 dinucleotide and 2 mononucleotide repeats for MSI testing.[2] Recent data suggests 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 towards including more mononucleotides and fewer dinucleotides in MSI testing panels. Many laboratories now use a commercially available kit for MSI testing that utilizes 5 mononucleotide markers.

MSI 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 or abnormal IHC 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,[3,4] 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.[5]

B. *MLH1* Promoter Hypermethylation Analysis and *BRAF* Mutational Analysis
Defective mismatch repair 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.[6] *BRAF* V600E mutations have been described in probands with monoallelic *PMS2* mutations.[7] Direct testing of *MLH1* promoter hypermethylation and/or the use of *BRAF* V600E mutational analysis prior to 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.[8]

C. *RAS* Mutational Analysis

The presence of a *KRAS* mutation has been shown to be associated with lack of clinical response to therapies targeted at *EGFR*, such as cetuximab[9] and panitumumab.[10] 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.[11] 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. A recent study has shown that *NRAS* mutation, like *KRAS* mutation, has influence on response to anti-EGFR therapy.[12] Although more studies are needed, these findings may lead to broad *KRAS* and *NRAS* panels to include codons 12, 13, 61, and 146 of both genes.

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 has been shown to play an important role in colorectal carcinogenesis and have been associated with *KRAS* mutation[13] and microsatellite instability.[14] *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.[15] *PIK3CA* mutations have been associated with resistance to anti-EGFR therapy in several studies,[16,17] but not in others.[18] 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.[17] By contrast, exon 9 *PIK3CA* mutations are associated with *KRAS* mutations and do not have an independent effect on cetuximab efficacy.[17] 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 prognosis 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.[19-22] Loss of *PTEN* has been found to co-occur with *KRAS*, *BRAF*, and *PIK3CA* mutations.[19,22] 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 tissues.[21] 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 (LCM): 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 and eosin (H&E) slide is examined under a light microscope and marked by a pathologist for subsequent tumor dissection and retrieval.

3. Manual without microscopic observation: H&E 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 employed for tissue retrieval.

References

1. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst.* 2004;96(4):261-268.
2. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res.* 1998;58(22):5248-5257.
3. Bellizzi AM, Crowder CD, Marsh WL, Hampel H, Frankel WL. Mismatch repair status in a cohort of rectal adenocarcinomas before and after chemoradiation. *Mod Pathol.* 2010;23:137A.
4. Radu OM, Nikiforova MN, Farkas LM, Krasinskas AM. Challenging cases encountered in colorectal cancer screening for Lynch syndrome reveal novel findings: nucleolar MSH6 staining and impact of prior chemoradiation therapy. *Hum Pathol.* 2011;42(9):1247-1258.
5. Shia J, Zhang L, Shike M, et al. Secondary mutation in a coding mononucleotide tract in MSH6 causes loss of immunoexpression of MSH6 in colorectal carcinomas with MLH1/PMS2 deficiency. *Mod Pathol.* 2013;26(1):131-138.
6. Domingo E, Niessen RC, Oliveira C, et al. BRAF-V600E is not involved in the colorectal tumorigenesis of HNPCC in patients with functional MLH1 and MSH2 genes. *Oncogene.* 2005;24(24):3995-3998.
7. Senter, L, Clendenning, M, Sotamaa, K, et al. The clinical phenotype of Lynch syndrome due to germline *PMS2* mutations. *Gastroenterology.* 2008;135(2):419-428.
8. Bessa X, Balleste B, Andreu M, et al. A prospective, multicenter, population-based study of BRAF mutational analysis for Lynch syndrome screening. *Clin Gastroenterol Hepatol.* 2008;6(2):206-214.
9. Lievre A, Bachet J-B, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res.* 2006;66(8):3992-3995.
10. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol.* 2008;26(10):1626-1634.
11. Allegra CJ, Jessup JM, Somerfield MR, et al. American Society of Clinical Oncology Provisional Clinical Opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. *J Clin Oncol.* 2009;27(12):2091-2096.
12. Douillard JY, Oliner K, Siena S, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med.* 2013;369(11):1023–1034.
13. Nosho K, Kawasaki T, Ohnishi M, et al. PIK3CA mutation in colorectal cancer: relationship with genetic and epigenetic alterations. *Neoplasia.* 2008;10(6):534-541.
14. Abubaker J, Bavi P, Al-Harbi S, et al. Clinicopathological analysis of colorectal cancers with PIK3CA mutations in Middle Eastern population. *Oncogene.* 2008;27(25):3539-3545.
15. Ogino S, Nosho K, Kirkner GJ, et al. PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer. *J Clin Oncol.* 2009;27(9):1477-1484.
16. De Roock, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol.* 2010;11(8):753-762.
17. De Roock, De Vriendt V, Normanno N, Ciardiello F, Tejpar S. KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. *Lancet Oncol.* 2011;12(6):594-603.
18. Prenen H, De Schutter J, Jacobs B, et al. PIK3CA mutations are not a major determinant of resistance to the epidermal growth factor receptor inhibitor cetuximab in metastatic colorectal cancer. *Clin Cancer Res.* 2009;15(9):3184-3188.
19. Laurent-Puig P, Cayre A, Manceau G, et al. Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. *J Clin Oncol.* 2009;27(35):5924-5930.
20. Frattini M, Saletti P, Romagnani E, et al. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. *Br J Cancer.* 2007;97(8):1139-1145.

21. Loupakis F, Pollina L, Stasi I, et al. PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer. *J Clin Oncol*. 2009; 27(16):2622-2629.
22. Sartore-Bianchi A, Di Nicolantonio F, Nichelatti M, et al. Multi-determinants analysis of molecular alterations for predicting clinical benefit to EGFR-targeted monoclonal antibodies in colorectal cancer. *PLoS One*. 2009;4(10):e7287.