COLLEGE of AMERICAN PATHOLOGISTS

Template for Reporting Results of Biomarker Testing of Specimens From Patients With Carcinoma of the Endometrium

Version: Endometrium Biomarkers 1.2.0.0 **Template Posting Date:** August 2019

This biomarker template is not required for accreditation purposes.

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Summary of Changes

V1.3.0.0
Added HER2 Reporting
Changed IHC Interpretation to include “for Mismatch Repair”

Biomarker Reporting Template

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.

Template posting date: August 2019

Note: *Use of this template is* ***NOT*** *required for accreditation purposes.*

Select a single response unless otherwise indicated.

RESULTS

Estrogen Receptor (ER) Status (Note A)

___ Positive
    Percentage of cells with nuclear positivity: ___ %
___ Negative
    ___ Internal control cells present and stain as expected
    ___ Internal control cells absent[#]
    ___ Other (specify): _____
___ Equivocal[##]
    ___ Internal control cells present; no immunoreactivity of either tumor cells or internal controls
    ___ Other (specify): _____

[#] *When a tumor is negative but no internal control cells are present, the pathologist must exercise judgment as to whether the assay can be interpreted as a true negative. This should include consideration of histologic type and grade, cold ischemia and fixation times, and the status of external controls, as well as if testing is performed on archived (weeks) unstained tissue sections. If the pathologist decides that hormone receptor status cannot be determined, the test should be reported as such and repeated on another block or specimen.*

[##] *Technical issues prevent the test from being reported as positive or negative. This may occur if specimen handling was inadequate, if artifacts (crush or edge artifacts) make interpretation difficult, or if the analytic testing failed.*

Progesterone Receptor (PgR) Status (Note A)

___ Positive
    Percentage of cells with nuclear positivity: _____ %
___ Negative
    ___ Internal control cells present and stain as expected
    ___ Internal control cells absent[#]
    ___ Other (specify): _____
___ Equivocal[##]
    ___ Internal control cells present; no immunoreactivity of either tumor cells or internal controls
    ___ Other (specify): _____

[#] *When a tumor is negative but no internal control cells are present, the pathologist must exercise judgment as to whether the assay can be interpreted as a true negative. This should include consideration of histologic type and grade, cold ischemia and fixation times, and the status of external controls, as well as if testing is performed on archived (weeks) unstained tissue sections. If the pathologist decides that hormone receptor status cannot be determined, the test should be reported as such and repeated on another block or specimen.*

[##] *Technical issues prevent the test from being reported as positive or negative. This may occur if specimen handling was inadequate, if artifacts (crush or edge artifacts) make interpretation difficult, or if the analytic testing failed.*

HER2 by Immunohistochemistry (Note B)
___ Negative (score 0)
___ Negative (score 1+)
___ Equivocal (score 2+)
Percentage of cells with uniform intense complete membrane staining: ____ %
___ Positive (score 3+)
Percentage of cells with uniform intense complete membrane staining: ____ %
___ Cannot be determined (indeterminate) (explain): _____

***HER2* by in situ Hybridization**
___ Negative (not amplified)
___ Positive (amplified)
___ Cannot be determined (indeterminate) (explain): _____

Number of invasive cancer cells counted: _____

___ **Dual probe assay**
HER2 :CEP17 ratio: _____
Average number of *HER2* signals per cell: _____
Average number of CEP17 signals per cell: _____

___ **Single probe assay**
Average number of *HER2* signals per cell: _____

Heterogeneous signals
___ Not identified
___ Present
Percentage of cells with amplified HER2 signals: _____ %

Immunohistochemistry (IHC) Testing for Mismatch Repair (MMR) Proteins (select all that apply) (Note C)
___ **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 for Mismatch Repair (MMR) Proteins

___ 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 is indicated (the presence of *MLH1* methylation suggests that the tumor is sporadic and germline evaluation is probably not indicated; absence of *MLH1* methylation suggests the possibility of Lynch syndrome, and sequencing and/or large deletion/duplication testing of germline *MLH1* is indicated)[#]

___ Loss of nuclear expression of MSH2 and MSH6: high probability of Lynch syndrome (sequencing and/or large deletion/duplication testing of germline *MSH2* is indicated, and, if negative, sequencing and/or large deletion/duplication testing of germline *MSH6* is indicated. If both are negative, sequencing and/or large deletion/duplication testing of germline *EPCAM* is indicated.)[#]

___ Loss of nuclear expression of MSH6 only: high probability of Lynch syndrome (sequencing and/or large deletion/duplication testing of germline *MSH6* is indicated)[#]

___ Loss of nuclear expression of PMS2 only: high probability of Lynch syndrome (sequencing and/or large deletion/duplication testing of germline *PMS2* is 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.*

Microsatellite Instability (MSI) (Note D)

___ 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 – Equivocal

Percentage of tumor cells present in specimen: _____%

***MLH1* Promoter Methylation Analysis (Note E)**

___ *MLH1* promoter methylation present

___ *MLH1* promoter methylation absent

___ Cannot be determined (explain): _____

p53 Expression (Note F)

___ Normal expression

___ Abnormal strong diffuse overexpression (>90%)

___ Abnormal null expression (complete loss of expression)

___ Cannot be determined (explain): _____

METHODS

Dissection Method(s) (select all that apply) (Note G)
___ 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.*

Estrogen Receptor Primary Antibody
___ SP1
___ 6F11
___ 1D5
___ Other (specify): _____

Progesterone Receptor Primary Antibody
___ 1E2
___ 636
___ 16
___ SP2
___ 1A6
___ 1294
___ 312
___ Other (specify): _____

HER2 by Immunohistochemistry Method
___ Food and Drug Administration (FDA) cleared (specify test / vendor): _____
___ Laboratory-developed test

HER2 Primary Antibody
___ 4B5
___ HercepTest
___ A0485
___ SP3
___ CB11
___ Other (specify): _____

HER2 by in situ Hybridization Method
___ Food and Drug Administration (FDA) cleared (specify test / vendor): _____
___ Laboratory-developed test

Number of Microsatellite Instability (MSI) markers tested (specify): _____

***MLH1* Promoter Methylation Method**
___ Methylation-specific real-time polymerase chain reaction (PCR)
___ Other (specify): _____

p53 Primary Antibody
___ DO-1
___ Other (specify): _____

Gene names should follow recommendations of The Human Genome Organisation (HUGO) Nomenclature Committee (www.genenames.org; accessed June 26, 2019).

All reported gene sequence variations should be identified following the recommendations of the Human Genome Variation Society (http://varnomen.hgvs.org; accessed June 26, 2019).

Explanatory Notes

A. ER and PgR Immunohistochemistry

Hormone receptor expression is occasionally assessed on primary invasive endometrial carcinomas at the request of the treating clinician in order to predict response to endocrine therapy. Guidelines for reporting results of hormone receptor testing in breast carcinomas published by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) require recording specific preanalytic and analytic variables that can affect test results.[1] Such information has not been required for endometrial carcinomas. However, details regarding assay validation or verification should be available in the laboratory. Any deviation(s) from the laboratory's validated methods should be recorded. Appropriate positive and negative controls should be used and evaluated.

Hormone receptor status is typically performed in formalin-fixed, paraffin-embedded tissue sections by immunohistochemistry (IHC). Only nuclear staining is considered positive. There are many tissue and technical variables that can affect test results, and the assays must be validated to ensure their accuracy. External proficiency testing surveys for estrogen receptor (ER) and progesterone receptor (PgR) for breast cancer are available from the CAP and other organizations and may be useful tools to help ensure that assays perform as expected. To avoid false-negative results, appropriate internal and external controls should be positive. In the endometrium, benign endometrial glands, endometrial stroma, or myometrium can serve as internal control tissue. If internal controls are not present, consider repeating the test on another specimen (if available). Reasons for false-negative results include the following:

- Exposure of tumor cells to heat (eg, carcinomas transected by using cautery during surgery)
- Prolonged cold ischemic time, which may result in antigenic degradation. One hour or less is preferable
- Under- or overfixation; fixation for at least 6 hours in buffered formalin is recommended, and prolonged fixation can also diminish immunoreactivity
- Type of fixative: ER is degraded in acidic fixatives such as Bouin's and B-5; formalin should be buffered to ensure pH range between 7.0 and 7.4
- Decalcification, which may result in loss of immunoreactivity
- Non-optimized antigen retrieval or use of old (weeks) tissue sections
- Type of antibody
- Dark hematoxylin counterstain obscuring faintly positive diaminobenzidine (DAB) staining

False-positive results occur less frequently. Rare reasons would be the use of an impure antibody that cross-reacts with another antigen or misinterpretation of entrapped normal or hyperplastic cells as invasive carcinoma. False-positive tests can also be generated by image analysis devices that mistakenly count overstained nuclei.

Reporting Guidelines

There are currently no outcome-driven consensus opinions that have been developed for the reporting of the results of immunohistochemical assays for ER and PgR for endometrial cancer. In absence of robust data, the CAP recommends using a similar reporting format that is used for reporting the results of immunohistochemical assays for ER and PgR for breast cancer (Table 1).[2]

As there is a wide range of receptor levels in individual cancers, a uniform reporting scheme using the proportion of positive cells as well as the intensity of immunoreactivity is recommended:

- Number of positive cells: The number of positive cells can be reported as a percentage or within discrete categories.
- Intensity: Refers to degree of nuclear positivity (ie, pale to dark). The intensity can be affected by the amount of protein present, as well as the antibody used and the antigen retrieval system. In most cancers, there is heterogeneous immunoreactivity with pale to darkly positive cells present.

Table 1.
Reporting Results of Estrogen Receptor (ER) and Progesterone Receptor (PgR) Testing

Result	Criteria	Comments
Positive	Immunoreactive tumor cells present (≥1%)	The percentage of immunoreactive cells may be determined by visual estimation or quantitation. Quantitation should be provided by reporting the percentage of positive cells in the entire section. If there is significant regional variation, that too should be reported.
Negative	<1% immunoreactive tumor cells present	

References
1. Hammond EH, Hayes D, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *Arch Pathol Lab Med.* 2010;134(6):907-922.
2. Fitzgibbons PL, Dillon DA, Alsabeh R, et al. Template for reporting results of biomarker testing of specimens from patients with carcinoma of the breast. *Arch Pathol Lab Med.* 2014;138(5):595-601.

B. HER2 (*ERBB2*) Testing

Scientific rationale: A subset of breast carcinomas (approximately 15% to 20%) overexpress human epidermal growth factor receptor 2 (HER2; HUGO nomenclature *ERBB2*). Protein overexpression is usually due to gene amplification. Assays for gene copy number, mRNA quantity, and protein generally give similar results; gene amplification correlates with protein overexpression in about 95% of cases. In a small subset of carcinomas (probably <5%), protein overexpression may occur by different mechanisms. Overexpression is both a prognostic and predictive factor.

Clinical rationale: HER2 status is primarily evaluated to determine patient eligibility for anti-HER2 therapy. It may identify patients who have a greater benefit from anthracycline-based adjuvant therapy.

Methods: HER2 status can be determined in formalin-fixed paraffin-embedded tissue by assessing protein expression on the membrane of tumor cells using IHC or by assessing the number of HER2 gene copies using in situ hybridization (ISH). When both IHC and ISH are performed on the same tumor, the results should be correlated. The most likely reason for a discrepancy is that 1 of the assays is incorrect, but in a small number of cases there may be protein overexpression without amplification, amplification without protein overexpression, or marked intratumoral heterogeneity.

HER2 (ERBB2) Testing by Immunohistochemistry

Factors altering the detection of HER2 (ERBB2) by IHC have not been studied as well as for ER and PgR. It is recommended that tissue be fixed in buffered 10% formalin for at least 6 hours unless another fixative has been validated. External proficiency testing surveys for HER2 are available from the CAP and other organizations. These surveys are invaluable tools to ensure that the laboratory assays are working as expected.

False-positive IHC results for HER2 may be due to:
- Edge artifact. This is usually seen in core biopsies, where cells near the edges of the tissue stain stronger than in the center, possibly because antibody pools at the sides. Specimens with stronger staining at the edge of the tissue should be interpreted with caution.
- Cytoplasmic positivity, which can obscure membrane staining and make interpretation difficult.
- Overstaining (strong membrane staining of normal cells). May be due to improper antibody titration (concentration too high).
- Misinterpretation of ductal carcinoma in situ (DCIS). High-grade DCIS is often HER2 positive. In cases with extensive DCIS relative to invasive carcinoma (particularly microinvasive carcinoma),

HER2 scoring may mistakenly be done on the DCIS component. Care must be taken to score only the invasive component.

False-negative IHC results for HER2 may be due to:
- Prolonged cold ischemia time.
- Tumor heterogeneity. When a negative result is found, but only a small biopsy sample was tested, repeat testing on a subsequent specimen with a larger area of carcinoma should be considered, particularly if the tumor has characteristics associated with HER2 positivity (ie, tumor grade 2 or 3, weak or negative PgR expression, increased proliferation index).
- Improper antibody titration (concentration too low)

False-negative and false-positive results can be reduced by paying attention to the following:
- Tissue controls. External controls must stain as expected. There are no normal internal controls for HER2 protein assessment by IHC.
- Correlation with histologic and other biomarker results. If the HER2 test is negative by IHC, but the tumor has characteristics associated with HER2 positivity (see above), repeating the test by ISH should be considered.

Reporting guidelines: ASCO and CAP have issued recommendations for reporting the results of HER2 testing by IHC (Table 4).[1]

Table 4. Reporting Results of HER2 Testing by Immunohistochemistry (IHC)

Result	Criteria
Negative (Score 0)	No staining observed *or* Membrane stating that is incomplete and is faint/barely perceptible and within ≤10% of tumor cells
Negative (Score 1+)	Incomplete membrane staining that is faint/barely perceptible and within >10% of tumor cells*
Equivocal (Score 2+)†	Weak to moderate complete membrane staining in >10% of tumor cells *or* Circumferential membrane staining that is intense but within ≤10% of tumor cells*
Positive (Score 3+)	Circumferential membrane staining that is complete and >10% of tumor cells*

* Readily appreciated using a low-power objective and observed within a homogeneous and contiguous population of invasive tumor cells.

† Must order reflex test (same specimen using ISH) or order a new test (new specimen if available, using IHC or ISH).

HER2 Testing by In Situ Hybridization

Fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH), and silver-enhanced in situ hybridization (SISH) studies for *HER2* determine the presence or absence of gene amplification. Some assays use a single probe to determine the number of HER2 gene copies present, but most assays include a chromosome enumeration probe (CEP17) to determine the ratio of HER2 signals to copies of chromosome 17. Although 10% to 50% of breast carcinomas have more than 2 CEP17 copies, only 1% to 2% of carcinomas show true polysomy (ie, duplication of the entire chromosome).

Failure to obtain results with ISH may be due to the following:
- Prolonged fixation in formalin (>1 week)[2]
- Fixation in non-formalin fixatives[3]
- Procedures or fixation involving acid (eg, decalcification) may degrade DNA[4]
- Insufficient protease treatment of tissue

External proficiency testing surveys for HER2 by ISH are available from CAP and other organizations. These surveys are invaluable tools to ensure that the laboratory assays are working as expected.

Reporting guidelines: ASCO and CAP have issued recommendations for reporting the results of HER2 testing by ISH (Tables 5 and 6).[1]

Dual Probe ISH Group Definitions:
Group 1 = HER2/CEP17 ratio ≥2.0; ≥4.0 HER2 signals/cell
Group 2 = HER2/CEP17 ratio ≥2.0; <4.0 HER2 signals/cell
Group 3 = HER2/CEP17 ratio <2.0; ≥6.0 HER2 signals/cell
Group 4 = HER2/CEP17 ratio <2.0; ≥4.0 and <6.0 HER2 signals/cell
Group 5 = HER2/CEP17 ratio <2.0; <4.0 HER2 signals/cell

Table 5. Reporting Results of *HER2* Testing by In Situ Hybridization (single-probe assay)

Result	Criteria (single-probe assay)
Negative	• Average HER2 copy number <4.0 signals/cell • Average HER2 copy number ≥4.0 and <6.0 signals/cell and concurrent IHC 0, 1+ or 2+ • Average HER2 copy number ≥4.0 and <6.0 signals/cell and concurrent dual probe ISH Group 5
Positive	• Average HER2 copy number ≥6.0 signals/cell • Average HER2 copy number ≥4.0 and <6.0 signals/cell and concurrent IHC 3+ • Average HER2 copy number ≥4.0 and <6.0 signals/cell and concurrent dual probe ISH Group 1

Table 6. Reporting Results of *HER2* Testing by In Situ Hybridization (dual-probe assay)

Result	Criteria (dual-probe assay)
Negative	• Group 5
Negative* (see comment)	• Group 2 and concurrent IHC 0-1+ or 2+ • Group 3 and concurrent IHC 0-1+ • Group 4 and concurrent IHC 0-1+ or 2+
Positive*	• Group 2 and concurrent IHC 3+ • Group 3 and concurrent IHC 2+ or 3+ • Group 4 and concurrent IHC 3+
Positive	• Group 1

*For Groups 2-4 final ISH results are based on concurrent review of IHC, with recounting of the ISH test by a second reviewer if IHC is 2+ (per 2018 CAP/ASCO Update recommendations).

Comment for Group 2 Negative result: *Evidence is limited on the efficacy of HER2-targeted therapy in the small subset of cases with HER2/CEP17 ratio ≥2.0 and an average HER2 copy number <4.0/cell. In the first generation of adjuvant trastuzumab trials, patients in this subgroup who were randomized to the trastuzumab arm did not appear to derive an improvement in disease free or overall survival, but there were too few such cases to draw definitive conclusions. IHC expression for HER2 should be used to complement ISH and define HER2 status. If IHC result is not 3+ positive, it is recommended that the specimen be considered HER2 negative because of the low HER2 copy number by ISH and lack of protein overexpression.*

Comment for Group 3 Negative result: *There are insufficient data on the efficacy of HER2-targeted therapy in cases with HER2 ratio <2.0 in the absence of protein overexpression because such patients were not eligible for the first generation of adjuvant trastuzumab clinical trials. When concurrent IHC results are negative (0-1+), it is recommended that the specimen be considered HER2 negative.*

Comment for Group 4 Negative result: *It is uncertain whether patients with ≥4.0 and <6.0 average HER2 signals/cell and HER2/CEP17 ratio <2.0 benefit from HER2 targeted therapy in the absence of protein overexpression (IHC 3+). If the specimen test result is close to the ISH ratio threshold for positive, there is a high likelihood that repeat testing will result in different results by chance alone. Therefore, when IHC results are not 3+ positive, it is recommended that the sample be considered HER2 negative without additional testing on the same specimen.*

Important issues in interpreting ISH are the following:

- Identification of invasive carcinoma: A pathologist should identify on the hematoxylin and eosin (H&E) or HER2 IHC slide the area of invasive carcinoma to be evaluated by ISH.
- Identification of associated DCIS: In some cases, DCIS will show gene amplification, whereas the associated invasive carcinoma will not. ISH analysis must be performed on the invasive carcinoma.

Some cancers have a low level of HER2 expression as determined by equivocal results by both IHC and ISH analysis. Repeat testing may be helpful to exclude possible technical problems with the assays but often does not result in definitive positive or negative results.

Either the number of *HER2* genes or the ratio of *HER2* to *CEP17* can be used to determine the presence of amplification. In the majority of carcinomas, both methods give the same result. In unusual cases, the two methods give different results, usually due to variation in the number of *CEP17* signals. Some studies have shown that chromosome 17 abnormalities can lead to alterations of the *HER2/CEP17* ratio, potentially leading to equivocal or incorrect ISH results.[5] In such cases, gene copy number may be a more accurate reflection of HER2 status. If there is a second contiguous population of cells with increased HER2 signals/cell, and this cell population consists of more than 10% of tumor cells on the slide (defined by image analysis or by visual estimation of the ISH or IHC slide), a separate counting of at least 20 non-overlapping cells must also be done within this cell population and also reported. An overall random count is not appropriate in this situation.

References

1. Wolff AC, Hammond MEH, Allision KH, et al. HER2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. In press
2. Selvarajan S, Bay B-H, Choo A, et al. Effect of fixation period on HER2/neu gene amplification detected by fluorescence in situ hybridization in invasive breast carcinoma. *J Histochem Cytochem.* 2002;50(12):1693-1696.
3. Willmore-Payne C, Metzger K, Layfield LJ. Effects of fixative and fixation protocols on assessment of Her-2/neu oncogene amplification status by fluorescence in situ hybridization. *Appl Immunohistochem Mol Morphol.* 2007;15(1):84-87.
4. Brown RS, Edwards J, Bartlett JW, Jones C, Dogan A. Routine acid decalcification of bone marrow samples can preserve DNA for FISH and CGH studies in metastatic prostate cancer. *J Histochem Cytochem.* 2002;50(1):113-115.
5. Gunn S, Yeh IT, Lytvak I, et al. Clinical array-based karyotyping of breast cancer with equivocal HER2 status resolves gene copy number and reveals chromosome 17 complexity. BMC Cancer. 2010;10:396.

C. Mismatch Repair Immunohistochemistry Testing

Immunohistochemical (IHC) testing for DNA MMR protein expression (ie, MLH1, MSH2, MSH6, and PMS2 expression) is performed on formalin-fixed, paraffin-embedded tissue. Loss of DNA MMR protein expression is likely to be due to mutation (either genetic or somatic) in one of the mismatch repair genes.[1-3] This information will help identify the gene that is most likely to have a mutation (eg, a patient whose tumor shows loss of MSH2 and MSH6 expression, but retention of MLH1 and PMS2 expression, may 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.[4] Other possible sources of

discordance include low tumor volume in the MSI sample. Note that loss of MSH6 protein expression may occur in absence of MSI-H phenotype.

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).

References
1. Haraldsdottir S, Hampel H, Tomsic J, et al. Colon and endometrial cancers with mismatch repair deficiency can arise from somatic, rather than germline, mutations. *Gastroenterology*. 2014;147(6):1308-1316.
2. Ligtenberg MJ, Kuiper RP, Chan TL, et al. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. *Nat Genet.* 2009;41(1):112-117.
3. Geurts-Giele WR, Leenen CH, Dubbink HJ, et al. Somatic aberrations of mismatch repair genes as a cause of microsatellite-unstable cancers. *J Pathol.* 2014;234(4):548-559.
4. McConechy MK, Talhouk A, Li-Chang HH, et al. Detection of DNA mismatch repair (MMR) deficiencies by immunohistochemistry can effectively diagnose the microsatellite instability (MSI) phenotype in endometrial carcinomas. *Gynecol Oncol.* 2015;137(2):306-310.

D. Microsatellite Instability Testing

Detection of hereditary defective mismatch repair has clinical implications for treatment of the affected patient and family members. Patients with a microsatellite instability-high (MSI-H) phenotype in their cancer tissues 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.[1-3] 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 endometrial cancers (about 15% of cases) due to somatic abnormalities, usually hypermethylation of the *MLH1* gene promoter.

MSI testing protocols are similar to those developed for colon cancer. These are briefly summarized here, but more complete details are available in the separately issued "Template for Reporting Results of Biomarker Testing of Specimens From Patients With Carcinoma of the Colon and Rectum."[4] Testing 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. 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 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.

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 (or somatic) mutation.

References
1. Haraldsdottir S, Hampel H, Tomsic J, et al. Colon and endometrial cancers with mismatch repair deficiency can arise from somatic, rather than germline, mutations. *Gastroenterology*. 2014;147(6):1308-1316.
2. Ligtenberg MJ, Kuiper RP, Chan TL, et al. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. *Nat Genet.* 2009;41(1):112-117.
3. Geurts-Giele WR, Leenen CH, Dubbink HJ, et al. Somatic aberrations of mismatch repair genes as a cause of microsatellite-unstable cancers. *J Pathol.* 2014;234(4):548-559.

4. Bartley AN, Hamilton SR, Alsabeh EP, et at. Template for Reporting Results of Biomarker Testing of Specimens From Patients With Carcinoma of the Colon and Rectum. http://www.cap.org/ShowProperty?nodePath=/UCMCon/Contribution%20Folders/WebContent/pdf/cp-colorectalbiomarker-14.pdf. Published December 2014. Accessed May 25, 2016.

E. *MLH1* Promoter Methylation Analysis

Defective mismatch repair in sporadic endometrial cancer is most often due to inactivation of the *MLH1* gene promoter by methylation (epigenetic silencing). Most laboratories utilize a methylation-specific real-time polymerase chain reaction (PCR) assay to determine the presence of methylation.

F. p53 Expression

The distinction between endometrioid and serous type endometrial carcinomas is typically based on morphologic evaluation. Analysis for *p53* gene mutations can occasionally be useful for diagnostically challenging tumors which are not morphologically distinguishable between endometrioid and serous phenotypes. The vast majority of serous type endometrial carcinomas exhibit mutations in *p53*. While most low-grade endometrioid endometrial tumors are not associated with *p53* mutations, a significant subset of high-grade endometrioid tumors are; thus, any ancillary testing for the presence of a *p53* mutation should be performed with an awareness of the limitations of the result with respect to providing a conclusive answer as to exact tumor type.[1-2] On occasion, *p53* testing may be requested for treatment purposes.

Extent of p53 specific nuclear immunostaining can be used to assess *p53* gene integrity in endometrial carcinoma. Normal endometrial glands with an intact *p53* gene express the protein at low levels, reaching a threshold of immunohistochemical detection (positive staining) in only a small percentage of cells. Generally this is 1% to 5% of nuclei, but may increase under conditions of cellular damage or repair. Two different staining patterns are each considered diagnostic of abnormalities of the *p53* gene itself. Most common are mutations resulting in a qualitatively abnormal p53 protein that stabilizes the p53 complex, resulting in intense nuclear staining in >90% of affected cells. In most cases that harbor mutations in *p53* that are associated with overexpression, intense nuclear staining is present in over 90% of affected cells. Second is genomic damage causing loss of expression, with complete absence of protein in all affected cells. The latter null phenotype must be distinguished from a failed stain. Low levels of expression within internal control tissues (stroma, or nonmalignant epithelium) can be used for this purpose. It should be noted that p53 expression is significantly affected by non-optimized antigen retrieval or use of archival (weeks) tissue sections.

References

1. Cancer Genome Atlas Research Network. Integrated genomic characterization of endometrial carcinoma. *Nature*. 2013;497(7447):67-73.
2. Hoang LN, McConechy MK, Kobel M, et al. Histotype-genotype correlation in 36 high-grade endometrial carcinomas. *Am J Surg Pathol.* 2013;37(9):1421-1432.

G. 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.

- Laser capture microdissection (LCM): Use of a laser-equipped microscope to isolate and retrieve specific cells of interest from a histopathologic region of interest.
- 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.
- Manual without microscopic observation: H&E slide is examined without a microscope and marked by a pathologist for subsequent tumor dissection and retrieval.
- Cored from block: Area of interest is cored from a paraffin-embedded tissue block.
- Whole tissue section: No tumor enrichment procedure employed for tissue retrieval.