



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

Version: Endometrium Biomarkers 1.2.0.1 Template Posting Date: September 2019

This biomarker template is not required for accreditation purposes.

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Summary of Changes

v1.2.0.1

Updated the Background Documentation (Notes)

v1.2.0.0

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: September 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 ($\geq 1\%$)	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

There are currently no guidelines that have been developed for the reporting of the results of HER2 testing on endometrial cancer. In the absence of such data, the CAP recommends using a similar reporting format that is used for reporting the results of HER2 testing for breast cancer.¹

HER2 (ERBB2) Testing by Immunohistochemistry**Table 2. Reporting Results of HER2 Testing by Immunohistochemistry (IHC)**

Result	Criteria
Negative (Score 0)	No staining observed <i>or</i> Membrane staining that is incomplete and is faint/barely perceptible and within $\leq 10\%$ 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 <i>or</i> Circumferential membrane staining that is intense but within $\leq 10\%$ 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**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 3. Reporting Results of *HER2* Testing by In Situ Hybridization (single-probe assay)

Result	Criteria (single-probe assay)
Negative	<ul style="list-style-type: none"> • Average <i>HER2</i> copy number <4.0 signals/cell • Average <i>HER2</i> copy number ≥4.0 and <6.0 signals/cell <u>and</u> concurrent IHC 0, 1+ or 2+ • Average <i>HER2</i> copy number ≥4.0 and <6.0 signals/cell <u>and</u> concurrent dual probe ISH Group 5
Positive	<ul style="list-style-type: none"> • Average <i>HER2</i> copy number ≥6.0 signals/cell • Average <i>HER2</i> copy number ≥4.0 and <6.0 signals/cell <u>and</u> concurrent IHC 3+ • Average <i>HER2</i> copy number ≥4.0 and <6.0 signals/cell <u>and</u> concurrent dual probe ISH Group 1

Table 4. Reporting Results of *HER2* Testing by In Situ Hybridization (dual-probe assay)

Result	Criteria (dual-probe assay)
Negative	<ul style="list-style-type: none"> • Group 5
Negative	<ul style="list-style-type: none"> • Group 2 <u>and</u> concurrent IHC 0-1+ or 2+ • Group 3 <u>and</u> concurrent IHC 0-1+ • Group 4 <u>and</u> concurrent IHC 0-1+ or 2+
Positive	<ul style="list-style-type: none"> • Group 2 <u>and</u> concurrent IHC 3+ • Group 3 <u>and</u> concurrent IHC 2+ or 3+ • Group 4 <u>and</u> concurrent IHC 3+
Positive	<ul style="list-style-type: none"> • Group 1

References

1. Wolff AC, Hammond MEH, Allison KH, et al. *HER2* testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. *Arch Pathol Lab Med.* 2018;142:1364–1382

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 al. 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.