



Template for Reporting Results of Biomarker Testing of Specimens From Patients With Carcinoma of Gynecologic Origin

Version: 1.0.0.0

Protocol Posting Date: December 2022

The use of this protocol is recommended for clinical care purposes but is not required for accreditation purposes.

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With guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees.

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Accreditation Requirements

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. This template is not required for accreditation purposes.

Summary of Changes

v 1.0.0.0

- New Protocol

Reporting Template**Protocol Posting Date: December 2022****Select a single response unless otherwise indicated.****CASE SUMMARY: (Gynecologic Biomarker Reporting Template)****TEST(S) PERFORMED**

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 (e.g., 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.

Gene names should follow recommendations of The Human Genome Organisation (HUGO) Nomenclature Committee (www.genenames.org; accessed October 12, 2022).

All reported gene sequence variations should be identified following the recommendations of the Human Genome Variation Society (<http://varnomen.hgvs.org/>; accessed October 12, 2022).

+Testing Performed on Block Number(s) (specify): _____**+Specimen Type**

___ Biopsy / curettage

___ Resection

___ Other (specify): _____

+Appropriate Controls Verified

___ Yes

___ No

___ Other (specify): _____

Immunohistochemical Tests Performed (Note [A](#)) (select all that apply)___ Estrogen Receptor (ER) Status (Note [B](#))**Estrogen Receptor (ER) Status**

___ Positive

Percentage of Cells with Nuclear Positivity: _____ %**Average Intensity of Staining**

___ Weak

___ Moderate

___ Strong

___ Negative (less than 1%)

___ Progesterone Receptor (PgR) Status (Note [B](#))**Progesterone Receptor (PgR) Status**

___ Positive

Percentage of Cells with Nuclear Positivity: _____ %**Average Intensity of Staining**

___ Weak

___ Moderate

___ Strong

___ Negative (less than 1%)

___ HER2 Status (for uterine serous carcinoma only) (Note [C](#))

HER2 Status*# No staining in tumor cells* Negative (score 0)#*## Faint / Barely perceptible, incomplete membrane staining in any proportion, or weak complete staining in less than 10% of tumor cells* Negative (score 1+)##*### Strong complete or basolateral / lateral membrane staining in less than or equal to 30%, or weak to moderate staining in greater than or equal to 10% of tumor cells* Equivocal (score 2+)###*#### Strong complete or basolateral / lateral membrane staining in greater than 30% of tumor cells* Positive (3+)#### Cannot be determined (indeterminate) (explain): _____ Mismatch Repair (MMR) Protein Status (Note [D](#))**Mismatch Repair (MMR) Protein Status (select all that apply)** MLH1**Nuclear MLH1 Expression** Intact Loss Other (specify): _____ PMS2**Nuclear PMS2 Expression** Intact Loss Other (specify): _____ MSH2**Nuclear MSH2 Expression** Intact Loss Other (specify): _____ MSH6**Nuclear MSH6 Expression** Intact Loss Other (specify): _____ Background non-neoplastic tissue / internal control shows intact nuclear expression**+Additional Comment (specify percentage loss or other):** _____**Immunohistochemistry (IHC) Interpretation for Mismatch Repair (MMR) Proteins# (Note [D](#))***# There are exceptions to these 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.* No loss of nuclear expression of MMR proteins: low probability of microsatellite instability-high (MSI-H) phenotype# 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 promoter 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, genetic counseling is recommended# Loss of nuclear expression of MSH6 only: high probability of Lynch syndrome, genetic counseling

is recommended#

___ Loss of nuclear expression of PMS2 only: high probability of Lynch syndrome, genetic counseling is recommended#

___ Other (specify): _____

___ p53 Status (Note [E](#))

p53 Status

___ Normal expression (wild type)

___ Abnormal expression (mutated)

___ Overexpression (strong, diffuse basilar nuclear expression in greater than 90% of cells)

___ Null (lack of nuclear or cytoplasmic expression)

___ Cytoplasmic staining, diffuse (with or without nuclear expression)

ADDITIONAL TESTS PERFORMED

+HER2 by in situ Hybridization (Note [C](#))

"Number of Observers" and "Number of Invasive Tumor Cells Counted" are required only when Negative or Positive is selected.

___ Negative (not amplified)

___ Positive (amplified)

___ Cannot be determined (indeterminate) (explain): _____

___ Not performed

___ Pending

Number of Observers: _____

Number of Invasive Tumor Cells Counted: _____ cells

Method (select all that apply)

___ Not applicable (not performed)

___ Dual probe assay

+Average Number of HER2 Signals per Cell: _____

+Average Number of CEP17 Signals per Cell: _____

+HER2 / CEP17 Ratio: _____

___ Single probe assay

+Average Number of HER2 Signals per Cell: _____

+Aneusomy (as defined by vendor kit used)

___ Not identified

___ Present (explain): _____

+Heterogeneous Signals

___ Not identified

___ Present

+Percentage of Cells with Amplified HER2 Signals

___ Specify percentage: _____ %

___ Other (specify): _____

___ Cannot be determined

+Microsatellite Instability (MSI) Interpretation (Note E)

The presence of MSI-H / deficient mismatch repair may also be an indication for additional testing for Lynch syndrome and genetic counselling.

- MSI-Stable (MSS)
- MSI-Low (MSI-L)
 - + 1-29% of the markers exhibit instability
 - + 1 of the 5 NCI or mononucleotide markers exhibits instability
 - + Other (specify): _____
- MSI-High (MSI-H)
 - + Greater than or equal to 30% of the markers exhibit instability
 - + 2 or more of the 5 NCI or mononucleotide markers exhibit instability
 - + Other (specify): _____
- MSI-Cannot be determined (explain): _____
- Pending

+MLH1 Promoter Methylation Analysis (Note G)

- MLH1 promoter methylation present
- MLH1 promoter methylation absent
- Cannot be determined (explain): _____
- Pending

+Image Analysis

- Not performed
- Performed
 - +Specify Method:** _____
 - +Biomarkers Scored by Image Analysis (select all that apply)**
 - ER
 - PgR
 - HER2 by IHC
 - HER2 by ISH
 - Other (specify): _____

Explanatory Notes

A. Biomarker Testing in Gynecologic Carcinoma

Biomarker testing in gynecologic malignancies is an evolving practice with numerous candidates under investigation for targeted therapies.^{1,2,3,4} Only a few of these tests have status in clinical guidelines or recommendations. Many of these markers have a major role in the diagnostic assessment of tumor type. American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) guidelines for breast cancer tissue ischemia and fixation for testing ER, PgR, and HER2 have been proposed for gynecologic tumors and may be adopted but are not currently required.^{5,6}

References

1. Jiang P, Jia M, Hu J, Huang Z, Deng Y, Hu Z. A nomogram model involving immunohistochemical markers for predicting the recurrence of stage I-II endometrial cancer. *Front Oncol.* 2021; 10:586081.
2. Gülseren V, Kocaer M, Özdemir IA, Çakır I, Sancı M, Güngördük K. Do estrogen, progesterone, p53, and Ki67 receptor ratios determined from curettage materials in endometrioid-type endometrial carcinoma predict lymph node metastasis? *Curr Probl Cancer.* 2020; 44(10):100498.
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B. 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.^{1,2} 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.³ This information has not been required for endometrial carcinomas and is optional. However, 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. In the endometrium, benign endometrial glands, endometrial stroma, or myometrium may serve as internal control tissue. If internal controls are not present, consider repeating the test on another specimen with internal controls (if available). Reasons for false-negative results include the following:

- Exposure of tumor cells to heat (e.g., carcinomas transected by using cautery during surgery)
- Prolonged cold ischemic time, which may result in antigenic degradation

- Under- or over-fixation; fixation for at least 6 hours in buffered formalin is recommended; 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 (weeks) old 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 modified reporting format similar to that used for reporting the results of immunohistochemical assays for ER and PgR for breast cancer (Table 1).⁴

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 (e.g., 10-20%).
- Intensity: Refers to degree of nuclear positivity (i.e., 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 (greater than or equal to 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 should also be reported.
Negative	Less than 1% immunoreactive tumor cells present	

References

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C. HER2 (ERBB2) Testing

The HER2 (ERBB2) gene is located on chromosome 17 and codes for a tyrosine kinase receptor from the epidermal growth factor receptor (EGFR) family. This protein is critical in signaling pathways that regulate cell division, proliferation, differentiation, and apoptosis. There are currently no consensus guidelines that have been developed for reporting of the results of HER2 testing in endometrioid carcinoma, but HER2 has been proposed as a biomarker, and testing is recommended by the National Comprehensive Cancer Network (NCCN) guidelines for advanced or recurrent uterine serous carcinoma.^{1,2,3} Heterogeneity of expression within a tumor is a common problem. Unlike the patterns seen in breast carcinoma, staining is usually **lateral or basolateral** and spares the apical portion of cells (U-shaped membranous pattern).³ Approximately 25-30% of uterine serous carcinoma will show HER2 overexpression and/or gene amplification, but divergent results between the two testing methods are common and concordance may be less than 50%.^{4,5,6} Although there is an increased progression-free survival in patients with homozygous HER2 protein expression treated with targeted chemotherapy, the overall response rate is low, and most uterine serous carcinomas tend to show heterogeneous HER2 expression. This has implications for treatment and negative HER2 results on small samples might erroneously exclude patients from HER2 targeted therapy.⁷

In the absence of conclusive data, CAP suggests using a similar reporting format as that used for reporting the results of HER2 testing for breast cancer, with modifications below.^{4,5,8,9}

Table 2. Reporting Results of HER2 Testing by Immunohistochemistry for Endometrial Serous Carcinoma

Result	Criteria
Negative (Score 0)	No staining observed
Negative (Score 1+)	Incomplete membrane staining that is faint/barely perceptible in any proportion of cells or Weak complete staining in less than 10% of tumor cells*
Equivocal (Score 2+) [†]	Intense complete or basolateral/lateral membrane staining in 30% or less tumor cells* Weak to moderate staining in greater than or equal to 10% of tumor cells*
Positive (Score 3+)	Intense complete or basolateral/lateral membrane staining in over 30% 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

The propensity for HER2 heterogeneity in uterine serous carcinoma is mirrored by intratumoral HER2 heterogeneity in fluorescent in situ hybridization (FISH) studies. FISH is performed on tumors with a 2+ IHC score, using the largest tumor area with HER2 immunoreactivity for testing.⁵ Currently, there are no recommendations to perform FISH in lieu of IHC testing. Based on a large clinical trial, only HER2/CEP17 ratios of greater than or equal to 2.0 are considered amplified and serve as baseline guidance for

reporting pending further studies.⁴ Several unanswered questions remain, such as whether endometrial biopsy or hysterectomy is the optimal specimen, whether multiple specimens should be tested, whether metastases should be tested, and if so, how to clinically manage discordant results.

Table 3. Reporting Results of HER2 Testing by Fluorescent In Situ Hybridization (single-probe assay)⁵

Result	Criteria (single-probe assay)
Negative	<ul style="list-style-type: none"> · FISH HER2/CEP17 ratio less than 2.0 and · Average HER2 copy number less than 6 per nucleus
Positive	<ul style="list-style-type: none"> · FISH HER2/CEP17 ratio greater than or equal to 2.0 or · FISH HER2/CEP17 ratio less than 2.0, copy number equal to or greater than 6 per nucleus

References

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D. Mismatch Repair (MMR) Immunohistochemistry Testing

Screening for the autosomal dominant Lynch syndrome can be performed by universal MMR testing of endometrioid carcinoma, one of the most common presentations. Immunohistochemical (IHC) testing for

DNA MMR protein expression (i.e., *MLH1*, *MSH2*, *MSH6*, and *PMS2* expression) is performed on formalin-fixed, paraffin-embedded tissue.

MMR testing in endometrioid carcinoma has been included in the molecular classification system for endometrioid carcinoma proposed by the World Health Organization.¹ MMR-deficient patients are eligible for immune checkpoint inhibitors, although in many cases, treatment is currently restricted to clinically challenging cases.²

If the results of DNA MMR IHC and MSI (microsatellite instability) testing are discordant (e.g., 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.¹ Another possible source of discordance is low tumor volume in the MSI sample. Note that loss of *MSH6* protein expression may occur in absence of MSI-H phenotype.^{3,4} Microsatellite instability is a primary pathway of endometrial carcinogenesis and should be considered when all MMR protein expression is intact.

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 non-neoplastic epithelial cells.^{4,5} Loss of expression of *MLH1* may be due to Lynch syndrome or methylation of the *MLH1* promoter region (as occurs in sporadic MSI carcinoma).

Patients with patterns reflecting a high likelihood of Lynch syndrome should be referred for consultation with a geneticist. No loss of nuclear expression of MMR proteins indicates a low probability of microsatellite instability-high (MSI-H). Loss of DNA MMR protein expression is likely to be due to mutation (either genetic or somatic) in one of the mismatch repair genes.^{6,7,8} This information will help identify the gene that is most likely to have a mutation (e.g., a patient whose tumor shows loss of *MSH2* and *MSH6* expression, but retention of *MLH1* and *PMS2* expression, may have an *MSH2* germline mutation). Loss of nuclear expression of *MLH1* and *PMS2* should be triaged for *MLH1* methylation studies. The presence of *MLH1* methylation suggests a sporadic tumor rather than a germline mutation, and further germline testing is likely not indicated. Absence of *MLH1* methylation suggests Lynch syndrome and sequencing and/or large deletion/duplication testing of germline *MLH1* is indicated. Loss of nuclear expression of *MSH2* and *MSH6*, loss of *MSH6* only, or loss of *PMS2* only all have a high probability of Lynch syndrome and genetic counseling should be considered.

References

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E. p53 Expression

p53 is a tumor suppressor protein that induces expression of p21, a cyclin-dependent kinase inhibitor that is involved in the arrest of cellular proliferation at the G1 phase. Essentially, p53 regulates cell proliferation, DNA repair, apoptosis, and genetic stability. Inactivation of p53 occurs through mutations of *TP53* or inactivation of p53 through binding proteins, resulting in dysregulated growth. Mutations result in abnormal cellular expression of the protein (overexpression or lack of expression) that can be detected by immunohistochemical methods. In gynecologic malignancies, p53 expression is frequently used as a diagnostic tool but can be employed as a marker for targeted chemotherapy. Mutations of *TP53* occur more commonly (~90%) in serous carcinoma than in endometrioid carcinoma (~10-40%) and are associated with significantly poorer outcomes.^{1,2}

Recently the WHO has included *TP53* evaluation into its molecular classification of endometrial carcinoma.³ The vast majority of serous-type endometrial carcinomas exhibit mutations in *TP53*. While most low-grade endometrioid endometrial tumors are not associated with *TP53* mutations, a significant subset of high-grade endometrioid tumors are; thus, any ancillary testing for the presence of a *TP53* mutation should be performed with an awareness of the limitations of the IHC result with respect to providing a conclusive answer as to classification.^{4,5} On occasion, p53 testing may be requested for treatment purposes, but sequencing of the *TP53* gene may be more appropriate to select patients for targeted therapy.⁶

Extent of p53 specific nuclear immunostaining can be used to assess *TP53* gene integrity in endometrial carcinoma. Normal endometrial glands with an intact *TP53* 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 *TP53* 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 *TP53* 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 a 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 old) tissue sections.

References

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F. 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 (e.g., *MLH1*, *MSH2*, *MSH6*, or *PMS2*) or an altered *EPCAM* (*TACSTD1*) gene.^{1,2,3} 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 endometrioid carcinoma (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.⁵

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.

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G. 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.