

CANADIAN ASSOCIATION OF PATHOLOGISTS
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Title

CAP-ACP Clinical Immunohistochemistry Checklists: Part I and Part II

Authored by

CAP-ACP National Standards Committee for High Complexity Laboratory Testing

Foreword

Similar to any other laboratory testing, the value of the laboratory test is in its ability to provide reliable results that can be used clinically. Clinical immunohistochemistry provides results to pathologists (Class I IHC tests) and to oncologists (Class II IHC tests). Therefore, both diagnostic work up and patient stratification for appropriate therapies often depend on results of IHC testing. CAP-ACP National Standards Committee for High Complexity Laboratory Testing (former CAP-ACP National Standards Committee/Immunohistochemistry) was charged by the CAP-ACP to work on improving standards in clinical immunohistochemistry. The Committee has prepared CAP-ACP Clinical Immunohistochemistry Checklists: Part I and Part II with the following purposes:

- To decrease risk to health and safeguard patient safety
- To bridge the gap between published guidelines and their implementation in laboratory practice
- To introduce uniform standards for clinical immunohistochemistry laboratory practice
- To promote high standards for quality assurance in clinical practice
- To facilitate communication between clinical immunohistochemistry laboratories

Invitation for participation in Immunohistochemistry Standards Development

An important aspect of the Checklists and other documents developed by the CAP-ACP National Standards Committee for High Complexity Laboratory Testing (former CAP-ACP National Standards Committee/Immunohistochemistry) is that all are living documents. After the documents are published/presented and there was an opportunity for reviewing, all comments and suggestions from the members of the CAP-ACP, other laboratory physicians, and other interested parties will be given serious consideration. All CAP-ACP National Standards Committee for High Complexity Laboratory Testing documents are expected to be revised periodically.

Please send your comments to cap@rcpsc.edu :



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Immunohistochemistry (IHC) Testing and Reporting Guidelines Checklist Class I and Class II (Part I)

1. The checklist is intended to be a guide to laboratories to ensure they meet a minimum standard of practice to perform Immunohistochemistry testing.
2. The Checklists address all components of the Class I and Class II IHC tests including specimen management, instrumentation, quality control (QC) and quality assurance (QA), proficiency testing (PT), reagent and method selection, and reporting of results.
3. Class II IHC tests are ER, PR, HER2, Ki-67, CD20, and CD117. Each Class II test has additional unique set of parameters that need to be considered. These are provided in IHC Checklists Part II.

RECOMMENDED PUBLISHED GUIDELINES:

General:

1. Torlakovic EE, Riddell R, Hewlett B, Banerjee D, El-Zimaity H, Glynn G, Pilavdzic D, Dawe P, Magliocco A, Barnes P, Berendt R, Cook D, Gilks B, Williams G, Perez-Ordonez B, Wehli B, Swanson PE, Otis CN, Nielsen S, Vyberg M, Butany J. Best practice recommendations for standardization of immunohistochemistry tests. Canadian Journal of Pathology 2009;1(2):14-25.
2. Brown RW. Histologic preparations- common problems and their solutions. College of American pathologists. 2009. Page 5

Specific:

See CAP-ACP Class II Checklists (Part II).

The proposed scope and content included here were addressed by the CAP National Standards Committee/Immunohistochemistry (NSC/IHC) members and external consultants and were presented to the CAP Executive Committee. This is a living document and will continue to evolve as more data and information become available. The opinions expressed and arguments employed herein do not necessarily reflect the official views of the organization or its members.

Checklist

Specimen Management- Pre-analytical component:

- The laboratory has a written policy/procedure on the submission of pathology specimens to ensure specimen integrity.

Fixation and Processing:

- The laboratory monitors and documents time before fixation ("ischemic time")
- Exposure to surface drying or excessive heat (e.g. cautery damage) is monitored and documented if present.
- Larger samples are sliced at 5-10mm intervals after appropriate gross inspection and margin designation.
 - *Fixation of up to 10 days is acceptable if the above three points are met. However, fixation time longer than 72 hours generates additional requirement for IHC test validation (see below).*
- Specimens are placed into appropriate volume (15 to 20:1) fixative promptly and time into fixative documented.²
 - *The recommended fixative is 3.7 - 4% w/v aqueous formaldehyde in phosphate buffer pH 7.4 nominal (range 7.2-7.6).*
 - *Other commercial fixatives commonly known as 10% neutral buffered formalin (NBF) have a pH ranging between 6.8-7.0 and may prove adequate, but the fixative should be tested and validated for each antibody*
 - *The pH of each lot of fixative should be monitored and documented. The storage temperature should be monitored and documented.*
 - *A minimum fixation time for samples up to 4 mm thick is 18 – 24 hours at 37°C - 25°C respectively. Samples ranging from 5 mm – 1.0 cm require 36 - 48 hours.*
 - *For some antibodies shorter fixation times e.g. 8 hours may suffice however, the laboratory should validate this with a sufficient sample size (e.g. 25-100 specimens) in parallel with the recommended minimum time. For Class II IHC test, sample size is determined by statistical power analysis based on the type of samples included (expected positives and expected negatives).*
 - *Longer fixation times of up to 7-10 days are acceptable; however fixation times longer than 72 hours may require adjustment of the antigen retrieval step during IHC staining and also require revalidation. It is recommended that during optimization and validation of new antibodies, controls are used that reflect the total fixation time range of 8 hours - 10 days.*
 - *Other fixatives may be used for some purposes but should be fully validated in parallel against the recommended fixative.*

Decalcification

- Decalcification is performed only after adequate fixation.

- Decalcified samples use separate controls which are fixed and decalcified in the same manner as the test.
- Separate validation procedures for each antibody are performed.
- The type of decalcifier, the temperatures (not to exceed 37°C) and the length of time in the fluid are monitored and documented.

Tissue Processing

- The tissue samples are processed in the traditional manner through ascending grades of alcohol, xylene, and into paraffin wax.
 - *If a laboratory uses such a processor in 'xylene free' mode, or uses non-conventional reagents, this process should be validated in parallel against tissue processed in the conventional manner.*
 - *If other forms of tissue processor e.g 'microwave' processors are used, these should be validated in parallel against a conventional processor using conventional reagents.*
- The fixative included on the tissue processor is of the same formulation as the original fixative.
 - *The time in the processing fixative is included in the documented total fixation time.*
- If elevated temperatures are used during processing as an aid to reduce processing times they are monitored and documented.
 - The temperature (37°C optimal) for fixative, alcohol and xylene does not exceed 40°C.
- The temperature for paraffin wax does not exceed 60°C and temperatures are monitored and documented.

Microtomy

- Sections are cut at a standard thickness for each test, this may vary from test to test.
 - *For nuclear markers 3 to 4 µm sections are recommended.*
 - *For IHC test that do not require 3-dimensional information, section thickness up to 4 µm is recommended.*
 - *Section thickness should be monitored and documented.*
- Sections are floated on distilled water with no additives and picked up on positively charged slides or slides otherwise treated for IHC purposes.
- Mounted slides should be dried and 'baked' on in a standard manner which is monitored, documented and validated.

Instruments/equipment maintained and monitored by qualified staff:

- All maintenance is documented.
- All thermal equipment: refrigerators, freezers, ovens and water bath, etc. are monitored.
- Freezers and fridges have remote alarms that are monitored
 - Automated defrost freezers and fridges should not be used

- Appropriate environments for the optimum operation of equipment is monitored and maintained.
- Thermometers are NIST calibrated.
- Pipettors are regularly calibrated and verified for accuracy at least every six months.
- IHC staining machines are maintained and calibrated as per the manufacturers specifications.

Quality Control:

Positive controls:

- Internal positive controls according to published guidelines whenever possible.
- The selection of tissues for external positive controls as appropriate according to published guidelines.
- Procedures and processes are documented, current, accurate and controlled.
- All tissue controls are prepared to the same parameters as the test.
- It is advised that the appropriate positive control should be on the same slide as the test for both Class I and Class II tests.
- Full controls are run with each batch of tests. A single control within a batch of test slides is appropriate for Class I tests only.
- Class II antibodies must have the positive control on the same slide as the test.
- Mechanisms are in place to detect false staining either non-specific or specific (e.g. endogenous peroxidases, endogenous biotin)

Negative Controls:

- Negative Reagent Controls:
 - Negative controls are prepared from the same block and stained by the same methodology as the test section except with the substitution of the primary antibody.
 - Substitution includes an isotype or negative serum from the same species as the primary antibody used in the test

- Negative Tissue Controls:
 - Negative tissue controls should be prepared the same as the patient sample but should lack the target antigen (where applicable).
 - Internal negative controls are preferred whenever possible. Pathologists ordering the test would consider using blocks that include tissues known to be negative for the respective marker.
 - External negative tissue control is often available as a part of external positive control for many markers, in particular when multitissue controls are used.
 - Control results are recorded and monitored and procedures exist that define corrective actions if sub standard staining is identified.

Quality Assurance:

- The laboratory uses standard operating procedures (SOPs) to ensure consistency of service.
- Laboratories performing HER2 staining should have ready access to in situ hybridization for HER2 gene analysis.
- Laboratories performing Class II tests must perform a sufficient number of tests so that an accurate descriptive statistical analysis can be performed for the purpose of test validation and internal audit.
- The laboratory maintains a statistical analysis of positivity rates amongst its patient population for specific Class II markers (e.g. ER, PR and HER2).

Reagent and Method Selection:

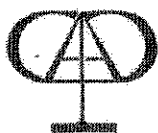
- There is a documented process for method selection.
- Established protocols exist for the development, evaluation and interpretation of new methods.
- Methods selected have been described in peer reviewed publications or have been developed 'in-house' and extensively validated and verified.
- Validation studies are fully recorded and the blocks, slides and documents are retained at least as long as such protocols are in use and longer if required by the accreditation organization.
- New lot numbers of each reagent are verified against existing reagents, using control blocks kept for that purpose, before being placed into use.
- All appropriate information is recorded including:
 - Clone
 - Date put into use
 - Dilution
 - Expiry Date (Note: reagents past their expiration date should never be used for test optimization and validation)
 - Antigen Retrieval: pre-treatment conditions specified (none, proteolysis or HIER),
 - Detection System
 - Specificity and staining patterns
 - Control tissue(s) (Note: principles of both, positive and negative control selection are defined and strictly followed)

Proficiency Testing:

- The laboratory participates in an External Proficiency Testing (EPT)/External Quality Assurance (EQA) program and maintains documentation of the results.
- There are appropriate processes in place to take action should there be any failure based on the current published guidelines and testing criteria of the respective EPT programs.

Reporting:

- Class II tests are reported according to current national and/or international guidelines (reference the guidelines).
- The testing criteria including preanalytical preparation, the clone used for testing, and methods should be included in the report.
- If the specimen was not prepared according to the guidelines (including pre-analytical and analytical phase), this is included in the report by specifying the deviation (reference the guidelines) and specifying if the test was validated for the purpose of the specific deviation or not.



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Class II Immunohistochemistry (IHC) Testing and Reporting Guidelines Checklist

Estrogen Receptor (ER)
Progesterone Receptor (PR)

Canadian Association of Pathologists – Association canadienne des pathologistes (CAP-ACP) IHC Test Classification: Class II Tests

| Current | In Consideration | For Discussion |
|---|------------------|----------------|
| Estrogen Receptor (ER) | C4d | NPM1 |
| Progesterone Receptor (PR) | DOG1 | FOXP1 |
| Human Epidermal Growth Factor Receptor 2 (HER2) | MSI markers | GCET1 |
| Proliferation Marker Ki-67 | | IgG/IgG4 |
| CD117 | | c-Myc |
| CD20 | | |

REFERENCES FOR ER/PR:

1. Lester SC, Bose S, Chen YY, Connolly JL, de Baca ME, Fitzgibbons PL, Hayes DF, Kleeer C, O'Malley FP, Page DL, Smith BL, Tan LK, Weaver DL, Winer E; Members of the Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with invasive carcinoma of the breast. Arch Pathol Lab Med. 2009 Oct;133(10):1515-38.
2. Lester SC, Bose S, Chen YY, Connolly JL, de Baca ME, Fitzgibbons PL, Hayes DF, Kleeer C, O'Malley FP, Page DL, Smith BL, Weaver DL, Winer E; Members of the Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with ductal carcinoma in situ of the breast. Arch Pathol Lab Med. 2009 Jan;133(1):15-25.
3. Yaziji H, Taylor CR, Goldstein NS, Dabbs DJ, Hammond EH, Hewlett B, Floyd AD, Barry TS, Martin AW, Badve S, Baehner F, Cartun RW, Eisen RN, Swanson PE, Hewitt SM, Vyberg M, Hicks DG; Members of the Standardization Ad-Hoc Consensus Committee. Consensus recommendations on estrogen receptor testing in breast cancer by immunohistochemistry. Appl Immunohistochem Mol Morphol. 2008 Dec;16(6):513-20.
4. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, Fitzgibbons PL, Francis G, Goldstein NS, Hayes M, Hicks DG, Lester S, Love R, Mangu PB, McShane L, Miller K, Osborne CK, Paik S, Perlmutter J, Rhodes A, Sasano H,

Schwartz JN, Sweep FC, Taube S, Torlakovic EE, Valenstein P, Viale G, Visscher D, Wheeler T, Williams RB, Wittliff JL, Wolff AC. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol. 2010 Jun 1;28(16):2784-95.

5. Ibrahim M. UK-NEQAS Recommended Best Methods, In: "Run 76, The Breast Hormonal Receptor Module: ER." Immunocytochemistry, 2008;6(3).

6. Dodson A, Ibrahim M. UK-NEQAS Recommended Best Methods, In: Run 75, The Breast Hormonal Module: PR. Immunocytochemistry. 2007;6(2):74-78.

7. NordiQC Recommended Best Methods, In, "Estrogen Receptor Alpha (ER), Run B5 2008". <http://www.nordiqc.org/Run-23-B5/Assessment/Assessment-ER.htm>

The proposed scope and content included here were addressed by the CAP National Standards Committee/Immunohistochemistry (NSC/IHC) members and external consultants and were presented to the CAP Executive Committee. This is a living document and will continue to evolve as more data and information become available. The opinions expressed and arguments employed herein do not necessarily reflect the official views of the organization or its members.

CLASS II IHC CHECKLIST¹

Hormone Receptors: Estrogen Receptor (ER) and Progesterone Receptor (PR)

This is test: Introduction Modification

Date of introduction or modification: _____ (day) _____ (month) _____ (year)

The protocol also used as Class I IHC testing: Yes No

Is different protocol necessary for Class I IHC testing: Yes No

Laboratories tests \geq 250 cases/year (recommended) Yes No

1. Preanalytical Component

See Class I and Class II Checklists, Part I.

2. Analytical Component

2.1. Positive controls (select all that apply):

- internal (normal breast tissue; it is highly recommended to select those paraffin blocks for hormone receptor staining that also include internal positive control, specifically benign breast tissue should always be evaluated and its presence and description included in the report)
- external (recommended)
- commercial source
- cell lines
- internally designed TMA
- pathologist who selected/designed the controls: _____

2.2. Internal positive controls:

- benign breast tissue processed in the same cassette with tumor (recommended)
- not controlled in gross room (not recommended)

2.3. External positive controls:

- one per run (not recommended)
- one on each test slide (recommended)
- none (not recommended)

2.3. External positive controls:

- normal cervix
- normal cervix and positive breast ca
- negative, weak positive, and strong positive breast ca
- other: _____
- other (in-house TMA design with): _____

¹ Throughout this document check *ALL* that applies.

2.4. Negative controls:

- internal only (it is highly recommended to select those paraffin blocks for hormone receptor staining that also include internal negative control; internal negative control like stromal cells should always be evaluated and its presence and description included in the report)
- internal and external (recommended)
- external only

2.5. Positive and negative controls results are recorded daily by:

- laboratory technologist (control log documents)
- pathologist (included in pathology reports)
- laboratory technologist and pathologist (recommended)

2.6.A Primary antibody clone for ER:

- SP1
- 6F11
- 1D5
- ER.2.123+1D5 cocktail
- Other: _____

2.6.B Primary antibody clone for PR:

- 1294
- 1A6
- 312
- Other: _____

2.7.A Primary antibody source for ER: _____

2.7.B Primary antibody source for PR: _____

2.8.A Primary antibody dilution for ER: _____

2.8.B Primary antibody dilution for PR: _____

2.9. Validation:

- internal validation (recommended)
- external validation (recommended)

3. Post-analytical Component

3.1. Scoring:

- results are reported quantitatively according to published guidelines (recommended)
- results are reported as H Score (recommended)
- results are reported as Allred Score
- results are reported as both, H Score and Allred Score
- FDA-approved image analysis results are reported
- results for ductal carcinoma in situ are reported according to published guidelines

Useful resources:

Table 4, From: Arch Pathol Lab Med. 2009 Oct;133(10):1515-38.

Figure 6, From: Arch Pathol Lab Med. 2009 Oct;133(10):1515-38.

Table 6, From: Arch Pathol Lab Med. 2009 Oct;133(10):1515-38.

Table 3, From: Arch Pathol Lab Med. 2009 Jan;133(1):15-25

3.2. Internal audit for pathologists:

- never; only group audit is done annually or bi-annually
- every 6 months
- annually (recommended; this does not apply to breast pathology subspecialty practice)
- biannually

3.3. Internal departmental/institutional audit:

- annually (recommended)
- biannually
- last audit result for ER: _____ (% positive cases) _____ (time period)
- last audit result for PR: _____ (% positive cases) _____ (time period)

3.4. Image Analysis is used for interpretation: No Yes

If yes, please state how was the system calibrated and how results are validated internally and externally:

3.5. Laboratory participates in following EQA programs for this test (mark all that apply):

- College of American Pathologists
- NordiQC
- UK NEQAS
- clQC
- QMP-LS
- Other: _____

3.6. Laboratory is accredited by (mark all that apply):

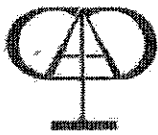
- Provincial College of Physicians and Surgeons
- OLA
- College of American Pathologists
- Accreditation Canada
- Other: _____
- Laboratory is NOT accredited; State what is used as a basis for TEST CERTIFICATION:

- Internal validation AND external validation AND daily QC monitoring with proof of acceptable internal and external performance of positive and negative controls AND EQA

results with achieved concordance $\geq 90\%$ with reference value or kappa-value $\geq .80$ (state EQA provider):

Other: _____

| Approved by: | Signature: | Date: |
|------------------|------------|-------|
| IHC Director | | |
| IHC Technologist | | |
| | | |



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Class II Immunohistochemistry (IHC) Testing and Reporting Guidelines Checklist

Human Epidermal Growth Factor Receptor 2 (HER2)

Canadian Association of Pathologists – Association canadienne des pathologistes (CAP-ACP) IHC Test Classification: Class II Tests

| Current | In Consideration | For Discussion |
|---|------------------|----------------|
| Estrogen Receptor (ER) | C4d | NPM1 |
| Progesterone Receptor (PR) | DOG1 | FOXP1 |
| Human Epidermal Growth Factor Receptor 2 (HER2) | MSI markers | GCET1 |
| Proliferation Marker Ki-67 | | IgG/IgG4 |
| CD117 | | c-Myc |
| CD20 | | |

REFERENCES FOR HER2:

1. Hanna W, O'Malley FP, Barnes P, Berendt R, Gaboury L, Magliocco A, Pettigrew N, Robertson S, Sengupta S, Têtu B, Thomson T. Updated recommendations from the Canadian National Consensus Meeting on HER2/neu testing in breast cancer. *Curr Oncol*. 2007 Aug;14(4):149-53.
2. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF; American Society of Clinical Oncology; College of American Pathologists. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol*. 2007 Jan 1;25(1):118-45. Epub 2006 Dec 11.
3. Lester SC, Bose S, Chen YY, Connolly JL, de Baca ME, Fitzgibbons PL, Hayes DF, Kleer C, O'Malley FP, Page DL, Smith BL, Tan LK, Weaver DL, Winer E; Members of the Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with invasive carcinoma of the breast. *Arch Pathol Lab Med*. 2009 Oct;133(10):1515-38.
4. Sauter G, Lee J, Bartlett JM, Slamon DJ, Press MF. Guidelines for human epidermal growth factor receptor 2 testing: biologic and methodologic considerations. *J Clin Oncol*. 2009 Mar 10;27(8):1323-33. Epub 2009 Feb 9. Review.

5. UK-NEQAS Recommended Best Methods, In, "Run 76, The Breast HER2 Module." Immunohistochemistry, 2008;6(3):139-144.
6. Walker RA, Bartlett JM, Dowsett M, Ellis IO, Hanby AM, Jasani B, Miller K, Pinder SE. HER2 testing in the UK: further update to recommendations. J Clin Pathol. 2008 Jul;61(7):818-24. Epub 2008 Apr 1. Review.
7. NordiQC Recommended Best Methods, In, "HER-2, Run B7 2009". <http://www.nordiqc.org/Run-26-B7/Assessment/assessment-B7-HER-2.htm>

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CLASS II IHC CHECKLIST²

Human Epidermal Growth Factor Receptor 2 (HER2)

This is test: Introduction Modification

Date of introduction or modification: ____ (day) ____ (month) ____ (year)

The protocol also used as Class I IHC testing: Yes No

Is different protocol necessary for Class I IHC testing: Yes No

Laboratories tests \geq 250 cases/year (recommended) Yes No

1. Preanalytical Component

See Class I and Class II Checklists, Part I.

2. Analytical Component

2.1. Positive controls (select all that apply):

- external (recommended)
- commercial source
- cell lines
- internally designed TMA
- pathologist who selected/designed the controls: _____

2.2. Internal negative controls:

- not controlled in gross room (not recommended)
- controlled in gross room (recommended)

2.3. External positive controls:

- one per run (not recommended)
- one on each test slide (recommended)
- none (not recommended)

2.3. External positive controls:

- only tissues/tumors whose HER2 status has been validated by more than one method are used for controls (recommended)
- multitissue block with tumour samples with negative, equivocal and positive results are included in every IHC run (recommended)
- validated cell lines from IHC FDA-approved kits are used in every IHC run
- validated cell lines (in-house) are used in every IHC run
- both, cell lines and tumour samples with negative, equivocal and positive results are used in every IHC run
- other: _____

² Throughout this document check *ALL* that applies.

2.4. Negative controls:

- internal normal duct (recommended; internal negative control should always be evaluated and its presence and description included in the report)
- internal and external (recommended)
- external only

2.5. Positive and negative controls results are recorded daily by:

- laboratory technologist (control log documents)
- pathologist (included in pathology reports)
- laboratory technologist and pathologist (recommended)

2.6. Primary antibody clone:

- mAb SP3
- pAb A0485
- HercepTest
- PATHWAY®
- Other: _____

2.7. Primary antibody source: _____

2.8. Primary antibody dilution: _____

2.9. Validation:

- internal validation (recommended)
- external validation (recommended)

3. Post-analytical Component

3.1. Scoring:

- results are reported quantitatively according to published guidelines (recommended)
- if membranous staining is present on normal breast tissue, the interpretation is appropriately adjusted (recommended)
- cases with cytoplasmic staining are referred for FISH testing (recommended)
- only invasive component of the tumor is scored
- if DCIS component is scored, it is reported separately from invasive component
- FDA-approved image analysis results are reported
- there is a system in place to ensure that repeat HER2 IHC testing will be done in a larger specimen, if negative result is obtained on a small biopsy specimen

Useful resource for scoring:

Table 6, From: Arch Pathol Lab Med. 2009 Oct;133(10):1515-38.

3.2. Internal audit for pathologists:

- never; only group audit is done annually or bi-annually
- every 6 months
- annually (recommended; this recommendation does not apply to breast pathology subspecialty practices)
- biannually

3.3. Internal departmental/institutional audit:

- annually (recommended)
- biannually
- last audit result for HER2: _____ (% positive cases) _____ (time period)

3.4. Image Analysis is used for interpretation:

- No Yes

If yes, please state how was the system calibrated and how results are validated internally and externally:

3.5. Laboratory participates in following EQA programs for this test (mark all that apply):

- College of American Pathologists
- NordiQC
- UK NEQAS
- cIQc
- QMP-LS
- Other: _____

3.6. Laboratory is accredited by (mark all that apply):

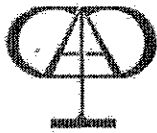
- Provincial College of Physicians and Surgeons
- OLA
- College of American Pathologists
- Accreditation Canada
- Other: _____

Laboratory is NOT accredited; State what is used as a basis for TEST CERTIFICATION:

Internal validation AND external validation AND daily QC monitoring with proof of acceptable internal and external performance of positive and negative controls AND EQA results with achieved concordance $\geq 90\%$ with reference value or kappa-value $\geq .80$ (state EQA provider):

Other: _____

| Approved by: | Signature: | Date: |
|------------------|------------|-------|
| IHC Director | | |
| IHC Technologist | | |



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**Class II Immunohistochemistry (IHC) Testing and Reporting
Guidelines Checklist**

CD117

**Canadian Association of Pathologists – Association canadienne des pathologistes
(CAP-ACP) IHC Test Classification: Class II Tests**

| Current | In Consideration | For Discussion |
|---|-------------------------|-----------------------|
| Estrogen Receptor (ER) | C4d | NPM1 |
| Progesterone Receptor (PR) | DOG1 | FOXP1 |
| Human Epidermal Growth Factor Receptor 2 (HER2) | MSI markers | GCET1 |
| Proliferation Marker Ki-67 | | IgG/IgG4 |
| CD117 | | c-Myc |
| CD20 | | |

REFERENCES FOR CD117:

- 1.. Sarlomo-Rikala M, Kovatich AJ, Barusevicius A, Miettinen M. CD117: a sensitive marker for gastrointestinal stromal tumors that is more specific than CD34. *Mod Pathol.* 1998 Aug;11(8):728-34.
2. Wong NA, Melegh Z. Antigen retrieval and primary antibody type affect sensitivity but not specificity of CD117 immunohistochemistry. *Histopathology.* 2009 Apr;54(5):529-38.
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4. Fletcher CD, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, Miettinen M, O'Leary TJ, Remotti H, Rubin BP, Shmookler B, Sobin LH, Weiss SW. Diagnosis of gastrointestinal stromal tumors: A consensus approach. *Hum Pathol.* 2002 May;33(5):459-65. Review.
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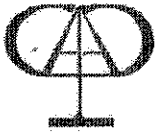
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CANADIAN ASSOCIATION OF PATHOLOGISTS
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CLASS II IHC CHECKLIST³

Test: CD117

This is test: Introduction Modification

Date of introduction or modification: ____ (day) ____ (month) ____ (year)

The protocol also used as Class I IHC testing: Yes No

Is different protocol necessary for Class I IHC testing: Yes No

Laboratories tests \geq 250 cases/year (recommended) Yes No

1. Preanalytical Component

See Class I and Class II Checklists, Part I.

2. Analytical Component

2.1. Positive controls (select all that apply):

- internal (it is highly recommended to select those paraffin blocks for CD117 staining that also include internal positive control; if there is evidence of benign tissue with predictable levels of CD117 expression, it should always be evaluated and its presence and description included in the report)
- external (recommended)
- commercial source
- cell lines
- internally designed TMA
- pathologist who selected/designed the controls: _____

2.2. External positive controls:

- one per run (not recommended)
- one on each test slide (recommended)
- none (not recommended)

2.3. External positive controls:

- only tissues/tumors whose CD117 is predictable (recommended)
- multitissue block with histologically normal small intestine or appendix, desmoid tumor, and two gastrointestinal tumors (one with moderate and strong expression of CD117) (recommended)
- validated commercial or in-house cell lines with established degree of CD117 expression
- other: _____

³ Throughout this document check *ALL* that applies.

2.4. Negative controls:

- internal only (it is highly recommended to select those paraffin blocks for CD117 staining that also include internal negative control; internal negative control should always be evaluated and its presence and description included in the report)
- internal and external (recommended)
- external only

2.5. Positive and negative controls results are recorded daily by:

- laboratory technologist (control log documents)
- pathologist (included in pathology reports)
- laboratory technologist and pathologist (recommended)

2.6. Primary antibody clone:

- rmAb YR145
- pAb A4502
- Other: _____

2.7. Primary antibody source: _____

2.8. Primary antibody dilution: _____

2.9. Validation:

- internal validation (recommended)
- external validation (recommended)

3. Post-analytical Component

3.1. Scoring

- external positive control is first evaluated; if multitissue block with desmoid is used in external positive control, desmoid tumor and smooth muscle should be negative
- moderate/strong diffuse pancytoplasmic +/- membranous pattern is recorded as "positive" for tumors
- diffuse "dotlike" immunostaining pattern only is also scored as positive
- when weak, cytoplasmic, granular pattern is detected, desmoid-type fibromatosis is excluded first

3.2. Internal audit for pathologists:

- never
- every 6 months
- annually (recommended; this recommendation does not apply to gastrointestinal subspecialty practices)
- biannually

3.4. Image Analysis is used for interpretation:

- No Yes

If yes, please state how was the system calibrated and how results are validated internally and externally:

3.5. Laboratory participates in following EQA programs for this test (mark all that apply):

- College of American Pathologists
- NordiQC
- UK NEQAS
- cIQc
- QMP-LS
- Other: _____

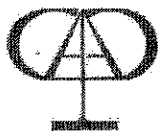
3.6. Laboratory is accredited by (mark all that apply):

- Provincial College of Physicians and Surgeons
- OLA
- College of American Pathologists
- Accreditation Canada
- Other: _____
- Laboratory is NOT accredited; State what is used as a basis for TEST CERTIFICATION:

Internal validation AND external validation AND daily QC monitoring with proof of acceptable internal and external performance of positive and negative controls AND EQA results with achieved concordance $\geq 90\%$ with reference value or kappa-value $\geq .80$ (state EQA provider):

Other: _____

| Approved by: | Signature: | Date: |
|------------------|------------|-------|
| IHC Director | | |
| IHC Technologist | | |



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Class II Immunohistochemistry (IHC) Testing and Reporting Guidelines Checklist

Canadian Association of Pathologists – Association canadienne des pathologistes (CAP-ACP) IHC Test Classification: Class II Tests

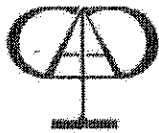
| Current | In Consideration | For Discussion |
|---|------------------|----------------|
| Estrogen Receptor (ER) | C4d | NPM1 |
| Progesterone Receptor (PR) | DOG1 | FOXP1 |
| Human Epidermal Growth Factor Receptor 2 (HER2) | MSI markers | GCET1 |
| Proliferation Marker Ki-67 | | IgG/IgG4 |
| CD117 | | c-Myc |
| CD20 | | |

REFERENCES FOR Ki-67:

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CLASS II IHC CHECKLIST⁴

Test: Proliferation Marker Ki-67

This is test: Introduction Modification

Date of introduction or modification: _____ (day) _____ (month) _____ (year)

The protocol also used as Class I IHC testing: Yes No

Is different protocol necessary for Class I IHC testing: Yes No

Laboratories tests \geq 250 cases/year (recommended) Yes No

1. Preanalytical Component

See Class I and Class II Checklists, Part I.

2. Analytical Component

2.1. Positive controls (select all that apply):

- internal (it is highly recommended to select those paraffin blocks for Ki-67 staining that also include internal positive control; if there is evidence of benign tissue with predictable levels of Ki-67 expression, it should always be evaluated and its presence and description included in the report)
- external (recommended)
- commercial source
- cell lines
- internally designed TMA
- pathologist who selected/designed the controls: _____

2.2. External positive controls:

- one per run (not recommended)
- one on each test slide (recommended)
- none (not recommended)

2.3. External positive controls:

- only tissues/tumors whose Ki-67 status is predictable (recommended)
- multitissue block with benign tonsil, histologically normal small intestine, and histologically normal skin (recommended)
- validated commercial or in-house cell lines with established Ki-67 proliferation fraction
- both cell lines and multitissue block tonsil/small intestine/skin are used in every IHC run
- other: _____

2.4. Negative controls:

- internal only (it is highly recommended to select those paraffin blocks for Ki-67 staining that also include internal negative control; internal negative control should always be evaluated and its presence and description included in the report)

⁴ Throughout this document check *ALL* that applies.

- internal and external (recommended)
- external only

2.5. Positive and negative controls results are recorded daily by:

- laboratory technologist (control log documents)
- pathologist (included in pathology reports)
- laboratory technologist and pathologist (recommended)

2.6. Primary antibody clone:

- mAb 7B11
- mAb MIB1
- rmAb 30-9
- rmAb SP6
- Other: _____

2.7. Primary antibody source: _____

2.8. Primary antibody dilution: _____

2.9. Validation:

- internal validation (recommended)
- external validation (recommended)

3. Post-analytical Component

3.1. Scoring

3.1.1 General:

- the percentage positive nuclei is recorded by using cell counter (recommended)
- the percentage of positive cells is estimated visually (see Figure 6, From: Arch Pathol Lab Med. 2009 Oct;133(10):1515-38) (not recommended for most tumors)
- FDA-approved image analysis results are reported

3.1.2 Malignant Lymphoma:

- cell counter is used to record percentage of positive cells in two different representative HPFs of tumor by counting a minimum of 100 neoplastic cells in each field to a total of 200 neoplastic cells (recommended)
- only neoplastic component of the tumor is scored
- in mantle cell lymphoma, the scoring is done only if 5 independent HPFs (at 400X) are available for evaluation and only in primary diagnosis samples before any treatment and is not done in bone marrow samples
- in mantle cell lymphoma, residual germinal centers, hot-spots, and proliferating T-cells are excluded from counts (see Figure 4, from J Hematopathol 2009;2:103-111)

3.1.3. Carcinoma:

- in carcinomas, invasive tumor proliferation fraction is evaluated and reported separately from in situ component
- in breast carcinoma, specific guidelines for specific patient population and tumor types are followed (state reference or other source of guidelines, e.g. ref. 4 indicates counting 2,000 tumor cells at the periphery of tumor):

in lung neuroendocrine carcinoma specific guidelines for specific patient population and tumor types are followed (state reference or other source of guidelines, e.g. ref. 7):

other carcinoma (state, organ, tumor type, and reference published guidelines):

3.1.4. Melanoma:

Not used as Class II test (recommended)

3.1.5. Other (e.g. Endocervical Biopsy):

Scoring of Ki-67 proliferation index is done according to current published guidelines for the specific organ/tumor/purpose (state reference or other source of guidelines):

Useful resources:

Figure 6, From: Arch Pathol Lab Med. 2009 Oct;133(10):1515-38.).

Figure 4, From: J Hematopathol 2009;2:103-111)

3.2. Internal audit for pathologists:

- never
- every 6 months
- annually (recommended)
- biannually

3.4. Image Analysis is used for interpretation:

No Yes

If yes, please state how was the system calibrated and how results are validated internally and externally:

3.5. Laboratory participates in following EQA programs for this test (mark all that apply):

- College of American Pathologists
- NordiQC
- UK NEQAS
- cIQc
- QMP-LS
- Other: _____

3.6. Laboratory is accredited by (mark all that apply):

- Provincial College of Physicians and Surgeons
- OLA
- College of American Pathologists
- Accreditation Canada
- Other: _____
- Laboratory is NOT accredited; State what is used as a basis for TEST CERTIFICATION:

Internal validation AND external validation AND daily QC monitoring with proof of acceptable internal and external performance of positive and negative controls AND EQA results with achieved concordance $\geq 90\%$ with reference value or kappa-value $\geq .80$ (state EQA provider):

Other: _____

| Approved by: | Signature: | Date: |
|------------------|------------|-------|
| IHC Director | | |
| IHC Technologist | | |