Validation of ImmunoCytochemical Staining & Implementation of On-Slide Controls

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Disclosures

• I do not have any financial interest, arrangement or affiliation with one or more organizations that could be perceived as a direct or indirect conflict of interest in the content of this presentation.
• I am not a cytopathologist

Objectives

• Develop an approach to the validation of immunohistochemical studies performed on cytology specimens
• Understand the need for appropriate on-slide controls
• Develop an approach to preparation and use of appropriate on-slide controls
Immunohistochemistry at LHSC 2018

- ~60,000 IHC
- ~200 different antibodies
- >90% on formalin-fixed paraffin embedded (FFPE)

Immunochemistry at LHSC 2018

- 2810 ICC (5%) performed on cytology specimens
- Goal to validate all but start with more common Abs
- 105 different antibodies used on cytology specimens
  - TTF1, MOC31, BerEP4, Calretinin, p40, CK7, CK20, WT1, CD10, LCA, CD30 = 10%
  - CD3, Syn, Chromogranin, CK19 = 10%
  - CKAE1/3, BerEP4, CD3, CD20 = 22%
  - 53 antibodies < 10%

The Problem

- How to validate immunohistochemical studies on cytology specimens?
- Differences compared to IHC on FFPE:
  - Alcohol based fixative or combination
  - Tissue handled differently - centrifuged, clotting agents, monolayer
- Cannot assume that optimized and validated protocols for FFPE tissues will perform equally on cytology specimens

Validation Guideline Statements

1. Laboratories must validate all IHC tests before placing into clinical service.
   Note: Such means include but are not necessarily limited to:
   - Correlating the new test's results with the morphology and expected results
   - Comparing the new test's results with the results of prior testing of the same tissue with a validated assay in the same laboratory
   - Comparing the new test's results with the results of testing the same tissue validation set in another laboratory using a validated assay
   - Comparing the new test's results with previously validated non-immunohistochemical tests
   - Testing previously graded tissue challenges from a formal proficiency testing program (if available) and comparing the results with the graded responses.
Perform IHC/ICC, using validated protocols for FFPE, on collected tissues that have undergone matched processing methods. Score % cells staining in quartiles on matched processing methods. Score intensity of cells staining as 1 if FFPE, >1 if < FFPE. Concordance if % and intensity of staining match. Discordance – consider enhanced protocol, different antibody.

Prospective Validation Map

Normal & Tumour Tissues

Formalin Tissue Cassette

Formalin Minced Tissue Cell Block

CytoLyt Tissue Cassette

CytoLyt Minced Tissue Cell Block

Formalin ThinPrep

CytoLyt ThinPrep

Perform IHC/ICC, using validated protocols for FFPE, on collected tissues that have undergone matched processing methods. Score % cells staining in quartiles on matched processing methods. Score intensity of cells staining as 1 if FFPE, >1 if < FFPE. Concordance if % and intensity of staining match. Discordance – consider enhanced protocol, different antibody.

Protein

CK AE1/AE3

Whole Tissue - Tonsil

p40

Whole Tissue - Tonsil

CD20

Whole Tissue - Tonsil

CD3

Whole Tissue - Tonsil

Ki-67

Whole Tissue - Tonsil
**CD10 Enhanced Protocol**

Whole Tissue - Tonsil

**Bcl6 Enhanced Protocol**

Whole Tissue - Tonsil

**Unable to Improve ALK1**

Suboptimal – Avoid ALK1 on CytoLyt fixed material

**p16 CytoLyt FNAB**

Suboptimal – Avoid p16 on CytoLyt fixed material
Strong, nuclear and cytoplasmic staining in ≥ 70%.

How Many Cases Are Needed For Validation?

3. For initial analytic validation of nonpredictive factor assays, laboratories should test a minimum of 10 positive and 10 negative tissues. When the laboratory medical director determines that fewer than 10 validation cases are sufficient for a specific marker (e.g., rare antigens), the rationale for that decision needs to be documented.

Note: The validation set should include high and low expresses for positive cases when appropriate and should span the expected range of clinical results (expression levels) for markers that are reported quantitatively.

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7. If IHC is regularly done on cytologic specimens that are not processed in the same manner as the tissues used for assay validation (e.g., alcohol-fixed cell blocks, air-dried smears, formalin-processed specimens), laboratories should test a sufficient number of such cases to sustain that assays consistently achieve expected results. The laboratory medical director is responsible for determining the number of positive and negative cases and the number of predictive and nonpredictive markers to test.

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Cost of Validation

- ~200 Antibodies
- 10 cases per antibody - Class I antibodies
- 20 cases per antibody - Class II antibodies
- Average cost per slide - $18 (majority, others $23, $45)
- 2000 x $18 = $36,000 (conservative estimate)

Validation Plan

- Prospective
  - Good for majority of antibodies
- Retrospective
  - For difficult to obtain tissues

Retention Formalin Tissue

Repeat IHC on archival cell block tissue on new platform

Compare original staining to staining on new platform

FFPE resection specimen serves as control for expected IHC staining

Score % cells staining in quartiles on matched cell block IHC

Score intensity of cells staining as 1 if > FFPE, <1 if < FFPE

Concordance if % and intensity of staining match or greater

Discordance – consider enhanced protocol, different antibody

Continue to look for prospective specimens

Implementing Validated IHC

- Keep track in spreadsheet of protocols, cases and concordance results
- Cross over to OMNIS from Autostainer when 90% concordance
- Inform cyto/pathologists of validated antibodies
- Disclaimer for non-validated antibodies

Control Issues

Canadian Association of Pathologists—Association canadienne des pathologistes National Standards Committee/Immunohistochemistry

Best Practice Recommendations for Standardization of Immunohistochemistry Tests

- Time to deliver results
- Interpretation of results
- Quality assurance measures

CME/GAM

Control issues
It is recommended that appropriate positive & negative controls are placed on the same slide as the patient material that is being tested.

If an unexpected negative result is encountered, the presence of an external positive control on the same slide as a patient's sample will greatly decrease the number of repeated tests and will demonstrate that the analytic component of the IHC testing was valid.

External negative controls are used to confirm the specificity of the test (if everything is brown, nothing is really brown).
External Positive Controls
Are valid only if they are fixed and prepared in the same manner as the tissue samples that are tested in the assay.

Cytology Specific Control Tissue Issues
It is not only inappropriate to use positive controls that are processed differently from tested samples, but it may be diagnostically misleading.

Because processing of cytologic samples is often substantially different from the processing of histologic samples, immunocytochemical tests require different QC/QA measures with an emphasis on the use of appropriate positive and negative controls prepared under the same conditions.

Validation of Punch Biopsy Control Tissues from CytoLyt Fixed Whole Tissue Sections vs from Formalin Fixed Whole Tissue Sections for On-Slide External Controls

Because most of ICC is performed on cell blocks and IHC concordance has been demonstrated between tissue sections and cell blocks generated from the same tissue, CytoLyt fixed tissue sections can be used as on-slide controls.

Punch biopsy tools for making multi-tissue controls

Preparation of Tissue for Multi-Tissue Control Blocks

Validation of Punch Biopsy Control Tissues from CytoLyt Fixed Whole Tissue Sections vs from Formalin Fixed Whole Tissue Sections for On-Slide External Controls

4 mm 3 mm 2 mm
Conclusions

- With appropriate validation of immunostaining protocols and use of appropriately prepared control tissues, immunostaining can be performed with confidence on cytological tissue preparations.