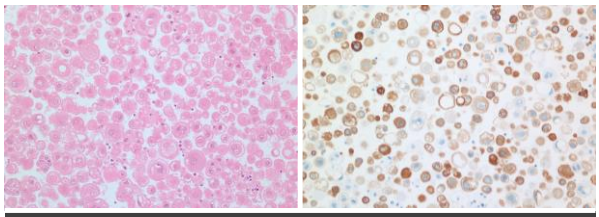


Validation of ImmunoCytochemical Staining & Implementation of On-Slide Controls

Bret Wehrli

Disclosures

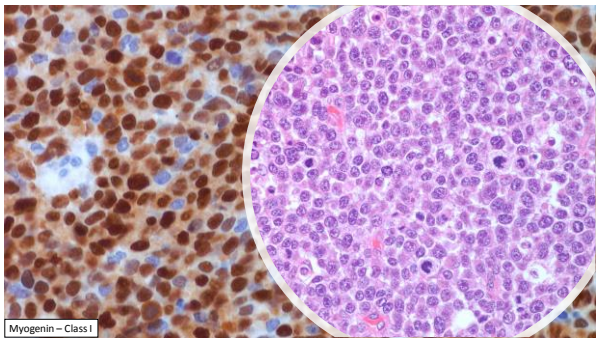
- I do not have any financial interest, arrangement or affiliation with one or more organizations that could be perceived as a direct or indirect conflict of interest in the content of this presentation.
- I am not a cytopathologist

From Autostainer to OMNIS:
Revalidation of IHC at LHSC

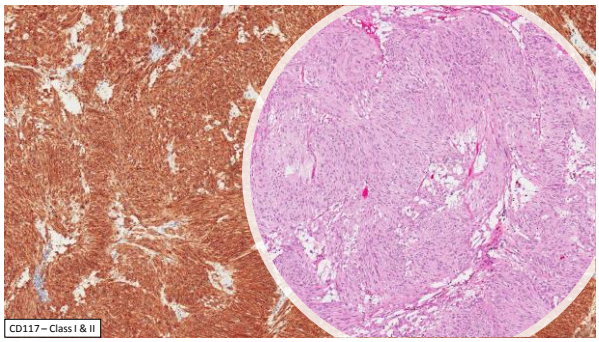
Dr. B. Wehrli, MD, FRCPC
Director of Immunohistochemistry, London Health Sciences Centre, London, Ontario, Canada

Objectives

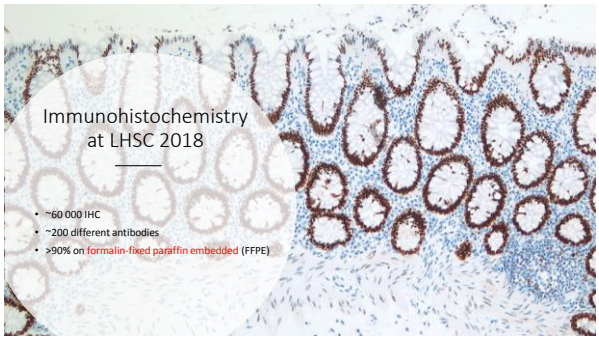
- Develop an approach to the validation of immunohistochemical studies performed on cytology specimens
- Understand the need for appropriate on-slide controls
- Develop an approach to preparation and use of appropriate on-slide controls



Myogenin – Class I

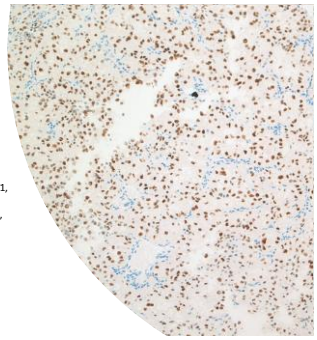


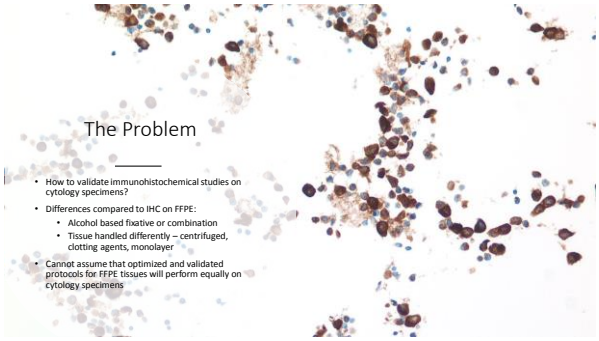
CD117 – Class I & II



Immunocytochemistry at LHSC 2018

- 2810 ICC (5%) performed on cytology specimens
- Goal to validate all but start with more common Ab
- 105 different antibodies used on cytology specimens
  - TTF1,MOC31,BerEP4,Calretinin,p40,CK7,CK20,WT1, CKAE1/3,CD20 = 50%
  - CD3,Syn,Chromo,CD5,CD10,CDX2,CyclinD1,CK5/6, CD23,Mammoglobin = 22%
  - 53 antibodies < 10x





CAP Laboratory Improvement Programs

Principles of Analytic Validation of Immunohistochemical Assays

Guideline From the College of American Pathologists Pathology and Laboratory Quality Center

Patrick L. Fitzgibbons, MD; Linda A. Bradley, PhD; Lisa A. Fatheree, BS, SCT(ASCP); Randa Alsabeh, MD; Regan S. Fulton, MD, PhD; Jeffrey D. Goldsmith, MD; Thomas S. Haas, DO; Rouzan G. Karabakhtsian, MD, PhD; Patti A. Loykasek, HT(ASCP); Monna J. Marolt, MD; Steven S. Shen, MD, PhD; Anthony T. Smith, MLS; Paul E. Swanson, MD

(*Arch Pathol Lab Med.* 2014;138:1432–1443

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 tissues 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; or
   Testing previously graded tissue challenges from a formal proficiency testing program (if available) and comparing the results with the graded responses.

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Validation Guideline Statements

6. When possible, laboratories should use validation tissues that have been processed by using the same fixative and processing methods as cases that will be tested clinically.

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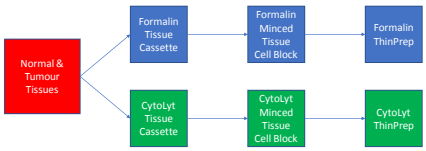
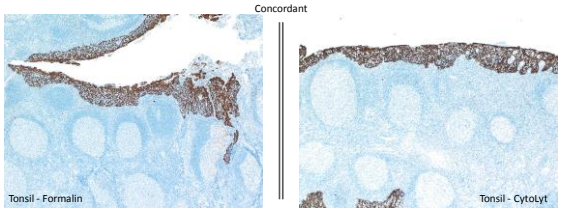
Prospective Validation Map



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

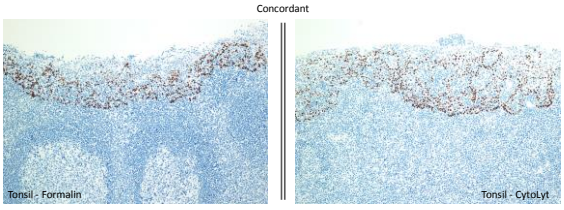
Concordant



CK AE1/AE3

Whole Tissue - Tonsil

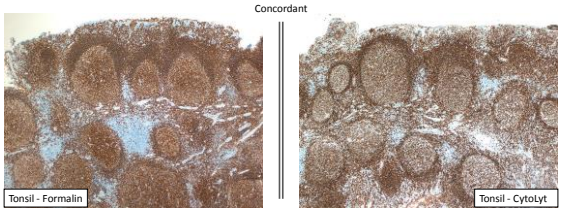
Concordant



p40

Whole Tissue -Tonsil

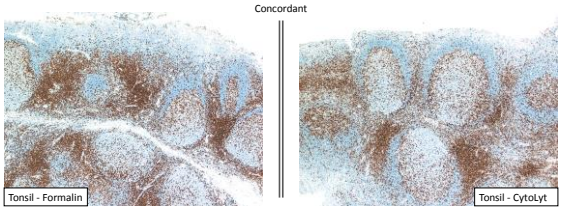
Concordant



CD20

Whole Tissue - Tonsil

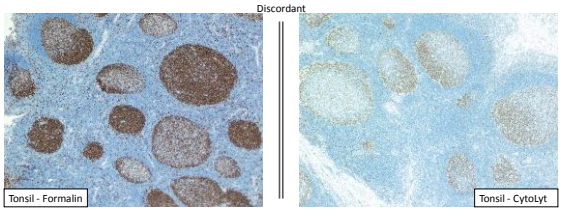
Concordant



CD3

Whole Tissue - Tonsil

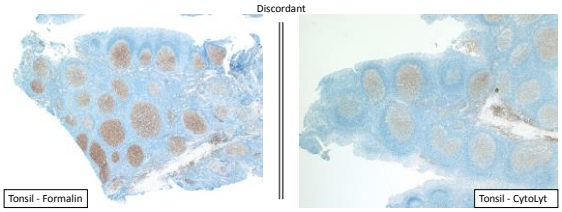
Discordant



Ki-67

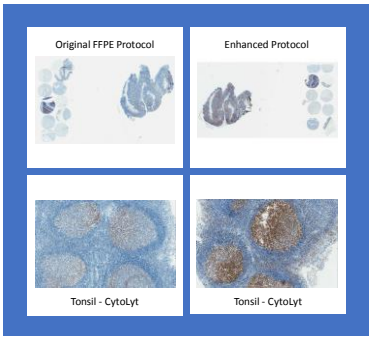
Whole Tissue - Tonsil

Discordant

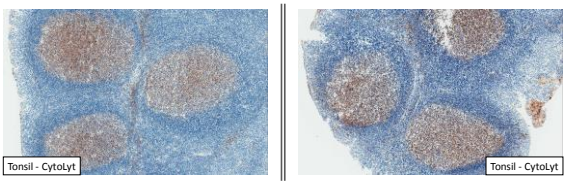


CD10

Whole Tissue - Tonsil

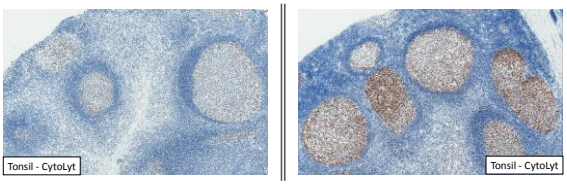


Ki-67 Enhanced Protocol



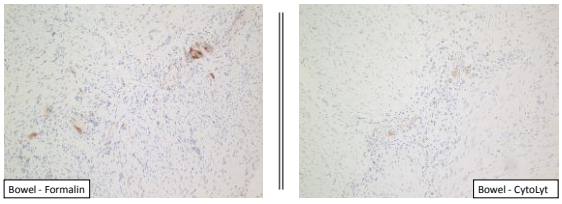
CD10 Enhanced Protocol

Whole Tissue - Tonsil



Bcl6 Enhanced Protocol

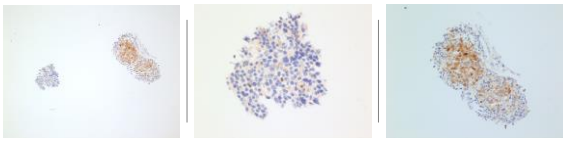
Whole Tissue - Tonsil



Unable to Improve ALK1

Suboptimal – Avoid ALK1 on CytoLyt fixed material

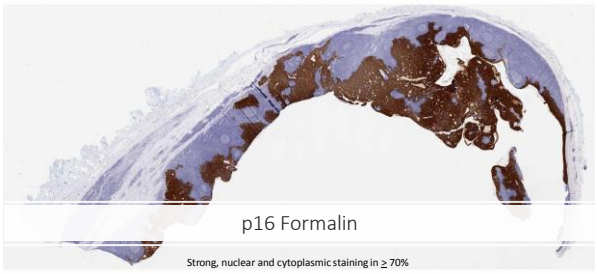
Neck Mass – Metastatic Squamous Cell Carcinoma, Unknown Primary



Weak to moderate, variable staining

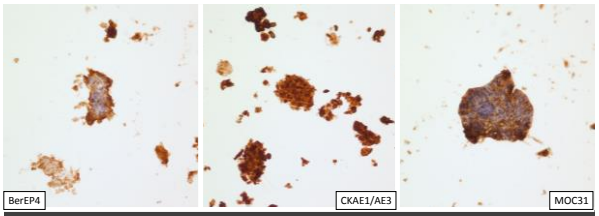
p16 CytoLyt FNAB

Suboptimal – Avoid p16 on CytoLyt fixed material

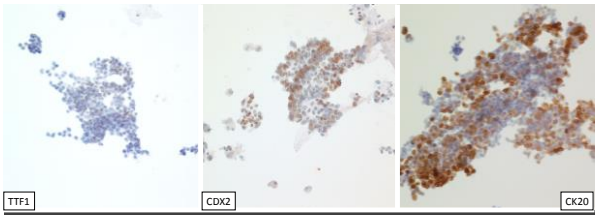


p16 Formalin

Strong, nuclear and cytoplasmic staining in ≥ 70%



ThinPrep ICC



ThinPrep IHC

How Many Cases Are Needed For Validation?

7. If IHC is regularly done on cytologic specimens that are not processed in the same manner as the tissues used for assay validation (eg, alcohol-fixed cell blocks, air-dried smears, formalin-postfixed specimens), laboratories should test a sufficient number of such cases to ensure 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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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 20 validation cases are sufficient for a specific marker (eg, rare antigen), the rationale for that decision needs to be documented.
   Note: The validation set should include high and low expressors 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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How Many Cases Are Needed For Validation?

4. For initial analytic validation of all laboratory-developed predictive marker assays (with the exception of HER2/*neu*, ER, and PgR), laboratories should test a minimum of 20 positive and 20 negative tissues. When the laboratory medical director determines that fewer than 40 validation tissues are sufficient for a specific marker, the rationale for that decision needs to be documented.
   Note: Positive cases in the validation set should span the expected range of clinical results (expression levels). This recommendation does not apply to any marker for which a separate validation guideline already exists.
5. For a marker with both predictive and nonpredictive applications, laboratories should validate it as a predictive marker if it is used as such.

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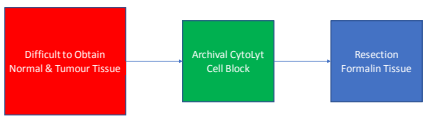
Cost of Validation

- ~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

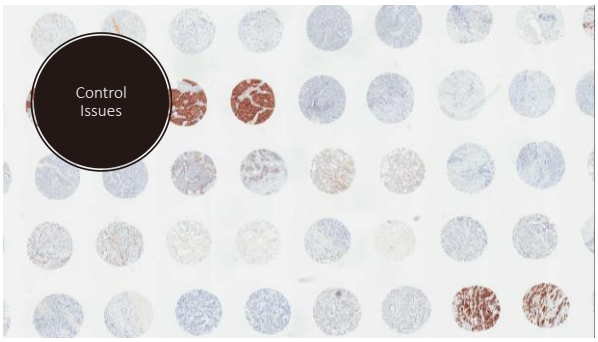
Retrospective Validation Map



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

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



CME/SAM

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

Best Practice Recommendations for Standardization of Immunohistochemistry Tests*

Emina Emilia Torlakovic, MD, PhD,[1] Robert Riddell, MD, FRCPath, FRCPC,[2] Diponkar Banerjee, MBChB, FRCPC, PhD,[3] Hala El-Zimaity, MD, MS, FRCPC,[4] Dragana Pilavdzic, MD, FRCPC,[5] Peter Dawe, MS,[6] Anthony Magliocco, MD, FRCPC,[7] Penny Barnes, MD, FRCPC,[8] Richard Berendt, MD, FRCPC,[9] Donald Cook, MD, FRCPC,[10] Blake Gilks, MD, FRCPC,[11] Gaynor Williams, MD, PhD,[12] Bayardo Perez-Ordonez, MD, FRCPC,[13] Bret Wehrli, MD, FRCPC,[14] Paul E. Swanson, MD,[15] Christopher N. Otis, MD,[16] Søren Nielsen, HT, CT,[17] Mogens Vyberg, MD,[17] and Jagdish Butany, MBBS, MS, FRCPC[13]

Positive Controls

REVIEW ARTICLE

Standardization of Positive Controls in Diagnostic Immunohistochemistry: Recommendations From the International Ad Hoc Expert Committee

Emina E. Torlakovic, MD, PhD,† Søren Nielsen, HT, CT,‡§ Glenn Francis, MBBS, FRCPA, MBA, FFSc (RCPA),‖¶# John Garratt, RT,‡** Blake Gilks, MD, FRCPC,†† Jeffrey D. Goldsmith, MD,‡‡ Jason L. Hornick, MD, PhD,*§§ Elizabeth Hyjek, MD, PhD,* Merdol Ibrahim, PhD,‖‖ Keith Miller, FIBMS,‖‖ Eugen Petcu, MD, PhD,‖ Paul E. Swanson, MD,¶¶## Xiaoge Zhou, MD,*** Clive R. Taylor, MD, PhD,‡‡‡ and Mogens Vyberg, MD‡§*

Negative Controls

REVIEW ARTICLE

Standardization of Negative Controls in Diagnostic Immunohistochemistry: Recommendations From the International Ad Hoc Expert Panel

Emina E. Torlakovic, MD, PhD,†‡ Glenn Francis, MBBS, FRCPA, MBA, FFSc (RCPA),§‖¶ John Garratt, RT,†‡# Blake Gilks, MD, FRCPC,†‡** Elizabeth Hyjek, MD, PhD,* Merdol Ibrahim, PhD,†† Rodney Miller, MD,‡‡ Søren Nielsen, HT, CT,§§‖‖ Eugen B. Petcu, MD, PhD,§ Paul E. Swanson, MD,¶¶ Clive R. Taylor, MD, PhD,## and Mogens Vyberg, MD§§‖‖*

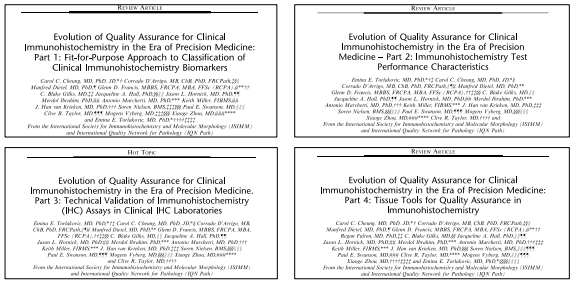
On-Slide Controls

RESEARCH ARTICLE

An Audit of Failed Immunohistochemical Slides in a Clinical Laboratory: The Role of On-Slide Controls

Carol C. Cheung, MD, PhD, JD,† Clive R. Taylor, MD, DPhil,‡ and Emina E. Torlakovic, MD, PhD†*

Quality Assurance Guidelines for Clinical Immunohistochemistry



REVIEW ARTICLE

Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 1: Fit-for-Purpose Approach to Classification of Clinical Immunohistochemistry Biomarkers

REVIEW ARTICLE

Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine – Part 2: Immunohistochemistry Test Performance Characteristics

HOT TOPIC

Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine. Part 3: Technical Validation of Immunohistochemistry (IHC) Assays in Clinical IHC Laboratories

REVIEW ARTICLE

Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 4: Tissue Tools for Quality Assurance in Immunohistochemistry

General IHC Tissue 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)

On-Slide Positive & Negative Control Tissues

Control tissues may be placed on slide prior to or after application of patient tissue

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

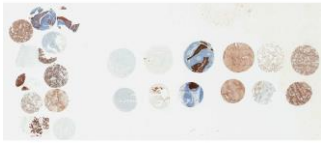
It is not only inappropriate to use positive controls that are processed differently from tested samples, but it may be diagnostically misleading

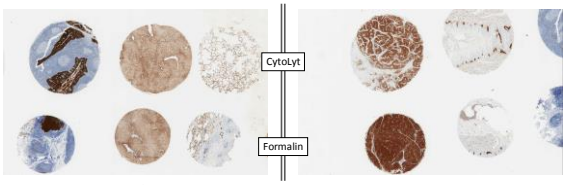
Cytology Specific Control Tissue Issues

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

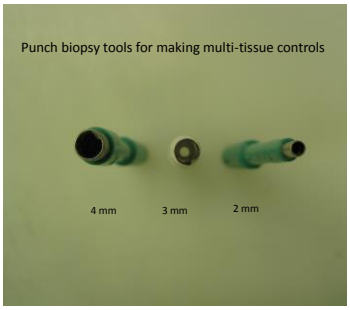
Because most of ICC is performed on cell blocks and IHC concordance has been demonstrated between tissue sections and cell blocks generated from the same tissue, Cytolyt fixed tissue sections can be used as on-slide controls.

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

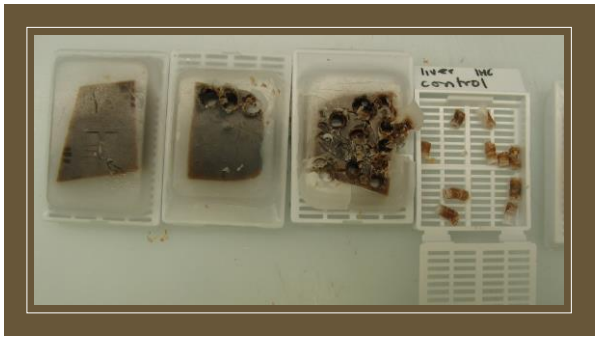


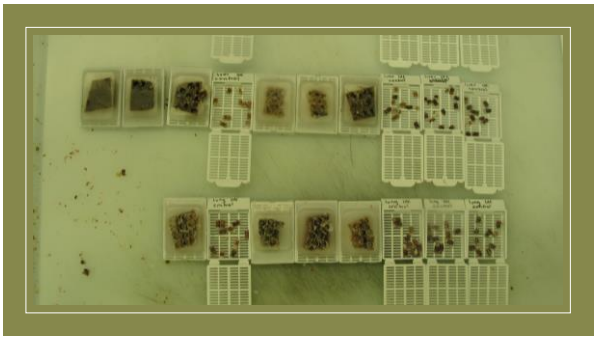


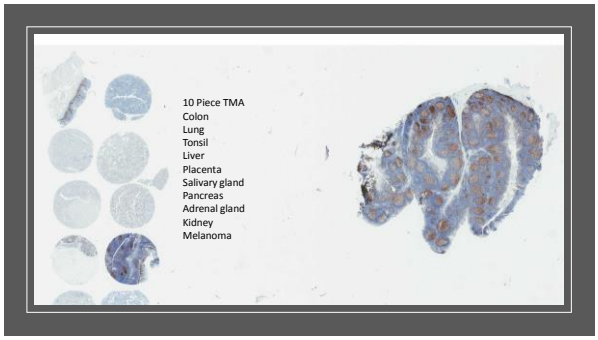
Validation of Punch Biopsy Control Tissues from CytoLyt Fixed Whole Tissue Sections vs from Formalin Fixed Whole Tissue Sections for On-Slide External Controls



Preparation of Tissue for Multi-Tissue Control Blocks







Conclusions

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