Common Misinformation's in Immunohistochemistry

Tri State Meeting
May 3, 2017

Steven Westra
Reagent Product Specialist
Leica Biosystems

Misinformation in Immunohistochemistry

- Flood of New Markers
- Diagnostic to Predictive
- The More The Better
- Tissue Microarray Hoax
- IHC Quantification

General Comments

- Some antibody's not new
- New (better) clones to old antigens
- Clones / working conditions: personal experience/preference
- Several excellent vendors, not one endorsed for antibodies

Unique IHC profiles

- HCC: CD34, Collagen IV
- Meningioma: EMA, PR
- Hemangioblastoma: S100, EGFR
- Barrett's esophagus: CDX2
- Hydatidiform mole: p57, FISH (ploidy)
- Hairy cell leukemia: CD20, CD25, TRAcP
- Fibromatosis: β-catenin (nuclear), SMA
- Glomus tumor: SMA (not desmin), +/- SNP

Unique IHC profiles

- Endometrioid CA: vimentin, β-catenin
- PML: SV40, p53
- Monophasic SS: Keratin, bcl2, CD99, β-catenin
- Solitary fibrous tumor: CD34, β-catenin
- PEComas: SMA, melanocytic markers
- Kaposi: HHV8
- Follicular dendritic cell tumor: CD21, CD35
- Inflammatory MFB tumor: SMA (filamentous), +/-EBV, +/-ALK
- Merkel cell tumor: Ck20+, Synaptophysin+, TTF-
- Cervical SIL: p16, MIB-1

Summary

- Avoid novel antibodies (except for experimentation) because:
  - Lack of specificity
  - Lack of track record
  - You don't want to be the Guinea Pig
- Acquire new antibodies only when specificity is verified and reproduced by serious investigators
- Acquire multi-purpose antibodies

Common Misinformations in Immunohistochemistry

- Preanalytic Aspects
- Analytical Factors
- Postanalytic Issues

CE Update—Anatomic Pathology II

Immunoperoxidase: Part I. The Technique and Its Pitfalls

Mehrdad Nadji, MD, and Azorides R. Morales, MD — Lab Medicine, 1983

The American Journal of Dermatopathology 8(1): 32–38, 1986.

REVIEW ARTICLE

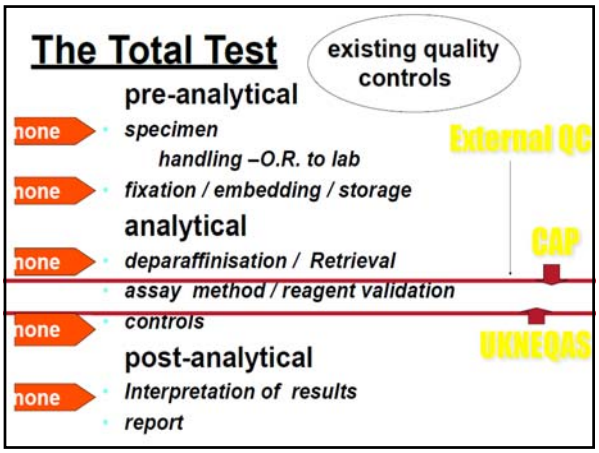
Immunoperoxidase Techniques

I. Facts and Artifacts

Mehrdad Nadji, M.D.

WARNING!

Practicing pathology accelerates aging!



Preanalytical Aspects

- Tissue Factors
  - Fixation
  - Processing
  - Decalcification
  - Storage

Myth:

- Formalin is the best fixative for immunohistochemistry.

Fact

- The best fixative for immunohistochemistry is no fixative. The next best is a mixture of alcohol and formalin.

	Advantage	Disadvantage
Formalin	• Better preservation of tissue morphology • Long term storage if paraffin embedded.	• Can mask epitope and reduce antigenicity. • Can affect expression of post translational modifications
Alcohol	• Better preservation of antigenicity. • Better suited for the study of DNA, RNA, and post translational modifications.	Can distort nuclear and cytoplasmic detail.

Myth:

- *A minimum of 6 hours is needed for formalin fixation.*

Fact:

- *A one hour fixation is quite adequate for small biopsies such as prostate and breast cores.*

Myth:

- *Overnight tissue processing is superior to rapid microwave technique.*

Fact:

- *Fixation is the most important factor in IHC; processing is not a determining factor.*

Pre-Analytic

Effects of Fixation/Processing

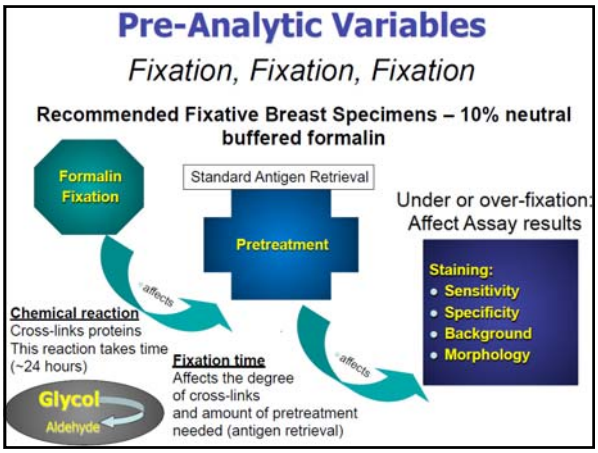
- Change in acidophilic/basophilic properties
- Destruction or masking of antigen epitopes
- Change in morphology
- **All of these effects are minimized by optimal formalin fixation**

	What is it	Effect
Underfixation	Incomplete fixation, immersion in fixative too short	• Can result in autolysis and target protein degradation • Can produce staining artifacts when dehydrating alcohols are used on under-fixed tissues
Overfixation	Excessive fixation, immersion in fixative too long	• Can result in epitope masking • Can produce strong non-specific staining.

R.Eisen, M.D.
1/25/2010
IHC-Course

Essentials for Q/C- Pretest variables

- Proper grossing, fixation and cutting of tissues
- Fixation: Under fixation is the biggest obstacle today given the use of antigen retrieval techniques- loss of antigen expression due to under fixation cannot be corrected
- Grossing: assuring that the appropriate regions of a tumor/ specimen are sampled; including benign tissue with tumor in the block for retention of normal internal controls (ER)



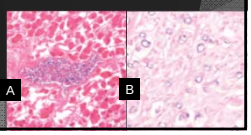
Molecular

**Over-fixed/under-digested tissue: Tissue morphology looks excellent with weak/no signal and low signal/background ratio due to poor probe accessibility.
*Under-fixed/over-digested tissue: Poor tissue morphology (tissue appear faded with loss of cell borders), loss of RNA due to protease over-digestion.

Pre-Analytic

Processing Reality

- "Over-processing" simply does not occur in any reasonable time frame
- Poor-processing is usually the result of:
  - Short fixation in formalin
  - Refixation in alcohol
- Under-fixation and processing is the norm
  - Under-fixation is the root cause of all problems



Myth:

- *Decalcification adversely affects IHC results.*

Fact:

- *Decalcification has no ill effects, provided that specimen is adequately fixed first.*

Preanalytical Aspects

- Tissue Factors
  - Fixation
  - Processing
  - Decalcification
  - Storage

Myth:

- *Unstained recuts of control tissue can be stored indefinitely.*

Fact:

- *Depending on the antigen, recut slides lose their reactivity anywhere from a few weeks to a few months.*

Myth:

- *Paraffin blocks lose their antigens with prolonged storage*

Fact

- *Not if the cut surface is re-covered by molten paraffin.*

Preanalytical Factors

- Estimated Preanalytical Variables: 61
- Those With Published Studies: 27
  - Many with conflicting conclusions!

Misinformation in Immunohistochemistry

- Preanalytic Aspects
- Analytical Factors
- Postanalytic Issues

Analytical Aspects

- Immunohistochemistry
  - Antigen Retrieval
  - Primary Antibody
  - Detection System
  - Control Slides

Myth:

- *Antigen retrieval should be universally applied to all IHC protocols.*

Fact:

- *The use of antigen retrieval depends on the fixative and the primary antibody.*

Myth:

- *Antigen retrieval corrects poor or inadequate fixation.*

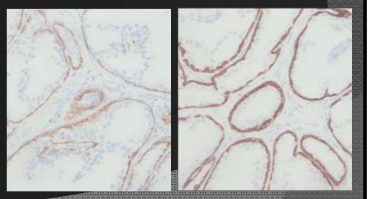
Fact:

- *Antigen retrieval only corrects OVER fixation not UNDER fixation.*

Analytic

Optimize retrieval methods

- ✗ The same retrieval technique is used for all primaries on the assumption that there is a successful universal HIER method.



Analytic

Antigen Retrieval

- Not all antibodies need antigen retrieval
- All antibodies are different
- Each antibody has to be accessed on an individual basis to determine the best antigen retrieval
- Even if an antibody does not require AR its dilution performance is often enhanced

Myth:

- *Post fixation of slides corrects under fixation for optimal IHC results.*

Fact:

- *Post fixation does not salvage antigen loss caused by initial delayed or inadequate fixation.*

Analytical Aspects

- Immunohistochemistry
  - Antigen Retrieval
  - Primary Antibody
  - Detection System
  - Control Slides

Primary Antibodies

- Monoclonal
  - Mouse
  - Rabbit
- Polyclonal
  - Rabbit
  - Goat
  - Others

Myth:

- *Rabbit monoclonals are more sensitive than mouse monoclonals.*

Fact:

- *Monoclonals are monoclonals; larger animals do not yield more sensitive reagents.*

Analytical Aspects

- Immunohistochemistry
  - Antigen Retrieval
  - Primary Antibody
  - Detection System
  - Control Slides

Myth:

- *More sensitive detection systems are preferable.*

Fact:

- *There is a trade off between higher sensitivity and higher quality IHC.*

Analytical Aspects

- Immunohistochemistry
  - Antigen Retrieval
  - Primary Antibody
  - Detection System
  - Control Slides

IHC Controls

- Known positive: A known positive tissue
- Known Negative: A known negative tissue
- Antibody Control: Replacement of antibody

Question:

What are the best positive and negative controls?

Answer:

The best positive and negative controls are internal controls.

Antibody selection

Before Optimizing Antibodies

- Determine appropriate control tissue - Is the antigen present?
  - Read the specification sheet for that antibody
  - Call the manufacturer for information about antibody
  - Look up Journal articles

Myth:

- *Antibody control is substitution of primary antibody with a buffer solution.*

Fact:

- *Antibody control is replacement of the primary antibody by an antibody with a different specificity.*

Misinformation in Immunohistochemistry

- Preanalytic Aspects
- Analytical Factors
- Postanalytic Issues

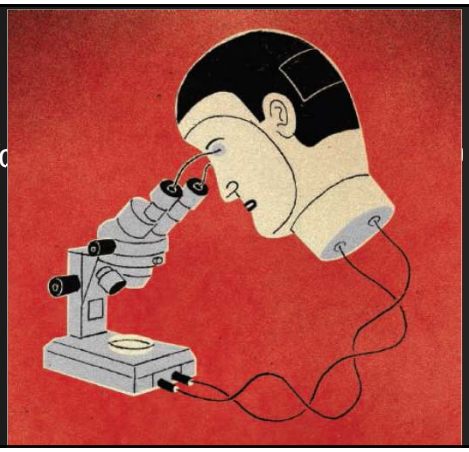
Myth:

- *IHC stains gradually lose their intensity in storage.*

Fact:

- *Not if DAB is used as the chromogen.*

Yo



Marker flood: how useful are they?

UM/JHS Lab, 2010

Potentially useful by literature	179
Commercially available	112
Tested in our laboratory	76
Selected for routine use	31

The More, the Better?

- Diagnostic IHC by buckshot approach
- Diagnostic IHC by pinpoint aiming

IHC: Potential Abuses

- Unnecessary utilization
- Irrational large panels

Why large IHC panels are used:

- Lack of cell specific markers
- Subjectivity of panel selection
- Experience of the observer
- ? Financial incentive (never)

IHC of Tissue Microarrays

To save time and money 

It is not representative 

Advantages

- Study of a great number of samples on the same slide;
- Uniform methodology for all samples on the same slide;
- Utilization of small quantities of tissue for the studies (minimizing the consume of material reagents);
- Reduction of costs in the reagents and time of processing;
- Preservation of the donor paraffin block for further analysis.

Disadvantages

- Inability to control the variability in the sampling and processing of the tissue before the analysis;
- Loss of tissue during the cut due to the area reduction;
- Poor characterization of the heterogeneity of a tumor (for example), can be solved by increasing the samples from the tumor different areas.

Optimization: Titrations & Controls

Sausage/Multi Tumor Block/Tissue Microarray

- Contain anywhere from 10 to 80 pieces of tissue in one block
- Time consuming to make, but will save time in the long run
- Include both strong, weak and if possible intermediate expression of antigen
- A skin punch biopsy needle is used to cut out representative tissue
  - 2 to 6 mm depending on personal preference, the size of the original sample in the block and the number of pieces in the block

wickipedia.org

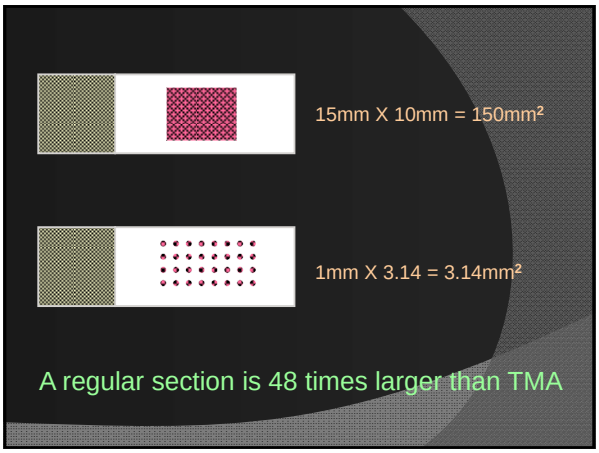
Optimization: Titrations & Controls

Tissue Control

4 multi-tissue control blocks for approximately 180/220 markers

- Block 1: Appendix, hepatocyte, tonsil, pancreas
- Block 2: Brain, striated muscle, skin, melanoma
- Block 3: Lung, prostate, placenta, thyroid
- Block 4: Thymus, bone marrow, Hodgkin Lymphoma, tonsil

- NordiQC



Diagnostic to Predictive

- Diagnostic IHC has been practiced as an art.

- Predictive IHC has to be used as a science.

IHC for predictive markers requires:

Standardization!

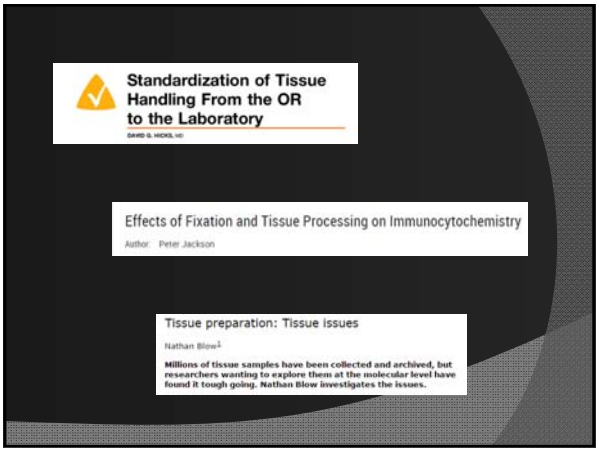
Standardization!

Standardization!

Standardization!

Where are we now with standardization?

Tissue Handling	NO
Tissue Fixation	NO
Tissue Processing	NO
Immunohistochemistry	Y/N
Image Analysis	YES



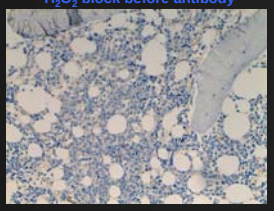
Antibody Diluent Matters

- Monoclonal antibodies are more susceptible
- Phosphate buffered saline (PBS) based diluents was always recommended
- Studies showed that it decreases reactivity of most of the monoclonal the antibodies
- Special antibody diluents are now commercially available

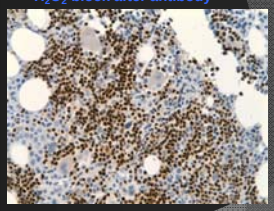
Analytic

Peroxidase Blocking

H_2O_2 block before antibody



H_2O_2 block after antibody



- Some antibodies (PAX-5) can be effected by the peroxidase blocking step
- Denaturing the epitope of recognition
- Simply switch these step so the antibody is incubated before the H_2O_2 block

IHC Quantification

- Without standardization of pre-analytical factors the results are unreliable.

- Are there biological or clinical reasons to quantify IHC?

Conclusion

- Avoid novel antibodies except for experimentation
  - Lack of track record, don't want to be the guinea pig
  - Lack of specificity
- Acquire new antibodies when specificity established and verified by experienced pathologists
- Acquire multipurpose antibodies
- Economic volume justification
- Cocktail as needed

Thank you