NATIONAL SOCIETY FOR HISTOTECHNOLOGY

32ND ANNUAL SYMPOSIUM / CONVENTION

SEPTEMBER 8TH – 13TH 2006

PHOENIX ARIZONA

Ken Green Immuno Lab

Division of Anatomical Pathology

Health Care Corporation of St. Johns

Eastern Health

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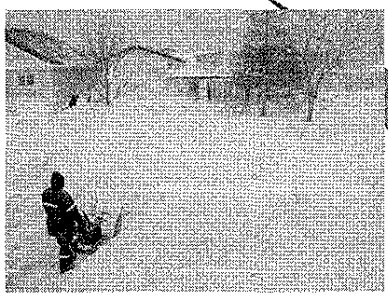
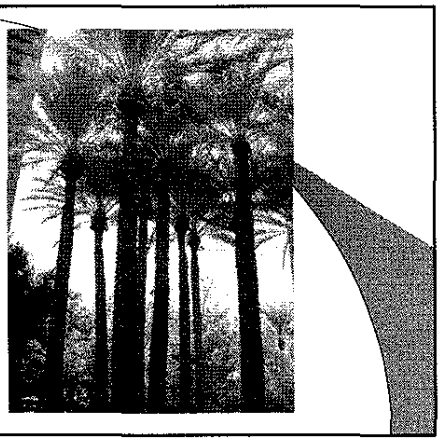
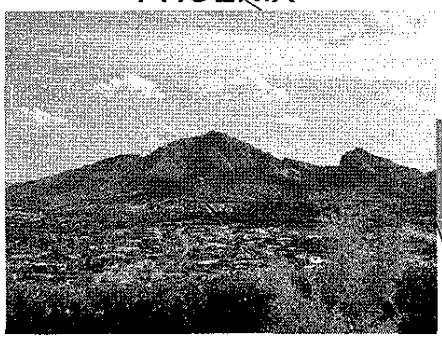
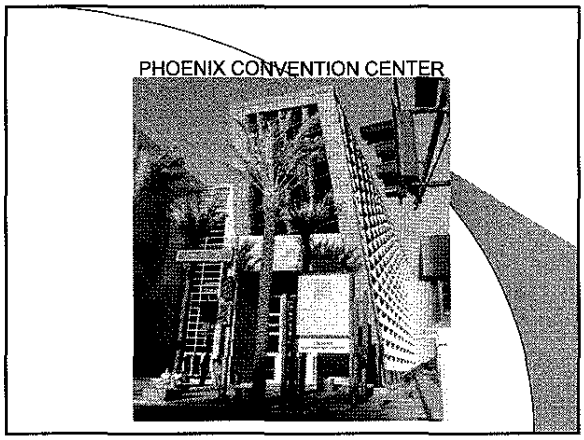
NEWFOUNDLAND





PHOENIX





8:00AM Saturday September 9th 2006
Introduction to Immuno Histrochemistry

Maria A. Geraci – Erck

Schering – Plough Research Institute

Lafayette, New Jersey

8:00AM Sunday September 10th 2006
Her 2 What's New?

Anita Ostrander
Pathology Manager
Seattle Cancer Care Alliance
Seattle, Washington

8:00AM Monday September 11th 2006

How to Make a New Antibody Work for Immuno Histochemistry

Chris M. Van Der Loos PHD
Dept. of Pathology
Amsterdam, Netherlands

Alton Floyd PHD
Consultant
Edwardsburg, Michigan

8:00AM Tuesday September 12th 2006
procedure Manuals

Quality System Essentials and Document Control

Peggy A. Wenk
Sharon E. Scalise

Anatomic Pathology
Royal Oak Michigan

8:00AM Wednesday September 13th 2006
Challenges in Providing an IHC Service

Bryan R. Hewlett
Quality Management Program, Laboratory Services
Ontario Medical Association
Toronto, Ontario

Neil M. Hand
Histopathology Dept.
Queens Medical Center
Nottingham England

HOYA

NSH

The National Society for Histotechnology is a non-profit organization, committed to the advancement of Histotechnology, its practitioners and quality standards of practice through leadership, education, and advocacy.

Overview of Convention

- All Histology and Pathology topics
- 53 Poster Sessions
- 100 Workshops
- Hundreds of vendors
- Histotechs and Pathologists
- Great network of contacts

Oncogenes

Today we understand that an oncogene "is a gene that when mutated or expressed at abnormally high levels contributes to converting a normal cell into a cancerous cell."

Oncogenes

- Axel Ullrich began studying a chemical found in the human body called epiderman growth factor (EGF).

Oncogenes

- Ullrich and Slamon discovered that the gene Her-2/new was connected to cancerous growth in both breast and ovarian cells.
- The Her-2/neu oncogene had not mutated like many other oncogenes, but instead produced the protein in very high levels. The Her-2/neu gene's protein appeared on the surface of breast cells as a receptor. Essentially , it receives signals telling the cell to grow and divide. When the her-2/neu gene is mutated it over expresses the protein and the cell grows rapidly and becomes cancerous.

Genentech Clinical Drug Trials

- Out of the results of the Phase II Clinical Trial came the thought that although Her-2/neu antibody might not "cure" cancer, it might become a type of 'cancer control" treatment. Traditionally chemotherapy drugs are toxic so physicians can administer them to patients for only a limited amount of time. It was thought that the Her-2/neu antibody might be given to patients for an indefinite period of time in order to delay progression of a patient's cancer.

Side Effects of Herceptin

- Side effects often occur during the first thretment with Herceptin. They include fever, chills, nausea, headaches, difficulty breathing, vomiting, and diarrhea. These side effects usually lessen or disappear with subsequent treatments with Herceptin.
- Herceptin can also cause significant damage to the heart muscle which can lead to heart failure.
- In patients with breathing difficulties or lung disease. Herceptin can cause serious breathing problems.

Herceptin Today

- Currently 25 to 30 percent of breast cancers over express Her-2. These patients usually have a more aggressive form of cancer and may relapse sooner after treatment than patients with cancers that do not overexpress Her-2

Hercepin

- Herceptin is a monoclonal antibody that is given intravenously to treat certain breast cancers.
- Herceptin targeted cancer cells that over express a protein called Her-2 which is found on the surface of cancer cells.
- We now understand that Herceptin works in three different ways.

How Herceptin Works

- It binds to the receptors on the surfaces of tumor cells. The receptors are pulled back into the cell and stop telling the cell to grow and divide.
- When given together with chemotherapy it prevents cells that are damaged from repairing themselves. These cells die and tumor growth is slowed.
- It attracts natural killer (NK) cells. These cells kill the tumor cells.

IHC Test

- If a patient's test is 3+ the cancer is considered Her-2 positive and the patient is a candidate for Herceptin
- If the patient's test is 0 or 1+ the cancer is considered Her-2 negative and the patient is not a candidate for Herceptin.
- If a patient's test is 2+ the cancer is borderline and usually the patient's physician will have the tumor tested with the FISH test.

FISH Test

- Fluorescence In Situ Hybridization.
- DNA probes "paint" the Her-2 genes in the tumor cells. If increased copies are detected the cancer is considered Her-2 positive, and the patient is a candidate for Herceptin.
- FISH results are either positive or negative.

IHC Disadvantages

- Fixation can play a large part in false relults when testing for Her-2
- If there is a delay in time after the tissue is removed for the body and before it is put into fixative, this can effect the IHC staining.
- If tissue is over fixed or under fixed it can affect IHC staining.

Antigen Retrieval

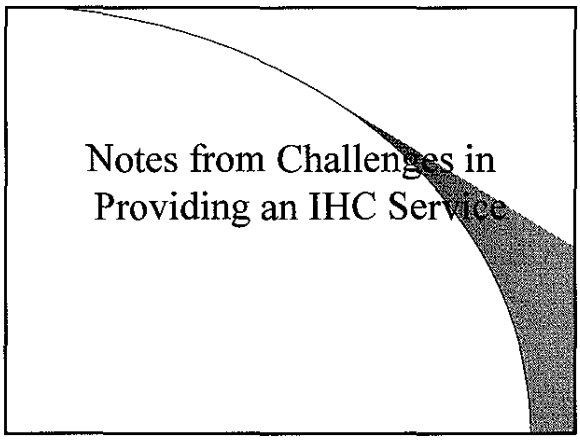
- Errors in Antigen Retrieval Procedures can also greatly effect Her-2 results.

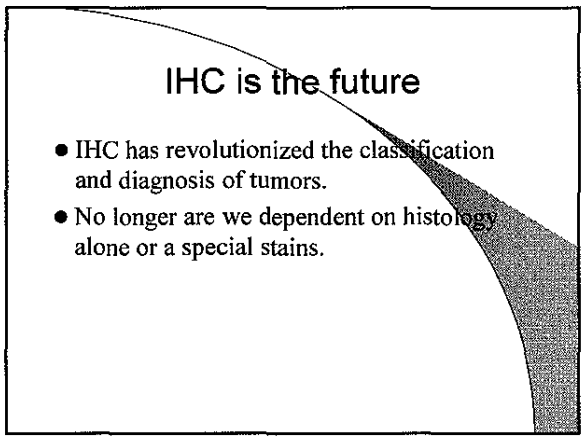
Breast Cancer Today

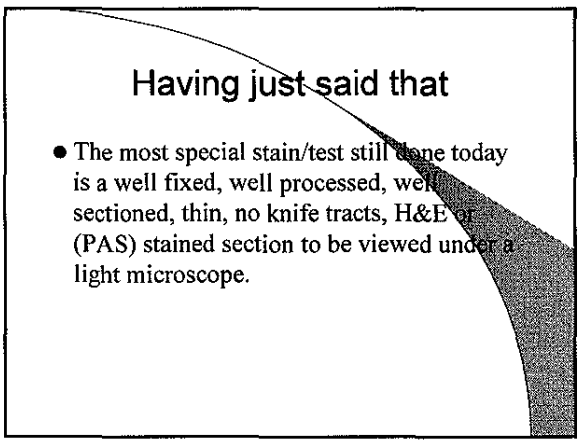
- It is the most common form of cancer found in women.
- It is the second leading cause of cancer deaths in women.
- In 2004 215,990 women in the U.S. were diagnosed with invasive breast cancer.
- In 2004 40,580 women in the U.S. died from breast cancer.

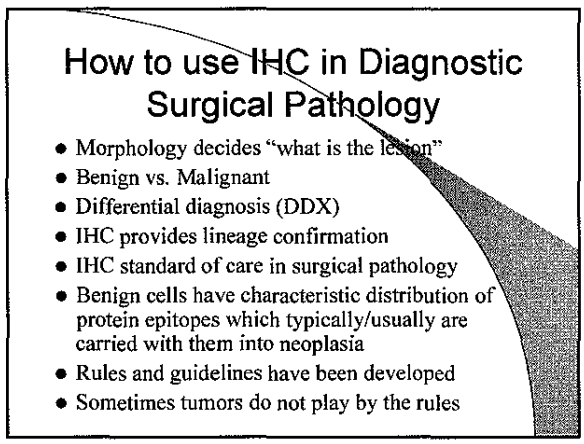
Future?

- Clinicians and Researchers predict that in just five years or six years entirely different methods of testing will be used.


Notes from Challenges in Providing an IHC Service


IHC is the future
IHC has revolutionized the classification and diagnosis of tumors.
No longer are we dependent on histology alone or a special stains.


Having just said that
The most special stain/test still done today is a well fixed, well processed, well sectioned, thin, no knife tracts, H&E or (PAS) stained section to be viewed under a light microscope.


How to use IHC in Diagnostic Surgical Pathology
Morphology decides "what is the lesion"
Benign vs. Malignant
Differential diagnosis (DDX)
IHC provides lineage confirmation
IHC standard of care in surgical pathology
Benign cells have characteristic distribution of protein epitopes which typically/usually are carried with them into neoplasia
Rules and guidelines have been developed
Sometimes tumors do not play by the rules

How to use IHC in Diagnostic Surgical Pathology

- Numerous antibodies and clones available
- Need to put in coherent order/use
- Pathologists do this by organizing by tumor type & how IHC can assist in different situations.
- May need to use multiple antibodies or panels to solve diagnostic dilemma
- Not all tumors +, or only certain % of tumors express antigen

10 Commandments of IHC

- 1. Start with a reasonable Differential Diagnosis (DDX)
- 2. "Never" use 1 Antibody in a DDX
- 3. Use a panel
- 4. Sensitivity must be high
- 5. Use Monoclonal ABS when possible
- 6. Control should match slide
- 7. Use your own database
- 8. If results are weird, investigate further
- 9. If a beginner or a problem arises, GET HELP!!
- 10. Send uncommon/rare cases to an expert

Why are there particular challenges in Immunohistochemistry?

- Results are affected by other staff/procedures
- Biological variation
- Sample collection
- Fixation/processing
- Section thickness

Why are there particular challenges in immunohistochemistry?

- Complex techniques
- IHC technically complex
- No aspect of this complexity should be ignored

Why are there particular challenges in immunohistochemistry?

- Little standardization?
- Many different protocols in use
- Sensitivity varies with small changes in protocol
- Specificity varies with small changes in protocol
- Wide range of reported results

Why are there particular challenges in immunohistochemistry?

New antobodies

- Mouse Monoclonal
- Rabbit Polyclonal
- Rabbit Monoclonal
- Double antigen staining
- Multiple antigen staining

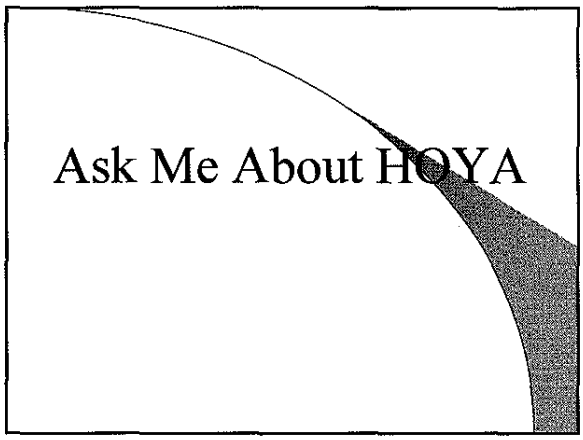
Setting technical quality specifications for staining

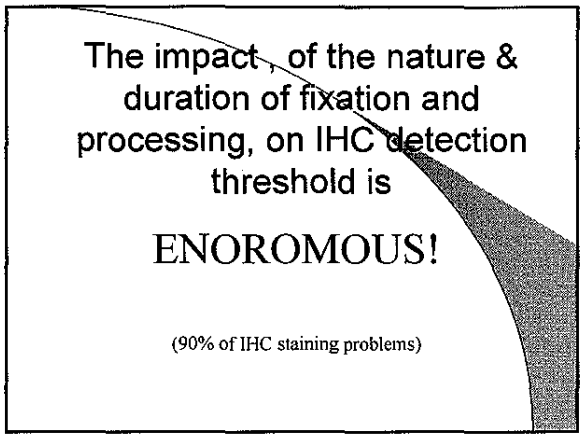
The Goal:-

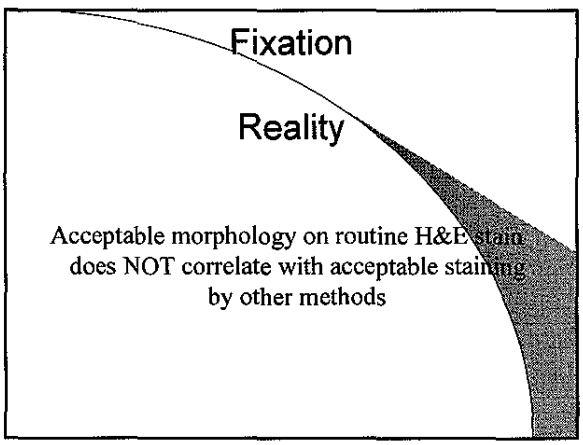
To ensure run-to-run reproducibility, evaluated against standardization control sections, days or weeks apart.

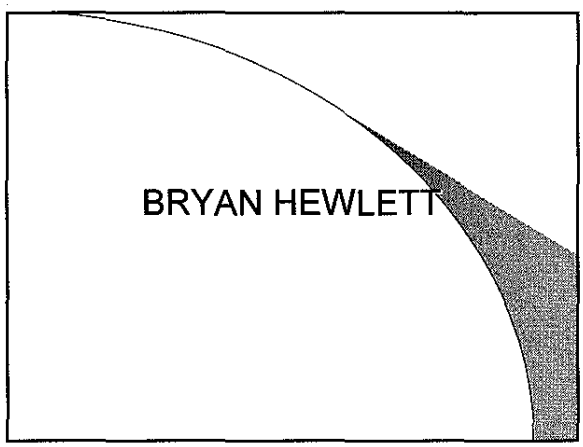
Factors effecting the IHC detection threshold

Biological variation
Sample Collection
Fixation/processing
Section thickness
Section pre-treatment
Antibody type/clone
Antibody-dilution
Sensitivity of detection reagents
Histochemical reaction


Ask Me About HOYA


The impact , of the nature & duration of fixation and processing, on IHC detection threshold is
ENOROMOUS!
(90% of IHC staining problems)


Fixation
Reality
Acceptable morphology on routine H&E stain does NOT correlate with acceptable staining by other methods


BRYAN HEWLETT

Formaldehyde fixation
How long will it take to fix?

The formaldehyde paradox.

Histologists have known for more than 70 years, that fixation of tissue in formaldehyde demonstrates a bizarre effect.

Namely, that formaldehyde is one of the fastest fixing agents to penetrate the tissue but one on the slowest to fix.

Formaldehyde fixation

For initial stabilization of fixation to occur binding time is crucial, not penetration time.

= 24 hours minimun for a
1.5 mm thick core biopsy
= 24 hours minimum for a
5mm thick tissue slice.

Binding time + penetration time = Reacation rate!

Fixation myths

Formaldehyde fixes at a rate of 1.0 mm/hour

Small pieces of tissue fix faster than larger pieces

The optimal fixation time for formaldehyde is 24 hours

Reality #1

No universal standard exists so what, exactly, Is a routinely formaldehyde-fixed, paraffin section?

Your routine or mine?

Routine formaldehyde fixation/processing;

Results in:-

Variable morphology and cell content
Variable shrinkage and hardening
Variable making/destruction of epitopes
Variable porosity
Variable basophilic/acidophillic relations
Variable intensity of stains

variable success/failure of IHC techniques!

Recommended fixation times

Standardize fixation times for all tissues requiring prognostic/predictive markers.!

(24 hour minimum in NBF)

BH

Pre-treatment

- May un-mask or improve the accessibility of epitopes
- May damage other epitopes
- May enhance unwanted cross-reactions
- Inappropriate pre-treatment may affect results
- May degrade morphology

Heat induced epitope recovery (HIER)

May un-mask or improve the accessibility to epitopes hidden by cross-linking or structural alteration of the protein during fixation by using heat and EDTA/citrate buffers.

Proteolytic digestion (PIER)

- May un-mask hidden protein cross-linking during fixation or otherwise to improve accessibility to epitopes by using enzymes.

Pre-treatment

There is NO standards for pre-treatment!

For PIER, standardize the time, temperature, and concentration.

For HIER standardize the time, temperature, and buffer concentration.

Myth

"Every antibody should have pre-treatment".

Reality

Each antigen-antibody is unique.

Antibody dilution

In general, the working dilution of an antibody should be the highest dilution which results in optimal specific staining with the least background.

Antibody dilution

Working dilution is determined by:

Fixation/processing
Pre-treatment used
Antibody type/clone
Incubation conditions
Sensitivity of detection reagents

Every lab is different

Factors affecting a IHC service

Methodologies
Equipment
Turnaround Time (TAT)
Quality Assurance
Staffing
Cost
Training/education

How to improve IHC

- Training
- Lectures
- Case studies
- Projects
- Presentations
- Teaching
- Money

IHC HCCSJ

3 step indirect (streptavidin) method

- Primary antibody against tissue antigen
- Secondary (which recognizes primary) will be tagged with biotin
- 3rd layer will be a strepavidin – enzyme complex allowing the streptavdin to recognize the biotin.
- Dab added to precipitate a color reaction

Immuno Lab at HCCSJ uses:

Ventana Benchmark

Ventana Benchmark XT

Automated systems to achieve the three step indirect (streptavidin) method.

Benchmark Automated System

- Depariffinization – on board
- Cell Conditioning (AR) – on board
- Individual thermo pad slide holders (can run AR and non AR simultaneously)
- Slides run at 42 degrees C
- 20 slide capacity
- 12 Antibody simultaneously
- Counter staining (Hematoxlyn) and bluing on Board.

Benchmark XT
All features of the Benchmark plus

- Temperature selectivity
- Primary antibody can be run at 37 degrees C 42 degrees c Room Temp
- 30 slide capacity
- Individual controlled thermo pad slide holders enabling adjacent slides to run at the selected temperature.

MYTH

Immunohistrochemistry is simply a Antigen / Antibody reaction

FACT

There is nothing simple about Immunohistochemistry

Technically complex procedure – 60 – 80 steps all being influenced by extraneous factors from specimen collection, fixation, processing, and cutting.

Resources

- Manufactures literature (package inserts)
- Immuno websites
- Network on contacts, people who use the same antibodies and equipment.
- Conferences (NSH)

BEWARE

HOYA