Precision Medicine requires Precision Diagnostics.

Many of the targets for personalized therapy are proteins.

IHC is, in theory, an ideal method for their detection and measurement.

------but we need to improve IHC performance, choice of controls and interpretation of results.

Disclosures; CRT –has consulting arrangements with for Philips, Agilent, PerkinElmer, Optra
**Theme**

Pathology is technology driven

“**The Age of the Microscope**”
180 years ago the microscope changed everything in medicine

“**The Age of the Intelligent Microscope**”
NOW - change of comparable magnitude is occurring today
– driven by two technologies

- **Molecular** (genetic) methods
- **Digital** computer analysis

From Magic to Molecules: An Illustrated History of Disease
Technology drives everything

Circa - 1700
Microscope invented

Circa - 1840
The microscope ‘invents’
Surgical Pathology

Better quality
Lower cost
Wider availability
The microscope—medical adoption was slow due to poor resolution and cost

Joseph Jackson Lister

Sir James Paget. 1854

‘Lectures on Surgical Pathology’, based on a series of 36 lectures given at the College of Surgeons 1847-1852.

Rudolf Virchow 1858

Both Classified cancers Depicted cancer cells

Der eigentliche Krebs hat auch Elemente von epithelialem Habitus, und Sie brauchen nur eben solche Punkte im Körper zu suchen, wo sich die Epithelzellen unregelmässig entwickeln, z. B. an den Harnwegen (Fig. 15), wo werden Sie dieselben sonderbaren, mit grossen Kernen und Kernkörperehen versehenen Bildungen antreffen, welche man als die spezifischen, polymorphen Krebszellen schildert. Der Krebs, das Cancroid oder Epithelium, die Perlgeschwulst oder das Cholesteatom, ja vielleicht das Dermoid, welches Haare, Zähne, Talgdrüsen produziert, wie sie im Eierstock so häufig vorkommen, alle
Ushered in the Age of the Microscope - for 150 years
THE Diagnosis was by H&E
- image analysis by mind and microscope !!! 1850 - 2017

Cajal  Maximow  Weigert
Hodgkin
Aschoff  Ehrlich  Lukes  Virchow
For 150 + yrs - H&E - formalin paraffin section – diagnostic opinion by a pathologist STILL TRUE IN 2017

80 years ago.
Immunofluorescent labeling – on frozen sections
Albert Coons, Astrid Fagraeus and others

Limited use in AP As Flu method loses GOLD STANDARD of morphology

40 + years ago.
IHC on FFPE tissue added in 1974
Taylor, Burns, Mason et al Oxford
Combined immunology with morphology note also first ‘multiplex’ IHC stain
From 1974 - 1998 IHC was just a ‘special stain’

SUDDENLY
THINGS CHANGED
-The ‘quality’ of IHC was no longer sufficient
-Quantification was at best an estimate

1998 – saw the first Companion Diagnostic
It marked the beginning of Precision Medicine.
A targeted therapeutic - is a ‘drug’ that---
- targets a specific molecule on a cell/tumor
- need to identify which patients respond

COMPANION DIAGNOSTIC - is a ‘classifier’

RESPONDERS V NON-RESPONDERS

KEY - Linked to defined therapeutic by data

eg. HER2
eg. HERCEPTIN therapy
eg. HERCEPT test
Detects
Response data

To use in this way IHC must be more than just a stain

Prognostic versus predictive value of biomarkers in oncology
Oldenhuis et al; 44, 946, 2008
How many companion diagnostics??? **Industry says “A LOT”**

**Progress of Companion Diagnostics**

**Approved US Drug and CDx Combinations**

- New Drug/New Test
- New Drug/Marketed Test
- Marketed Drug/New Test
- Marketed Drug/Marketed Test

- **Herceptin/HercepTest** (1998)
- **Erbitux/EGFR pharmDX** (2004)
- **Gleevec/c-KIT pharmDX** (2005)
- **Vectibix/EGFR pharmDX** (2006)
- **Xalkori/Vysis ALK Break Apart FISH Probe**
- **Erbitux/therascreen KRAS RGQ PCR**
- **Zelboraf/cobas BRAF V600 Mutation Test**
- **Perjeta/HercepTest**
- **Tarceva/cobas EGFR Mutation Test**
- **Gilotrif/therascreenE GFR RGQ PCR**
- **Kadcyla/HER2 FISH pharmDX**
- **Mekinist/THxID BRAF Kit**
- **Tafinlar/THxID BRAF**
- **Exjade/FerriScan R2 MRI**
- **Kadcyla/HercepTest**
- **Gilotrif/therascreenE GFR RGQ**

Companion diagnostics partnerships grew from 8 in 2008 to 90+ in 2014.

Bristol-Myers Squibb
How many will we need? ‘BUSINESS’ also says “A LOT”

Industry recognizes the opportunity and are willing to work with anyone

Roche to Acquire Ventana for $3.4 Billion
BY JEANNE WHALEN
January 23, 2008

After trying for seven months to win over Ventana Medical Systems Inc., Swiss drug giant Roche Holding AG said it reached an agreement to acquire the U.S. diagnostics company for $3.4 billion.

The deal advances Roche’s aim of diversifying more into machines and other tools that help diagnose and monitor disease. Ventana’s board approved the deal after Roche raised its offer by 19%.

Ventana makes a diagnostic disease. Roche believes that could lead to a greater share of market share in the future.

Roche has now agreed to buy 10x stock on signs of growth in the pharmaceutical industry. Meanwhile, sales of diagnostics firms are growing at their slowest pace in years, and are not expected to grow much in any quarters.

Roche’s prescription drug lines have fared better than those of many rivals, but the company is still hedging its bets by expanding more into diagnostics. In addition to Ventana, Roche has said it would acquire two other diagnostics companies: closely held NimbleGen Systems Inc., of Madison, Wis., for $272.5 million, and Beavers Corp., of Gaithersburg, Md., for $600 million.

Are diagnostics the new wonder drug on Wall Street?

Franz Humer

FEEDING FRENZY - but concerns at many levels
How many companion diagnostics??? Science also says “A LOT”

COMPANION DIAGNOSTICS detect these ‘target’molecules

Molecular classification of colon adenocarcinoma

Different cases of colon cancer

different mutated ‘driver’ genes

Different molecules targets for therapy

HOW WILL WE DO IT?

IHC  
FISH  
PCR  
NGS

ANY WAY WE CAN!!
Immunohistochemistry  
Fluorescent in situ hybrid  
Polymerase chain reaction  
Next generation sequencing
As a result - the role of pathology has changed

YEAR 2010
Morphologic classification of lung

- NSCLC: Adenocarcinoma
- NSCLC: Large cell
- NSCLC: Squamous cell
- SCLC

TODAY
Molecular classification of lung adenocarcinoma

- unknown
- EGFR
- EML4-ALK
- MEK
- FGFR4
- BRAF
- HER2
- PIK3CA
CANNOT do this by H&E
Thus in lung cancer alone –
Many Companion Dxs needed for molecular classification.

NSCLC: Adenocarcinoma
NSCLC: Squamous cell
NSCLC: Large cell

ALK
BRAF
HER2
MET
KRAS
MEK
PIK3CA
FGFR4
EGFR
PD-L1
unknown

Proteintech, Ventana, Dako, Biosource, CRT

Gu, Taylor
AIMM 2014
Up to this time IHC was used to produce 100 s of ‘special stains’ on FFPE tissues

Same rationale as for any other stain, --to produce a different color to assist cell / tissue recognition.

But over the past 100 years this approach has produced some very bad habits
Routine stain H & E

‘Special Stain’ - IHC CD30

No controls

The result is adjusted to ‘please the pathologist’

MUST have positive and negative controls

Should not be adjusted to ‘please the pathologist’

Reproducibility is poor

- run to run
- day to day
- lab to lab

Result

- IHC quality poor and variable
- quantification not possible
So - what is the problem?
IHC detects targeted protein –
BUT - IHC is just a stain -

Can we achieve
--- consistency and the quality
to turn IHC into a
‘quantitative’ assay??

Her2 result – current
+      ++      +++

Her2 result - quantitative
mean value surface expression
100   1000   10,000
attograms/cancer cell

To convert a ‘stain’ to an ‘assay’
Validation & Controls & interpretation
must be more rigorous

In Situ Proteomics – ISP
Measuring protein per cell
Can we achieve
-improved quality?
-true quantification?

Right now IHC has elements of witchcraft - labs ‘do their own thing’

1. Require a detailed strict protocol with controls

2. Require that we follow the protocol exactly

3. Require BETTER controls to assure that we are doing it
The problem is in detail---
Can we ‘control’ the UNCONTROLLED VARIABLES?

**Is the variation ‘real’ = biology?**
Or is it due to --

1. ‘Poor sample preparation’ - variable fixation
2. Variable section thickness
3. Variable IHC/AR Protocol / different labs
4. Variable chromogen development
5. Section heterogeneity
6. Variation in pathologist scoring / subjective

“scoring” Predictive Markers is crude

<table>
<thead>
<tr>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS THE SCORE ‘REAL’ ??</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Many ‘uncontrolled’ assay variables
How can we improve??

**Better controls would be a good start**

Taylor, Becker, AIMM 2011
Taylor  AIMM 2014, CTR 2015
Part Solution — turn the Anatomic Pathology lab into a Clinical Lab

**Clinical Lab**
- Highly automated
- Strict protocols
- Validated reagents
- Rigorously controlled
- Universal reference standards

**Regular AP lab**
- None of these

Multiple manual steps of IHC ‘stain’ are difficult to reproduce manually
Immunohistochemistry = Enzyme Linked ImmunoSorbent Assay

SAME REAGENTS
SAME PRINCIPLES

Sample prep uncontrolled
Partly automated
No universal reference standard
Poor reproducibility
Not quantifiable

Sample preparation controlled
Fully automated
Universal reference standard
Excellent reproducibility
Strictly quantitative
True we cannot control everything – but there are some possible approaches to improvement of IHC

The model - Convert IHC to an ELISA type approach on tissue
- turn a ‘qualitative stain’ into a ‘quantitative immunoassay’

1. Consider all phases of IHC - THE TOTAL TEST

2. PRE-ANALYTIC - Control or Qualify Sample Preparation

3. ANALYTIC - Use same control materials in all IHC labs

4. ANALYTIC - Produce a Quantifiable Reference Standard for calibration

5. POST–ANALYTIC - Score Predictive Markers by digital analysis
Within Lab and from Lab to Lab - Sample Preparation one of the biggest problems

PRE-ANALYTIC VARIABLES.

WARM ISCHEMIA – surgery, vessels clamped

COLD ISCHEMIA - (transport, fix?, gross schedule)

GROSSING – block size – penetration reagents

FIXATION – type, (formalin) freshness, pH, TOTAL TIME

PROCESSING, - alcohol stages, xylol (TIME) paraffin temp

STORAGE - as block

CUTTING – thickness, evenness, tears

TIME LAPSE to staining
Immunohistochemistry (IHC) to in situ proteomics (ISP)

- **a ‘stain’**
- **a measurement**

**THE TOTAL TEST** Standardization & Quantification in IHC
The Road to In Situ Proteomics.

**STANDARDIZE & CONTROL EVERYTHING**

- **Pre-analytic**
  - Fixation, processing, cutting

- **Analytic**
  - Retrieval
  - Reagents, Protocols, Basic controls

- **Post-analytic**
  - Interpretation, scoring, Reporting

THE TOTAL TEST: Standardization & Quantification in IHC
The Road to In Situ Proteomics.
Analytic - No Shortage of Reagents

Georges J. F. Köhler César Milstein
Nobel Prize in Physiology and Medicine
1984

Max Planck Institute
For Immunobiology
Freiburg.

British medical Council
Laboratory for Molecular
Biology, Cambridge

HYBRIDOMAS
Monoclonal antibodies

Or detection methods
Or retrieval methods
Or automated platforms
Or opinions

But there is a shortage of
VALIDATION: including lack of validated controls
“XXXX Abs Inc” (USA) has increased the number of validated IHC antibodies available in its catalog to more than 3,500. IHC antibodies extensively tested against formalin-fixed paraffin-embedded (FFPE) human tissues.

Immunohistochemistry can detect any protein encoded by the 21,000 genes in the human genome.”

Catalog includes 83,400 monoclonal and polyclonal Abs to 13,000 targets.

IHC Collection— “YYYY Abs Inc” (Taiwan) IHC collection of 8600+ antibodies targeting human genes, (tissue microarray for novel biomarker discovery), and 400+ antibodies in Pathology research.
<table>
<thead>
<tr>
<th>Run 96</th>
<th>Run 97</th>
</tr>
</thead>
<tbody>
<tr>
<td>365 participants</td>
<td>365 participants</td>
</tr>
<tr>
<td>Markers</td>
<td>Markers</td>
</tr>
<tr>
<td>SMA, CK</td>
<td>SMA, CD34/CD31</td>
</tr>
</tbody>
</table>

## Retrieval
- **Heat**:
  - 297 labs; 76% acceptable results
  - Enzymatic: 146 labs; 32% acceptable

## Retrieval reagents
- Mostly pH6 or 9

### Primary Antibodies
- **SMA**
  - 18 antibodies from 10 suppliers
- **CK**
  - 26 antibodies from 16 suppliers

### Primary Antibodies
- **SMA**
  - 20 antibodies from 9 suppliers
- **CD34/CD31**
  - 25 antibodies, 11 suppliers

## Detection Reagents
- 26 different detection reagents from 13 suppliers

## Detection Reagents
- 23 different detection reagents from 11 suppliers

## Autostainers
- 17 different instruments from 7 suppliers

## Autostainers
- 17 different instruments from 7 suppliers

## Chromogen+
- Great majority used DAB from 19 suppliers

## Chromogen+
- Great majority used DAB from 11 suppliers

---

**NordiQC & UK data - NO SHORTAGE OF PROTOCOLS**
What about the controls??

CONTROLS

To assure quality
MORE STANDARD CONTROLS

INTERPRETATION

AUTOMATION HELPS ACHIEVE THIS

PROTOCOL

Fixation
Primary ab
Secondary ab
Label
Chromogen
Method
AR
Background

MORE STANDARD CONTROLS

CONTROLS
National Institute of Standards & Technology
Reference Standard (control) - Requirements

Table 5  Summary of required characteristics of any reference standard that would provide a basis for accurate quantification of IHC on FFPE tissue

<table>
<thead>
<tr>
<th>Immunohistochemical reference standard: requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>It must be subjected to all of the same rigors of sample preparation (ischemia, transport, fixation) as the “test” tissue</td>
</tr>
<tr>
<td>It must be integrated into all steps of the test (assay) protocol, including evaluation of the result</td>
</tr>
<tr>
<td>It should contain a known amount of the reference standard protein</td>
</tr>
<tr>
<td>It should be universally available to all laboratories performing the assay</td>
</tr>
<tr>
<td>It should be inexhaustible and inexpensive</td>
</tr>
</tbody>
</table>

Taylor CR. **Quantitative In Situ Proteomics**--Cell Tissue Res. 2015; 360:109-120.
‘in house’ control tissue (block)
– is in fact an ‘external’ tissue control
-- has similar but not same FFPE as test tissue
-- a finite amount of control tissue block(s)
- so a lab must make **new controls all the time**
- and **every lab in fact has different controls**
-- therefore not quantifiable

**So what are the possibilities??**
1998. HercepTest – included Cell lines as ‘RUN’ controls
To assure greater consistency
- in labs
- and among labs

POTENTIAL NEW CONTROLS
Cell line controls should be validated in the context of their use
Note – do not control pre-analytic phase

Bioengineered Cell lines. PDL-1 by IHC
High
medium
neg

Courtesy Farah Patell-Socha
Horizon Discovery, Cambridge, UK.

HercepTest
Interpretation manual
Dako
WHAT CHOICES do we have? -- Existing types of ‘controls’

- Tissue block - ‘known’
- Sausage block
- Micro-tissue array
- Cell line cytoprep.
- Cell line block
- Peptide ‘dots’
- Faux Tissue

All provide some control of assay
--- But limited control of sample prep
--- Cell lines, and ‘spots’ –potentially quantify

Histoid MC7 + FSF
Breast ca section

E cadherin
ER
HER 2 Ki-67

3D. FAUX TISSUE
Mimics morphology
Potentially -
-- Quantifiable
-- universal

POTENTIAL NEW CONTROLS
Improved validated cell line controls – retaining morphology

Courtesy Dr A Imam
StatLabs, Texas.

Human Breast Cancer Histoid: An In Vitro 3-Dimensional Co-culture Model That Mimics Breast Cancer Tissue

Pavinder Kaur, Brenda Ward, Baisakhi Saha, Lillian Young, Susan Groschen, Geza Techy, Yani Lu, Roscoe Atkinson, Clive R. Taylor, Marylou Ingram, and S. Ashraf Imam

Molecular Pathology Program (PK, BS, SAI) and In Vitro System/Tissue Engineering Program (BWW, GT, MI), Huntington Medical Research Institutes, Pasadena, California, Departments of Pathology (LY, RA, CRT) and Preventive Medicine (SG), University of Southern California Keck School of Medicine, Los Angeles, California, and Department of Population Sciences (YI), City of Hope, Duarte, California
POTENTIAL NEW CONTROLS

Tissue Internal Controls have been used for years to inform on ‘quality’ – but we can do better

**Plasma cells**  Ig , K, L  
Internal controls  
Taylor and Burns, 1974

**VIMENTIN**  
Used as fixation guide  
Batttifora et al

**Estrogen Receptor**  
ER on residual normal breast  
Serves as internal fixation and method control


Torlakovic et al. Getting controls under control - the time is now for immunohistochemistry. J Clin Path. 2015: 0; 1-4Online 10.1136/jclinpath- 2014-202705
Having improved the IHC method - the biggest challenge remains - performing multiple Companion Diagnostics for many (all) cancers and SCORING THEM

COMPANION DIAGNOSTICS
HOW WILL WE DO IT?

IHC
FISH
PCR
NGS

Immunohistochemistry – retains morphologic cell ID
-----which is lost in
Polymerase chain reaction
Next generation sequencing

--- it is not just the expressed proteins
---- but also Immune cells
- lymphocytes/macrophages
and their activation

Gu, Taylor
Applied Immunohistochem Mol Morphology Jan, 2014
MANY different cancers
Many different drugs
EACH REQUIRING DIFFERENT approved TEST
- or different LDT

**PD-1 inhibitors:** Examples of drugs that target PD-1 include:

- Pembrolizumab (Keytruda)
- Nivolumab (Opdivo)

Melanoma, NSC lung cancer, colon cancer, Kidney, bladder, head and neck cancer Hodgkin and NH lymphomas

**PD-L1 inhibitors:** Examples of drugs that target PD-L1 include:

- Atezolizumab (Tecentriq)
- Avelumab (Bavencio)
- Durvalumab (Imfinzi)

HUGE PROBLEM for LABS

Assessment for Targeted Therapy Testing in Cancer: Urgent Need For Realistic Economic and Practice Expectations.

Yaziji, Taylor AIMM 2017
An approved Companion DX test – is validated vs clinical outcome

An FDA approved PDL-1 assay requires -
Validated method, reagents, controls & scoring – (manual in this case).

Challenges -
--Identification and scoring of cancer cells
--in some tests - Identification and scoring of immune cells
--Reproducibility
Look at the problem in the context of PD-L1

Many different antibodies
Many different approved tests

‘scoring systems’ - very complex
-differs among tests
-Is a cell positive? – threshold intensity
-Score Percentage positive?
--Semi-quantitative at best
-May include other difficult tasks
such as presence of immune cells

Scoring system - MUST be reproducible
First problem – percentage requires ‘counting’ the number of cancer cells that show ‘positive staining’ & TOTAL cancer cells by eye.

How many cancer cells per section? - --- up to 2,000,000 total cells per section or per field (magnification)? - calculate $\pi r^2$ if tumor cell 20u diameter then = 10,000 tumor cells per x 10 field

= 600 cells per x 40 field (varying with cell size, mix of tumor versus stroma)

% = ‘positive cancer cells’
total cancer cells

We put down a number -but really we just guess?

% = ‘positive cancer cells’
total cancer cells

tissue (or whole slide image –WSI) 3 x 2 cms.

x 4. diam 5mm

x 10. diam 2.0mm

x 20. diam 1 mm

x 40. diam 0.5mm = 500µm
How many cancer cells?

Cannot count – so estimate that half are cancer cells?
- 600 X ½
-- about 300

But IF the denominator is:
- 330 (not 300)
- then ’15’ should not be Rx
or if 270
– then 14 should be Rx

How many total cancer cells
Denominator?

How many positive Ca cells?
Numerator?

% = positive cell count
300

What is the ‘score’?
PD-L1 Threshold - 5%
Does the patient get treated or not?

ONE HIGH POWER X40 FIELD
Percentage positive = numerator: +ve cancer cells
denominator: total ca cells

PDL-1 membrane stain
-- so count the positive cancer cells
15 cells = 5 % threshold
14 - no treatment
15 -- $100,000 Rx

But note -- we have only ‘scored’ 600 cells among maybe 2,000,000 or < 0.0003%
Second problem: distinguish cancer cell from immune cells - by eye

How many of the positive cells are cancer cells?

Adeno Ca

PD-L1 - brown
CD 68 – red
40% are macrophages

Is this a positive test?
>1%?  > 5% ?

Squamous ca

PD-L1 - brown
p63 – Ca cell nuclei - red
Negative test
Positive Ca cells = 0

From Taylor AIMM 2014
Third problem - also need to evaluate immune cells by type and number

Cases with CD8 cells do well with PD-L1 Rx
Identify immune cells by phenotype
determine location in relation to tumor

How do we detect them? MULTIPLEX IHC is effective
Include fluorescent methods gives DIRECT INFORMATION

Tissue Extract methods LOSE SPATIAL information
NGS RNA
PCR Proteomics

Leads to notion of two categories of cancer

-- require very different therapeutic approaches

<table>
<thead>
<tr>
<th>Immunogenic</th>
<th>non immunogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘inflamed’</td>
<td>‘silent’</td>
</tr>
<tr>
<td>Response suppressed</td>
<td>Immune cells absent</td>
</tr>
</tbody>
</table>

**Figure 1: Two Distinct Mechanisms of Immune Resistance**

(A) Inflamed tumors express high levels of pro-inflammatory innate and adaptive immune signals, such as chemokines for T-cell recruitment, but negative immune regulators dominate, including Foxp3+ T-regulatory cells, programmed death 1 ligand (PD-L1), and IDO; (B) non-inflamed tumors express few chemokines and have few tumor-infiltrating lymphocytes, due to poor effector cell trafficking. They have high expression of vascular markers and abundant macrophages and fibroblasts. From Gajewski et al. Curr Opin Immunol. 2011. [9] Used with permission.
Inflamed vs ‘silent’ cancers
Pathologists need to make the distinction - HOW??

REQUIRES MULTIPLE MARKERS

Ligand pairs
PD 1  PDL 1
CD40  CD40L
CTLA-4  CD 86
OX40  OX40L
GITR  GITR L

Tissue Section
Digital multiplex IHC  FISH
Retains ---
Tumor cell ID
Cell relationships
Cell numbers

Immune Cell phenotypes
CD3  CD4  CD8  CD25
CD20  CD68  FoxP1 etc

Patrick A. Ott et al.  
Clin Cancer Res 2013;19:5300-5309
Multiplex IHC may help solve these problems because it can do all of these things at the same time

- Detect BIOMARKER expression
- Achieve better cell ID Immune cell phenotyping
- Quantification = counting Accurate scoring
- Quantification = amount --comparing intensity versus internal standard

Courtesy - Cliff Hoyt PerkinElmer, 2015
PD-1 Blockade with Pembrolizumab in Advanced Merkel-Cell Carcinoma

Paul T. Nghiem, M.D., Ph.D., Shailender Bhatia, M.D., Evan J. Lipson, M.D.,

Merkel CA cells – orange-nse
PDL1 - green
CD8 T cells - yellow
CD68 macrophages – red

Post Rx in responder
Tumor reduced
PDL-1 reduced
CD 8 increased
DIGITAL PATHOLOGY - BIOMARKERS
What NEW THINGS are possible?
‘rehabilitate’ fluorescence by restoring morphology – virtual H&E

Displayed in familiar ways . . .

Spectral composite

Simulated H&E

Simulated IHC

PD-L1

Foxp1

CD8

CD34
Analyzed quantitatively...

- **Spectral composite**
- **tumor/stroma pattern recognition**
- **Mask**
  - Green = tumor
  - Red = stroma

**Expression scoring**

- **PD-L1**
  - H-score: 185
  - 0-1: 11.31%, 1+ to 2: 29.36%, 2+ to 3: 22.32%, 3+: 37.00%
  - Negativity: 99.16%, Positivity: 0.84%

- **Foxp1**
  - H-score: 185
  - 0-1: 11.31%, 1+ to 2: 29.36%, 2+ to 3: 22.32%, 3+: 37.00%
  - Negativity: 99.16%, Positivity: 0.84%

- **CD8**
  - H-score: 185
  - 0-1: 11.31%, 1+ to 2: 29.36%, 2+ to 3: 22.32%, 3+: 37.00%
  - Negativity: 89.41%, Positivity: 10.59%

- **CD34**
  - H-score: 185
  - 0-1: 11.31%, 1+ to 2: 29.36%, 2+ to 3: 22.32%, 3+: 37.00%
  - Negativity: 99.79%, Positivity: 0.21%
Multiplex IHC
-- phenotype ID
multiple cell types
--- “score ‘ them
-- assess spatial relationships
at the same time
YOU CANNOT READ THESE SLIDES

- Microscope
  -- glass slide

- Computer
  -- WSI

Obstacles to digital pathology

RESOLUTION

SCANNING (acquisition, display) SPEED

IMAGE (file) STORAGE / SHARING / VIEWING

Apps for scoring (counting), quantification, analysis, metrics
Acceptance by pathologists

HARDWARE COSTS
SOFTWARE costs - access

REGULATORY and REIMBURSEMENT

THIS CAN !! with your help

with your help
Conclusions. ---diagnostic review by WSI was not inferior to microscope slide review.

Press Information

April 13, 2017

Philips receives FDA clearance to market Philips IntelliSite Pathology Solution for primary diagnostic use in the US.
A Large Multicenter, Retrospective Non-Inferiority Study to Evaluate Diagnostic Concordance between Optical vs Digital Microscopic Diagnoses in 2000 Surgical Pathology Cases

April 2017
FDA approved
For Primary Diagnosis

Whole slide imaging (WSI), also known as digital pathology, is an emerging technology. It involves the acquisition of images from stained tissue sections on glass slides. The images are converted into digital images that can be viewed on a monitor. Digital pathology can elevate collaboration between labs/specialists and allows easy consultation across distances. The digitized images can be archived and accessed in a moments time. The digital pathology platform (Philips IntelliSite Pathology Solution) used in this study is intended for in vitro diagnostic use as an aid to the pathologist to view, review and diagnose digital images of surgical pathology slides. Before substituting the time-honored, familiar and versatile microscope with digital microscopy, several vital concerns need to be addressed. The most critical issue is whether pathologic diagnoses rendered using WSI are comparable to (i.e. non-inferior to) pathologic diagnoses made by optical microscopy. Although several studies comparing digital vs optical microscopy in diagnosis have been conducted, these studies have been single or small multicenter studies, sampling a single organ or lacking control adjudication. This large multicenter non-inferiority study compares microscopy to WSI reads of 2000 surgical pathology cases from 20 different organ systems (54 subtypes) with 16 reading pathologists from 4 institutions.

CLINICAL STUDY DESIGN

- 4 Clinical sites
- 27 Pathologists for:
  - Case enrollment (4)
  - Validation (4)
  - Reading (16)
  - Wash out MD and MO (3)
- 2,000 Cases
- 16,000 Reads:
  - 4 Pathologists per case
  - Read MO and MD
  - Wash out adjudicators

RESULTS

- Figure 2: Difference in major discordance rate digital - optical (%)
- Figure 3: Difference major discordance rates (MD - MO) by organ
- Figure 4: Difference major discordance rates (MD - MO) by reading pathologist

CONCLUSIONS

- Manual Digital is non-inferior to Manual Optical for primary diagnosis in surgical pathology
- Manual Digital is non-inferior to Manual Optical across a wide range of organ systems and pathologists

DISCLOSURE

This study was sponsored by Philips Digital Pathology Solutions
So now the H&E scan is approved - what other riches are to be found in the old H&E??
THE TREND - “cloud based” -- Optra
No special software; any hardware you like
All you need is THE NET and a BROWSER

Like Google Maps

Where are the nearest restaurants?

Where are the nearest Cancer cells?

Camelyon 16 challenge data set

Detecting Cancer Metastases on Gigapixel Pathology Images
Yun Liu et al Martin C. Stumpe. GoogleBlog 2017

Courtesy Anagha Jadhav, OptraScan
Camelyon 16 challenge data set

32 entries from 23 teams

Winner Andrew Beck et al Beth Israel
AI beat the pathologist standard

(AI + Pathologist) > Pathologist

Univeristiy of Warwick
This revolution will affect us
Not just the hardware
But the software
Pathology ‘Apps’.

WHY is this important?
REVOLUTION
Just as 150 years ago

Horus.
Seeing Eye
Path PAD 2020
The ‘intelligent’ microscope

Digital assistance for pathologist

PDL-1 score

H&E diagnose

Segment Cancer cells

6-plex score

Co-localize

Ki67 score

HER2 score
Summary - IHC - to improve quality and to quantify - what must be done?

CONTROL - preparation-fixation (qualify tissues)
DEFINE - Analytes (protein targets)
VALIDATE - Reagents
VALIDATE / STANDARDISE - Total Method as a whole
DEVELOP - uniform ‘shared’ control systems
DEVELOP – quantitative internal reference standards
DEVELOP - standard interpretation/scoring by computer

and ALL OF THESE STEPS REQUIRE IMPROVED CONTROLS
and all require ‘monitoring’

--Total Test Concept--
Companion Diagnostics and digital pathology
Selected personal references


Taylor CR. Quantitative In Situ Proteomics; a proposed pathway for quantification of immunohistochemistry at the light-microscopic level. Cell Tissue Res. 2015; 360:109-120.
