

# Validating algorithms under CLIA and GLP requirements

27 July 2010

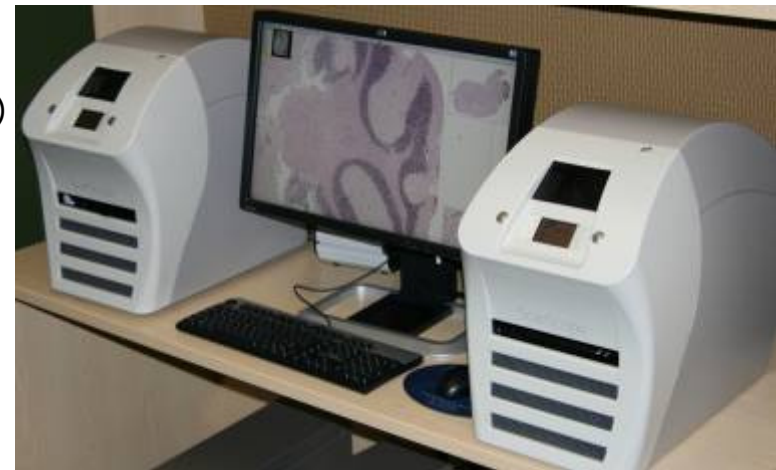
Steve Potts, Ph.D. MBA

CEO

Flagship Biosciences LLC

# Flagship Biosciences

- A digital pathology and image analysis service provider
  - Slide scanning & hosting
  - Advanced image analysis
  - Digital pathology conferencing
- A combination of technology, talent, experience, expertise
- Fast and secure IT infrastructure
- GLP and CLIA-compliant histology and pathology
  - EPL, Inc (GLP histology and pathology)
  - EPL Archives (GLP archives)
  - Vitro Molecular (CLIA and IHC development)
- Biomarker development
  - EMT companion diagnostic
  - Histone profiling (PrognosDx)



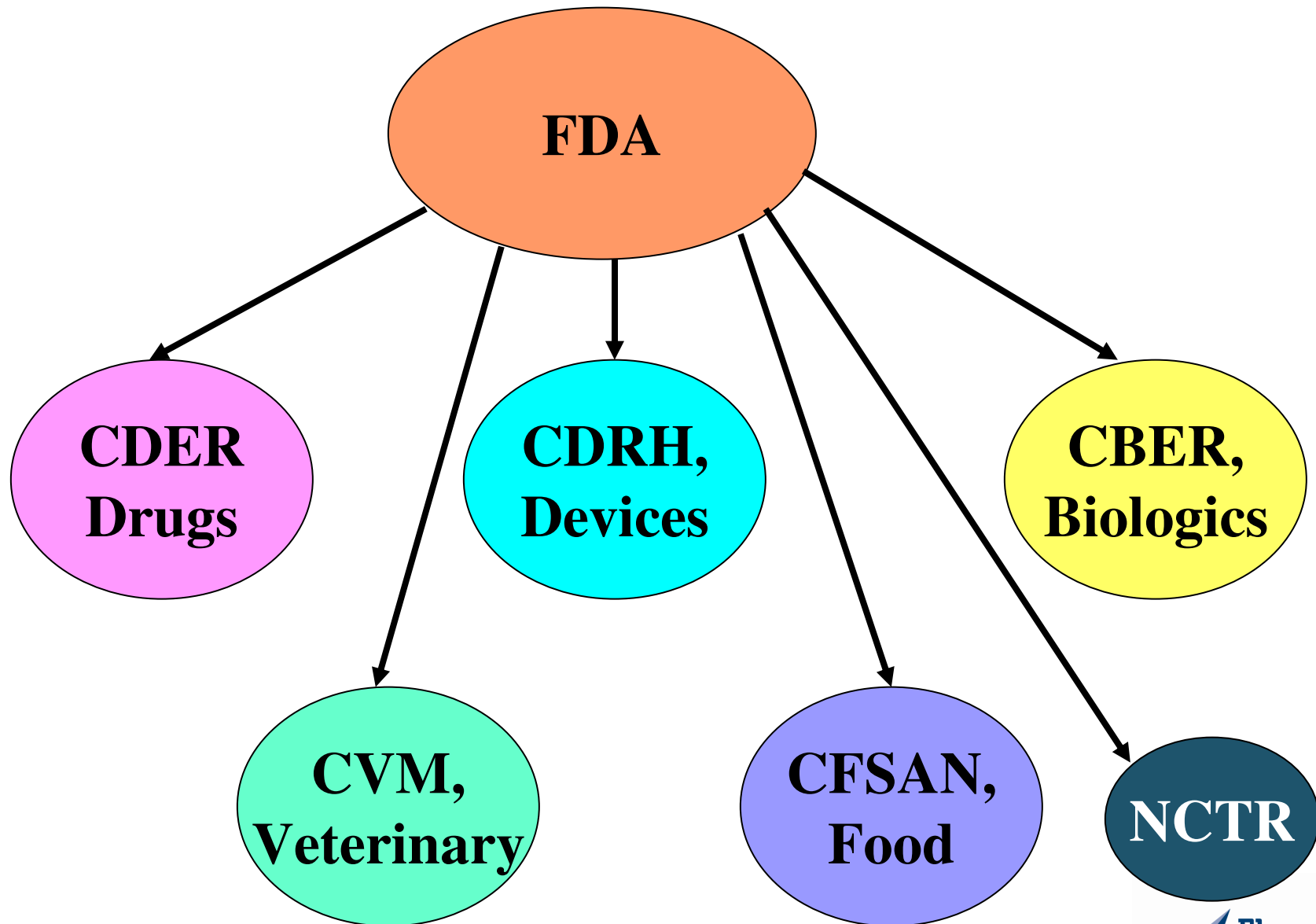
# Disclosure

- Flagship Biosciences has no financial connection (other than being a customer) to Aperio
  - We use Aperio scanners for hardware, Aperio software for cell and area measurements and Visiopharm software for advanced image analysis and stereology.
  - Also developing our own in-house algorithms with the Aperio SDK and other technologies
- Flagship is a pathologist-owned company seeking to meet practical needs and gaps we see in the industry NOW

# 3 kinds of people in this audience

- Clinical –  
*“I know more than I want to know about CLIA, but what is GLP?”*
- Preclinical (safety studies in pharma)  
*“I know all I want to know about GLP, but what is CLIA?”*
- Academic Research and Pharma Discovery Groups  
*“I don’t care about GLP or CLIA...”*

# Regulatory groups involved



# Outline

- Vendor validation gap with CLIA (clinical workflows)
  - Medical device manufacturer regulations for DP
  - Clinical lab regulations under CLIA
- Vendor validation gap with GLP (preclinical safety studies)
  - State of adoption of digital slides in preclinical
  - Peer review & image analysis
  - Validation required in GLP
- The path forward:
  - SOPs for in-house GLP image analysis
  - IA validation service for CLIA

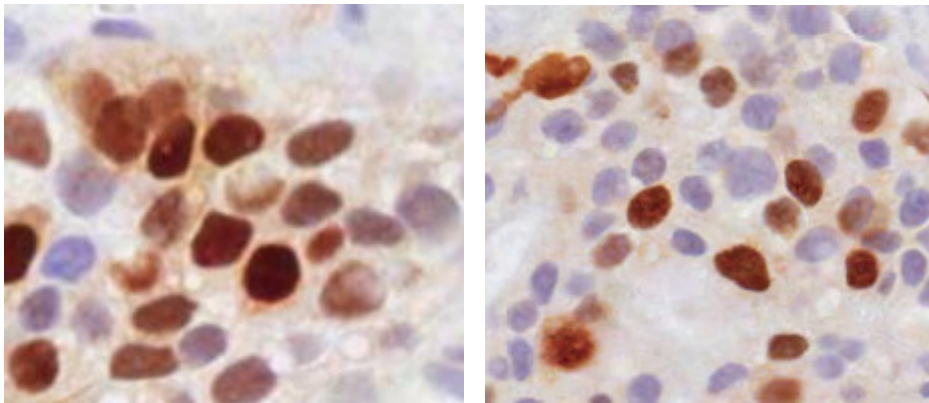
# Vendor validation gap with CLIA (clinical)

- Medical device manufacturers can only market specific uses of the technology
- Aperio example:
  - *"The exact intended use and label indication for each clearance is available at <http://www.aperio.com/other/regulatory.asp>."*
  - IHC HER2 Image Analysis
  - IHC HER2 Manual Read of Digital Slides
  - IHC ER/PR Image Analysis
  - IHC PR Breast Tissue Manual Read of Digital Slides
  - IHC HER2 Breast Tissue Tunable Image Analysis

# Vendor validation gap with CLIA (clinical)

**Nuclear algorithm** tuned for ER / PR

- What about when you are counting Ki67?
  - Need to modify averaging radius for more blurring effect to compensate for pixelated Ki67 staining
  - Need to modify curvature threshold for less side-by-side cell segmentation
- But no validation is supplied out of the box
- What about inflammatory cells, TUNEL, many other cell counting applications... ?



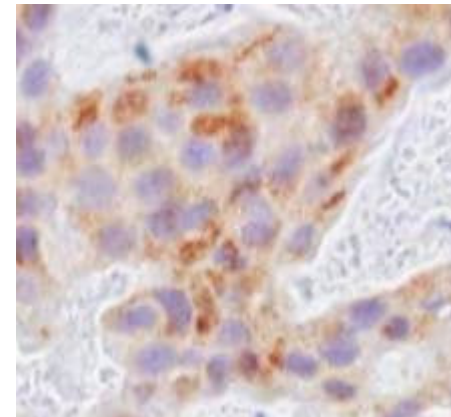
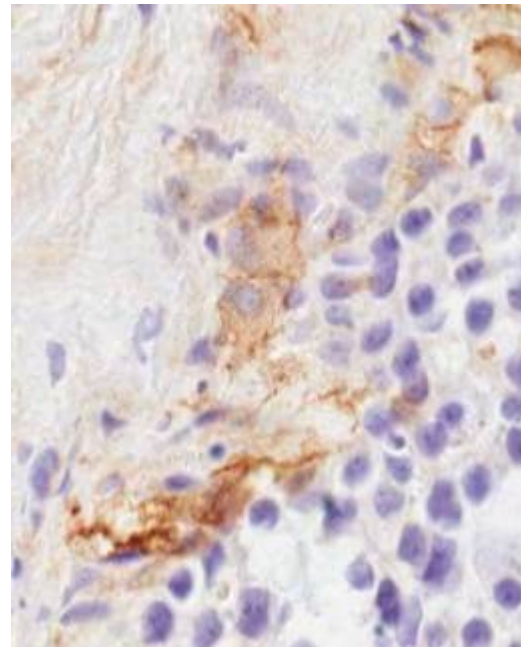
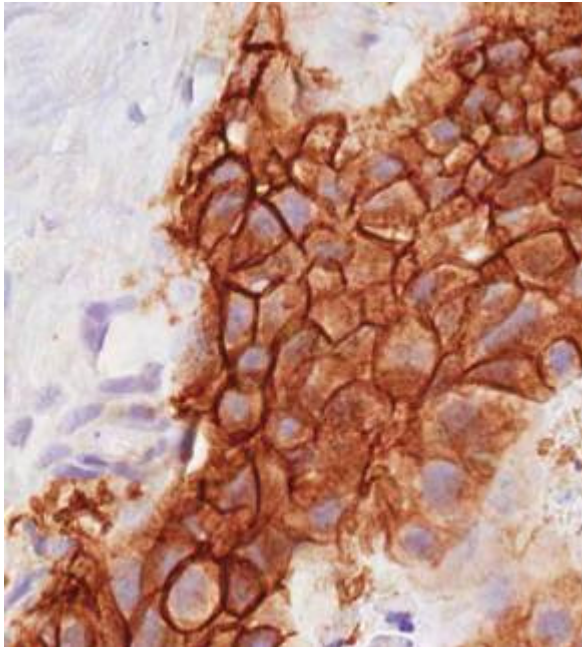
A cleared algorithm validated for PR (left) will need to be adjusted for the classic “speckling” and “spotty” staining commonly seen in Ki67 (right)

Algorithms	
Select Algorithm	Import Macro...
<b>'Nuclear v9' Parameters</b>	
View Width	1000
View Height	1000
Overlap Size	100
Image Zoom	1
Markup Compression Type	0 - Same as processed image
Compression Quality	30
Classifier Neighborhood	0
Classifier	0 - None
Class List	
Averaging Radius (um)	1
Curvature Threshold	2.5
Segmentation Type	2 - Cytoplasmic Rejection
Threshold Type	1 - Edge Threshold Method
Edge Trimming	1 - Weighted
Lower Threshold	0
Upper Threshold	230
Min Nuclear Size (um <sup>2</sup> )	20
Max Nuclear Size (um <sup>2</sup> )	1000000
Min Roundness	0.1
Min Compactness	0
Min Elongation	0.1
Remove Light Objects	0
Clear Area Intensity	240
Nuclear Stain (Red)	0.696858
Nuclear Stain (Green)	0.643073

# Vendor validation gap with CLIA (clinical)

## Membrane algorithm tuned for HER2

- What about EGFR...or phosphomarkers...or your novel membrane targets?



P53 staining in advanced breast cancer

An algorithm validated and cleared for HER2 (left) will need to be modified for use in EGFR (right), and may not be appropriate



**US Food and Drug Administration**

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**IMPORTANT DISTINCTION:**

**Medical Device Manufacturers are regulated by FDA**

Can only promote FDA cleared or approved products in the clinical market.

**Clinical Laboratories are regulated by CLIA (not FDA)**

Have a choice of using non-FDA cleared or approved test systems (see 493.1253)  
– a question of the laboratory's comfort level.

# Medical Device Manufacturers Regulations

- **US Food and Drug Administration (FDA)**



# Digital Pathology FDA 510k Clearances Immunohistochemistry (IHC)

[www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm](http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm)

## ScanScope XT System (Aperio)

2009/08	K080564	Breast	Her2/neu	Dako	<b>Tunable Image Analysis - System</b>
2008/10	K080254	Breast	PR	Dako	Reading on Monitor - System
2008/08	K073667	Breast	ER/PR	Dako	Image Analysis - System
2007/12	K071671	Breast	Her2/neu	Dako	<b>Reading on Monitor - System</b>
2007/10	K071128	Breast	Her2/neu	Dako	Image Analysis - System

## PATHIAM (Bioimagine)

2009/02	K080910	Breast	Her2/neu	Dako	Image Analysis - System
2007/02	K062756	Breast	Her2/neu	Dako	Image Analysis - SW

## VIAS (Tripath)

2006/09	K062428	Breast	<b>P53</b>	Ventana	Image Analysis - System
2006/04	K053520	Breast	<b>Ki-67</b>	Ventana	Image Analysis - System
2005/08	K051282	Breast	Her2/neu	Ventana	Image Analysis - System
2005/05	K050012	Breast	ER/PR	<b>Ventana</b>	Image Analysis - System

## ARIOL (Applied Imaging)

2004/03	K033200	Breast	ER/PR	Dako	Image Analysis - System
2004/01	K031715	Breast	Her2/neu	Dako	Image Analysis - System

## ACIS (Clariant/Chroma Vision)

2004/02	K012138	Breast	<b>ER/PR</b>	Dako	Image Analysis - System
2003/12	K032113	Breast	<b>Her2/neu</b>	Dako	<b>Image Analysis - System</b>

## QCA (Cell Analysis)

2003/12	K031363	<b>Breast</b>	<b>ER</b>	<b>Dako</b>	<b>Image Analysis - SW</b>
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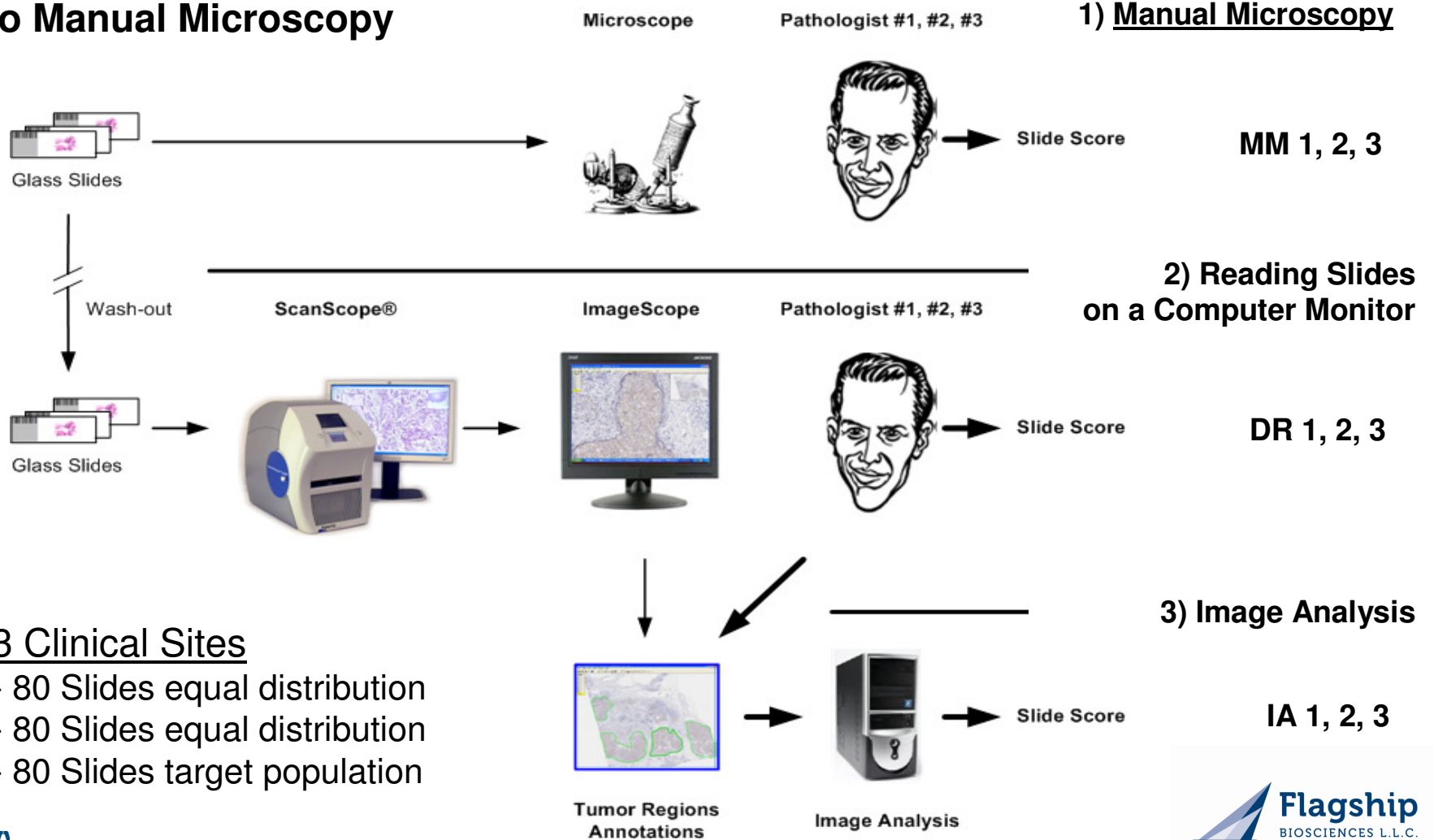
**Date 510(k) Number      Tissue      Stain      Reagent      Application**



# IHC Study Design Example Breast – HER2 – Dako

[www.aperio.com/pathology-events/webinar\\_clinical.asp](http://www.aperio.com/pathology-events/webinar_clinical.asp)

## I. Substantial Equivalence to Manual Microscopy



### 3 Clinical Sites

- 80 Slides equal distribution
- 80 Slides equal distribution
- 80 Slides target population

www.....





## Hematology and Pathology Devices Advisory Committee – 22&23 October 2009

[www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/MedicalDevices/MedicalDevicesAdvisoryCommittee/HematologyandPathologyDevicesPanel/ucm146748.htm](http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/MedicalDevices/MedicalDevicesAdvisoryCommittee/HematologyandPathologyDevicesPanel/ucm146748.htm)

**2 Day Advisory Panel meeting on Digital Pathology Whole Slide Imaging (WSI)**  
to gather expert information for how to ensure that  
the current standards of Safety and Effectiveness of routine surgical pathology  
will not be compromised if WSI is used instead of the conventional light microscope.

***Is digital WSI a class II device (req. 510k), or a class III device (req. PMA)?***

***How should a manufacturer validate the performance of a digital WSI system?***

Presentation and discussions about:

- Principles of light microscopy (the reference method)
- Principles of digital WSI
- Possible criteria and studies for analyzing the accuracy and reproducibility of the diagnostic performance of pathologists
- Objective and subjective aspects of reading slides

Status-Quo: FDA has not yet released any guidelines or provided a clear path  
on what manufacturers have to do to get FDA clearance/approval  
for digital pathology systems for primary diagnosis.



## **STATEMENT by the College of American Pathologists**

Potential Public Health Implications for not establishing proper validation.

### ***Test Conditions:***

- Focus on use of the tool in the context of how a diagnosis is rendered in clinical practice.
- All slides from each case using each modality (vs. “representative” slide)

### ***Sample Size:***

- Large sample size to detect small performance differences.

### ***Specimen Types:***

- Different specimen types require different capabilities and validation procedures.

### ***Diagnostic Spectrum:***

- Challenge set that reflects the spectrum of diagnostic labels and secondary measures.

### ***Evaluation Criteria:***

- Intra-pathologist variability, not accuracy of diagnosis.

Caution: FDA approval for primary diagnosis for a specific tissue application may result in significant off-label use.

# Lab Regulations

- **US Food and Drug Administration (FDA)**
- **Clinical Laboratory Improvement Amendments (CLIA) '88**
- **College of American Pathologists (CAP)**
  
- **American Society of Clinical Oncology (ASCO)/CAP**  
**New IHC HER2 and ER/PR Guidelines**

# **Clinical Laboratory Improvement Amendments (CLIA)' 88**

All Clinical Labs need a CLIA license to operate and to be able to bill Medicare & Medicaid

## **Sections in the CLIA standard that apply to Digital Pathology**

### **493.1105 Retention Requirements**

- (a)(6) Test reports
- (a)(7) Slide, block, and tissue retention

### **493.1251 Procedure Manual**

- (b)(5) Calibration and calibration verification
- (b)(6) Reportable range for test results for the test system
- (c) Manufacturer's test system instructions or operator manuals

### **493.1252 Test Systems, Equipment, Instruments, Reagents, Materials, and Supplies**

### **493.1253 Establishment and Verification of Performance Specifications**

- (b)(1) Verification of performance specifications
- (b)(2) Establishment of performance specifications

### **493.1254 Maintenance and Function Checks**

### **493.1255 Calibration and Calibration Verification Procedures**

### **493.1256 Control Procedures**

### **493.1291 Test Report**

## College of American Pathologists (CAP)

Almost all Clinical Labs seek CAP accreditation, requires inspection based on check-list

### CAP Anatomic Pathology Checklist\* that applies to Digital Pathology

#### **#1 Procedure Manual**

ANP.07328

#### **#2 Quality Management**

ANP.10050

ANP.10200 & 10250

#### **#3 Surgical Pathology Reports**

ANP.12500

#### **#4 Immunologic and Molecular Methods – Predictive Markers**

ANP.22988

ASCO/CAP HER2 guidelines added (ANP.22989-999)

ANP.22997

ANP.22999

#### **#5 Instruments and Equipment**

#### **ASCO/CAP Guidelines are becoming part of the Check-List**

A committee has been formed that is looking into adding **Digital Imaging** and **Image Analysis** to the Check-List.



**ASCO/CAP Guidelines – IHC HER2 Breast [2007]\***

***Approximately 20% of current HER2 testing may be inaccurate***

***HER2 on all invasive breast cancers***

***No Gold Standard for HER2***

***I. Preanalytic (Tissue Preparation)***

***II. Analytic (Staining)***

95% concordance with another validated test (FISH, ISH, IHC) for pos. and neg. cases

***III. Postanalytic (Interpretation)***

Modified Scoring Scheme for 3+ with > 30% (vs. 10%) threshold for 3+ cells

Equivocal reflex testing with another test (gene vs. protein)

\* Guideline for HER2 Testing in Breast Cancer  
[www.flagshipbio.com](http://www.flagshipbio.com) - Vol 131, January 2007

## ASCO/CAP Guidelines – IHC ER/PgR Breast [2010]\*

***Up to 20% of current IHC determinations of ER/PR testing worldwide may be inaccurate (false negative or false positive)***

***ER/PR on all invasive breast cancers and breast cancer recurrences***

***No Gold Standard for ER/PR  
(clinically validated = clinical benefit from endocrine therapy)***

***“Image Analysis holds promise for improving inter- and intra-observer reproducibility, but controversy exists about how imaging should be implemented at this time.”***

\* Breast Cancer Hormone Receptor Guideline, IHC  
[www.flagshipbio.com](http://www.flagshipbio.com)  
Hormonal and Immunohistochemical Lab Med (2010)

## ***I. Preanalytic (Tissue Preparation)***

- *Tissue handling*      **Large, preferable multiple core biopsies of tumor preferred**  
**Time from Tissue Removal to Fixation < 1 hour**  
**Oriented, inked for surgical margin assessment,**  
**and sectioned at 5 mm intervals**  
**Small portion of tumor and fibrous normal tissue together (if possible)**  
**to ensure normal breast elements are available as an int. pos. control**
  
- *Type of fixative*      **10% NBF (neutral buffered formalin)**  
**Alternatively – validation against NBF fixation**
  
- *Duration of fix.*      **Duration of Tissue Fixation > 6 hours and < 72hours**  
**Adequate volume of fixative (opt. 10x specimen)**

## ASCO/CAP Guidelines – IHC ER/PgR Breast [2010]

### II. Analytic (Staining)

#### - Antibodies

#### Clinically-Validated Antibodies with published reports\*

ER: 1D5, 6F11, SP1, 1D5+ER.2.123

PR: 1294, 1A6, 312

\*\*\*No research use only, investigational use only,  
nor laboratory-developed antibodies.

#### \*Note:

FDA clearance  
not sufficient  
e.g. PR 363

#### \*\*\*Note:

Enforcing FDA  
clearances and  
approvals

#### Alternatively – show concordance with a clinically validated assay

90% concordance ER/PR pos. / 95% concordance ER/PR neg.

#### - Control samples

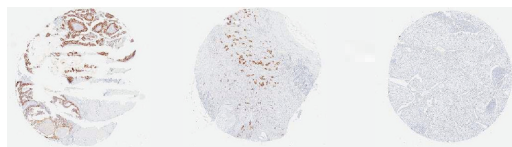
#### Pos. and Neg. Batch Control incl. intermediate reactivity

#### ➤ Monitor assay performance

Score Percentage and Intensity \*\*

#### \*\*Note:

Use of Image Analysis suggested.



#### On-slide external controls and internal normal epithelial elements

#### ➤ Validate proper assay performance



## ASCO/CAP Guidelines – IHC ER/PgR Breast [2010]

### III. Postanalytic (Interpretation)

#### - Reporting

#### Accessioning

Patient identification  
Physician identification  
Date of procedure  
Clinical indication for biopsy  
Specimen site and type  
Collection time  
Time sample placed in fixture  
Type of fixture  
Fixation duration

#### IHC Report

Patient identification\*  
Physician identification\*  
Date of service\*  
Specimen site and type\*  
Specimen identification (case and block nb)  
Fixative  
Cold ischemia time (time: removal - fixation)  
Duration of fixation  
Staining method  
- Primary antibody and vendor  
- Assay details and other reagents/vendors  
- References supporting validation of assay  
Status of FDA approval  
Controls (high, low-level, neg., int. elements or normal breast tissue)  
Adequacy of sample for evaluation  
Results\*  
- Percentage and Intensity  
- Interpretation  
- Pos., Neg., uninterpretable  
- Internal and external controls (pos. neg. not present)  
- Standard assay conditions met/not met  
- Optional score  
- Comment:  
- Explain reason for uninterpretable result  
- Unusual conditions  
- Provide correlation with histologic type of tumor

[www.flagshipbio.com](http://www.flagshipbio.com)

\*minimum, others need to be available in lab

### **III. Postanalytic (Interpretation)**

- *Internal quality control and validation*

**Comprehensive quality control program for all aspects of the total test**

- **Validation of test method before reporting patient results (Re-Validation of test whenever there is a significant change to the test system)**
- Standard Operating Procedures with appr. elements and sign-off
- Qualifications, responsibilities, and training of personnel
- Proper labeling of samples and reagents
- Equipment calibration, maintenance, QC, and remedial actions
- Internal QA plan for entire testing process and evidence that it is followed
- Quality of tests for interpretation
- Ongoing competency assessment of technologists and pathologists
- Report adequacy and quality
- Recordkeeping for entire test process and record retention
- Accurate, timely submission of results
- **Periodic Trend Analysis (based on patient population)**
- **Regular, Ongoing assay reassessments at least semiannually**

**Mandatory Proficiency Testing (>90%) twice per year**

**CAP Lab Accreditation – inspection every other year, self every year  
CAP Certification program for Pathologists**

## ***Definition of VALIDATION OF A TEST***

... “There is **no universally acceptable procedure** for validating tests. The process for validating tests must take into account the purpose for which a test is **intended** to be used, **claims** made about the test, and the **risks** that may prevent the test from serving its intended purpose or meeting performance claims. Even **FDA-approved and FDA-cleared tests require limited revalidation** in clinical laboratories (a process often referred to as verification) to establish that local implementation of the test can reproduce a manufacturer’s validated claims. Tests that use reagents or equipment that have not been validated (such as RUOs or IUOs) typically pose increased risks that require more extensive validation, as do tests used in more loosely controlled settings. The determination of whether a test has been adequately validated requires **professional judgment.**”

## Recommendations for validating estrogen and progesterone receptor immunohistochemistry assays.\*

Referenced by **ASCO/CAP ER/PgR Guideline**

### Initial Test Validation

GOAL:

**Reasonable**, but not absolute, **assurance** that a **test is performing as intended**.

METHOD:

Test Results to be **compared with a standard** – ideally **clinical outcome**, but not practical.

Compare to:

- **Another laboratory** that validated against **clinical outcome**
- Another laboratory **conforming with ASCO/CAP guidelines** and having performed proper validation.
- Another laboratory with an **alternative, clinically validated method**.
  
- Tissue challenges used in a **Proficiency-Testing Program**
- **Validation tissue** provided by an organization such as CAP or the NIST

If the laboratory wants to use special techniques for scoring, such as image analysis, they must use them in the validation study.

\* Validating ER and PgR Immunohistochemistry Assays  
Fritzgibbons et al, Arch Pathol Lab Med (in press)  
[www.flagshipbio.com](http://www.flagshipbio.com)

## Recommendations for validating estrogen and progesterone receptor immunohistochemistry assays.

How many tests will a lab have to do as part of the initial validation? **“Five won’t cut it”**

### FDA cleared or approved Test

#### 40 Specimens

≥ 20 Negative (< 1%)

≥ 20 Positive (≥ 1%)

incl. ≥ 5 Weak Positive (1-10%)

Not more than 10 specimens per run

Runs on multiple days with multiple personnel

Acceptance Criteria\* (with 1% cut-off)

≥ 90% Positive Percent Agreement

≥ 95% Negative Percent Agreement

or use **verification procedure**  
from **package insert**

### Lab developed or modified Test

#### 80 Specimens

≥ 40 Negative (< 1%)

≥ 40 Positive (≥ 1%)

incl. ≥ 10 Weak Positive (1-10%)

Not more than 20 specimens per run

Runs on multiple days with multiple personnel

Acceptance Criteria\* (with 1% cut-off)

≥ 90% Positive Percent Agreement

≥ 95% Negative Percent Agreement

## **Recommendations for validating estrogen and progesterone receptor immunohistochemistry assays.**

### **Ongoing Assay Assessment**

**Monitor pos. and neg. ER rates (Trend Analysis) - compare to expectations - 2x / year**

**Monitor concord. between ER/PR results and gene expression  $\geq 95\%$  (if performed)**

**External Proficiency Testing Program  $\geq 90\%$**

**Monitor ER/PR results by pathologist – acceptable variations est. by Lab Dir. – 2x / year**

### **Pathologist Skill Validation**

(not participated in the initial test validation)

#### **40 Specimens**

$\geq 20$  Negative ( $< 1\%$ )

$\geq 20$  Positive ( $\geq 1\%$ )

some Weak Positive (1-10%)

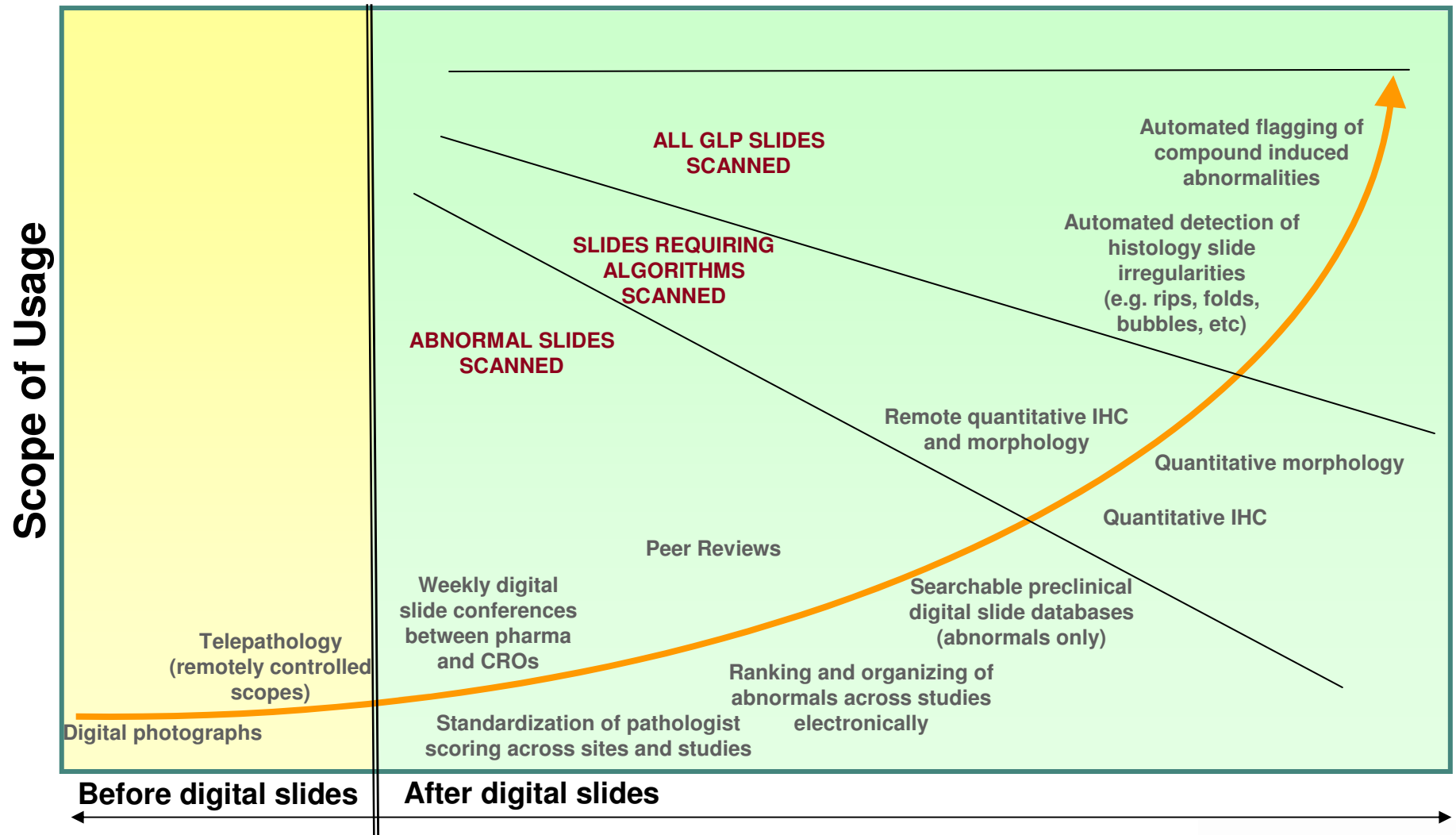
Acceptance Criteria (with 1% cut-off)

$\leq 2$  incorrect assessments

# Outline

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  - State of adoption of digital slides in preclinical
  - Peer review & image analysis
  - Validation required in GLP
- The path forward:
  - SOPs for in-house GLP image analysis
  - IA validation service for CLIA

# Adoption in preclinical toxicology



# Information on whole slide imaging in GLP

## ■ Resources for GLP

- Premarket specification to industry of what was required from digital pathology vendors in December 2007 (PDF)
- Overview of GLP and IQ/OQ/PQ with digital pathology (PDF)
- Invited presentation at PhRMA (PDF) (Amgen, GSK, Aperio)
- Invited presentation at New York Academy of Sciences (PDF) (Biogen-Idec, Aperio)
- ACVP 2008 Annual Meeting (PDF)
- Poster presentation at STP (PDF)
- Poster presentation at ESTP (PDF)
  
- For an overview of the 21 CFR Part 11 and equivalent geographic regulations, please see the following:
  - US 21 CFR Part 11 (PDF)
  - European Union Annex 11 (English) (PDF)
  - European Union Annex 11 (German) (PDF)
  - Japanese ERES guidelines (Japanese) (PDF)

- Available at <http://www.aperio.com/applications/pharma-biotech-glp.asp>

# PhRMA presentation 2008

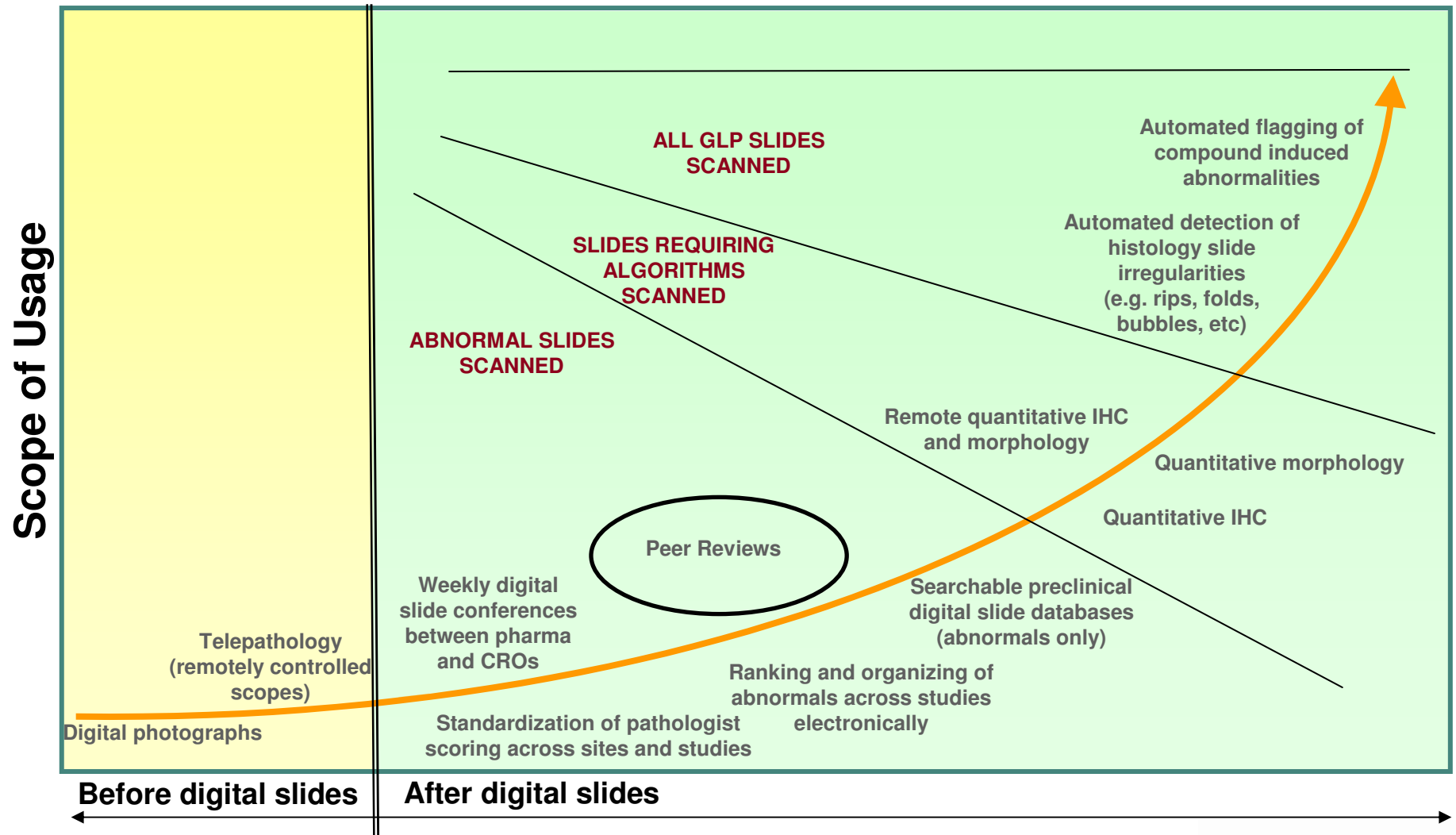
## Validation of Pathology Imaging Systems – A Pathologist’s View of Use of Imaging Systems in GLP studies

**Dr Ian Pyrah, BVM&S, PhD, MRCVS, FRCPath**  
**Executive Director, Pathology**  
**Amgen**

**Pharma meeting “Bioresearch Modernization – the Quality Path  
Forward” September 15<sup>th</sup> 2008**

**Washington DC**

# Emerging need for peer review validation



# VIPER: Validation of peer reviews

- Flagship Biosciences is leading a consortium of 6 pharmas and one medical device pathologist participants to look at workflow and validation of whole slide images in peer reviews
  - Workflow
  - Pathologist time versus glass slides
  - 20x versus 40x?
  - Randomization and blinding strategies
  - Validation approaches
  - International bandwidth issues
- Kickoff meeting ACVP Dec 2010
- Next meeting STP June
- Donated primate study with full dual peer review



# Collaboration with EPL in peer reviews



**Flagship**  
BIOSCIENCES L.L.C.

Raising the digital standard

Home Company Services Industry Therapeutics Tissues R

## EPL and Flagship Biosciences Form a Strategic Alliance to Provide Preclinical Pathology and Quantitative Services

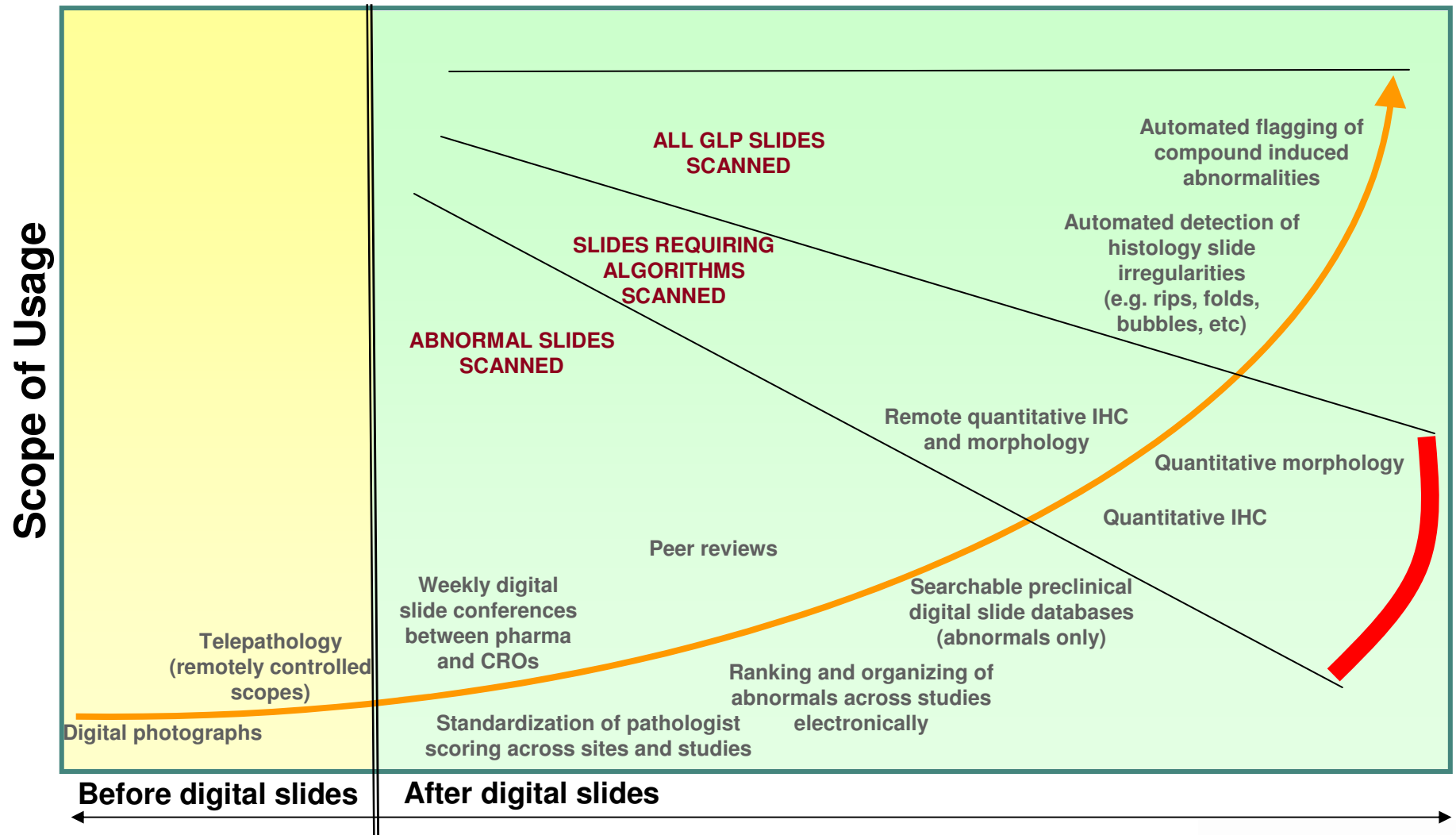
*Alliance offers a full pathology solution to pharmaceutical and biotechnology companies*

Flagstaff, AZ – April 2, 2010 – Flagship Biosciences LLC, a provider of digital pathology services, announced today it has formed a strategic alliance with Experimental Pathology Laboratories, Inc. (EPL), a leading toxicologic pathology contract laboratory known worldwide for excellence in providing GLP preclinical pathology for both industry and government.

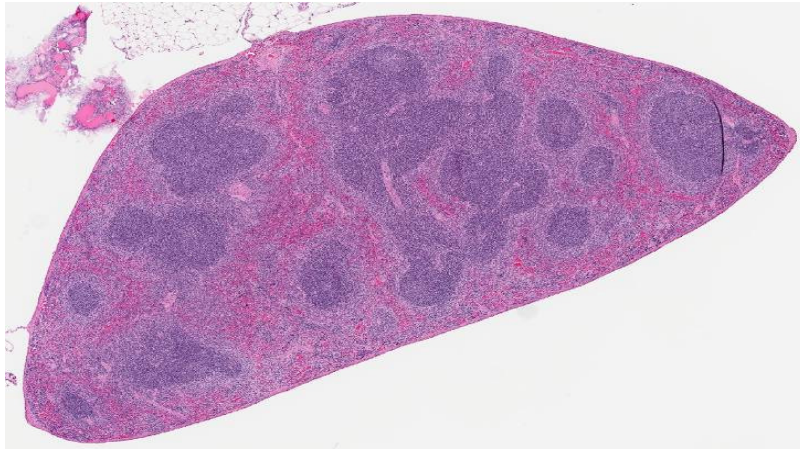
The goal of the alliance is two-fold – to eliminate geographic restrictions on simultaneous multi-site or remote site slide examination by using whole slide imaging (called telepathology) and to advance pathology towards a more quantitative discipline through whole-slide image analysis. Flagship provides digital pathology services, including slide scanning services, digital hosting, whole slide archiving, searchable databases, and quantitative pathology. These services will enhance EPL's renowned world-class GLP offerings, eventually enabling its pathologists to eliminate geographic limitations for peer reviews and other activities. A consortium called VIPR (Virtual Imaging in Peer Reviews) has already been formed by a group of pharmaceutical companies and Flagship; the expertise of EPL's pathologists will be essential to investigate and eliminate the roadblocks that remain in using digital slide images for remote peer reviews.



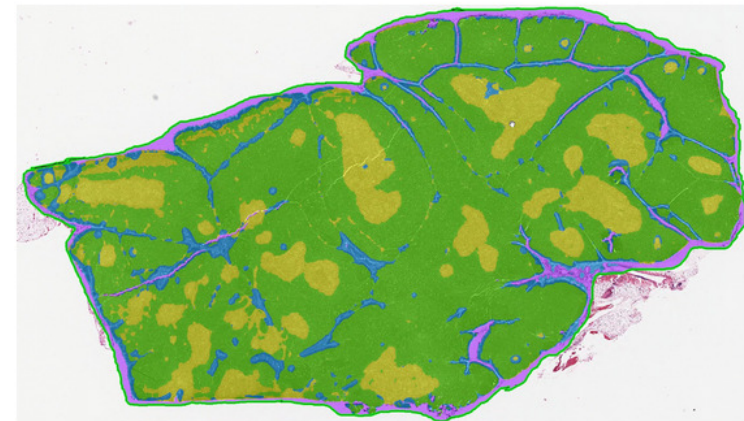
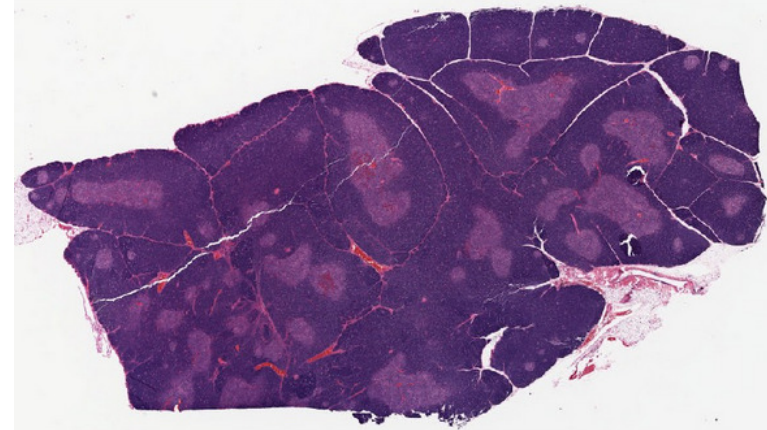
# Emerging use of image analysis in GLP



# Image analysis quantitation in preclinical



Periaarteriolar lymphoid tissue (green) and red pulp (red) in a mouse spleen.



Thymus cortex to medulla ratio analysis

# Vendor validation gap with GLP

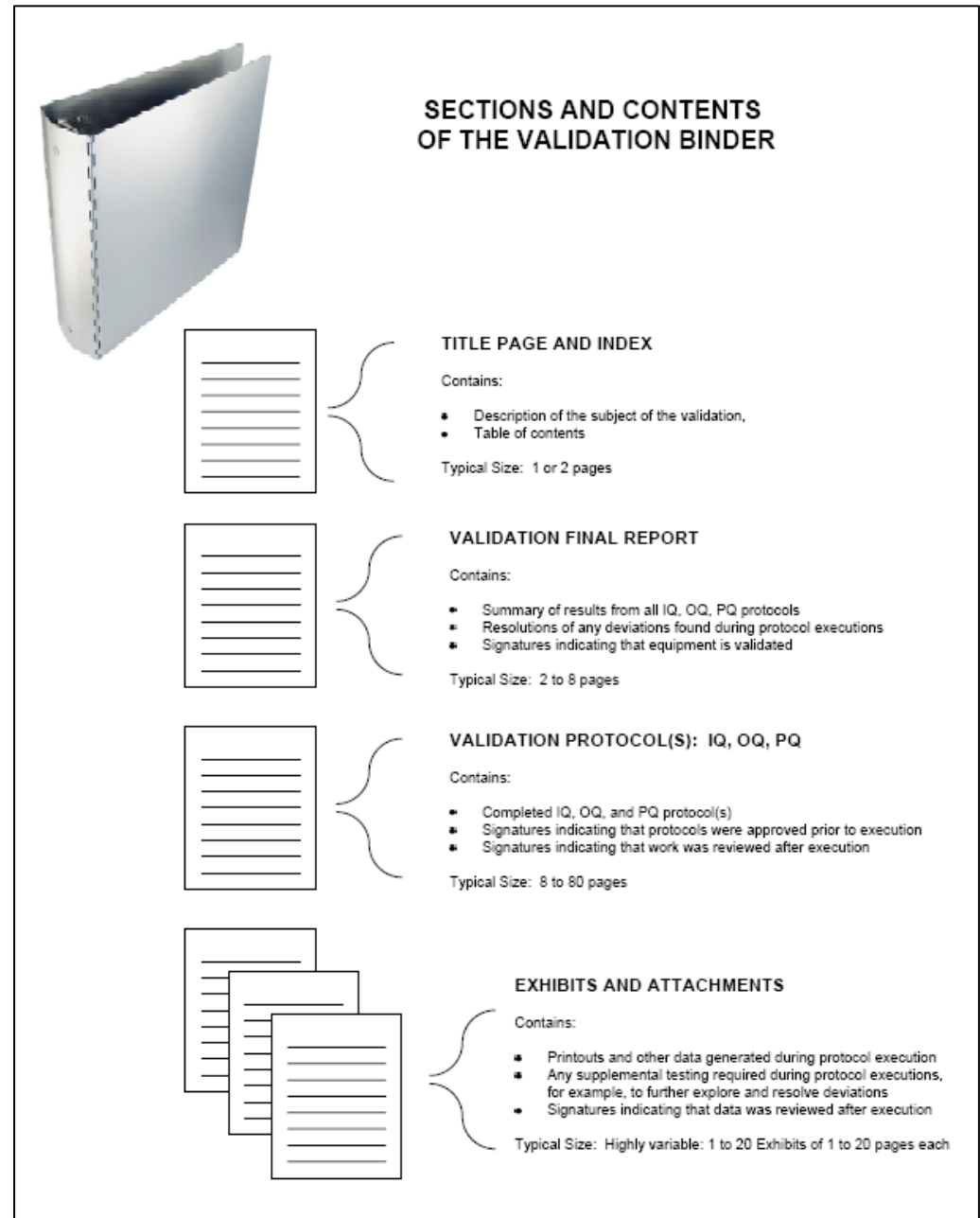
- For GLP environment, some vendors supply IQ/OQ/PQ validation process
  - This does not cover image analysis
- Emerging area is use of IA on toxicology studies, to supplement historic minimal/mild/moderate/marked qualitative scoring with computer assisted scoring
  - Both area and cell based, highly diverse solutions
    - See Flagship's [image analysis blog](http://www.flagshipbio.com) at [www.flagshipbio.com](http://www.flagshipbio.com)
- No out-of-the-box algorithms from any vendor, the problem is too diverse

# 21 CFR 11 Functionality

<b>Category</b>	<b><u>Details</u></b>
System logins and passwords	Timeouts, password rules enforcement, administrator privileges, etc.
Access control and user privileges	Configurable roles (pathologist can sign off, technician can scan and QA slides, etc.)
Auto detection of data tampering	Images and bar codes cannot be tampered with, 1D & 2D barcoding. IMAGE SIGNATURE
Comprehensive audit log	Application and file level logging
Electronic signature records	Complete sign-off workflow

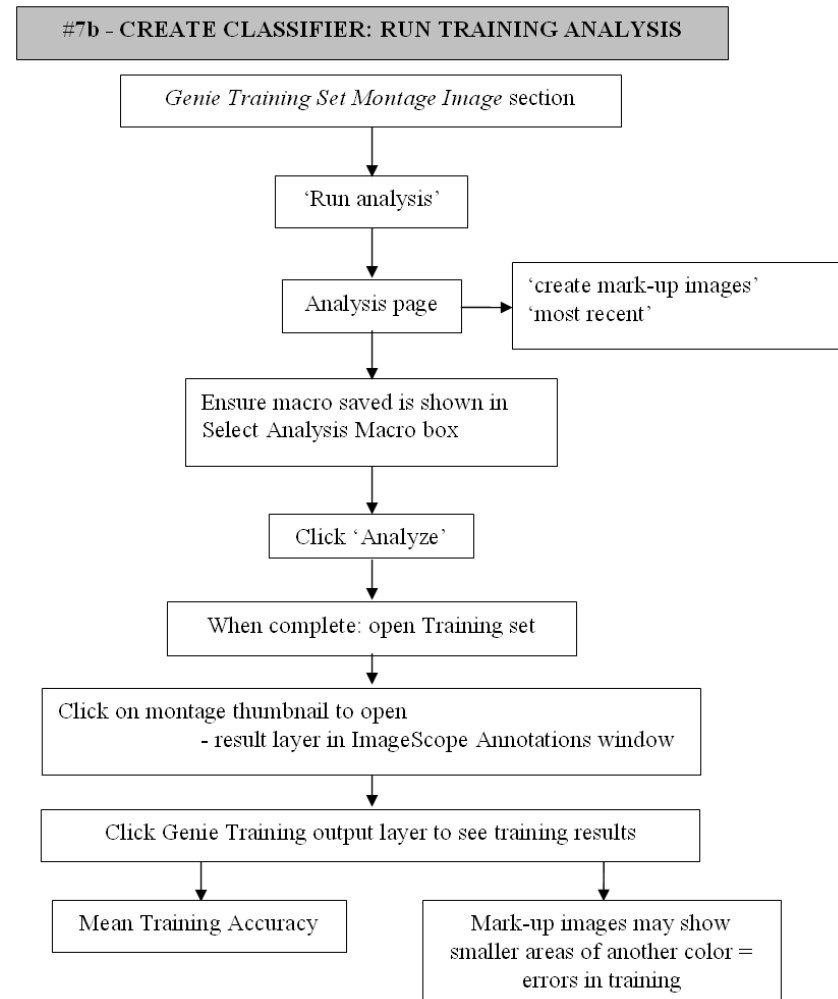
# IQ/OQ/PQ validation

- IQ: Have all system components been installed correctly?
- OQ: Do system components operate correctly?
- PQ: Does the entire system perform as expected?



# SOP for pattern recognition

- Write a workflow for your image analysis process
- Then re-write as an SOP
- Validation steps should follow the SOP steps

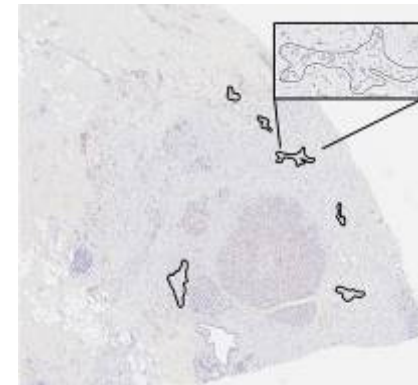


Workflow courtesy Dave Young, [Flagship Biosciences](http://www.flagshipbio.com)

# Tissue variability and acceptable error

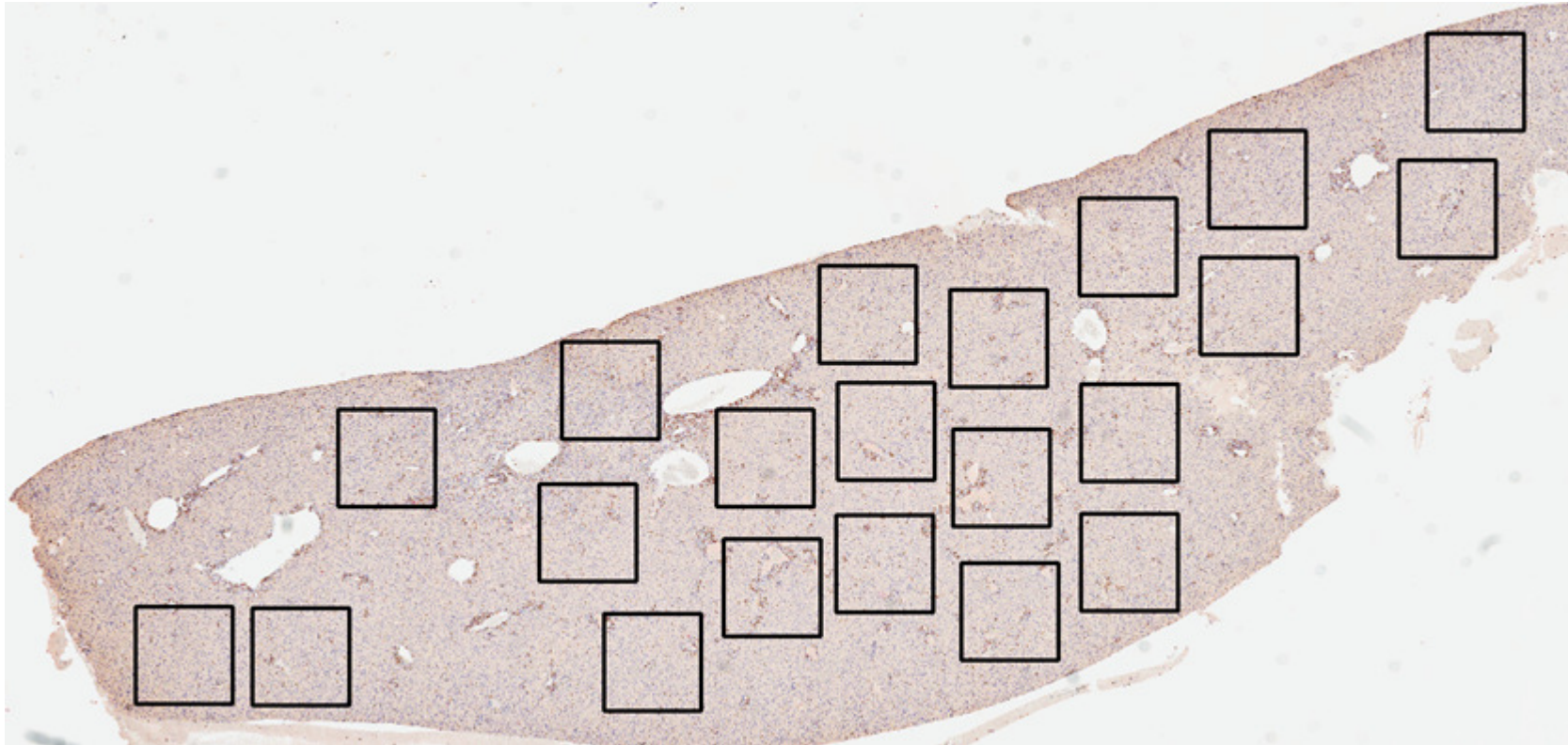
Tissue variability & acceptable error

- Find out what your manual accepted error rate is before digital slides and IA
- Estimate your error rates on image analysis projects, record them, make it part of the SOP
- Is it better to perfectly analyze small “representative” areas or accept a level of error and run the entire tissue section?
  - Measure intratissue variability rates



# Measuring intratissue variability rates

Tissue variability & acceptable error



Courtesy [Frank Voelker, Quantitation in Toxicologic Pathology, STP meeting 2008](#)

# Example intratissue variability rates

Tissue variability & acceptable error

Tissue	Count	Number of sections sampled in a tissue	Number of tissues	Biological variability across a tissue section (average coefficient of variation)
<b>Variation of individual regions across a single tissue section</b>				
Mouse xenografts	Microvessel count (manual)	10	9	46%
Mouse xenografts	Microvessel area (automated)	10	9	37%
Mouse livers	Neutrophil counts	8	2	16%
Rat livers	T lymphocyte counts	21	2	32%
Human breast	HER2 H Score	7 to 11	5	19%
Human breast	ER H Score	6 to 10	5	41%
Human breast	PR H Score	6 to 11	5	63%
<b>Variation of different composite regions averaged across a single tissue section</b>				
Human breast	HER2 (+3,+2,+1,0 scoring)	15-20 averaged as one composite region	180	17%
Human breast	ER percent positive cells	15-20 averaged as one composite region	180	11%
Human breast	PR percent positive cells	15-20 averaged as one composite region	180	33%

# Emerging need for stereology

- Random 2D sampling is not enough
- Europe is leading in this area
- Stereology will be required in GLP settings and is already moving to the clinic in Europe
  
- See Bob Dunstan's webinar (Biogen-Idec) on use of stereology (June 9,2010)
  - [www.visiopharm.com](http://www.visiopharm.com)

# Slide Storage

## If you use image analysis, you will need to treat the slide as raw data.

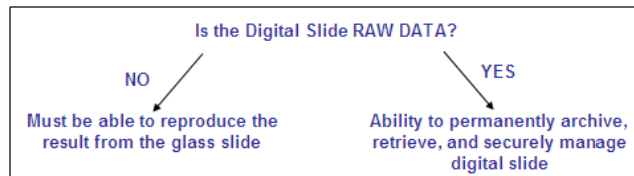
### Raw data and vendor responsibilities

There is substantial debate in the industry regarding the definition of raw data as it pertains to whole slide images.<sup>1,2</sup> The GLP regulations, 21 CFR Part 58, Section 7b state:

*“Only the signed and dated final report of the pathologist comprises raw data respecting the histopathological evaluation of tissue specimens.”*

*“... § 58.190(a) requires histopathological blocks, tissues, and slides to be retained as specimens. ... so final report can be reconstructed by ... by a second pathologist or by a team of pathologists.”*

However, with emerging expansion in the use of quantitative image analysis, the digital slide will be considered raw data because glass slides fade over time and the exact numerical data obtained upon reanalysis will not duplicate those obtained from the original digital image unless the slide can be stored so no tissue or staining degradation will occur. Vendors must prepare for this eventuality. There are a different set of responsibilities placed on the vendor depending on whether the digital slide is considered raw data.

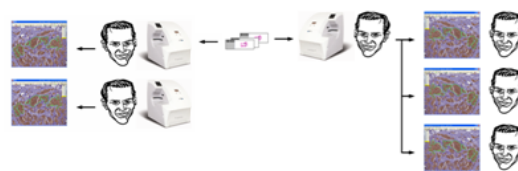


Different raw data assumptions mean different requirements from digital pathology solution providers. Technology must be available for both scenarios

### Reproducing the result from the glass slide

Regardless of whether the digital slide is considered raw data, there is agreement by all that the use of a digital image must result in the same histological diagnosis as the glass slide, or if image analysis is used, the result must be able to be recreated from the glass slide. The question of the ability to produce a consistent pathologic diagnosis was examined in depth in a multisite study with 780 IHC slides across three geographic sites.<sup>3</sup> The variability between 3 pathologists (interpathologist), between one pathologist at different times (intrapathologist), between 3 ScanScopes (interscanner), and between one ScanScope at different times (intrascanner) is listed below. These type of studies reinforce the concept that results from evaluation of a digital slide emulate those from a glass slide.

Measurement	Variability	SD
Inter-pathologist	Different pathologists	10.03%
Intra-pathologist	Same pathologist, different days	2.69%
Inter-scanner	Different scanners	0.80%



[Dunstan, Potts, NYAS Preclinical GLP 2007](#)

[Pyrrah, Blackmer, Potts, PhRMA Preclinical Session 2007](#)

# Outline

- Vendor validation gap with CLIA (clinical workflows)
  - Medical device manufacturer regulations for DP
  - Clinical lab regulations under CLIA
- Vendor validation gap with GLP (preclinical safety studies)
  - State of adoption of digital slides in preclinical
  - Peer review & image analysis
  - Validation required in GLP
- The path forward:
  - Preclinical: SOPs for in-house GLP image analysis
  - **Clinical and clinical trials: IA validation service for CLIA**

# Flagship training and IA validation services

- Requires a close dialog between image analysis experts and pathologists willing to embrace image analysis
- Completed a virtual training trial run: 6 week session (two hours each Thursday) on image analysis training (led by pathologists and IA experts)
  - <http://www.flagshipbio.com/products-page/virtual-instructor-led-training/>

# Flagship training and IA validation services

- CLIA image analysis validation service
  1. Multi-week session (two hours each Thursday on regulated image analysis concepts)
  2. 3<sup>rd</sup> party vendor independent validation of image analysis in a regulated setting
  3. Virtual exchange of digital slides and image analysis results between CLIA labs

## Guidance and Advice:

Dave Eberhard, MD (UNC / LabCorp)

Steve Schmechel, MD (Univ of Minnesota)

Hadi Yaziji, MD (Vitro Molecular)

Yaziji, H. et al. Appl Immunohistochem Mol Morphol. 2008 Dec;16(6):513-20. Consensus recommendations on estrogen receptor testing in breast cancer by immunohistochemistry.

- Please contact [pathservices@flagshipbio.com](mailto:pathservices@flagshipbio.com) for further information

# Conclusions

- We've reviewed the vendor validation gap with CLIA (clinical workflows)
  - Medical device manufacturer regulations for DP
  - Clinical lab regulations under CLIA
  - NEXT STEPS: IA validation service
- We've reviewed the vendor validation gap with GLP (preclinical safety studies)
  - State of adoption of digital slides in preclinical
  - Peer review & image analysis
  - NEXT STEPS: In-house SOPs for GLP IA, Stereology

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