Creating Collaborative Quality: Implementing Digital Pathology and Improving the Histotechnology Process
Speakers

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Creating Collaborative Quality

This workshop will address the issues and barriers that can destroy the implementation of a digital pathology solution into today’s Histology laboratory

• Gain an understanding of the importance of standardization and process improvement in the pre-analytical steps
• Learn to apply appropriate quality and process improvement techniques with an emphasis on digital pathology solutions
• Understand the value of quality improvement to the implementation process
What is Collaborative Quality?

- Reduction of Variables
- Data, Metrics and Measurement
- Process Analysis
- Process Structure
- Interoperability
Why Collaborative Quality?

- Conflict of Need and Current Processes
  - Shift in Specificity and Sensitivity
  - No shift in Process
  - Demand for Patient Centric Care
Why Collaborative Quality?

- Conflict of Need and Current Processes
  - Product Improvement – block/slide/stain
  - Process Improvement – procedure/standardization
  - People Improvement – training/competency
Why Collaborative Quality?

- Process Management
  - Standardization
  - Small Batch Processing
  - Continuous Work Flow
  - Continuous Improvement
Why Collaborative Quality?

• Nonconforming Event Management
  – Defect Identification
  – Documentation, Reporting & Analysis
  – Corrective Action Process
Why Collaborative Quality?

• Performance Management
  – Assessments
    • Internal
    • External
    • Technologists
    • Process
  – Accreditation
    • Proficiency Testing for Process
    • Performance Comparison
The Histology Laboratory Today

- The principles and techniques utilized have been around for 100’s of years since the 18\textsuperscript{th} century. How we perform routine and special stains has not changed much.
- The technology that we currently use has improved but has not been standardized between laboratories and not all labs are utilizing newer technologies.

- Reagents and Tools
- Equipment and Automation
- Barcoding

Minimal standardization of Pre-Analytic Variables and NOT well defined Interlaboratory Standards
The Histology Laboratory of the Future

• Technology and Automation will drive QUALITY
• More CLOSED systems
• Interoperability of results
• Collaboration of Pathologist, Technologist and Technology
• Meet the NEEDS and REQUIREMENTS of Patients

Empirical standardization of Pre-Analytic Variables and Interoperability of Results
The Histology Laboratory of the Future

• Move from Quality Control to Quality Assurance
• Systematic Requirements
• Validation of ALL Processes
• Documentation of Process
• Results WILL meet the Patient Requirements
The Histology Laboratory of the Future

• Control the Cost of Quality
  – Cost of Quality
    • Controllable – direct costs to ensure that only acceptable products and services reach the customer
    • Equipment - costs to invest
    • in equipment to measure, accept, or control a product or service

\[
\text{COQ} = \text{Cost of Units Produced (C x #units)} = \text{COQ}
\]

\[
\text{COPQ} = \text{Cost of Unit (C x #units) + Cost of Rework (Cx4 x #units)} = \text{COQ}
\]
The Histology Laboratory of the Future

• Controlled Histology/Pathology Method
  – Combined Quality Processes with Technology
    • Product Improvement – block/slide/stain
    • Process Improvement – procedure/standardization
    • People Improvement – training/competency
    • Consistency thru STANDARDIZATION
  • Patient Focused
  • Pathologist Satisfaction
  • Employee Satisfaction
  • Defined Method for Implementing Technology
One of the most important aspects to the successful implementation of a Digital Pathology Solution is the overall quality and consistency of the histology preparations. Changes need to be made to bring the Histology Laboratory to where it needs to be today and for the future.

1. Understand that there is variability in our process
2. Define where there is variability in our process
3. Understand how that variability effects what we do
4. Determine the limits – what is acceptable and what is unacceptable in our process – a range of
Expressing Histology in a New Way

- Accuracy
- Precision
- Specificity
- Robustness
- Range
- Quantification Limit
- Detection Limit
- Reference Standard
- Repeatability
- Linearity

These are our NEW TERMS

We need to define and understand these terms and how they apply to the histology process

This is what the lab of the future needs to determine and monitor

Digital Pathology IS the tool we can use
Where is our Critical Variability??

- Cold Ischemic Time
- Fixation Time
- Processing
- Embedding
- Sectioning
  - Thickness
  - Placement
  - Levels
- Staining
  - Routine
  - Special
  - Immunohistochemical

We need to ask ourselves the following questions:

What are the critical steps in the histology process?

How can we decrease the variability of those steps?

1st STEP – DEFINE STANDARDS!
Define standards for Histology Specimen Collection and Preparation – Pre Analytical Steps

Team Approach – include pathologists, grossing technicians, specimen entry individuals, possibly even nursing staff (individuals who collect the samples) etc., during this process.

- Start with your Nursing Manual – it should include basic sample collection guidelines on amount of fixative, time to fixative, etc.
- Lots of consults? – your reading slides prepared elsewhere – they need to be educated on your slide preparation standards

This applies to all use cases - research, clinical and education

To include defined **SPECIFIC** GUIDELINES for all aspects of the Pre analytical Steps.

- Specimen collection, Grossing, Processing, Embedding, Sectioning and Staining
Section Placement Defined

Basic Guidelines - center of the slide – not near any edges of the slide, vertical position.
Defined for each sample type – dependent upon scanner.
My preference is to have samples embedded in a row, I believe that this creates a better quality slide and also makes reviewing the slide easier for the pathologist or researcher.

If multiple samples are embedded in one cassette, each cassette is embedded identically for an entire study (this is more research based).
Define Work/Specimen Instructions

These are specific guidelines created for a particular specimen type that contain the following with multiple images:

- What paperwork is filled out
- How to label the block
- How to label the slide
- How to gross the sample
- What processing cycle is used
- How the sample is embedded
- How to section the samples
  - Section thickness
  - Section placement on slide
  - Number of sections per slide
  - Waterbath temp if required
  - How slides are dried
- What stains are used
- If slides are scanned or not
- Quality Control – how to evaluate the slides
Cassette Labeling:
- Premier study number: 2010-045
- Client study number: 02210-TP1
- Group and animal number: NV1-OS

Grossing:
- Place eye in 6mm spherical tissue mold with mark facing up. If the eye is too small for mold, place on edge of mold, hold gently with fingers and trim tissue.
- Use low profile microtome blade.
- OD: Place the eye with optic nerve facing away from you and cut on the right side.
- OS: Place the eye with optic nerve facing towards you and cut on the right side.
- Place the eye cut side down in cassette, place in 70% alcohol.
Rat Eyes

Processing:
- Cycle 2 (same day processing).
- Use propar as a replacement for xylene.

Embedding:
- Remove air-bubbles from tissue by gently rolling lens in paraffin before embedding.
- Place eye cut side down.
- OD: Place the eye with optic nerve facing towards you and place the cassette top to the left side.
- OS: Place the eye with optic nerve facing away from you and place the cassette top to the left side.

Slide Labeling (use Plus slides):
- Premier study number
- Client study number
- Animal number
- Slide number
- Stain

Sectioning:
- Collect Mid Ocular sections with optic nerve present in all sections.
- Collect 7 slides with 3 sections/slide (unless specified otherwise).
- DO NOT melt wax from unstained slides.
Staining:
- H&E staining of slides #1, #4, and #7 (unless specified otherwise).

(This sectioning and staining procedures are the most common ones and may be different depending on the researcher’s request.)

Quality Control:
- Check if optic nerve is visible.
- Check the quality of staining.
Quality Control and Standardization – H&E

What is acceptable and what is not???

“The Pathologist Variable”

- accept a wide range of staining intensity and variation
- no defined standard for the H&E
- every lab's H&E is different

Will this continue to be acceptable as technology moves forward and additional digital pathology solutions are utilized??

NO, NO and NO!
Quality Control and Standardization – H&E

What is acceptable and what is not???

“The Role of Technology”

• create defined and narrow standards
• use the empirical rule (68-95-99)
• strict acceptable standards for the H&E
• every lab will participate and be the same

This change will allow new technology and increased automation to move into the process and move forward additional digital pathology solutions!!

YES, YES and YES!
H&E Staining Protocol Variation – Rat Eye (5 microns)

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Hematoxylin Time – Hematoxylin Normal</th>
<th>Differentiation Time – 5% Glacial Acetic Acid</th>
<th>Eosin Time – Eosin Y - Alcoholic</th>
<th>Alcohol Time</th>
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<tr>
<td>0</td>
<td>30 seconds</td>
<td>10 minutes</td>
<td>10 seconds</td>
<td>50% - 5 minutes</td>
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<tr>
<td>1</td>
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<td>3 minutes</td>
<td>30 seconds</td>
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<td>1 minute</td>
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<td>6 minutes</td>
<td>30 seconds</td>
<td>3 minutes</td>
<td>95% - 30 seconds</td>
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<td>6</td>
<td>8 minutes</td>
<td>30 seconds</td>
<td>5 minutes</td>
<td>95% - 30 seconds</td>
</tr>
<tr>
<td>7</td>
<td>15 minutes</td>
<td>30 seconds</td>
<td>15 minutes</td>
<td>95% - 30 seconds</td>
</tr>
<tr>
<td>8</td>
<td>20 minutes</td>
<td>0 seconds</td>
<td>20 minutes</td>
<td>95% - 0 seconds</td>
</tr>
</tbody>
</table>

This study was designed to test the robustness of an H&E algorithm

Note: Red Text – standard H&E protocol
All slides were stained on a Sakura Tissue-Tek® Prisma™ Automated Slide Stainer
Reagents – Anatech – Hematoxylin Normal Strength and Eosin Y – Alcoholic, differentiated with 5% aqueous glacial acetic acid blued with 0.5% aqueous ammonium hydroxide

@dpatweet
#PV14
What Did We Learn??

• We were pretty surprised with the results
• Our current H&E staining reagents were quite robust (protocols created were not ordinary, 1 minute changes will not have much of an affect)
  – Poor quality H&E is not necessarily related to time in reagents but more due to overuse of reagents or poor quality control
  – Poor quality H&E is more related to improper placement of reagents in stainer.
  – Poor quality H&E is related to poor fixation and processing
Monitoring H&E Staining over Time

Traditionally we manage stain QC via a PASS/FAIL or YES/NO – “did it work or not” – Need to move beyond that, utilizing the technology (WSI) that we have……

• Study Design - stain several sections over the course of how often we changed our H&E stainer
• Example:
  – We change our stainer every 2 weeks – we are a low volume lab with respects to H&E.
  – Ran two slides daily – one human and one animal tissue
  – Utilized a Multi-tissue block
• Scan, Review and the next step would be to Analyze
Section Thickness

Critical Parameter that we have not really looked that closely at.

1. Can be extremely variable
   – Microtome, condition of microtome, etc.
   – Knife blade (new or old)
   – How cold the block is*
   – Turning the microtome wheel
   – Individual technique, use of freeze spray, blowing on the block

2. Study Completed – told to collect 4 micron sections when analyzed - actually ranged from 3 to 8 microns

* Clinical verses Research, number of sections collected per block, is image analysis involved
Section Thickness needs to be Consistent – Decrease this Variable

Two levels of same block of mouse pancreas
Same slide, same staining run
Sectioning error – leads to differences in staining intensities
Her 2 Staining and Section Thickness

3 microns – I_avg = 97

4 microns – I_avg = 110

5 microns – I_avg = 114

6 microns – I_avg = 124

@dpatweet #PV14
Variable Staining and/or Variable Section Thickness
Other WSI Use Cases in Histology
Sure we can monitor positive control staining and consistency via visual assessment but why stop there………………

Use the analysis tools available to generate numbers that can be statistically tracked over time. This will allow us to determine staining precision (consistency) and if staining is drifting over time.

We can use this technology as part of the IHC verification process by gaining numerical data on antibody or even detection system lot numbers.

We can even use this as a way to evaluate the consistency of staining between labs or between stainers or staining platforms.
Tracking IHC Positive Control Staining over Time
Image Analysis – Positive Pixel Count
### Expressing the Data

<table>
<thead>
<tr>
<th>Slide ID</th>
<th>Stain</th>
<th>Algorithm</th>
<th>Isp = Total Intensity of Strong Positive</th>
<th>Iavg = (Iwp+Ip+Isp)/(Nwp+Np+Nsp)</th>
<th>Positivity = NPositive/NTotal</th>
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Utilized 3 different skin control blocks over the course of 2 years

Scanned all control slides

Analyzed both entire image and select annotation area on a subset utilizing a positive pixel count algorithm

Graphed the $I_{avg}$ – Average intensity of the positive pixels as a Levy Jennings Chart
Area of Interest Over Time
Levey Jennings Chart 1

Laminin IHC - Human Skin (Selected Annotation Area)

Average Intensity

Control Over Time

UCL 186.181
CL 166.652
LCL 147.122

140 145 150 155 160 165 170 175 180 185 190
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

@dpatweet

#PV14
Levey Jennings Chart 2

Laminin IHC – Human Skin (Whole Image)

Average Intensity

Control Over Time

UCL
172.837

CL
155.356

LCL
137.876

132
137
142
147
152
157
162
167
172
177

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
Levey Jennings Chart 3

Laminin IHC – Human Skin – 3 different controls
(Selected Annotation Area)

Average Intensity

Control Over Time
Neurofilament – Same Control Block Over 2 years

Neurofilament IHC – Selected Annotation Area

Average Intensity vs. Time

- UCL: 167.8100
- CL: 162.2406
- LCL: 136.6713

Time:
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55

Average Intensity:
131.48 136.48 141.48 146.48 151.48 156.48 161.48 166.48 171.48
Other Methodologies

- Compare new lots of antibodies
- Compare the performance of two different stainers
- Compare the performance of two different laboratories
Other Methodologies

- Compare patient results
- Compare technologist performance
- Compare pathologist performance
- Compare and ensure performance of the process
Study Design – Sea Level to Altitude

• 24 different antibodies including 1 isotype control
  – range of cytoplasmic, membrane and nuclear localization
  – both low and high pH retrieval solutions
• Run multiple tissue blocks specific to each antibody that would cover a variety of staining intensities and patterns
• Scan slides and run image analysis
• Data analysis – Simple Linear Regression
Annotation Layers
# Simple Linear Regression Analysis

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## Statistics

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<tr>
<td>Fdist</td>
<td>0.000000</td>
<td>probability of higher F occurring by chance</td>
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</table>
Percent Positive Pixel Counts: Sea Level Vs. High Altitude

\[ y = 0.95x \quad R^2 = 0.93 \]

Intensity of Positive Pixels: Sea Level Vs. High Altitude

\[ y = 0.97x \quad R^2 = 0.99 \]
IHC protocol development

Same tissue and target
Different antibody clones and vendor
Able to review multiple slides at the same time at the same magnification
Basic Measurements and Counts
• Scan and maintain all documentation on IHC protocol development, initial verification/validation and new lot verification.
  – Most commercially available databases can upload documents into the same case folder that the images are stored in.

• Scan and maintain all positive controls for both special and IHC staining.

• Scan and maintain all H&E quality check slides.
Creating Collaborative Quality

- Standardization is essential to implementation of new technology
- Once implementation is completed, incorporate the new technology into the continual improvement process
- Merging the new technology with the process creates incremental change, but breakthrough may happen
- Incorporating new technology into the continual improvement process supports core elements
Creating Collaborative Quality

• Incorporating new technology into the continual improvement process supports the essential elements of
  – Efficiency
    • Identification
    • Reduction
    • Elimination
  – Evolution
    • Self reflection of process
    • Effectiveness of delivery
  – Feedback
    • Process
    • Customer
Creating Collaborative Quality

• Incorporating new technology into the continual improvement process supports the essential elements of
  – Reduction of variables
  – Collection of data
  – Create measurement and metrics
  – Process improvement
  – Interoperability

• Digital Pathology is the technology that supports specific performance indicators to ultimately reduce overall costs
Acknowledgements

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